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Une Approche Computationnelle pour l'Etude de Processus Morphogénétiques

de la motilité des cellules à la croissance des vaisseaux

MÉMOIRE

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**Habilitation à Diriger des Recherches
de l'Université Joseph Fourier – Grenoble I**

par

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Résumé

Les processus morphogénétiques sont les processus qui sous-tendent l'émergence de la forme. Leur étude consiste à décrire, comprendre et expliquer comment les formes apparaissent et évoluent. Pour cela, une approche computationnelle basée sur la simulation numérique, c'est à dire sur la réalisation d'expérience *in silico* a été utilisée et appliquée à deux cas morphogénétiques en particulier.

Le premier cas a concerné la motilité cellulaire, c'est à dire l'étude des mécanismes à l'origine des mouvements et de la forme de la cellule. Une plateforme de modélisation hybride a pour cela été développée. Elle couple un modèle continu des déformations membranaires qui résultent de la dynamique spatio-temporelle de l'actine à un modèle discret de type automate cellulaire pour rendre compte de la nature discrète des adhésions et des fibres du cytosquelette de la cellule. Plusieurs situations expérimentales ont été considérées de la migration de la cellule aux formes cellulaires observées sur des substrats patternés (c'est à dire qui présentent des motifs adhésifs contrôlés) et ont permis de développer progressivement les différents éléments de la plateforme.

Le second cas morphogénétique considéré a concerné la croissance vasculaire à travers différentes approches. Une approche expérimentale a eu pour but de mettre en évidence l'impact de la rigidité du substrat sur la formation de cordons vasculaires *in vitro*. La seconde approche a porté sur le développement d'un modèle computationnel hybride pour rendre compte de la croissance vasculaire dans le contexte de l'angiogenèse tumorale. Le modèle hybride couple une formulation continue pour décrire les processus de diffusion des différentes espèces moléculaires impliquées (tels que les facteurs de croissance ou l'oxygène) à une formulation discrète pour décrire la migration individuelle des cellules endothéliales formant les vaisseaux. La structure modulaire du modèle computationnel a de plus permis d'intégrer les mécanismes d'adaptation du diamètre des vaisseaux qui résultent des contraintes hémodynamique, c'est à dire liées au flux sanguin.

La plateforme computationnelle a été développée par la suite pour intégrer un module de croissance tumorale et un module sur les moyens d'actions thérapeutiques, dans le cadre du projet CATS pour Computer-Assisted Therapeutic Strategy. L'ambition de ce projet en cours de développement, est de mettre au point une tumeur virtuelle destinée à tester et à optimiser de nouveaux protocoles thérapeutiques.

Mots clés : morphogénèse, motilité, angiogenèse, tumeur virtuelle, systèmes complexes, modèles hybrides, plateforme computationnelle, simulation numérique.

"Systems biology...is about putting together rather than taking apart, integration rather than reduction. It requires that we develop ways of thinking about integration that are as rigorous as our reductionist programmes, but different....It means changing our philosophy, in the full sense of the term"

Denis Noble

1

Introduction générale

L'objet de ce premier chapitre est de présenter la thématique de recherche, ainsi que les concepts et les méthodes qu'elle utilise. Je me suis en particulier référée pour cette présentation aux ouvrages "L'invention des formes" d'Alain Boutot et "Vie artificielle - ou la biologie rencontre l'informatique" de Jean-Philippe Rennard. Je me suis également référée à la thèse sur la "Simulation des phénomènes complexes en Oncologie" de Gabriel Querrec, soutenue à l'Université de Bretagne Occidentale en 2005, qui donne une présentation très complète des systèmes complexes en biologie.

1.1 Les processus morphogénétiques

1.1.1 Définition et exemples

Les processus morphogénétiques sont les processus qui sous-tendent l'émergence (*genèse*) de la forme (*morpho*). Leur étude consiste à décrire, comprendre et expliquer comment les formes apparaissent et évoluent. La morphogénèse est l'élément clé du développement et donc de la biologie, c'est pourquoi sa définition est souvent réduite au seul contexte de l'embryogenèse. Dans ce mémoire, je ferai référence à la définition de ce terme dans son sens le plus général. Je l'appliquerai à tout élément biologique dont la forme change au cours du temps, c'est à dire qui présente une dynamique spatio-temporelle.

Je me suis intéressée à deux processus qui présentent cette propriété, *la motilité cellulaire* et *la morphogénèse vasculaire*. La motilité cellulaire désigne l'aptitude des cellules à se déformer, ce qu'elles font constamment pour maintenir l'intégrité de l'organe ou du tissu auquel elles appartiennent. Les cellules se déforment, migrent et se divisent pour s'adapter aux contraintes et à l'évolution de l'environnement. Dans le cas

de la motilité, la morphogénèse se situe à l'échelle subcellulaire dans la mesure où il s'agit de décrire la dynamique de molécules et de protéines qui contribuent à l'agencement de structures internes à la cellule et qui vont lui donner sa forme. Dans le cas de la morphogénèse vasculaire, nous nous situons à l'échelle supra cellulaire puisqu'il s'agit cette fois de décrire comment les cellules s'organisent les unes par rapport aux autres, migrent et se divisent pour former un réseau de vaisseaux. Le réseau vasculaire est une structure très dynamique avec d'une part l'apparition et la disparition des vaisseaux et d'autre part l'adaptation du diamètres de ces vaisseaux en fonction de l'évolution des contraintes versatiles qu'imposent le flux sanguin.

1.1.2 La biologie des systèmes complexes

L'étude des processus morphogénétiques a pour ambition de chercher à comprendre comment des éléments interagissent pour faire émerger une forme. *L'approche réductionniste* qui consiste à étudier individuellement les éléments d'un mécanisme pour en déduire le fonctionnement s'avère inadaptée dans le contexte de la morphogénèse. La raison en est que le comportement des éléments résulte des propriétés des interactions entre ces éléments plutôt que des propriétés des éléments eux-mêmes. On parle alors de propriété "émergente" lorsque le réductionnisme échoue.

L'approche adaptée pour traiter ce type de problème dit "complexe" est *l'approche systémique*. La biologie systémique consiste à aborder la biologie en tant que système et non comme une somme de composants indépendamment les uns des autres. C'est à partir de l'idée de rassemblement de composants et d'analyse des interactions que l'on cherche à comprendre la réalité.

L'étude de la morphogénèse s'inscrit dans celle des systèmes biologiques complexes. Les *systèmes complexes* restent difficiles à définir mais ils possèdent au minimum la propriété *d'émergence*, c'est à dire que la seule connaissance des composants du système ne permet pas de prédire l'observation. On attribue également aux systèmes complexes la propriété connexe *d'auto-organisation*. Elle traduit le fait que le système voit son entropie décroître et sa structure et son organisation se compliquer. L'auto-organisation trouve sa source dans la non-linéarité des processus et dans les bifurcations associées qui permettent à travers des mécanismes de transition de phases d'atteindre un état macroscopique stable. Enfin on peut ajouter les propriétés *de robustesse* et *d'adaptabilité*. La première traduit la capacité du système à conserver ses propriétés fonctionnelles lorsqu'il est soumis à différentes perturbations *via* des mécanismes de rétroaction. Alternativement l'adaptabilité permet au système d'ajuster son comportement ou sa forme aux conditions changeantes de l'environnement.

De part ses propriétés, un système complexe ne peut être mis en équations solvables et prédictives. Le système se définit en général à partir d'un grand nombre de paramètres dont chacun peut avoir une influence essentielle sur son comportement. Celui ci ne peut donc être prédit autrement que par l'expérience ou la simulation. La

biologie des systèmes fait appel à des méthodes mathématiques et à des outils informatiques pour modéliser les processus biologiques et concevoir de nouvelles expériences. Ce sont ces méthodes que je décris à présent.

1.2 L'approche computationnelle

1.2.1 Définition

Le terme "computationnel" fait référence à l'outil informatique. *L'approche computationnelle* se distingue donc de *l'approche mathématique* dans le sens où elle requiert nécessairement l'assistance d'un ordinateur. L'approche mathématique s'inscrit cependant dans le cadre des approches computationnelles puisque les équations mises en jeu pour décrire la plupart des processus biologiques impliquent de nombreux termes non-linéaires qui font que les systèmes d'équations à résoudre n'ont en général pas de solutions analytiques. Il est donc nécessaire d'utiliser la simulation numérique pour faire émerger ces solutions non prédictibles. La simulation numérique et l'informatique sont ainsi devenues des outils incontournables de la biologie systémique souvent renommée biologie computationnelle.

Du fait de l'omniprésence de l'outil informatique, nous maintiendrons la distinction entre l'approche mathématique et l'approche computationnelle par le fait que la première reste fondée sur un formalisme d'équations tandis que la deuxième peut s'en passer au moins partiellement. Pour cela l'approche computationnelle se fonde sur l'énoncé de *lois d'inférence*, qui permettent de décrire les différentes étapes d'un processus menant au résultat. En d'autres termes, l'approche computationnelle se fonde sur la définition d'un algorithme.

Un exemple typique de structure computationnelle est présenté dans la figure 1.1. Dans cette structure des modules de détection (en rouge), de décision (losanges gris) et d'action (en vert) sont définis et mis en relation pour conduire à une réponse. Le module de détection sonde l'état du système et/ou les conditions de l'environnement. Le module de décision pose une question dont la réponse est soit binaire (oui/non), soit donne lieu à la sélection d'une option parmi un choix d'options possibles pré-définies et discriminées par un seuil (par exemple une température, une concentration, etc). Enfin le module d'action ou module effecteur est un module qui traduit un changement d'état, qui enclenche un processus ou qui produit un résultat (soit intermédiaire, soit final). Une telle structure computationnelle est à la base du fonctionnement des automates cellulaires.

1.2.2 Les automates cellulaires

Les automates cellulaires peuvent être considérés comme une idéalisation mathématique d'un système physique dans lequel l'espace, le temps ainsi que les quantités physiques sont discrets. Les dynamiques des automates cellulaires sont interprétées

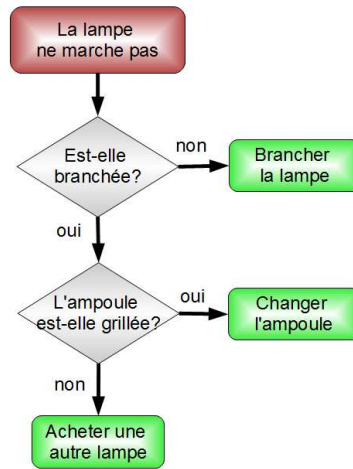


FIGURE 1.1: Exemple de diagramme d'inférence

en termes de règles microscopiques simples, capables d'exhiber le même comportement macroscopique que le système réel étudié. Ils offrent ainsi une approche intuitive et puissante pour représenter des phénomènes complexes, difficiles voire impossibles à modéliser par les approches classiques (équations différentielles ordinaires, équations aux dérivées partielles, etc). En outre, les automates cellulaires présentent un gros avantage du point de vue numérique à savoir, une bonne adéquation aux architectures des ordinateurs et des machines parallèles. Le fait de travailler avec des quantités booléennes prévient des instabilités numériques puisque les calculs se font d'une manière exacte. Il n'y a pas non plus de troncature ou d'approximation dans les dynamiques elles mêmes [1].

L'élément de base d'un automate cellulaire est la cellule. L'état de chaque cellule est une variable qui prend ses valeurs dans un ensemble fini de valeurs admissibles. Dans le cas le plus simple, chaque cellule peut avoir l'état binaire 1 ou 0. Dans des situations plus complexes, les cellules peuvent avoir différents états. Les cellules sont arrangées dans un réseau spatial régulier appelé espace cellulaire qui peut être une ligne dans le cas (1D), une grille dans le cas (2D). Dans le cas 2D, les cellules sont souvent arrangées selon un réseau rectangulaire mais d'autres arrangements (en cellules carrées, triangulaires ou hexagonales) sont parfois utilisés. Chaque cellule possède un voisinage qui correspond à l'ensemble des cellules voisines qui interagissent avec elle. L'état d'une cellule est mis à jour de manière synchrone à chaque pas de temps en utilisant un ensemble de règles qui décrivent les transitions d'états de l'instant t au temps $t + 1$. Ainsi l'état du réseau entier avance selon des pas de temps discrets.

L'un des exemples le plus célèbre d'automate cellulaire est "le jeu de la vie" imaginé par John Horton Conway en 1970. Cet automate assigne à chaque cellule l'état binaire 1 ou 0, signifiant que la cellule est soit vivante, soit morte. Des règles sont définies et déterminent l'évolution de la population, laquelle dépend de l'état initial qui a été défini. A chaque étape, l'évolution d'une cellule est entièrement déterminée par l'état

de ses huit voisines de la façon suivante (Fig. 1.2) :

- une cellule morte possédant exactement trois voisines vivantes devient vivante (elle naît).
- une cellule vivante possédant deux ou trois voisines vivantes le reste, sinon elle meurt.

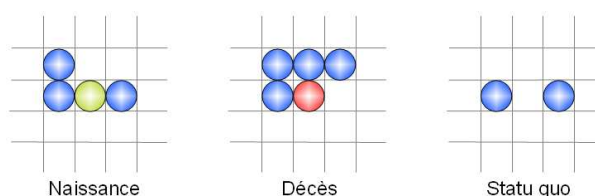


FIGURE 1.2: Conséquences des règles d'évolution du jeu de la vie

Malgré ces règles très simples, le jeu de la vie permet le développement de motifs extrêmement complexes.

1.2.3 Les modèles hybrides

Les modèles hybrides sont des modèles qui couplent des formalismes discrets (automates cellulaires) et continus (équations différentielles ordinaires, équations aux dérivées partielles, etc). En fonction du problème considéré, un formalisme peut être plus adapté que l'autre. On observe en général que les processus macroscopiques ont une nature plutôt continue tandis que les processus à plus petites échelles font ressortir la nature discrète. En biologie, l'intégration des niveaux d'échelles de la molécule à l'organe est indispensable.

La nature intrinsèquement multi-échelle des systèmes biologiques, à la fois dans l'espace, avec la multiplicité des niveaux d'organisation (molécule, cellule, tissu, organe) et dans le temps, rend extrêmement difficile de les modéliser de façon uniforme. C'est pourquoi les techniques de modélisation hybrides se sont particulièrement développées dans le contexte de la biologie systémique. Les modèles hybrides ont ainsi été développés et appliqués avec succès pour décrire les réseaux génétiques, les réactions biochimiques, les voies de signalisation, l'électrophysiologie cardiaque ou encore la croissance tumorale.

Je me suis intéressée pour la première fois aux modèles hybrides dans le contexte de la motilité cellulaire. Durant ma thèse, j'ai cherché à modéliser les mouvements membranaires d'une cellule déposée sur un substrat 2D, c'est à dire telle qu'on peut les observer dans les cultures *in vitro*. J'ai utilisé pour cela un modèle continu pour décrire la dynamique spatio-temporelle de l'actine dont la densité locale conditionne les mouvements de la membrane. Le modèle continu s'est révélé efficace sur ce point mais ne permettait pas de rendre compte des déplacements occasionnels de la cellule. Ceux-ci résultent de la formation d'une adhésion ponctuelle qui bloque le mouvement

de la membrane pour servir de point d'appui à la cellule. L'adhésion, qui est un entité ponctuelle à l'échelle de la cellule, si elle ne peut être décrite dans le formalisme continu, peut l'être en revanche très facilement dans un formalisme discret.

Le développement d'un modèle hybride pour rendre compte des divers aspects de la motilité cellulaire est ainsi présenté au chapitre suivant (chapitre 2). J'ai par la suite contribué au développement de modèles hybrides dans le contexte de la croissance vasculaire et en particulier de l'angiogenèse tumorale. Travaux que je présenterai dans le chapitre 3. Enfin dans le dernier chapitre (chapitre 4), je présenterai mon travail en cours, où la puissance des modèles hybrides pour aborder des problèmes complexes est mise en application dans le projet CATS pour "Computer Assisted Therapeutic Strategy". Ce projet a pour but de développer une tumeur virtuelle implémentée dans une plateforme de simulation et destinée à tester et optimiser de nouveaux protocoles thérapeutiques.

“Cell and tissue, shell and bone, leaf and flower, are so many portions of matter, and it is in obedience to the laws of physics that their particles have been moved, moulded and conformed. ... Their problems of form are in the first instance mathematical problems, their problems of growth are essentially physical problems, and the morphologist is, ipso facto, a student of physical science.”

D’Arcy Thompson, On Growth and Form (1917)

2

Motilité Cellulaire

2.1 Introduction

Le terme *motilité* désigne la capacité d’un élément à produire des mouvements. Ce terme se distingue du terme *mobilité* qui désigne le simple fait qu’un élément bouge. La motilité est cruciale puisqu’elle conditionne le bon ou mauvais fonctionnement de la cellule. Sur le plan physiologique, la migration cellulaire est primordiale dans l’embryogenèse ou dans les processus de réparation tissulaire (cicatrisation). Sur le plan pathologique, les processus d’inflammation ou d’invasion des cellules cancéreuses sont des exemples de comportements anormaux des cellules liés à un défaut dans la motilité.

La cellule est un objet complexe par excellence, qui peut être cependant grandement simplifié pour aborder le problème de la motilité. Celle-ci est essentiellement due au cytosquelette d’actine, où la dynamique de l’actine influence de plusieurs façons les forces de protrusion et de rétraction qui agissent sur la membrane cellulaire pour la déformer. La polymérisation des filaments d’actine peut créer une force protrusive sur la membrane [2]. Réciproquement les fibres d’actine tirent sur la membrane sur laquelle elles sont ancrées, grâce à leur propriété contractile que leur confère leur association avec la myosine [3]. Par conséquent, décrire la motilité consiste essentiellement à décrire les interactions entre le cytosquelette d’actine et la membrane cellulaire et les processus associés de régulation de ces interactions en relation avec les contraintes imposées par l’environnement.

L’interaction avec l’environnement, c’est à dire avec la matrice extra cellulaire, s’effectue à travers les adhésions. Les protéines transmembranaires que sont les intégrines interagissent avec les protéines de la matrice, comme la fibronectine ou le collagène,

pour établir le lien entre le cytosquelette et l'environnement, c'est à dire entre les milieux intra et extra-cellulaires. Les mouvements cellulaires se trouvent ainsi directement influencés par les propriétés physicochimiques de la matrice. De nombreuses études ont porté sur les effets de ces propriétés. Nous nous sommes nous mêmes particulièrement intéressés au rôle de la rigidité et de la topographie (relief) de l'environnement et avons démontré leurs effets différentiels entre deux lignées de cellules de type fibroblastique l'une normale et l'autre tumorale. La lignée tumorale s'est révélée beaucoup moins sensible à son environnement. Ce manque de sensibilité a été interprété par une difficulté pour les cellules à former des adhésions [4].

Sur la base de ces brèves considérations biologiques, que nous développerons par la suite, il est clair que modéliser la motilité cellulaire implique d'intégrer les éléments permettant de décrire les dynamiques d'évolution du cytosquelette et des adhésions et de les mettre en relation avec les informations en provenance de l'environnement. Bien que les éléments clés de la motilité soit identifiés, la description des différents processus impliqués reste difficile dans la mesure où ces processus se produisent à des échelles spatiales et temporelles très variées, depuis les interactions moléculaires aux échelles de la microseconde et du nanomètre, jusqu'à la migration cellulaire aux échelles de la minute et du micromètre. Les événements qui se produisent à chaque échelle sont très complexes en eux-mêmes. Cela explique pourquoi ils sont souvent considérés indépendamment les uns des autres en particulier d'une échelle à une autre. Un nombre important de modèles ont été proposés au cours de ces dix dernières années, associés à une diversité d'hypothèses et d'approches théoriques fondées sur des formalismes de nature discrète ou continue. On note que les approches discrètes sont le plus souvent liées aux événements se produisant aux plus petites échelles [5, 6] tandis que les approches continues (équations aux dérivées partielles couplées) restent associées aux plus grandes échelles où les concentrations ou populations (masses) importent plus que les individus [7, 8]. Les nombreux et différents modèles sont par conséquent souvent très spécifiques se focalisant sur un élément central à la fois [9]. Jusqu'à présent, il n'y a eu que très peu de tentatives d'unification des différents modèles existants, qui demeurent juste différentes facettes du même problème.

Un défi important de la biologie computationnelle moderne, est de parvenir à interfacer ces différents modèles. Cela nécessite de construire des structures computationnelles permettant d'intégrer différents types d'algorithmes afin d'être en mesure de manipuler la complexité qui émerge des interactions entre les nombreux processus de nature variée (biochimique, biomécanique, etc.) C'est à dire afin de générer des modèles d'ordre supérieur (plus intégrés). L'utilisation d'une structure modulaire offre la possibilité de traiter les différents processus soit de façon indépendante, soit de façon synergique. De telles structures modulaires sont particulièrement flexibles, puisque chaque module peut être développé isolément, avec des niveaux de raffinement différents d'un module à l'autre. Certains modules peuvent aussi être assimilés à des boîtes noires si les mécanismes sont mal définis, en attendant l'acquisition future de connaissances pour tester et valider les hypothèses permettant l'illumination progressives de ces boîtes.

Notre travail a ainsi été motivé par le développement d'une telle structure computationnelle modulaire pour explorer divers aspects de la motilité cellulaire. Après une présentation plus approfondie des acteurs de la motilité (section 2.2), la colonne vertébrale du modèle est présentée (section 2.3). L'ajout de différents modules nous a permis ensuite d'étudier deux différents aspects de la motilité. Le premier concerne la migration cellulaire spontanée, c'est à dire non-induite par une stimulation (section 2.4). Cette étude permet de poser les bases du module dédié à l'adhésion. Le second aspect considéré concerne les déformations de cellules sur substrats patternés et permet de développer le module dédié à la maturation et à l'organisation des fibres du cytosquelette (section 2.5).

2.2 Les acteurs de la motilité

La motilité cellulaire repose au coeur des interactions du trio cytosquelette, adhésion et environnement. Le cytosquelette d'actine est formé de fibres dont l'organisation dépend de la formation et de la localisation des adhésions, elles-mêmes dépendantes des propriétés physicochimiques de l'environnement extra cellulaire (Fig. 2.1).

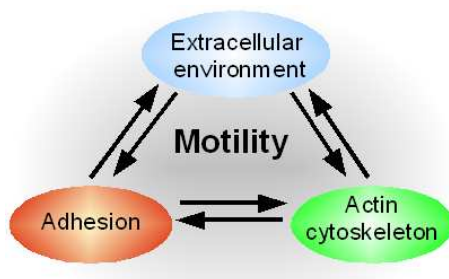


FIGURE 2.1: La motilité cellulaire résulte de l'interaction entre trois principaux acteurs : les adhésions, le cytosquelette d'actine et l'environnement extra cellulaire.

2.2.1 Les adhésions

Les adhésions sont formées par des complexes protéiques transmembranaires qui permettent de former un lien entre la cellule et son environnement qui comprend d'autres cellules et la matrice extra cellulaire. Plus que de simples liens physiques, les adhésions centralisent les signaux en les détectant, les coordonnant et les transmettant afin que la cellule réponde de façon adaptée. Les réponses cellulaires inadaptées sont ainsi souvent liées à un problème dans la cascade de signalisation. Notre étude est restreinte aux cas des adhésions avec la matrice extra cellulaire uniquement.

Les adhésions sont des structures très dynamiques qui évoluent en forme et en taille. Plus de 90 protéines distinctes ont été identifiées comme contribuant aux adhésions [11] certaines étant spécifiques d'un type d'adhésion particulier. Les différents types

d'adhésion peuvent ainsi être identifiés par leur composition moléculaire [12–14]. Trois types sont classiquement dissociés et constituent trois niveaux du processus de maturation (Fig. 2.2). Le type le plus primitif est le point d'adhésion ou précurseur d'adhésion (Adhesion Precursor, AP) qui se forme spontanément dès que les protéines transmembranaires que sont les intégrines entrent en contact avec les protéines de la matrice extracellulaire telles que la fibronectine ou le collagène. Les APs se forment préférentiellement à la périphérie cellulaire dans l'aire très motile qu'est le lamellipodium [15]. Ils ont une vie très éphémère en particulier lorsque le substrat *in vitro* n'est pas fonctionnalisé par des protéines matricielles. Les APs sont également très fragiles et se dissocient avec les mouvements de passage de la membrane. Ils peuvent également maturer en se regroupant pour former de petits agrégats appelés complexes focaux (Focal complex, FX) grâce au recrutement de protéines telles que la taline et la paxilline. Les FXs sont plus résistants et permettent l'ancrage de la membrane au substrat. Ils évoluent en recrutant progressivement de nouvelles protéines telles que l' α -actinine, FAK, VASP, Arp2/3 et vinculine. Ces protéines de signalisation et de liaison permettent aux adhésions de nucléer les filaments d'actine [16]. La stimulation mécanique de ces adhésions, sous la forme d'une tension croissante de la part des fibres du cytosquelette [17] induit la maturation des FXs en adhésions focales (Focal Adhesion, FA) qui s'opère *via* le recrutement additionnel de la zyxine et de la tensine. Cependant, une absence ou perte de tension sur les adhésions conduit au désassemblage de la structure protéique. Les FAs forment des points d'ancrage pour la cellule très solides, capables de supporter les fortes contraintes de traction développées lors de la translocation cellulaire. Ces points d'ancrage s'ils sont nécessaires à la migration peuvent également l'entraver si les adhésions sont trop fortes et ne cèdent pas à la translocation ce qui est le cas de l'adhésion fibrillaire. Celle-ci se développe à partir des résidus d'adhésions focales après translocation et se situe par conséquent sous la cellule dans des zones beaucoup moins périphériques que les autres types. L'adhésion fibrillaire n'est pas pour l'instant considérée dans notre modèle.

Note : Le terme "maturer" est utilisé tout au long de ce mémoire pour désigner le processus d'évolution progressif en plusieurs étapes distinctes des adhésions et des fibres.

2.2.2 Le cytosquelette d'actine

Bien que le cytosquelette cellulaire soit formé de trois types de filaments, les microtubules, les filaments intermédiaires et les microfilaments d'actine, seuls les microfilaments jouent un rôle important dans la motilité des cellules adhérentes. Les microfilaments mesurent de 1 à $2\mu\text{m}$ pour un diamètre de 5 à 7nm . La protéine d'actine dont ils sont faits est l'une des plus abondantes et représente environ 25% de la quantité totale des protéines cellulaires. Le filament d'actine est un polymère (actine-F) formé de monomères globulaires (actine-G). Les monomères s'assemblent pour former une double hélice de filaments. La polymérisation des filaments requiert des ions calcium et de l'énergie sous forme d'ATP. Les microfilaments sont polarisés avec une extrémité positive à partir de laquelle s'effectue l'assemblage des monomères aux filaments, c'est

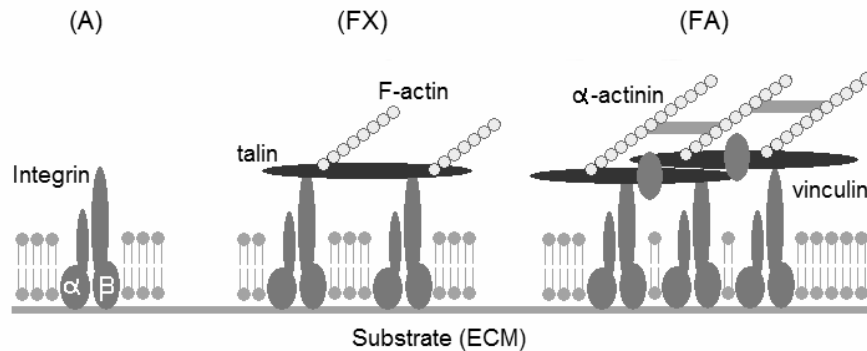


FIGURE 2.2: Trois types d'adhésion cellulaires sont distingués en fonction de leur niveau de maturation. Le premier type est le précurseur d'adhésion (A) formé par une unique intégrine. L'agrégation des intégrines conduit à la formation d'un complexe focal (FX). Il s'effectue par le recrutement de taline qui permet de connecter l'adhésion au cytosquelette cellulaire. Le recrutement ultérieur d' α -actinine et de vinculine (parmi beaucoup d'autres protéines) permet de structurer un complexe plus étendu qui résiste aux forces croissantes de traction. Il s'agit de la plaque focale (FA).

à dire la polymérisation et une extrémité négative de dépolymérisation. Dans la cellule, les filaments gardent une longueur plus ou moins constante à travers le processus de polymérisation / dépolymérisation où l'actine globulaire est immédiatement recyclée (actin turnover). De très nombreuses protéines sont associées à la dynamique de l'actine (Fig. 2.3) et à son organisation en une variété de structures : simple filament, réseau inter connecté de fibres ou câbles contractiles impliquant la myosine II et capables de générer des forces et de résister aux tensions extra cellulaires.

Les types de fibres

Les fibres du cytosquelette s'organisent en une variété de structures selon leur localisation, leur orientation (radiale ou transverse), leur taille, leur propriété contractile et leur connexion avec d'autres structures (autres fibres du cytosquelette ou adhésions). Trois types d'agrégats filamenteux ont été identifiés (Fig. 2.4) et décrits [19] :

les arcs transverses apparaissent dans le lamellipodium, c'est à dire à l'extrémité membranaire des cellules adhérentes. Ils se forment par l'assemblage de courts agrégats de filaments d'actine. La forte densité du réseau branché d'actine dans le lamellipodium, caractérisé par une dynamique rapide de polymérisation / dépolymérisation, contribue à générer de nombreux arcs transverses. L'incorporation de la myosine II dans les arcs transverses s'opère lorsque les arcs sont convectés par les flux cytoplasmiques en direction du centre de la cellule. Les arcs transverses ne forment pas de lien avec le substrat, néanmoins ils peuvent se lier à des structures qui le sont et contribuer ainsi à la génération de tension.

les fibres dorsales de tension résultent de l'élongation des filaments d'actine issus des adhésions focales. L' α -actinine qui est une protéine de liaison est alors impliquée

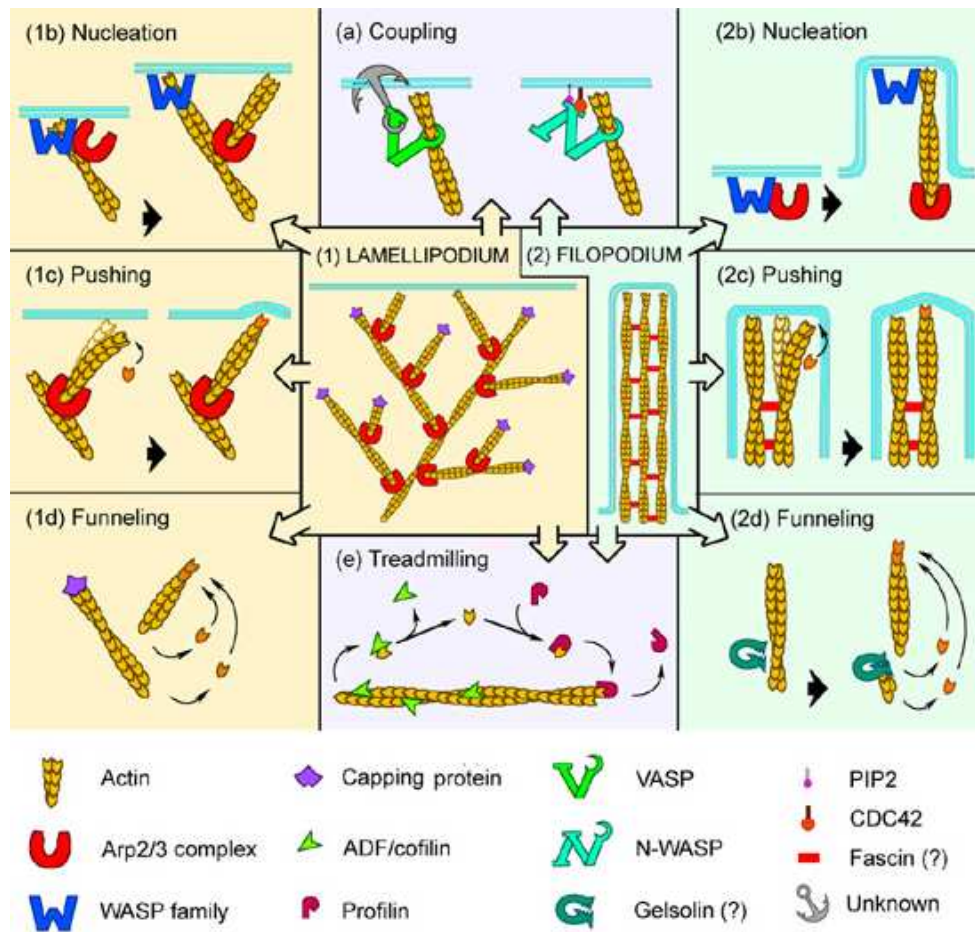


FIGURE 2.3: Aperçu des différents acteurs et de leurs rôles dans la dynamique et la structuration de l'actine. *Borisy and Svitkina, Current Opinion in Cell Biology, 2000.*

pour permettre l'agrégation des filaments. Une fibre dorsale est ainsi liée à une adhésion focale à une de ses extrémités tandis que l'autre extrémité s'allonge dans une direction préférentiellement radiale tout en s'élevant vers la face dorsale de la cellule [20]. Malgré le terme "de tension" associé à leur nom, les fibres dorsales ne sont pas contractiles. Cependant, elles transmettent la contractilité une fois connectée à une structure possédant cette propriété telle qu'une autre fibre contractile.

les fibres ventrales de tension formées principalement par l'addition de deux fibres dorsales. Les fibres ventrales sont par conséquent ancrées à leurs deux extrémités à une adhésion focale (Fig. 2.5) [21]. Ces fibres sont localisées sur la face ventrale de la cellule, c'est à dire la face en contact avec le substrat.

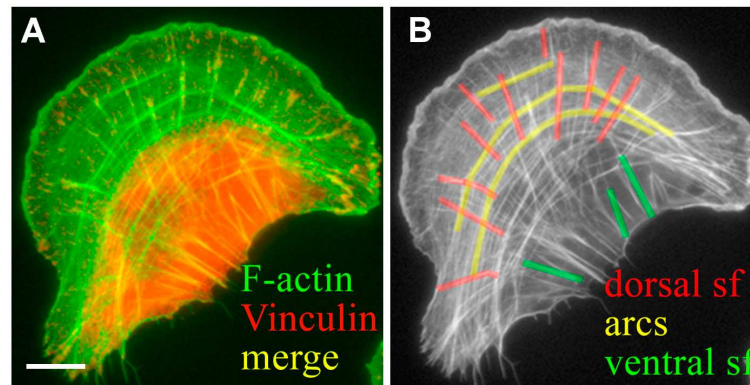


FIGURE 2.4: Visualisation des principales structures fibreuses d'actine : arcs transverses, fibres dorsales et ventrales. Le marquage de la vinculine identifie les adhésions focales. *Hotulainen and Lappalainen, Journal of Cell Biology, 2006.*

Actine et forme cellulaire

L'organisation de l'actine se traduit dans la forme de la cellule puisque c'est le cytosquelette d'actine qui sous tend la membrane (Fig. 2.5). Les deux structures les plus fréquemment observées sur la cellule en culture sur un substrat bidimensionnel sont le lamellipode et le filopode (Fig. 2.6). Le *lamellipode* est une large extension membranaire de faible épaisseur (moins de $0.5\mu\text{m}$) très riche en actine. Les filaments y sont organisés en un réseau planaire très dense formé de courts filaments inter connectés dont la direction principale pointe sur la membrane. Le lamellipode s'étend sous la pression exercée par la polymérisation des filaments. Cette structure est ainsi impliquée dans la migration cellulaire où le lamellipode s'étend *via* la polymérisation et se rétracte *via* les contractions du réseau d'actomyosine. Le filopode est une extension du lamellipode. Il s'agit d'un pic étroit de quelques micromètres de longueur formé par l'association de plusieurs filaments d'actine parallèles. Lamellipode et filopode sont des structures qui permettent à la cellule de sonder son environnement afin d'orienter ses déplacements vers le lieu le plus favorable.

2.2.3 L'environnement

L'environnement de la cellule détermine sa fonction et son devenir [22]. Il est aujourd'hui démontré que des cellules souches placées sur des substrats de rigidités différentes se différencient dans des lignées distinctes [23]. Les progrès récents dans les techniques de micro et nano fabrication ainsi que dans la chimie des surfaces permettent de construire des micro environnements cellulaires artificiels dont les caractéristiques mécanique, topographique et géométrique sont finement contrôlées. Plus spécifiquement, la capacité à manipuler la localisation et la forme des zones d'adhésion a permis d'accroître notre compréhension des mécanismes qui gouvernent la forme cellulaire [24]. De plus, la réalisation de substrats micro structurés avec des zones adhésives ponctuelles (discrètes) sous forme de micro-piliers adhésifs (Fig. 2.7) ou de patches

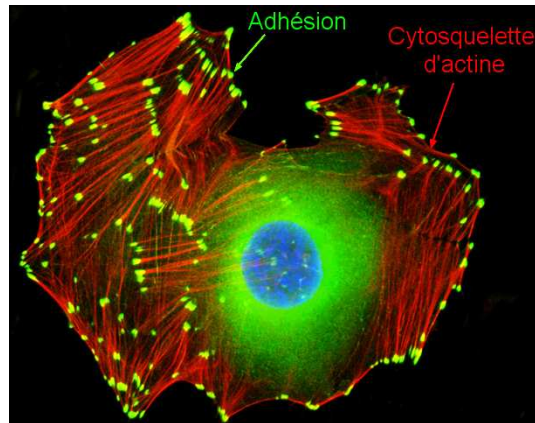


FIGURE 2.5: Fibroblaste 3T3 issu d'un embryon de souris, Olympus America Inc. Les structures d'intérêt sont rendues fluorescentes. L'ADN est marquée en bleu (sonde Hoechst 33342) et les filaments d'actine en vert (Alexa Fluor 488). Les adhésions focales apparaissent en jaune suite au marquage par anticorps de la vinculine. <http://www.olympusmicro.com/primer/techniques/fluorescence/gallery/cells/fibroblastindex>

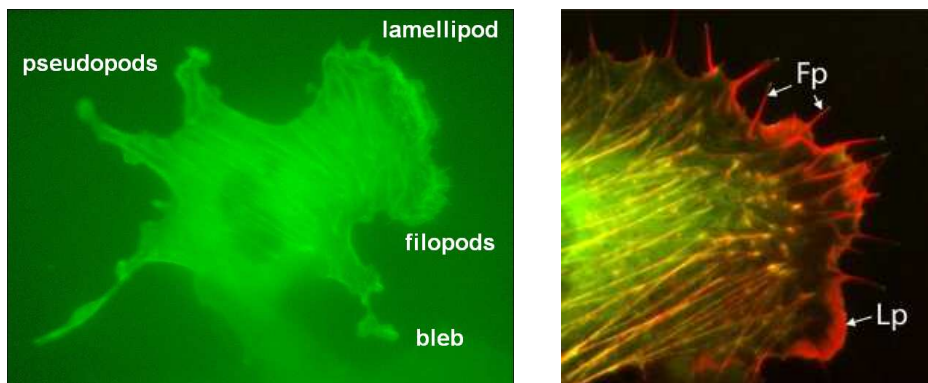


FIGURE 2.6: A droite, fibroblaste 3T3 dont le cytosquelette d'actine est rendu visible par un marquage à la GFP et qui montre les principaux types de déformation membranaires observables sur ce type de cellule (image réalisée par Olivier Collin, DynaCell, TIMC-IMAG). A gauche, image montrant les filopodes (Fp) qui se développent à partir du lamellipode (Lp). Vic Small, <http://www.imba.oeaw.ac.at/research/vic-small/research>

fonctionnalisés avec différentes protéines d'adhésion de la matrice intracellulaire (fibronectine, collagène, etc.) a permis de se rapprocher considérablement du contexte *in vivo*. Par exemple l'utilisation de patches adhésifs discrets a révélé la nature complexe de la protrusion cellulaire [25] : un filopode commence par croître jusqu'à atteindre une zone adhésive ; le filopode se consolide et la dynamique de l'actine déclenche la protrusion membranaire qui prend alors naissance à l'extrémité du filopode. La manipulation de la forme et de la taille des motifs adhésifs (formes en V, U ou T) montre comment les fibres de tensions du cytosquelette d'actine s'opposent à la tension membranaire en se formant au dessus des zones non adhésives (Fig. 2.8) [26, 27]. L'agrégation contrainte des adhésions focales sur les motifs adhésifs, qui forment les points d'ancrage des câbles d'actine, influence la polarisation de la cellule et sa forme. Ainsi, la structuration du

cytosquelette induite par la distribution des zones adhésives conditionne la réponse fonctionnelle de la cellule en influençant sa migration et/ou sa division.

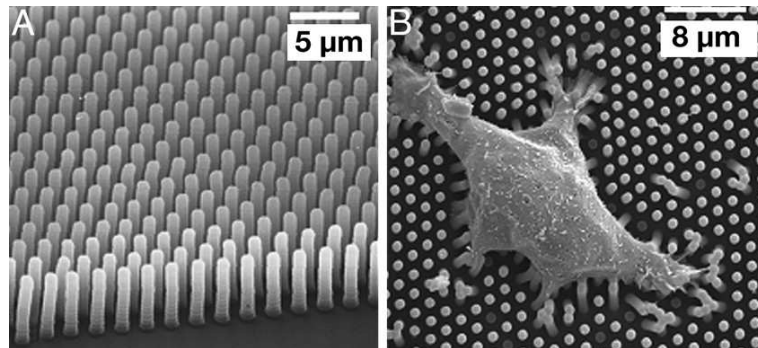


FIGURE 2.7: Tapis de picots déformables dont les déflexions permettent d'estimer les forces de traction exercées par les cellules. *Benoît Ladoux*, <http://www.msc.univ-paris-diderot.fr/lab513/Gallery.php>

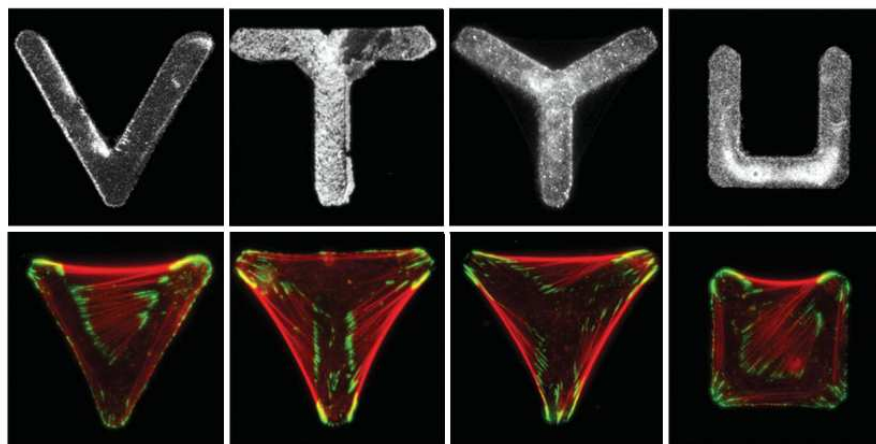


FIGURE 2.8: Motifs adhésifs qui permettent de mettre en évidence les schémas préférentiels d'organisation des fibres d'actine et des adhésions. *Théry et col. Cell Motility and the Cytoskeleton, 2006.*

2.3 Un modèle des déformations cellulaires

2.3.1 Hypothèses du modèle

Les déformations cellulaires résultent de la compétition entre forces de protrusion (dirigées vers l'extérieure de la cellule, F_{out}) et de rétraction (dirigées vers l'intérieure de la cellule, F_{in}) qui s'appliquent sur la membrane. Alors qu'il est clairement établi que la rétraction résulte de la traction des filaments d'actine ancrés à la membrane, à travers la contractilité du complexe d'actomyosine, l'origine de la force protrusive n'est pas complètement élucidée. Deux mécanismes ont été identifiés pour la protrusion, mais la façon dont ils interviennent l'un par rapport à l'autre n'est pas claire. Dans le premier mécanisme, les contractions cellulaires expulsent le cytoplasme contenu dans les pores du réseau cytosquelettique ce qui génère des flux cytoplasmiques qui poussent la membrane vers l'extérieur formant ainsi des excroissances membranaires (tel le bleb de la figure 2.6). Dans le second mécanisme, la polymérisation du cytosquelette d'actine au voisinage de la membrane crée une force qui pousse la membrane [2]. Dans ce cas, les fluctuations browniennes de la membrane suffisent pour permettre aux monomères d'actine de s'insérer entre la membrane et l'extrémité des filaments [28]. Il apparaît de plus en plus évident que ces deux mécanismes co-existent dans la cellule, l'un dominant l'autre selon l'état dynamique de la cellule. Ces états sont, soit l'état (passif) de repos caractérisé par des oscillations membranaires, soit l'état (actif) de migration où la cellule se polarise sous l'influence de facteurs externes. Par opposition, la migration passive s'effectue en l'absence de signaux explicites. Dans ce dernier cas, les deux mécanismes protrusifs se superposent et se manifestent par une alternance entre des phases migratoires rapides et orientées et des phases de déplacements lents et erratiques [29].

Le modèle général des déformations cellulaires étudié est dédié au cas migratoire passif, non induit (ou spontané). Le modèle computationnel proposé est basé sur la description de la dynamique intra-cellulaire de l'actine couplée aux mouvements de la membrane [30, 31]. Dans ce modèle, la polymérisation et la dépolymérisation de l'actine sont supposées auto-régulées autour d'une densité d'équilibre d'actine filamenteuse (actine-F). Les propriétés contractiles du réseau d'actomyosine et viscoélastiques du cortex d'actine sont les autres constituants fondamentaux du modèle. Les déformations cellulaires, c'est à dire les mouvements de la membrane, résultent de la rupture de l'équilibre entre les forces de protrusion et de rétraction qui s'exercent sur la membrane. Les variations de l'espace intracellulaire disponible, qui s'étend ou se contracte, influence la dynamique de l'actine qui en retour modifie les forces de protrusion et de rétraction sur la membrane. La boucle est ainsi bouclée.

Le modèle considère un domaine bi-dimensionnel annulaire, délimité d'un côté par le corps cellulaire et de l'autre par la membrane qui constitue une frontière libre. La largeur du domaine est représentée par $L(\theta, t)$ pour une direction θ ($0 \leq \theta \leq 2\pi$) et un temps t donné (Fig. 2.9). $a(\theta, t)$ représente la concentration d'actine libre dans le lamellipodium et $v(\theta, t)$ représente sa vitesse tangentielle de déplacement en θ et t .

Des conditions périodiques s'appliquent à ces variables compte tenu de la géométrie annulaire.

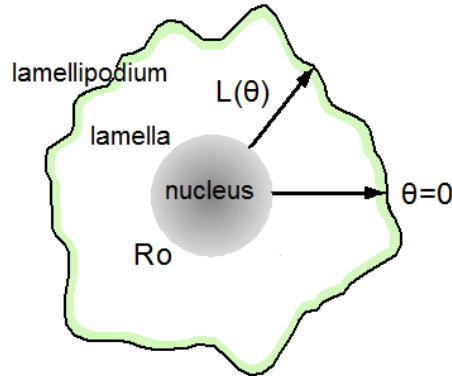


FIGURE 2.9: Représentation théorique de la cellule. Le noyau cellulaire est représenté par un domaine circulaire de rayon $R_0 = 7\mu m$. Deux zones sont distinguées : (i) le lamellipodium qui est la zone périphérique qui borde la membrane cellulaire et qui contient un réseau dense de filaments d'actine inter connectés, (ii) la lamelle qui est la zone située entre le lamellipodium et le noyau cellulaire. Le système de coordonnées polaires est utilisé. L'axe horizontal est l'axe de référence angulaire $\theta = 0$. $L(\theta)$ représente la distance entre la surface du noyau et la membrane évaluée selon la direction θ .

Le système normalisé d'équations aux dérivées partielles qui décrit les mouvements de la membrane est donné par :

$$\frac{\partial(La)}{\partial t} = -\frac{\partial}{\partial\theta}(Lav) + L(1-a), \quad (2.1)$$

$$av = \frac{\partial}{\partial\theta} \left[\mu a \frac{\partial v}{\partial\theta} + \sigma(a) - \kappa_c \right], \quad (2.2)$$

$$\delta(a) \frac{\partial L}{\partial t} = F_{out} + F_{in} + \kappa_m \quad (2.3)$$

$$\text{avec } \kappa_{c,m} = \tau_{c,m} \frac{\partial}{\partial\theta} \left(a \frac{\partial L}{\partial\theta} \right) \text{ et } \sigma(a) = \psi a^2 e^{-2a/a_{sat}}$$

Les deux premières équations décrivent la dynamique de polymérisation et dépolymérisation de l'actine et ses déplacements dans le lamellipodium. μ représente le coefficient de viscosité cytoplasmique, $\sigma(a)$ la contractilité du réseau d'actomyosine qui dépend localement de la densité d'actine et de sa densité de saturation a_{sat} (Fig. 2.15). κ_c est un terme de tension de courbure qui dépend de la rigidité τ_c de la structure filamenteuse du lamellipodium (cortex). La troisième équation décrit les mouvements de la membrane qui résultent d'un équilibre de force où $\delta(a)$, κ_m , F_{out} , F_{in} représentent respectivement les conditions d'adhérence de la cellule, la tension induite par la courbure membranaire, les forces de protrusion (outward) et de rétraction (inward).

2.3.2 Conditions pour générer des formes oscillantes

La méthode pour définir les paramètres permettant de générer un comportement donné, consiste à effectuer l'analyse de stabilité linéaire du système. Pour cela, on linéarise le système d'équations dans un premier temps. Par cette approximation, nous faisons l'hypothèse que les termes non-linéaires n'agissent pas de façon majeure sur le comportement général du système. Dans un second temps, une perturbation des variables a , L et v autour de leur valeur à l'état stationnaire homogène permet d'évaluer l'évolution du système pour des solutions de la forme $\exp(\lambda t + im\theta)$ où m désigne le mode de déformation.

Les solutions recherchées sont des formes cellulaires oscillantes qui caractérisent les mouvements de protrusion et de rétraction de la membrane. De telles solutions peuvent être obtenues à travers une déstabilisation de l'état stationnaire uniforme du système conduisant à une bifurcation de Hopf pour laquelle des cycles limites existent. La recherche d'une bifurcation de Hopf, contraint l'espace des solutions et par conséquent les paramètres physiques du modèle. Il en résulte que les paramètres β et γ , qui représentent respectivement la pression hydrostatique intracellulaire et l'élasticité du réseau d'actine, n'ont pas d'influence sur la détermination du mode de déformation. Les paramètres clés sont la viscosité cytoplasmique μ et l'élasticité du cortex (actine-membrane) τ . Une viscosité ou une rigidité accrue de la cellule conduit naturellement à une sélection de modes inférieurs, c'est à dire à une forte contrainte sur la déformation de la membrane, et donc à la génération d'un nombre limité de protrusions membranaires. Un autre paramètre important est celui qui régit la contractilité de l'actomyosine ψ et qui provient de l'expression de $\sigma(a)$. Le paramètre ψ permet lorsqu'il croît de sélectionner des modes de déformations plus grands c'est à dire un plus grand nombre de protrusions membranaires. Les effets de ces paramètres sur la stabilité du système d'équations sont représentés dans la figure 2.10.

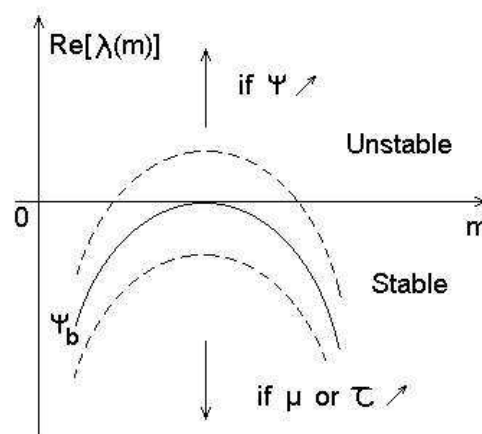


FIGURE 2.10: Diagramme de stabilité du système en fonction de l'évolution des paramètres clés que sont la viscosité cytoplasmique μ , la rigidité du cortex τ et la contractilité de l'actomyosine ψ .

2.3.3 Généralisation et simulation numérique

Le modèle représenté par le système d'équations aux dérivées partielles (2.1-2.3) a été étudié en détail sous sa forme généralisée où l'équation d'équilibre des forces qui s'appliquent sur la membrane, devient une équation de type "frontière libre" qui borne le domaine [32]. Cette nouvelle représentation mathématique (2.4-2.6) permet de "libérer" la membrane, c'est à dire de s'affranchir de la condition de faible courbure imposée par la formulation originale et de décrire des déformations membranaires de plus grande amplitude et plus variées.

$$\frac{\partial a}{\partial t} = D\Delta a + \nabla \cdot \left(a \frac{\partial \mathbf{v}}{\partial t} \right) + 1 - a, \quad (2.4)$$

$$\nabla \cdot (\sigma_{ve} + \sigma(a) + \sigma_p) = 0, \quad (2.5)$$

$$\delta(a) \frac{\partial L}{\partial t} = F_{out} + F_{in} + \kappa \quad (2.6)$$

On retrouve la même structure du modèle où σ_{ve} et σ_p représentent respectivement la contrainte visco-élastique et la contrainte de pression. Un terme additionnel de diffusion de coefficient D est introduit pour libérer les déplacements de l'actine dans le lamellipodium. Les termes d'adhésion $\delta(a)$ et de protrusion F_{out} sont des constantes positives et le terme de rétraction s'exprime par $F_{in} = -\gamma La$.

Le modèle sous sa forme généralisée permet de simuler un ensemble de déformations cellulaires réalistes dans la mesure où elles correspondent à une variété de comportements dynamiques effectivement observés expérimentalement.

Dans un premier temps, les paramètres physiques du modèle sont choisis de façon à sélectionner le mode $m = 1$ de déformation membranaire. La simulation numérique à partir de ce choix de paramètres conduit à la génération d'une onde de déformation en rotation (Fig. 2.11). Une telle onde a été reportée sur des cellules de types kératinocytes ou leucocytes [30, 33].

La sélection du mode supérieur $m = 4$ s'opère en diminuant la viscosité cytoplasmique μ et la rigidité du cortex τ . L'onde de rotation fait place à un mouvement membranaire pulsatile de protrusion / rétraction, synchronisé selon deux axes perpendiculaires (Fig. 2.12). Cette dynamique a été reportée pour des cellules de type fibroblaste [32, 34] pour lesquelles l'espace cytoplasmique est en effet plus vaste et plus dilué (moins visqueux). La rigidité du cortex (actine-membrane) est également moindre dans la mesure où l'actine est plus faiblement concentrée et de façon moins homogène au voisinage de la membrane.

Enfin la sélection de modes plus élevés $m \geq 4$ est obtenue en intensifiant la contractilité du cortex d'actomyosine ψ . Les simulations numériques montre un schéma de déformations coordonnées entre protrusions et rétractions membranaires très réaliste

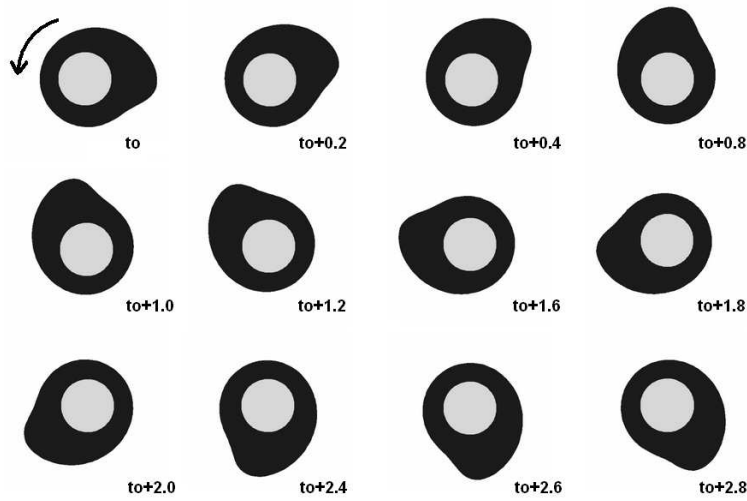


FIGURE 2.11: La sélection du mode de déformation membranaire $m = 1$ conduit à l'apparition d'une onde en rotation (unité de temps arbitraire).

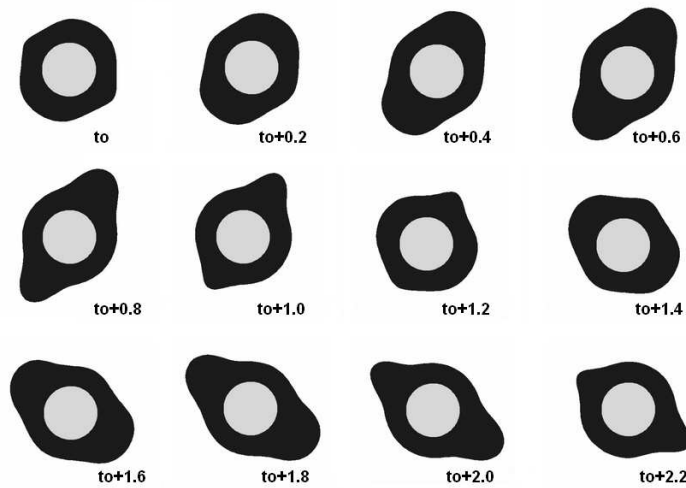


FIGURE 2.12: La sélection du mode de déformation membranaire $m = 4$ conduit à une pulsation de la cellule selon deux directions perpendiculaires (unité de temps arbitraire).

(Fig. 2.13). Nous avons observé un schéma de déformation très similaire sur des fibroblastes L929 (Fig. 2.14).

Le modèle continu des déformations membranaires est à présent hybridé par l'ajout successif d'éléments discrets pour rendre compte dans un premier temps de la migration cellulaire (section 2.4) et dans un second temps de la forme cellulaire sur substrats patternés (section 2.5).

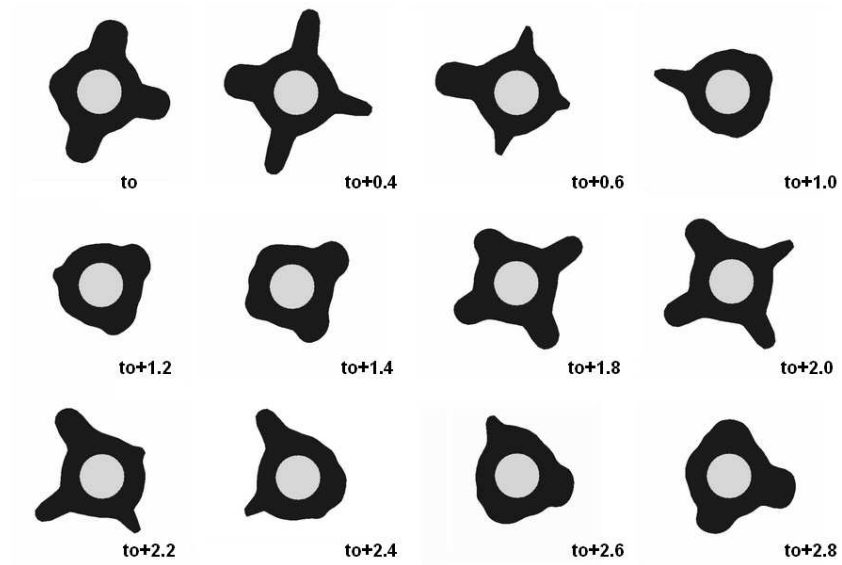


FIGURE 2.13: La sélection de modes $m \geq 4$ conduit à des mouvements coordonnés de protrusion et de rétraction de la membrane (unité de temps arbitraire).

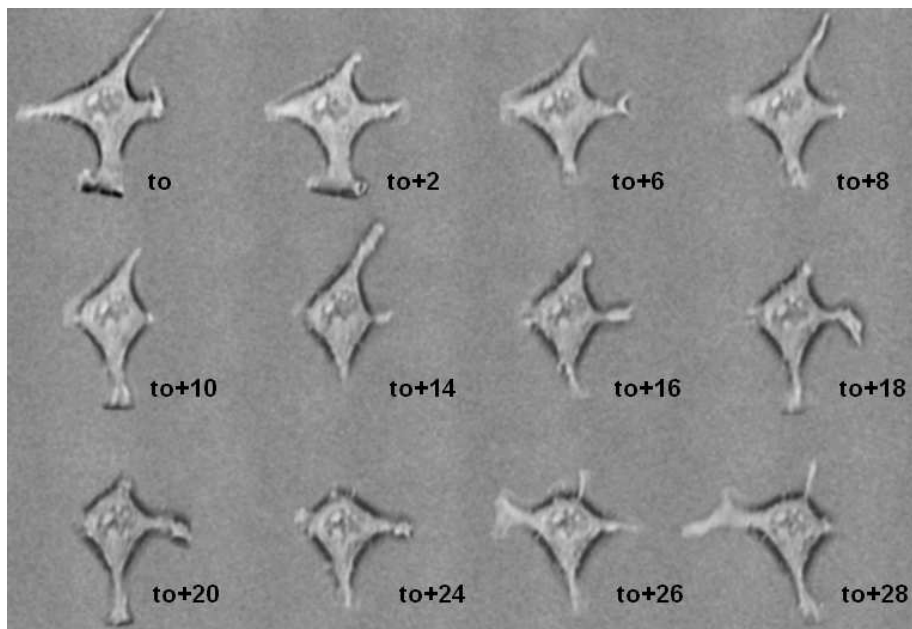


FIGURE 2.14: Séquence de déformation d'un fibroblaste L929 (temps indiqué en minutes).

2.4 Migration cellulaire spontanée

Ce travail a été réalisé en collaboration avec Eleni Mylona de l'Institut of Applied and Computational Mathematics d'Héraklion et Mark Chaplain du Département de Mathématiques de l'Université de Dundee dans le cadre du réseau européen Marie Curie Research Training Network (2002-2008) "Modelling, Mathematical Methods, and Computer Simulation of Tumour Growth and Therapy". Nos résultats ont été publiés dans le Journal of Theoretical Biology.

2.4.1 Motivation

La migration cellulaire est un processus hautement intégré où la dynamique de polymérisation / dépolymérisation de l'actine, la contractilité de l'actomyosine et la dynamique des adhésions sont étroitement liées. Nous avons proposé un modèle computationnel pour étudier le couplage entre ces différents éléments dans le contexte de la migration cellulaire spontanée, c'est à dire non stimulée par les conditions environnementales. Dans ce contexte particulier, les mouvements membranaires sont des oscillations qui résultent de la compétition entre la pression hydrostatique et la contractilité des fibres d'actine comme nous l'avons vu précédemment. Des points d'adhésions peuvent se former au cours des oscillations sous certaines conditions et les forces contractiles peuvent les amener à mûrir en adhésions focales. La polymérisation active de l'actine sur le front cellulaire, qui vient renforcer la protrusion due à la pression hydrostatique, permet de générer la force de traction nécessaire à la cellule pour se transloquer et avancer. Les simulations numériques de ce modèle ont tout d'abord pour but de montrer qu'il est possible de reproduire certains aspects les plus caractéristiques de la migration de fibroblastes. Sur la base de ce pré-requis, il devient possible d'étudier plus spécifiquement l'impact des différents paramètres temporels du modèle, tels que le temps de recyclage des protéines d'adhésion après translocation et les temps de vie des différentes adhésions, sur la vitesse de migration cellulaire et sur la nature de la trajectoire.

Cette étude est importante dans la mesure où elle étudie la migration non-induite. Elle contribue à une meilleure compréhension du potentiel de réaction de la cellule lorsqu'elle est soumise à un stimulus.

2.4.2 Hypothèses du modèle

La migration est un processus typiquement décrit en 3 à 5 étapes selon le niveau de détail considéré. Nous considérons ici trois étapes principales qui sont (1) la protrusion membranaire, (2) l'adhésion au substrat, (3) la translocation. Le modèle computationnel proposé se fonde sur l'intégration de ces trois processus pour permettre à la cellule d'explorer son environnement, que nous supposons ici parfaitement uniforme. Cette condition permet de s'affranchir de la description explicite du substrat.

Le modèle computationnel hybride est construit sur la base du modèle continu décrivant les déformations membranaires et décrit précédemment. Les éléments discrets, principalement pour gérer la formation ponctuelle des adhésions, sont intégrés comme il suit :

- la formation d’une adhésion est matérialisée par un accroissement du frottement de la membrane sur le substrat et s’exprime par $\delta(a) = a + \alpha\delta_{adh}$. α est un coefficient de friction et $\delta_{adh} = \delta_A + \delta_{FX} + \delta_{FA}$ avec $\delta_X = 1$ ou 0 selon s’il y a ou non une adhésion de type X au point considéré,
- la protrusion membranaire est principalement due à la pression hydrostatique β renforcée par la pression due à la polymérisation des filaments qui poussent la membrane au voisinage des adhésions focales (FA). La force protrusive résultante s’exprime par $F_{out} = \beta + \beta(a)\delta_{FA}$ (Fig. 2.15),
- la rétraction membranaire est due à la traction des filaments d’actine sur la membrane. L’intensité de cette force est supposée ici linéairement proportionnelle à la concentration d’actine et s’exprime par $F_{in} = -\gamma La$ où γ est le coefficient d’élasticité du réseau d’actine,
- le terme de tension membranaire κ reste inchangé par rapport au modèle de référence précédemment défini.

L’équation décrivant les mouvements membranaires s’expriment alors :

$$(a + \alpha\delta_{adh})\frac{\partial L}{\partial t} = \beta + \beta(a)\delta_{FA} - \gamma La + \kappa. \quad (2.7)$$

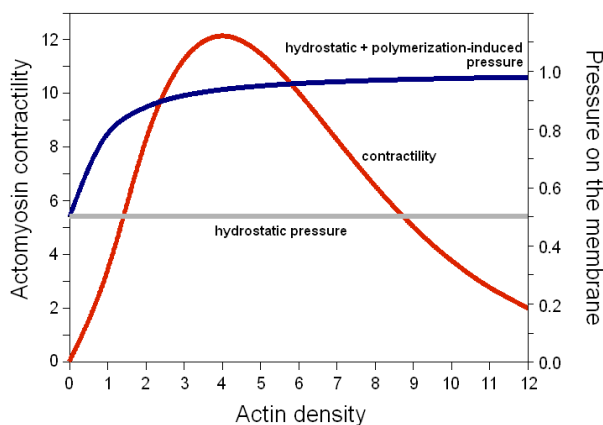


FIGURE 2.15: Profil de contractilité de l’actomyosine $\sigma(a) = \psi a^2 e^{-a/a_{sat}}$ (courbe rouge). La contractilité augmente jusqu’à atteindre la densité de saturation (a_{sat}). Au delà de ce point, la contractilité décroît en raison de l’effet de compaction des filaments. Profil de la pression qui s’exerce sur la membrane et qui est due à la combinaison de la pression hydrostatique β et de la pression induite par la polymérisation des filaments $\beta(a)$ (courbe bleue).

Nous faisons l’hypothèse que la probabilité de former une adhésion est plus grande dans une zone protrusive (telle que le lamellipode) puisque la surface de contact entre la cellule et le substrat est plus grande. Cela se traduit dans le modèle par l’identification de la zone où l’extension membranaire est maximum (L_{max}) et ce maximum doit

également être associé à une concentration d'actine suffisante (a_{thr}).

Chaque type d'adhésion correspond à un niveau de maturation caractérisé par sa durée de vie et sa résistance à la traction. Si la force de tension résultante (R_F) exercée sur le site adhésif est positive (c'est à dire si la force est une traction) alors AP mature en FX. Si cette condition n'est pas satisfaite durant la durée de vie de l'adhésion AP, alors l'adhésion se détache. De façon similaire, la maturation de FX en FA se produit si R_F appliquée sur l'adhésion atteint le seuil R_{thr} avant que la durée de vie de l'adhésion n'expire. Une fois FA formée, l'actine est recrutée autour de cette adhésion pour former et renforcer les fibres de tension. Les fibres en se contractant permettent de tirer la cellule en avant. La translocation n'est supposée se produire que lorsque la force exercée par les filaments atteint le seuil T_{thr} suffisant pour arracher la cellule aux adhésions périphériques. Avant qu'un nouveau cycle protrusion-adhésion-translocation ne commence, les protéines d'adhésion doivent être recyclées. Pendant cette période de recyclage dite "réfractaire", la cellule ne peut pas former de nouvelles adhésions.

Afin que l'automate puisse déterminer si une adhésion peut croître ou non, un critère de force est défini. On calcule pour cela la force résultante $R_F(\theta_i, t)$ qui s'exerce au temps t sur chaque adhésion i . Cette force résultante correspond à la somme des contributions de toutes les forces individuelles $F(\theta_j, t)$ qui équilibrent les mouvements de la membrane dans toutes les directions θ_j . Ces forces sont alors projetées sur la direction θ_i correspondant à l'adhésion i ($\theta_i = 2\pi i/m$, avec m le nombre de points définissant le contour membranaire), c'est à dire :

$$R_F(\theta_i, t) = \sum_{j=0}^m F(\theta_j, t) \cos(\theta_j - \theta_i), \quad (2.8)$$

$$\text{avec } F(\theta_j, t) \quad \text{telle que} \quad a \frac{\partial L}{\partial t} + F(\theta_j, t) = 0. \quad (2.9)$$

$F(\theta_j, t)$ est dérivée de l'équation d'équilibre des forces sur la membrane, négligeant le terme κ lié à la courbure membranaire puisque sa contribution est faible au regard des autres forces impliquées qui sont : (i) le terme de friction qui correspond à un accroissement de la tension au point d'adhésion, (ii) la tension passive des filaments d'actine modulée par l'extension membranaire et par la densité d'actine au point considéré, et (iii) la pression qui tend à repousser le corps cellulaire du site d'adhésion. $F(\theta_j, t)$ au site j et au temps t s'exprime par :

$$\begin{aligned} F(\theta_j, t) &= \delta_{adh}(\theta_j, t) \underbrace{\gamma_2 [L(\theta_j, t + \Delta t) - L(\theta_j, t)]}_{\text{tension liée à l'adhésion}} \\ &+ \underbrace{\gamma L(\theta_j, t) a(\theta_j, t)}_{\text{tension passive des filaments}} - \underbrace{[\beta + \beta(a) \delta_{FA}(\theta_j, t)]}_{\text{pression}} \end{aligned} \quad (2.10)$$

avec $\gamma_2 = \alpha/\Delta t$ le pas de temps du schéma numérique. On note que $F(\theta_j, t)$ dépend

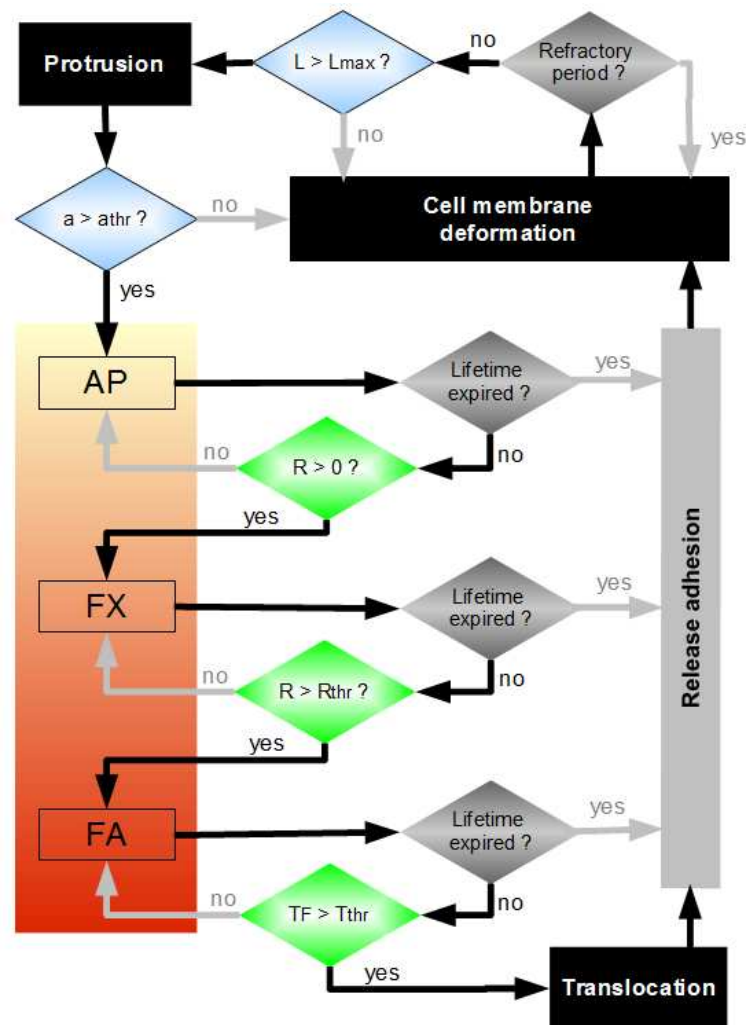


FIGURE 2.16: Diagramme d'inférence pour simuler la migration cellulaire. Les voies conduisant à la translocation cellulaire, c'est à dire à un pas en avant pour la cellule, sont figurées en noir. Les voies correspondant aux conditions alternatives sont figurées en gris.

fortement de la nature du site j . Par exemple, si j n'est pas un site d'adhésion alors seule la contribution passive demeure, c'est à dire $F(\theta_j, t) = \gamma L(\theta_j, t) a(\theta_j, t) - \beta$.

Le modèle suppose que les fibres de tension lient selon une direction radiale l'adhésion focale au corps cellulaire. La translocation, c'est à dire le déplacement du centroïde de la cellule, et la direction de migration θ_M résultent de la compétition entre les forces de traction exercées par chaque fibre de tension sur les adhésions focales. La force de traction résultante T_F portée par chaque fibre i est calculée à partir de l'expression :

$$T_F(\theta_i, t) = \sum_{j=0}^m \underbrace{\{\gamma_2[L(\theta_j, t + \Delta t) - L(\theta_j, t)]\}}_{\text{lié à l'adhésion}} + \underbrace{\{\gamma L(\theta_j, t)a(\theta_j, t)\}}_{\text{lié aux fibres}} \delta_{FA}(\theta_j, t) \cos(\theta_j - \theta_i) \quad (2.11)$$

Le déplacement noté \mathbf{r} du centroïde est simplement donné par $r = T_F(\theta_M, t)/k$. Cela correspond à un rappel élastique, avec k le coefficient élastique, par lequel les fibres de tension tirent brusquement une fois atteint le seuil de traction T_{thr} nécessaire à la translocation.

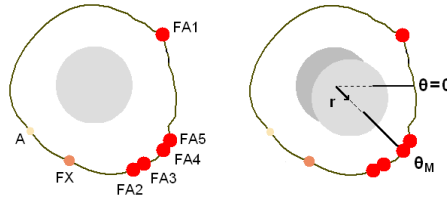


FIGURE 2.17: Image extraite d'une simulation montrant les 3 types adhésifs (AP, FX et FA) formés à la périphérie cellulaire. Seules les adhésions FA contribuent au calcul du déplacement cellulaire. Le centroïde de la cellule est tiré dans la direction θ_M qui correspond à la direction de plus grande traction de la part des filaments ancrés aux FA. Le vecteur \mathbf{r} représente le déplacement effectif du centroïde depuis sa position initiale (gris sombre) à sa position finale (gris clair).

2.4.3 Simulation numérique

Les simulations numériques permettent de visualiser simultanément les déformations membranaires, la trajectoire de la cellule et la dynamique de formation et de maturation des adhésions (Fig. 2.18). Les trajectoires simulées montrent une alternance entre phases de migration lentes et rapides avec des directions de persistance de la migration n'excédant pas une heure. Ces résultats sont en accord qualitatif avec des observations réalisées sur la lignée de fibroblastes 3T3 (collaboration avec E. Mylona).

Afin de valider le modèle, l'influence de l'adhésivité de la cellule au substrat (gouvernée par le paramètre α , éq 2.7), sur la vitesse de migration a été testée. Les résultats expérimentaux montrent une dépendance biphasique ("en cloche") où la vitesse croît avec la force d'adhésion jusqu'à ce que la force d'adhésion devienne trop forte et entraîne la décroissance de la vitesse jusqu'à empêcher la cellule de migrer [35]. Les simulations réalisées montrent que le modèle permet de reproduire ce comportement biphasique (Fig. 2.19).

Les influences des paramètres temporels du modèle (temps de recyclage des protéines d'adhésion et durées de vie des adhésions) sur la vitesse et la trajectoire de la cellule ont également été testées. Les résultats montrent que l'augmentation du temps de recyclage des protéines conduit à un ralentissement des cellules (Fig. 2.19). Cela vient

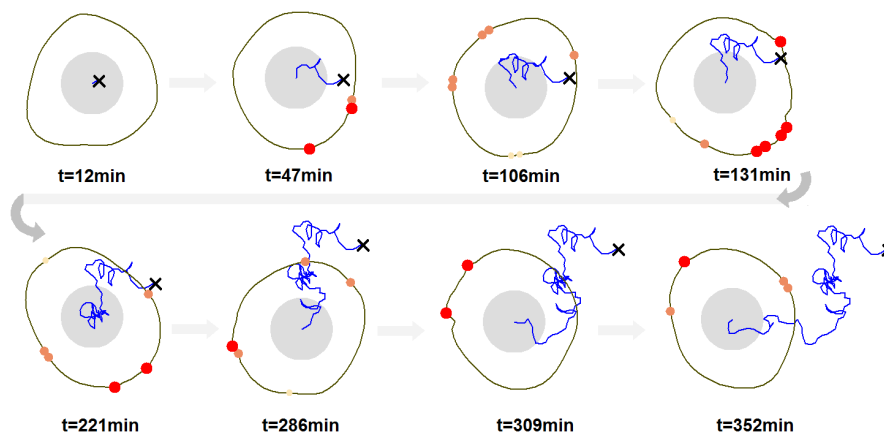


FIGURE 2.18: Simulation numérique des déformations membranaires et des déplacements de la cellule dont la trajectoire apparaît en bleu. Les différents types d'adhésions sont représentés (par taille croissante) : point focal (AP) en jaune, complexe focal (FX) en orange et plaque focale (FA) en rouge.

du fait que les cellules ont plus de temps pour retourner vers un état stable homogène. Il devient donc plus difficile de re-déstabiliser le système pour former de nouvelles adhésions. Tandis que pour un recyclage rapide des protéines, la cellule est toujours partiellement polarisée et les conditions de formation d'un point d'adhésion sont plus rapidement atteintes.

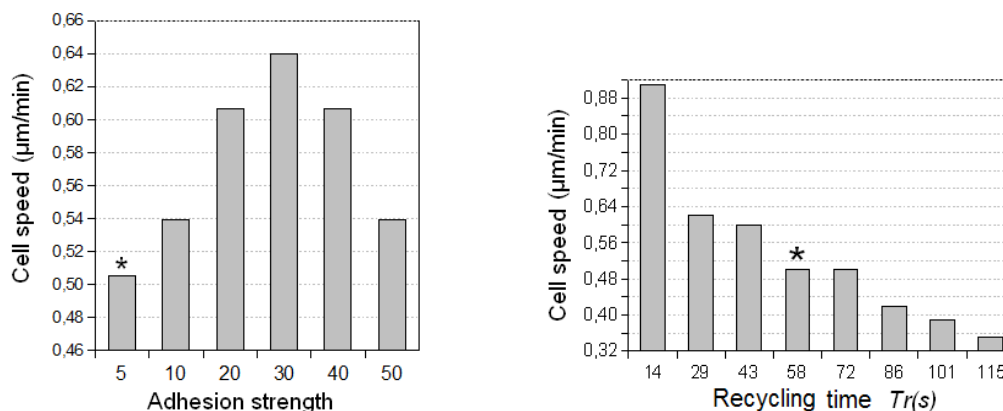


FIGURE 2.19: A gauche, évolution de la vitesse de migration en fonction du coefficient d'adhésion de la cellule au substrat. A droite, évolution de la vitesse de migration en fonction du temps de recyclage des protéines d'adhésion après translocation.

Les résultats montrent également qu'une modulation des temps de vie des différents types d'adhésion (AP, FX et FA) permet de générer des trajectoires avec des directions de migration plus ou moins persistantes (Fig. 2.20). En particulier, une durée de vie plus longue du point d'adhésion (AP) tend à favoriser le maintien d'une direction de persistance.



FIGURE 2.20: Comparaison de deux trajectoires qui correspondent à des durées de vie différentes pour les adhésions. Une durée de vie courte conduit à la trajectoire 1 plus erratique tandis qu’une durée de vie plus longue des adhésions conduit à la trajectoire 2 plus orientée (persistance).

2.4.4 Bilan

L’intégration d’un mécanisme simple de maturation des adhésions, fondé sur une stimulation mécanique d’intensité croissante, permet d’aborder la migration cellulaire et d’étudier les effets d’un certain nombre de paramètres.

Tout d’abord, l’augmentation du paramètre d’adhésivité montre une évolution ”en cloche” de la vitesse de migration conforme aux observations expérimentales, pré-requis indispensable à tout modèle de migration cellulaire.

Dans un second temps, le modèle permet surtout de se faire une meilleure idée de l’influence des nombreux paramètres temporels que sont le temps de recyclage protéique après rupture des adhésions et les temps de vie associés à chaque type d’adhésion. Les résultats obtenus sont conformes à ce qui était attendu et ne montrent rien de surprenant. Cependant les bases sont posées pour étudier les mécanismes qui coordonnent les différentes phases migratoires. Le modèle peut permettre à présent d’aborder un certain nombre de questions, et donne des pistes pour booster la migration en vue de favoriser par exemple la cicatrisation ou à l’opposé, l’inhiber pour neutraliser la dissémination des cellules cancéreuses.

Note : Le modèle computationnel a été implémenté sous forme de logiciel suite à plusieurs demandes qui m’ont été faites pour exploiter son potentiel à répondre à une variété de questions.

2.5 Déformations de cellules sur substrats patternés

Ce travail a été initié dans le cadre d’une collaboration avec le Laboratoire des Technologies de la Micro électronique (LTM - UMR CNRS 5129) qui possède la technologie pour réaliser des substrats patternés à l’échelle micrométrique. J’ai été partiellement détachée dans ce laboratoire sur la période 2007-2008, où j’ai été formée sur le protocole de réalisation des patterns en salle blanche. Patrick Schiavone et Tzvetelina Tzvetkova, tous deux en double rattachement TIMC-IMAG et LTM, ont contribué à l’émergence de ce projet ainsi que David Fuard du LTM.

Notre travail préliminaire a été présenté sous forme de poster à l'European Congress of Mathematical and Theoretical Biology (ECMTB) d'Edimbourg en Juillet 2008 et où il a été distingué par un prix de présentation.

Le modèle computationnel a ensuite été développé de façon significative par Céline Franco (2008-2009) dans le cadre de son stage de Master 2 "Ingénierie pour la Santé et le Médicament", option Modèles et Instruments en Médecine et Biologie.

Ce travail a fait l'objet d'un chapitre du livre "From Single Scale-Based Models to Multiscale Modeling" et d'une publication dans le journal "Mathematical Modelling of Natural Phenomena".

2.5.1 Motivation

L'observation de cellules en culture *in vitro* montre des oscillations membranaires avec des motifs spatio-temporels récurrents, comme nous l'avons vu précédemment. Dans ce contexte expérimental d'adhérence sur un substrat uniforme non fonctionnalisé, la dynamique du cytosquelette n'atteint jamais un état stationnaire puisque les adhésions fluctuent de manière aléatoire. Elles se font et se défont sans ancrages suffisamment longs temporellement et suffisamment forts pour permettre la mise en place du câblage des fibres de tension du cytosquelette qui permet la stabilisation de la forme cellulaire. Des patchs adhésifs de taille micrométrique permettent de limiter et de discrétiser la surface d'adhésion de la cellule au substrat [25]. Avec ces nouvelles conditions d'adhésion, la cellule devient capable de développer des liens plus forts avec le substrat à travers le mécanisme de regroupement des intégrines conduisant à la maturation rapide des adhésions. Les fibres de tension entrent alors en compétition les unes avec les autres et amènent la cellule à converger vers des formes d'équilibre stables qui dépendent essentiellement de la distance entre zones adhésives.

Afin de progresser dans la compréhension des liens qui unissent les processus de maturation des adhésions et des fibres de tension du cytosquelette, nous avons spécifiquement considéré un substrat constitué de patchs adhésifs discrets. Les patchs sont disposés selon un motif régulier afin de contrôler la position et la taille des zones adhésives de la cellule qui contraindront en retour la longueur des fibres de tension. En variant la distance inter-patchs, il devient ainsi possible d'étudier comment l'environnement adhésif conditionne la stabilité de la forme cellulaire et comment cela peut être relié à la régulation de la force cellulaire, c'est à dire à l'intensité de la force totale que la cellule est capable de développer et de maintenir.

Des expériences préliminaires ont été réalisées par Tzvetelina Tzvetkova pour observer l'étalement et la forme de fibroblastes 3T3 déposés sur des motifs hexagonaux de patchs adhésifs. Un exemple typique (Fig. 2.21) montre que la cellule a des difficultés à s'étaler et émet de courtes protrusions dans toutes les directions de l'espace pour sonder l'environnement (A). Les protrusions qui entrent finalement en contact avec des

zones adhésives permettent à la cellule de développer les points d'ancrage nécessaires à son étalement. Durant la phase d'étalement, la cellule continue de développer des protrusions pour poursuivre son étalement progressif de patches en patches (B). La forme cellulaire résultante est ainsi contrainte par la répartition des patches adhésifs ce qui conduit à une forme plus polarisée et géométrique qu'elle ne le serait naturellement (C). Ce sont ces observations qui ont servi de référence pour le développement du modèle.

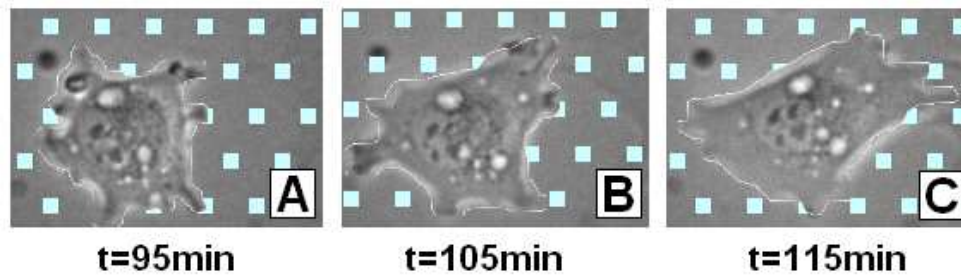


FIGURE 2.21: Séquence d'étalement d'un fibroblaste 3T3 sur un substrat patterné constitué de patches adhésifs carrés de $4\mu\text{m}$ de côté. La distance inter-patches est de $10\mu\text{m}$. Les patches adhésifs sont rendus visibles par post-traitement graphique avec PhotoFiltre. Le temps de référence $t = 0$ correspond à l'instant où les cellules ont été déposées sur le substrat.

2.5.2 Hypothèses du modèle

Le modèle computationnel est fondé sur le scénario proposé par Senju et Miyata pour décrire l'étalement cellulaire et l'organisation des fibres du cytosquelette. Les différentes étapes de ce scénario sont décrites dans la figure 2.22 et reprisent en détail dans les sections qui suivent.

Formation et maturation des adhésions et des fibres

La formation, la maturation et le devenir des adhésions et des fibres sont étroitement liés et ne peuvent donc être décrits indépendamment. Nous proposons ici un automate cellulaire pour décrire les dynamiques d'évolution des adhésions et des fibres, couplé au modèle d'EDP décrivant les déformations membranaires.

Trois types d'adhésions sont différenciées dans le modèle en fonction de leur niveau de maturation depuis le point d'adhésion (Adhesion Point, AP) à la plaque focale (Focal Adhesion, FA) en passant par le complexe focal (Focal complex, FX). Nous faisons l'hypothèse que les APs se forment spontanément lorsque la cellule entre en contact avec un patch adhésif (Fig. 2.23, étape 1). Les fluctuations thermiques peuvent rendre compte de l'énergie requise pour la formation de ces adhésions éphémères et faibles. Les APs se brisent facilement si la membrane se déplace trop vite au dessus d'elles. Nous supposons que l'AP mature en FX à travers un processus d'agrégation. L'agrégat croît jusqu'à atteindre une taille critique S_{crit} au delà de laquelle il devient FX (étape 2).

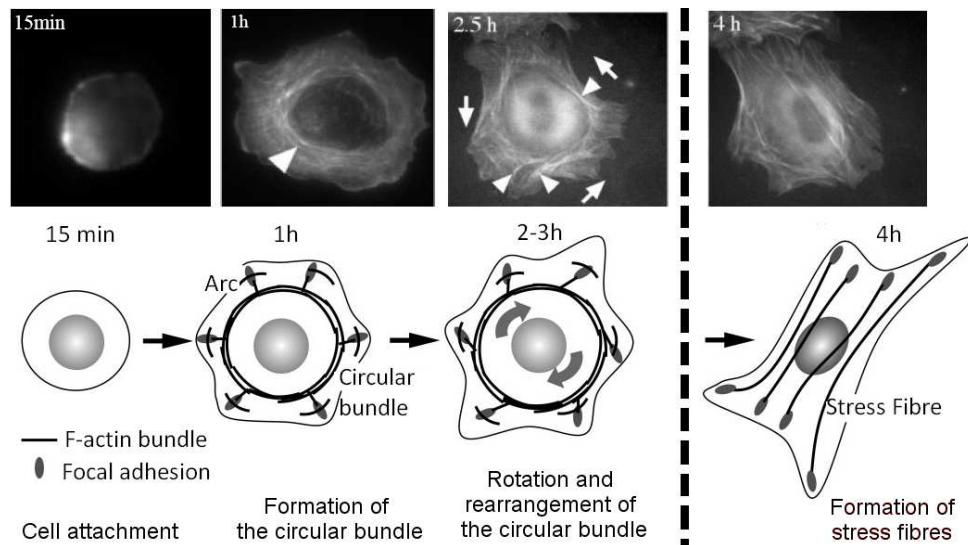


FIGURE 2.22: Scénario sur l'évolution du cytosquelette d'actine proposé par Senju et Miyata, 2008. Suite à la mitose, l'actine commence à polymériser et forme des filaments courts désorganisés. Les adhésions ne se sont pas encore formées d'où la forme ronde de la cellule. Après 1h, l'amas de fibres circulaires s'est formé ainsi que les fibres dorsales DF. Les extrémités de ces DFs sont liées au substrat par des adhésions focales FAs. Les courtes fibres transverses TFs apparaissent sous forme d'arcs à la périphérie cellulaire, puis sont entraînées par les flux cytoplasmiques jusqu'au centre de la cellule où elles s'assemblent (coalescent) pour former des amas circulaires contractiles. La tension qui se développe à partir de ceux-ci est transmise au substrat par les DFs, qui sont directement associées aux FA. La force de traction est dirigée radialement vers le noyau cellulaire. Dans ce scénario, les anneaux circulaires de fibres sont supposés réguler la morphologie cellulaire en empêchant l'étalement de la cellule. Lorsque les arcs de fibres transverses cessent de se former, l'amas de fibres circulaire commence à tourner. Les fibres de stress SFs sont formées lorsque la cellule est étalée sur le substrat.

Les FX sont des adhésions plus fortes qui ancrent la membrane au substrat et l'empêchent ainsi de glisser. De plus les FX initient la formation des fibres dorsales (DF) (étape 3a) qui croissent dans la direction radiale, c'est à dire vers le centre de la cellule. Les fibres transverses se forment très rapidement entre deux FX consécutives et non trop distantes (étape 3b) grâce à la disponibilité de nombreux fragments de fibres dans le lamellipodium.

Lorsqu'un FX est mécaniquement sollicité, il se renforce et mature en FA. Nous considérons que la sollicitation existe dès qu'une DF se lie à l'anneau contractile (étape 4). Dans ce cas, la tension générée par les contractions de l'anneau est transmise par la DF au FX et lui permette ainsi de mûrir en FA, tandis que parallèlement la DF devient une DSF (fibre dorsale de tension) (étape 5).

De manière similaire, lorsqu'une FA se forme, les fibres transverses (TFs) qui lui sont liées deviennent des fibres transverses de tension (TSFs) (étape 6). Les TSFs sont contractiles et tirent sur les adhésions auxquelles elles sont fixées à leurs deux extrémités, permettant ainsi la maturation des FXs en FA (étape 7).

Les fibres de tension DSFs ou TSFs sont contractiles et génèrent des forces. Si la force résultante générée R_F par ces fibres sur une FA est en dessous d'un certain seuil, alors la FA se désassemble, ce qui déclenche par cascade le désassemblage de toutes les fibres liées à cette adhésion.

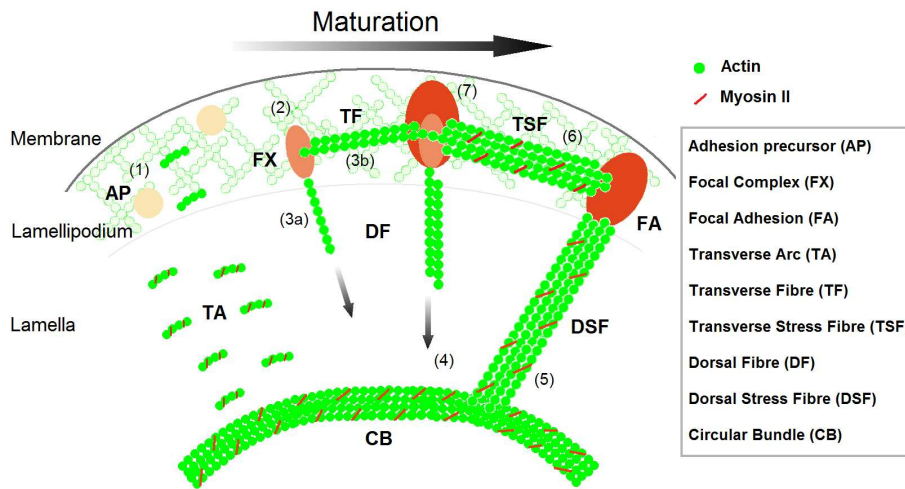


FIGURE 2.23: Sept étapes peuvent être distinguées pour décrire la maturation des adhésions et des fibres du cytosquelette. Les précurseurs d'adhésion (APs) se forment dans le lamellipodium (1). Simultanément, les arcs transverses (TAs) se forment au centre du réseau branché d'actine. Ils sont alors entraînés vers le centre de la cellule par le flux cytoplasmique, s'associant au passage à la myosine II qui leur confère leur contractilité, avant de se regrouper autour du noyau pour former un anneau de fibres contractile (CB). Une AP croît jusqu'à atteindre une taille critique au delà de laquelle elle devient un complexe focal (FX) (2). Les adhésions FXs servent de points de nucléation pour les fibres dorsales (DFs) (3a) et transverses (TFs) (3b) dans les directions respectivement radiale et tangente par rapport à l'anneau contractile. Une DF s'allonge jusqu'à atteindre l'anneau contractile (4). La connexion à cet anneau lui communique la propriété contractile et la fibre mature en fibre de stress (DSF) (5). Une fibre transverse (TF) liée à une plaque focale (FA) devient contractile et mature également en fibre de stress (TSF) (6). Une fibre TSF permet de connecter deux FAs distantes (7).

Ce processus couplé de maturation des adhésions et des fibres se traduit en langage computationnel à partir du diagramme d'inférence (flowchart) présenté en figure 2.24.

L'anneau contractile et la sélection des fibres

L'anneau contractile se forme par l'accumulation des arcs transverses à la base du lamellipode. Cela se produit durant les premiers stades de l'étalement cellulaire, soit environ une heure après le dépôt des cellules sur le substrat [36]. La contractilité de

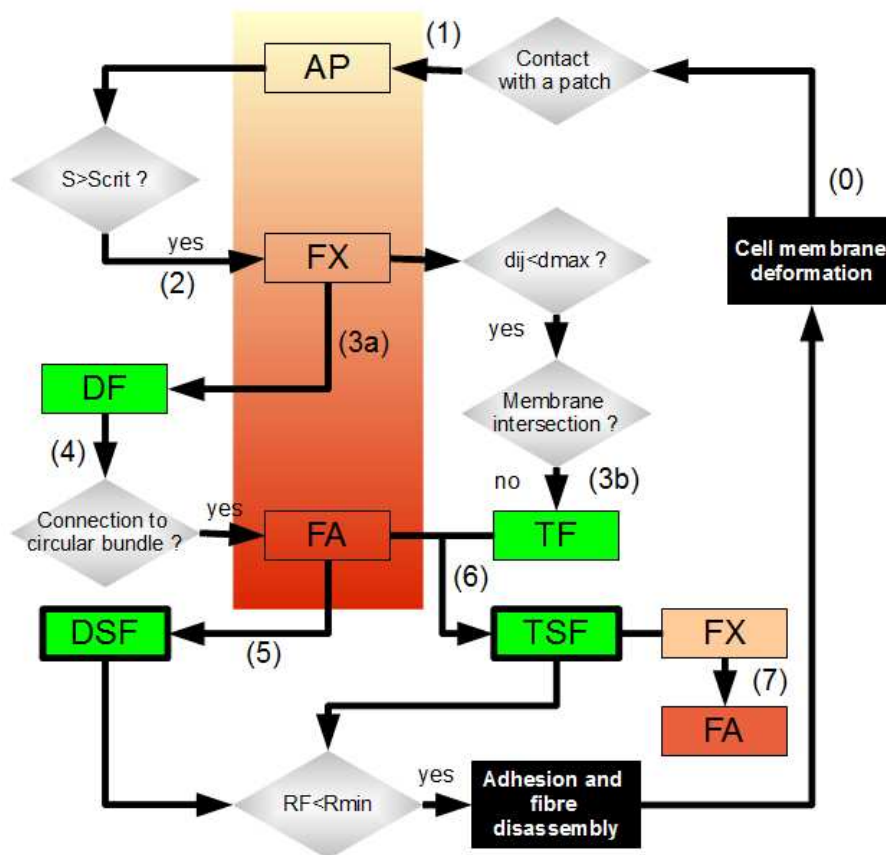


FIGURE 2.24: Diagramme d'inférence décrivant la formation et la maturation des adhésions et du cytosquelette d'actine. La membrane se déforme (0) et dès qu'elle entre en contact avec un patch adhésif, un point focal d'adhésion se forme (1). Lorsque l'agrégat de points focaux atteint une taille critique S_{crit} , il forme un complexe focal FX (2). Deux événements peuvent alors se produire. Dans le premier cas, une fibre dorsale DF croît à partir de FX (3a) jusqu'à atteindre l'anneau contractile (4). Ce contact, permet à la fibre dorsale DF d'exercer une force sur FX qui mature en plaque focale FA. Cette maturation déclenche la maturation de DF en fibre contractile DSF (5). Dans le second cas, après la formation de FX (2), si un autre FX est suffisamment proche (dans la limite de d_{max}) et si la membrane ne constitue pas un obstacle, une fibre transverse TS se développe alors spontanément entre les deux FXs (3b). Si TS est connectée à une plaque focale FA à l'une des ses extrémités, TF devient une fibre contractile TSF (6) et en retour, FX de l'autre extrémité de la fibre TSF devient une plaque focale FA (7). La force résultante R_F exercée par les fibres de stress (DSF et TSF) sur les sites d'adhésion déterminera leur évolution (8). Si cette force est trop faible (inférieure à R_{min}) alors l'adhésion et les fibres associées se désassemblent.

l'anneau régule la formation des adhésions focales durant le processus d'étalement cellulaire. La formation de l'anneau contractile n'est pas explicitement décrite par notre modèle. On a supposé que lorsque la cellule commence à s'étaler sur son substrat, les arcs transverses, générés dans le lamellipodium comme résidus de la dynamique du réseau branché d'actine, sont entraînés de la périphérie vers le centre cellulaire. Les

arcs fusionnent alors pour former l'anneau contractile en accord avec le scénario proposé par Senju et Miyata (2008). Cette structure apparaît très rapidement dans les simulations après quelques itérations seulement.

Le rôle de l'anneau contractile, comme démontré par Senju et Miyata (2008) est essentiel pour l'organisation des fibres de tension qui donneront sa forme finale stable à la cellule. Sur la base des observations expérimentales, nous intégrons dans notre modèle computationnel, l'élément clé de ce scénario qui est la maturation des fibres dorsales en fibres dorsales de tension une fois la connexion établie avec l'anneau contractile. Celui-ci transmet alors sa propriété contractile aux fibres qui peuvent alors solliciter mécaniquement les adhésions afin de les amener à leur tour à maturation.

A chaque fois qu'une fibre se connecte à l'anneau contractile, elle tire sur cette anneau induisant sa déformation ou sa rotation. Dans le modèle, nous décrivons l'anneau par une ellipse déformable. A chaque itération, plusieurs points du contour de l'ellipse peuvent ainsi être déplacés par la traction des fibres. Une nouvelle ellipse optimum est alors calculée sur la base d'une méthode de calcul des moindres carrés.

Comme l'anneau contractile se déplace et se déforme, certaines fibres qui lui sont liées à un moment donné peuvent se détacher, tandis que d'autres fibres se trouvant soudain plus proches de cette structure en mouvement peuvent s'y attacher. Afin de déterminer le devenir de chaque fibre, un ensemble de critères est défini comme il suit :

- nous évaluons tout d'abord l'angle de rotation de l'ellipse. S'il est supérieur à 45° alors on applique le second critère sinon les filaments peuvent se détacher ou s'attacher selon leur proximité avec l'anneau dans sa nouvelle position (Fig. 2.25(1)).
- Dans un second temps, si les fibres dorsales de tension (DSF) forment un angle inférieur à 45° avec l'axe principal de l'ellipse, alors les fibres sont maintenues sinon elles se désassemblent (Fig. 2.25(2)).

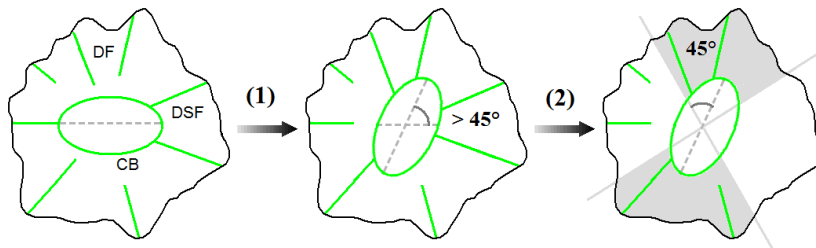


FIGURE 2.25: Représentation schématique de la cellule pour expliquer les règles d'assemblage et de désassemblage des fibres dorsales (DF). Les fibres dorsales croissent et se connectent à l'anneau contractile pour devenir à leur tour contractile (DSF). Ces fibres deviennent alors capables d'exercer des forces qui déforment l'anneau en une ellipse et la font tourner. (1) Si l'angle de rotation de l'ellipse est supérieur à ± 45 degrés, un autre critère est appliqué qui gouverne le désassemblages des fibres de stress dorsales (DSF). (2) Suite à la rotation de l'ellipse, si l'angle formé entre l'axe principal de l'ellipse et une DSF est à nouveau supérieur à ± 45 degrés alors la DSF est désassemblée (en dehors des zones grisées).

Calibrage spatio-temporel du modèle

La mise à l'échelle spatiale est basée sur la mesure effectuée sur des fibroblastes 3T3. Le rayon du noyau de ces cellules R_0 qui est relativement constant d'une cellule à une autre, a été choisi comme longueur de référence pour notre modèle afin d'établir la conversion entre pixels et micromètres. Les mesures donnent $R_0 = 7\mu m$.

La mise à l'échelle temporelle est plus délicate. Il est tout d'abord nécessaire d'identifier un processus avec une échelle de temps adéquate pour rendre compte à la fois des événements rapides et des événements longs dans la cellule. Nous avons choisi comme temps de référence, le taux d'élongation des fibres dorsales, dont la mesure expérimentale est de $0.34 \pm 0.15\mu m/min$ [19, 36]. A partir de cette valeur, nous identifions le temps avec le nombre d'itérations nécessaires dans la simulation pour faire croître une fibre sur la longueur de référence de $7\mu m$. Ce temps correspond à 21 minutes pour 600 itérations, ce qui permet d'assigner à l'itération numérique une durée de $0.03min$.

2.5.3 Simulation numérique

La première simulation montre l'étalement d'une cellule sur un substrat patterné avec une distance inter patches $d = 10\mu m$ (Fig. 2.26). La cellule est initialement ronde et s'étale en formant des points d'adhésion (APs) avec les patches du substrat (Fig. 2.26A). A ce stade précoce de l'étalement, les arcs transverses sont convectés vers le centre de la cellule pour former l'anneau contractile (ce phénomène n'est qu'imaginé mais pas décrit explicitement par le modèle). Les APs mûrissent en complexes focaux (FXs) par regroupement et dès que l'amas atteint la taille critique requise. Parallèlement des fibres transverses (TFs) se forment entre FXs consécutifs et les fibres dorsales (DFs) émergent des FXs pour croître radialement vers le centre de la cellule (Fig. 2.26B). Une fois que les DFs atteignent l'anneau contractile, les FXs mûrissent en plaques focales (FAs) et les fibres deviennent des DSFs. Toutes les TFs liées à des FAs deviennent à leur tour des TSFs (Fig. 2.26C). La maturation des adhésions et des fibres conduit ainsi à une cellule de forme polygonale contrainte où la membrane se trouve piégée par le réseau sous-jacent de TSFs (Fig. 2.26D).

La figure 2.27 présente les différents états cellulaires obtenus en faisant varier la distance inter patches de 10 à $14\mu m$. Deux cellules déposées avec une position aléatoire par rapport au motif du substrat sont présentées dans chaque cas. Les formes résultantes sont différentes pour un même cas tout en présentant des similitudes. Les cellules simulées ont au final des formes polygonales dictées par la position des adhésions. Lorsque le paramètre d augmente, les formes cellulaires sont de moins en moins contraintes, avec des portions de la membrane qui demeurent libres de se déformer. Ceci est d'autant plus visible pour les grandes distances inter patches $d = 20$ et $d = 24\mu m$ (Fig. 2.28). L'espacement des patches fait que la cellule a du mal à former des FAs et donc des fibres pour contraindre la forme de la membrane. Celle-ci effectue alors des mouvements alternés de protrusion et de rétraction sans pouvoir se fixer à un patch. Des adhésions

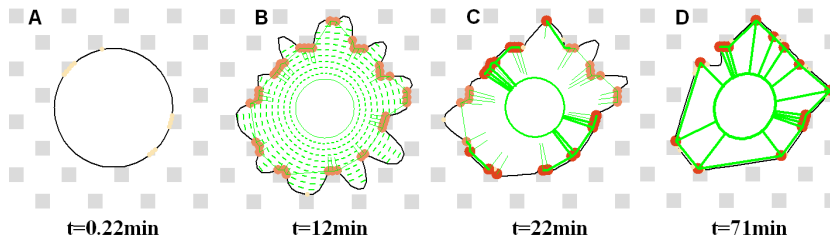


FIGURE 2.26: Simulation numérique de l'étalement d'une cellule sur un substrat patterné avec une distance inter patches $d = 10\mu m$. Les 4 phases représentées sont : (A) le dépôt de la cellule sur le substrat, (B) la formation de l'anneau contractile, (C) la maturation des adhésions et des fibres, (D) la forme résultante où la membrane est totalement contrainte. Les APs apparaissent en jaune, les FXs en orange et les FAs en rouge. Les fibres non contractiles sont représentées par des traits fins et les fibres contractiles par des traits épais.

transitoires peuvent se former, mais elles n'arrivent jamais à maturation puisque la membrane se rétracte avant qu'une fibre n'ait le temps de s'établir.

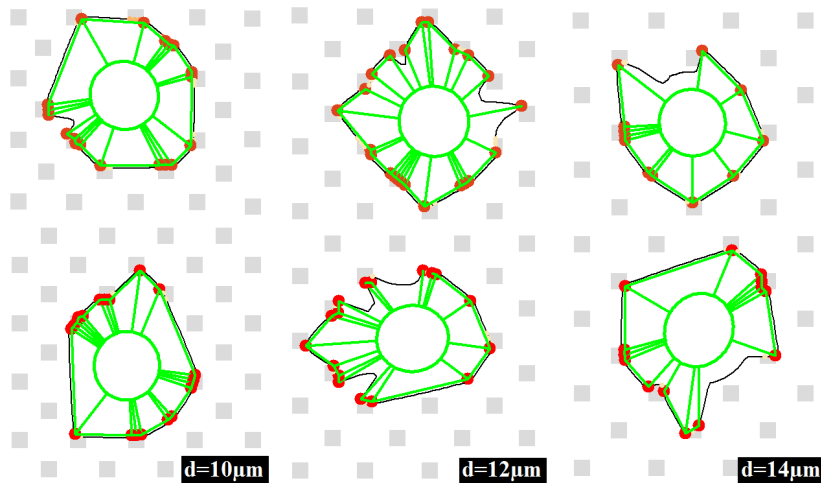


FIGURE 2.27: Morphologies résultantes simulées pour différentes valeurs de distance inter patches : $10\mu m$, $12\mu m$ et $14\mu m$ de gauche à droite.

2.5.4 Bilan

Le modèle computationnel que nous avons développé a permis pour la première fois de simuler les mécanismes de maturation simultanée des adhésions et des fibres, sur la base d'un scénario observé expérimentalement. Le modèle montre que le niveau d'extension membranaire admissible conditionne la morphologie de l'état stationnaire de la cellule pour une distance inter patches données. Les simulations ont également permis de montrer que le choix des paramètres liés d'une part à la durée de vie des adhésions et d'autre part à la vitesse de croissance des fibres conditionne la forme cellulaire et sa stabilité.

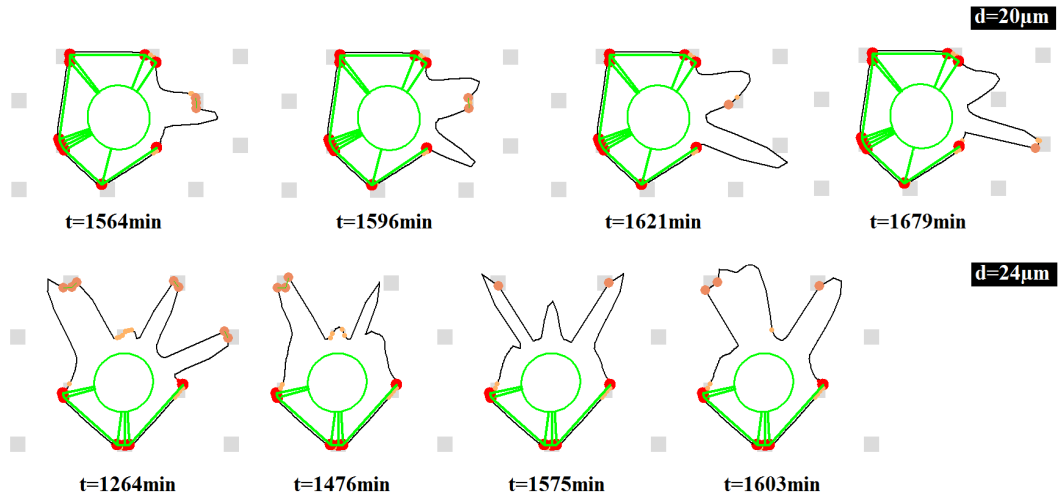


FIGURE 2.28: Séquences temporelles des morphologies résultantes oscillantes simulées pour les distance inter patches $d = 20$ et $d = 24\mu m$.

Le rôle de la contractilité dans la maturation des adhésions et des fibres a également été exploré ainsi que les conséquences sur le potentiel pour la cellule à générer des forces. Les résultats montrent que l'intensité des forces de traction tend à augmenter lorsque la distance entre les patches est plus grande par contre la contractilité ne modifie pas de façon significative le niveau de force généré.

Le modèle se révèle ainsi un bon outil d'investigation d'un certain nombre de paramètres cellulaires qui peuvent être testés expérimentalement. Une limitation majeure du modèle est l'utilisation de lois phénoménologiques pour décrire la maturation des adhésions et des fibres de tension ce qui limite la finesse de description des mécanismes. Un raffinement du modèle computationnel peut cependant s'envisager assez facilement, grâce à sa conception modulaire et imbriquée. Ainsi nous envisageons la prise en compte explicite des acteurs et des mécanismes de régulation de la dynamique de l'actine (Arp2/3, cofilin, gelsolin, etc.) que nous avons brièvement évoqués en section 2.2.2.

2.6 Conclusion

Ces travaux ont permis de mettre en place les briques essentielles d'un modèle computationnel qui permet d'aborder la motilité dans toute sa complexité. Nous nous sommes en premier lieu intéressés à la dynamique des déformations membranaires dont les équations forment le coeur du modèle autour duquel des éléments additionnels ont pu être tour à tour greffés. Nous avons ainsi commencé par ajouter un module pour gérer la formation des adhésions, ce qui a permis de rendre compte de la migration de la cellule. Puis nous avons complété le modèle en intégrant un module qui gère de façon plus complète la description des différentes structures de fibres. Ceci nous a permis de générer de nouvelles morphologies cellulaires contraintes par un environnement adhésif

imposé.

Nous avons parallèlement contribué à mettre au point un protocole expérimental pour réaliser les substrats patternés dans le but de valider le modèle théorique. Cependant nous n'avons pas pu aller au delà de cette mise au point par manque de financement. Toutes nos nombreuses tentatives de soumission de projets sur ce sujet ont échouées malgré des rapports très positifs. De nombreuses équipes se sont lancées sur la brèche ouverte par les progrès des micro technologies dans la réalisation de substrats contrôlés, mais nous n'avons pas été assez rapidement compétitifs sur le plan expérimental, bien que notre avance sur le plan théorique tienne toujours.

Cet axe de recherche est à présent pour moi sur un plan secondaire. Il reste cependant actif au service de l'axe cancer et du projet CATS décrit au chapitre 5.



3

Morphogenèse Vasculaire

3.1 Introduction

L'angiogenèse, se définit comme le développement de vaisseaux sanguins à partir d'un réseau pré-existant [37]. L'angiogenèse se distingue de la vasculogenèse qui correspond à la mise en place d'un réseau vasculaire, lors du développement embryonnaire, par différenciation des angioblastes, cellules précurseurs de celles formant les vaisseaux sanguins : les cellules endothéliales. Ce processus se produit chez l'adulte, dans des contextes physiologiques lors de la cicatrisation tissulaire, ou encore lors des processus inflammatoires. Dans de nombreux contextes pathologiques, l'angiogenèse joue un rôle aggravant. En cancérogénèse, par exemple, l'irrigation d'une tumeur solide par les vaisseaux sanguins fournit une source de nutriment qui permet la croissance tumorale et permet le développement de métastases. L'angiogenèse intervient également lors de certaines affections oculaires graves et de maladies inflammatoires. Le contrôle de l'angiogenèse représente donc un grand enjeu clinique, ce qui explique les nombreux travaux pour mieux connaître les facteurs régulant ce phénomène.

L'angiogenèse est un processus multifactoriel, régulé à la fois par des facteurs solubles (facteurs de croissance) et par les propriétés mécaniques de la matrice extra cellulaire caractérisée entre autres par sa rigidité. Folkman et Haudenschild (1980) ont montré que les modèles expérimentaux d'angiogenèse *in vitro* fournissaient une méthode contrôlable permettant l'étude de la formation des cordons vasculaires. L'hypothèse sous-jacente est que si les modèles *in vitro* reproduisent les caractéristiques *in vivo* dans le contexte de l'angiogenèse, alors ils peuvent fournir des informations sur les mécanismes de formation des vaisseaux.

Dans ce chapitre, nous présentons deux approches distinctes de l'angiogenèse. La

première est une approche expérimentale et étudie spécifiquement l'influence de la rigidité de l'environnement sur l'émergence de cordons vasculaires. La seconde est une approche théorique qui concerne le développement d'un modèle computationnel mettant en avant l'organisation vasculaire induite par les facteurs de croissance angiogénique dans le contexte tumoral.

L'émergence de l'auto-organisation est à la source de mon intérêt pour ces deux études qui ont été menées indépendamment l'une de l'autre à des époques différentes et dans des contextes également distincts. Une inter-fécondation des résultats de ces deux études seront l'objet de développements futurs que je ne ferai qu'évoquer en conclusion de ce chapitre.

3.2 Morphogenèse de cordons vasculaires *in vitro*

Les expériences présentées ont pu être réalisées grâce au financement apporté par une ATIP Jeunes Chercheurs (2005). Je me suis pour cela investie dans la réalisation des expériences en acquérant les bases de la culture cellulaire.

*L'analyse des images a été l'objet du stage de Master 2 "Modèles et Instruments en Médecine et Biologie" de Ghizlane Meskaoui (2006-2007). Ce travail a été présenté à plusieurs workshops et a fait l'objet d'une publication dans *Microvascular Research*.*

3.2.1 Motivation

La formation de cordons vasculaires peut être observée *in vitro* en ensemençant des cellules sur des biogels. Les conditions requises pour que ce processus d'angiogenèse *in vitro* se produise, ont été intensivement étudiées. Cependant, jusqu'à présent, seules les structures capillaires résultantes ont été caractérisées à travers des méthodes morphométriques. Une caractérisation de la dynamique, c'est à dire de l'évolution au cours du temps de ce processus était manquante. Nous avons par conséquent étudié spécifiquement cet aspect dynamique, en extrayant à partir de séquences d'images, les paramètres morphodynamiques pertinents. Dans cette étude, nous nous sommes spécialement intéressés à l'influence de la rigidité du biogel sur la formation des cordons vasculaires. Nos résultats montrent que ce paramètre influence fortement la dynamique du processus et que des conditions optimum, dans le contexte de nos expériences, peuvent être identifiées.

3.2.2 Le modèle expérimental

Les expériences ont été effectuées en utilisant la lignée de cellules endothéliales Eahy 926, ensemençées à raison de 20000 cellules/cm² sur des gels de fibrine obtenus à partir de la polymérisation du fibrinogène. Des gels de différentes concentrations en fibrinogène ont été considérés en faisant varier la densité de monomères de 1 à 3mg/ml. Plus la concentration en fibrinogène est grande et plus le gel est rigide. On désigne

par "flexible", "intermédiaire" et "rigide" les gels de 1mg/ml , 2mg/ml et 3mg/ml de fibrinogène respectivement.

3.2.3 Observations

L'observation *in vitro* du processus angiogénique montre l'apparition progressive de lacunes qui émergent de l'auto-organisation des cellules (Fig. 3.1a-b). Les lacunes croissent et fusionnent (Fig. 3.1c) jusqu'à ce qu'un réseau de cordons se forme à leur intersection (Fig. 3.1d).

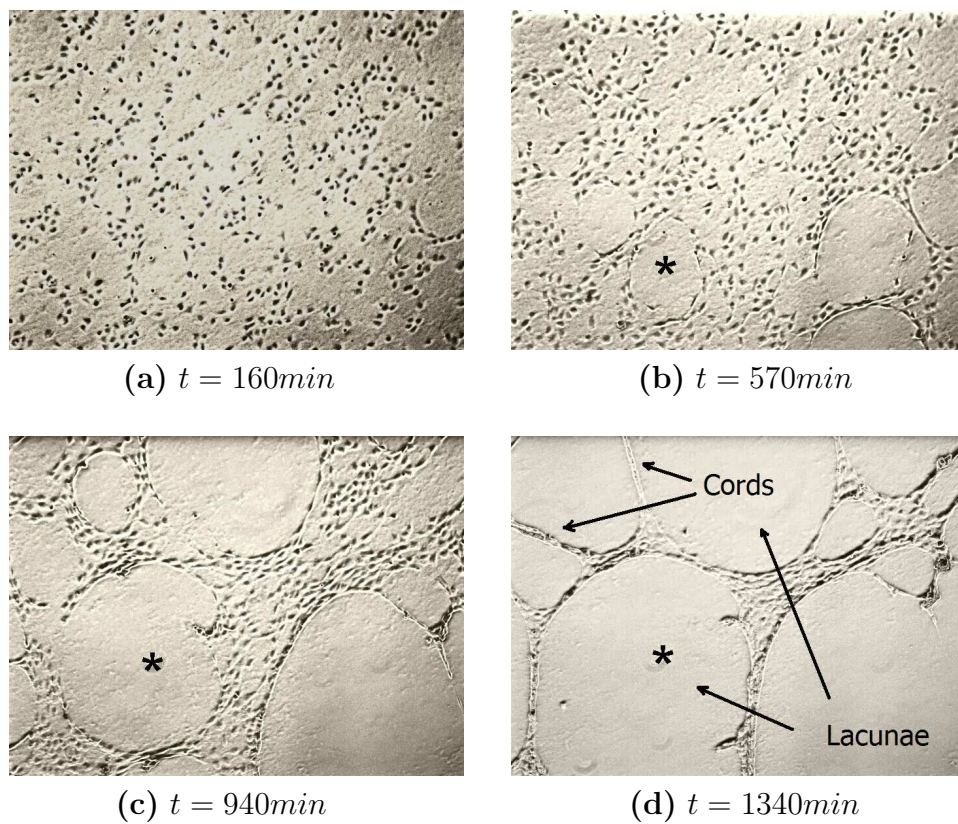


FIGURE 3.1: Images en contraste de phase montrant 4 étapes successives du processus de formation de cordons vasculaires *in vitro* sur un gel de rigidité intermédiaire.

La dynamique du processus morphogénétique (croissance du réseau de cordons) est évaluée à partir du suivi temporel de 3 paramètres des lacunes : la vitesse de croissance de leur surface, leur indice de forme (rapport grand axe sur petit axe) et la surface associée.

L'évaluation de la vitesse de croissance moyenne des surfaces (Fig. 3.2a) montre un profil biphasique d'évolution en fonction de la rigidité pour les deux premiers intervalles de temps $[0,700]$ et $[700,1200]$ minutes. Lorsque le processus est plus avancé dans le temps on observe que les lacunes continuent de croître uniquement pour le gel flexible.

Pour les rigidités intermédiaires les données sont manquantes pour les temps avancés puisque le processus est déjà arrivé à son terme. L'évolution temporelle de l'indice de forme des lacunes (Fig. 3.2b) montre qu'il croît au cours du temps quelque soit la rigidité du gel, ce qui traduit la déformation progressive des lacunes sous l'effet des forces de tensions exercées par les cellules sur le gel. Les déformations apparaissent toujours plus importante dans le cas du gel de rigidité intermédiaire. La mise en correspondance de l'indice de forme (Fig. 3.2a) et de la surface de la lacune (Fig. 3.2b) montre que pour les rigidités intermédiaires, la surface de la lacune atteint rapidement son maximum ($t=1020\text{min}$) puis décroît ($t=1500\text{min}$) tandis que l'indice de forme continue de croître. Cela signifie que les forces de tensions exercées par les cellules tendent le réseau qui atteint une configuration d'équilibre mécanique. Pour les gels flexibles, ni la déformation, ni la surface des lacunes n'évoluent avant des temps longs ($t=2000\text{min}$). Dans ce cas, le gel est trop flexible pour pouvoir transmettre les forces de tension entre les cellules et aucun réseau stable n'émerge. Pour les gels rigides, les déformations des lacunes (Fig. 3.2b) sont beaucoup plus faibles que pour les gels intermédiaires alors que les surfaces (Fig. 3.2c) sont similaires dans les deux cas. Cela indique que le gel oppose trop de résistance à la déformation, bien que les forces développées soient importantes, puisque les lacunes se déchirent et fusionnent, retrouvant ainsi temporairement des formes circulaires (traduites par la brusque chute de l'indice de forme à $t=1500\text{min}$) avant de se retendre par la suite ($t=2000\text{min}$).

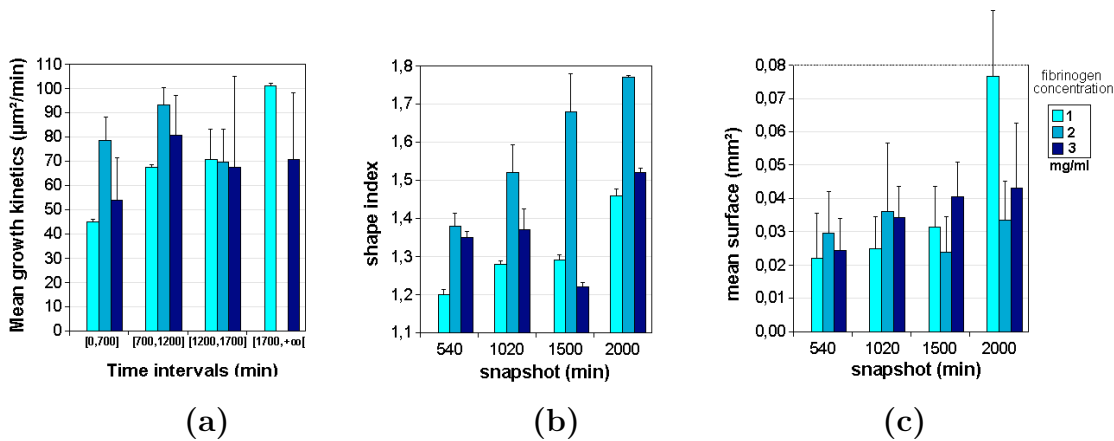


FIGURE 3.2: (a) Vitesse moyenne de croissance des lacunes pour chaque concentration en fibrinogène (de 1 à 3 mg/ml) et pour 4 intervalles de temps successifs, (b) indice de forme moyen et (c) surface moyenne des lacunes, évalués pour chaque concentration en fibrinogène (de 1 à 3 mg/ml) et pour 4 instants au cours du processus de formation des cordons.

Notre étude a permis d'identifier quatre phases dans la morphogénèse des cordons qui sont (i) l'apparition des lacunes, (ii) la croissance et les premières fusions de lacunes, (iii) la déformation des lacunes, c'est à dire l'étirement des cordons et (iv) la stabilisation et/ou la rupture du réseau de cordons vasculaires. Notre étude montre également qu'il existe une rigidité optimale du biogel pour l'obtention d'un réseau stable de cordons vasculaires. Il s'agit de la rigidité intermédiaire pour laquelle les forces de traction

générées par les cellules endothéliales et la résistance passive du biogel s'équilibrent rapidement. Cet équilibre explique la formation d'un réseau capillaire stable à la fin du processus angiogénique. Dans le cas des biogels flexibles et rigides (faible résistance du biogel et forte traction cellulaire respectivement), cet équilibre est difficile à atteindre. Ce qui conduit à des structures cellulaires peu connectées.

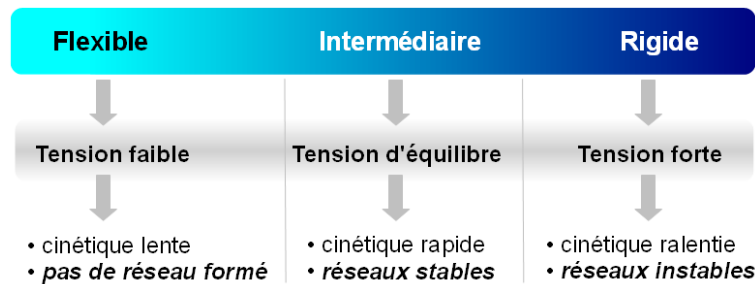


FIGURE 3.3: Schéma de synthèse des dynamiques de formation des réseaux de cordons vasculaires en fonction de la rigidité du biogel.

3.2.4 Rigidité apparente *versus* densité protéique du biogel

Dans l'étude précédente, portant sur l'influence de la rigidité du substrat sur la formation de cordons vasculaires *in vitro*, la rigidité a été modulée en faisant varier la concentration en fibrinogène pour réaliser le biogel de fibrine. Or si ce procédé permet effectivement de faire varier la rigidité de la matrice, il fait également varier la densité de protéines d'adhésion. Il devient donc difficile de déterminer, dans quelle mesure les deux paramètres rigidité et adhésivité interviennent respectivement dans le processus de formation des cordons.

Un modèle expérimental identique au précédent est utilisé. C'est à dire l'utilisation de cellules endothéliales de la lignée EaHy926ensemencées sur un substrat de fibrine. La rigidité apparente du substrat varie en fonction de l'épaisseur de celui-ci. On utilise la rigidité de substrat intermédiaire (correspondant à une concentration en fibrinogène de 2mg/ml) pour laquelle nous avons précédemment obtenu les meilleurs résultats quant à la génération d'un réseau de cordons vasculaires stables. Le gel est alors coulé dans une boîte de pétri inclinée de 10 degrés afin d'obtenir un gradient d'épaisseur, c'est à dire un gradient de rigidité pour lequel la densité de protéines d'adhésion est constante car fixée par la densité en fibrinogène.

Les images montrant la formation du réseau de cordons ont été acquises 7h après ensemencement des cellules sur le gel. La totalité de la boîte de Pétri a été scannée manuellement par tranches successives orientées dans la direction du gradient de rigidité. La structure du réseau de cordons le long du gradient d'épaisseur du gel est visible à partir de la reconstitution d'une tranche par superposition des images. Les images montrent que la structure du réseau varie avec la rigidité apparente du substrat. Le nombre de lacunes diminue avec la rigidité, par contre leur taille augmente. Dans la

zone plus rigide, les forces de tension exercées par les cellules sur le gel peuvent s'exprimer pour tendre et faire fusionner les lacunes entre-elles d'où leur nombre plus faible et leur taille plus grande. Ce résultat correspond aux conclusions de notre précédente étude et tend à confirmer que la rigidité du gel plutôt que la densité de sites d'adhésion est le facteur prépondérant dans la morphogénèse vasculaire.

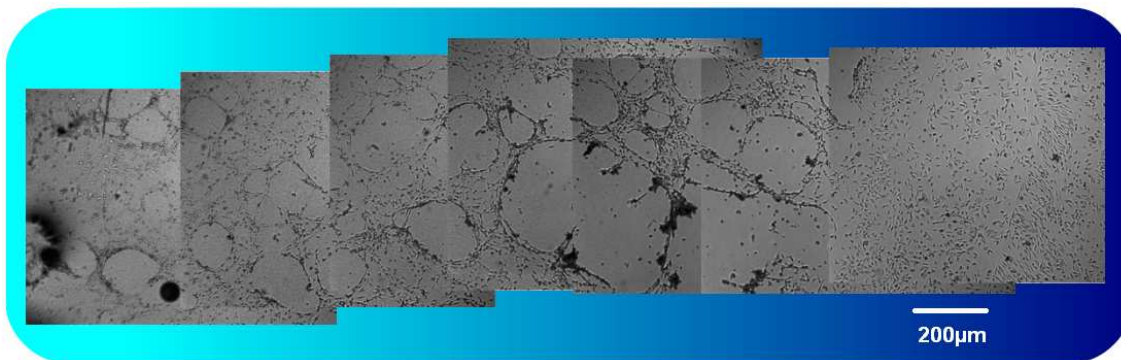


FIGURE 3.4: Reconstitution d'une tranche de l'échantillon qui montre l'état de formation d'un réseau de cordons vasculaires 7h après ensemencement des cellules sur une matrice de fibrine présentant un gradient de rigidité apparente : gel épais à gauche de faible rigidité apparente jusqu'à un gel fin à droite de forte rigidité apparente.

3.3 Modélisation de l'angiogenèse tumorale

Ce travail a été initié à l'Institut d'Ingénierie du pétrole de l'Université Heriot-Watt d'Edimbourg où j'ai effectué mon postdoc. L'institut se spécialise dans la modélisation mathématique et la simulation numérique de la circulation de fluides (pétrole et eau) et de gaz dans les réseaux poreux (roches) afin de trouver les moyens d'optimiser les méthodes d'extractions du pétrole piégé dans la roche. La transposition des outils logiciels de simulations développés par l'ingénierie pétrolière au cas de l'angiogenèse a été amorcée par Steven McDougall sur la base de l'analogie entre le cas géologique et biologique. Dans les deux cas il s'agit de décrire la circulation d'un fluide (pétrole / sang) dans un réseau complexe (roches / réseau vasculaire) à la différence importante que le cas géologique traite d'objets inertes et le cas biologique d'objets vivants c'est à dire en perpétuelle évolution donc nécessairement bien plus complexes.

Le projet a été réalisé en collaboration étroite avec Mark Chaplain et Alexander Anderson du département de Mathématiques de l'Université de Dundee, spécialistes de la modélisation mathématique de la croissance tumorale et de l'angiogenèse. Ce projet a bénéficié du financement de plusieurs organismes britanniques EPSRC et du "Medical Research Council" dans le but d'encourager les échanges entre disciplines, ainsi que d'un financement dans le cadre du réseau européen RTN sur la thématique "Using Mathematical Modelling and Computer Simulation to Improve Cancer Therapy".

3.3.1 Motivation

De nombreux modèles décrivant l'angiogenèse tumorale ont été développés pour décrire la croissance des vaisseaux. Cependant, aucun de ces modèles ne prenait en compte la circulation du sang dans ces vaisseaux qui est pourtant fondamentale pour leur fonctionnalisation. Notre objectif a donc été de proposer pour la première fois un modèle mathématique de l'angiogenèse tumorale prenant en compte un certain nombre de mécanismes régulateurs associés à la circulation sanguine à travers le réseau vasculaire en croissance. La prise en compte des propriétés rhéologiques du sang rend en effet possible l'évaluation et l'optimisation de l'efficacité des thérapies (chimiothérapie). L'observation de la circulation et de la diffusion des médicaments au sein du réseau vasculaire permet ainsi de mettre en évidence l'importance de la structure du réseau (architecture, densité et connectivité) sur l'efficacité de la délivrance par perfusion des médicaments et de leur acheminement jusqu'aux tissus tumoraux.

3.3.2 Hypothèses du modèle

Le processus d'angiogenèse se déclenche lorsque la tumeur atteint une taille critique de quelques millimètres au delà de laquelle elle épuise les ressources en oxygène et nutriments disponibles dans les tissus avoisinants. A ce stade, les cellules tumorales deviennent hypoxiques et se mettent à produire un facteur de croissance angiogénique (VEGF) qui diffuse dans les tissus jusqu'à être détecté par les cellules endothéliales qui forment la paroi des vaisseaux situés à proximité. Au contact de ce facteur de croissance, les cellules endothéliales se mettent à migrer en direction de la tumeur en formant des tubes, qui s'interconnectent pour former le réseau vasculaire (Fig 3.5).

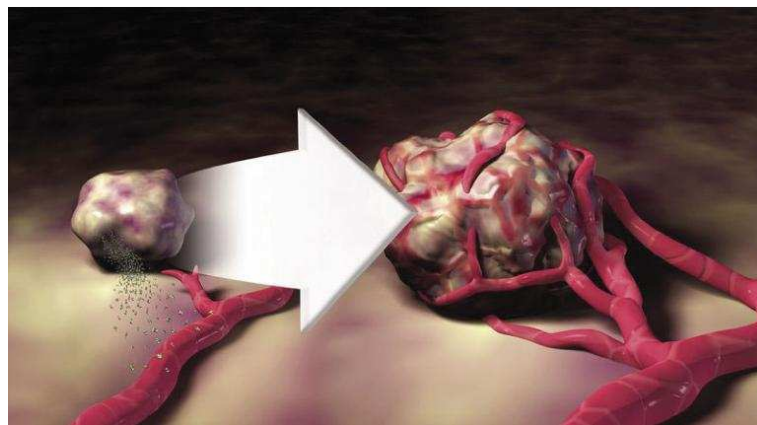


FIGURE 3.5: Evolution tumorale du stade avasculaire au stade vasculaire *via* l'émission de VEGF.

Les cellules endothéliales qui forment les parois des vaisseaux se désolidarisent les unes des autres et migrent à travers le tissu en remontant vers la source de VEGF qui stimule également leur prolifération. Des germes se forment alors progressivement sur les vaisseaux proches de la tumeur et croissent guidés par le gradient de VEGF. Les vaisseaux en croissance forment des branches et des boucles en se reconnectant entre

eux, il s'agit du processus d'anastomose.

Durant leur migration, les cellules endothéliales produisent des métallo protéases (MMPs) qui sont des enzymes permettant de dégrader la matrice extra cellulaire, en particulier les fibres de fibronectine. Les cellules peuvent alors progresser plus facilement à travers le tissu en traçant des voies que les autres cellules peuvent emprunter.

Un modèle hybride de la croissance vasculaire

Le modèle que nous utilisons pour décrire l'angiogenèse s'inspire de celui proposé par Anderson et Chaplain (1998). Les cellules endothéliales (n) migrent selon la combinaison de 3 composantes (i) une composante aléatoire par diffusion, (ii) une composante chimiotactique qui correspond à la réponse des cellules au gradient de facteur angiogénique de type VEGF (c) produit par la tumeur et (iii) une composante haptotactique qui traduit la sensibilité des cellules au gradient de fibronectine (f) qui est une des protéines de la matrice extra cellulaire avec laquelle les cellules endothéliales se lient par l'intermédiaire de récepteurs membranaires spécifiques. L'équation de conservation des cellules endothéliales est donnée par :

$$\frac{\partial n}{\partial t} = \underbrace{D\nabla^2 n}_{\text{diffusion}} - \underbrace{\nabla \cdot (\chi(V)n\nabla V)}_{\text{chimiotaxie}} - \underbrace{\rho\nabla \cdot (n\nabla f)}_{\text{haptotaxie}} \quad (3.1)$$

D est le coefficient de diffusion des cellules endothéliales, $\chi(V) = \chi/(1 + \delta V)$ caractérise la sensibilité des cellules au gradient de VEGF et ρ leur sensibilité au gradient de fibres matricielles. Les évolutions des concentrations de VEGF (V), de fibronectine (f) et de métallo protéases MMPs (m) sont données par :

$$\frac{\partial V}{\partial t} = -\eta n_i V, \quad (3.2)$$

$$\frac{\partial f}{\partial t} = \beta n_i - \gamma m f, \quad (3.3)$$

$$\frac{\partial m}{\partial t} = \alpha n_i + v\nabla^2 m - \nu m. \quad (3.4)$$

n_i représente une valeur booléenne (1 ou 0) qui indique la présence ou l'absence d'un germe de cellules endothéliales au point considéré de la grille de simulation. Les paramètres β et α caractérisent respectivement les taux de production de fibronectine et de MMPs par les cellules endothéliales et η leur taux de consommation du VEGF. La dégradation de la fibronectine s'opère via les MMPs et est caractérisée par le taux de dégradation γ . Les MMPs une fois produites diffusent localement avec un coefficient de diffusion v et se dégradent spontanément avec un taux ν .

Afin de représenter la migration individuelle des cellules endothéliales plutôt que l'évolution d'une densité, l'équation de conservation (3.1) est discrétisée pour conduire à une équation de la forme :

$$n_{i,j}^{t+1} = n_{i,j}^t P_0 + n_{i+1,j}^t P_1 + n_{i-1,j}^t P_2 + n_{i,j+1}^t P_3 + n_{i,j-1}^t P_4 \quad (3.5)$$

où i et j sont les positions de la cellule endothéliale selon l'axe horizontal et l'axe vertical respectivement de la grille de simulation et t désigne l'instant considéré. La migration de chaque cellule individuelle est déterminée par l'ensemble des coefficients P_i qui définissent les probabilités qu'a la cellule de rester à sa place (P_0) ou de se déplacer à gauche (P_1), à droite (P_2), en haut (P_3) ou en bas (P_4) (Fig. 3.6). Ces coefficients sont fonction du poids de chacune des composantes migratoires (aléatoire, chimiotactique et haptotactique), c'est à dire des conditions locales en densités de VEGF et de fibres matricielles. Ainsi par exemple, une plus forte densité de VEGF dans l'une des 4 directions de migration possibles donnera un poids plus fort à la probabilité associée à cette direction.

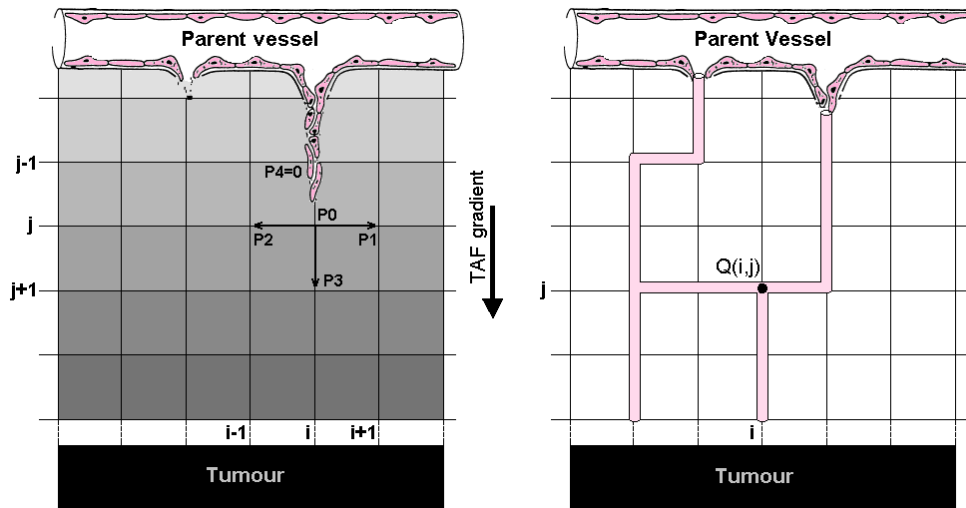


FIGURE 3.6: A gauche, la grille utilisée pour la simulation numérique de la croissance vasculaire. La direction de migration de chaque germe est définie de façon probabiliste, ou chaque probabilité dépend des conditions locales en facteur de croissance et en fibres matricielles. A droite, la grille utilisée pour définir les vaisseaux. Ceux-ci se définissent sur un axe horizontal ou vertical de la grille. A chaque vaisseau est assigné un rayon afin de pouvoir simuler le flux sanguin. A chaque noeud du réseau est appliquée la loi de conservation du flux (flux entrants = flux sortants).

Lois de branchement et anastomose

Le processus de branchement correspond à la formation de deux nouveaux germes à partir d'un germe existant. Les deux germes croissent ensuite de façon indépendante. Nous supposons dans ce modèle que la probabilité de branchement dépend uniquement de la concentration locale en VEGF, puisque ce facteur stimule également la prolifération. Ainsi plus la concentration en VEGF est élevée, ce qui est le cas lorsque l'on se rapproche de la source tumorale, plus la probabilité de branchement est grande.

Le processus d'anastomose correspond à la formation de boucles par la rencontre et la fusion de deux germes. Il est possible que lors de la rencontre des deux germes, l'un subsiste et poursuive sa croissance. L'anastomose s'accroît à l'approche de la tumeur, puisque les vaisseaux en croissance se font plus denses.

Propriétés rhéologiques sanguines

Le sang est un milieu biphasique complexe, composé de très nombreux constituants (Fig 3.7). La phase solide contient essentiellement les éléments cellulaires : (i) les globules rouges (érythrocytes) impliqués dans le transport de l'oxygène et du dioxyde de carbone ; (ii) les globules blancs (leucocytes) impliqués dans les mécanismes de défense immunitaires de l'organisme contre les bactéries et les virus, et (iii) les plaquettes impliquées dans la coagulation. Ces éléments solides sont transportés dans le plasma qui constitue la phase fluide. Le plasma est une solution contenant des électrolytes et des molécules organiques tels que les métabolites, les hormones, les enzymes, les anticorps et autres protéines. Une mesure de la phase solide est donnée par l'hématocrite, qui représente la fraction de volume de globules rouges contenus dans le sang ceux-ci étant prédominants. La valeur moyenne de l'hématocrite chez l'humain est d'environ 45%. En raison de sa nature biphasique, le fluide sanguin ne se comporte pas de façon linéaire et sa viscosité mesurée pour différentes vitesses de flux dans les micro vaisseaux varie. Pour un flux donné Q dans un capillaire cylindrique de rayon R , la viscosité mesurée est dite "apparente" et est déterminée par l'expression suivante :

$$\mu_{app} = \frac{\pi R^4}{8L} \cdot \frac{\Delta P}{Q} \quad (3.6)$$

où L désigne la longueur du capillaire et ΔP la chute de pression axiale. La viscosité apparente relative (paramètre sans dimension) est alors définie en divisant la viscosité apparente par la viscosité du plasma, soit : $\mu_{rel} = \mu_{app}/\mu_{plasma}$

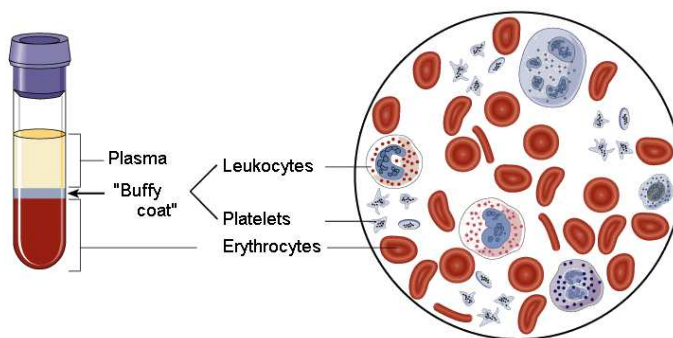


FIGURE 3.7: Représentation de la composition sanguine : phase fluide et phase solide.

La viscosité plasmatique est typiquement de 1.2 cp. La mesure directe de la viscosité sanguine est difficile à accomplir avec précision. Pries et col. (1996) ont proposé une approche alternative fondée sur la comparaison de la distribution du flux entre un système expérimental et un modèle théorique intégrant les mêmes propriétés. L'expression mathématique complètement empirique qui rend le mieux compte des données

expérimentales est la suivante :

$$\mu_{rel}(R, H_D) = \left[1 + (\mu_{0.45} - 1)f(H_D) \left(\frac{2R}{2R - 1.1} \right)^{27} \right] \left(\frac{2R}{2R - 1.1} \right)^2 \quad (3.7)$$

où $\mu_{0.45}$ est la viscosité qui correspond à la valeur moyenne normale soit ($H_D = 0.45$), R est le rayon du vaisseau et $f(H_D)$ est une fonction de l'hématocrite. Ces termes sont définis par :

$$\mu_{0.45} = 6e^{-0.17R} + 3.2 - 2.44e^{-0.06(2R)^{0.0645}}, \quad (3.8)$$

$$f(H_D) = \frac{(1 - H_D)^C - 1}{(1 - 0.45)^C - 1}, \quad (3.9)$$

$$C = (0.8 + e^{-0.15R}) \left(-1 + \frac{1}{1 + 10^{-11}(2R)^{12}} \right) + \left(\frac{1}{1 + 10^{-11}(2R)^{12}} \right). \quad (3.10)$$

La viscosité apparente est représentée dans la figure 3.8 pour plusieurs valeurs de l'hématocrite et pour une gamme de rayons de vaisseaux typique de l'échelle microvasculaire. On remarque que la viscosité apparente augmente lorsque le rayon décroît puisqu'il y a alors plus de frottement de la part des globules rouges sur la paroi des vaisseaux. Cette évolution dépend évidemment aussi du taux de globules rouges (hématocrite).

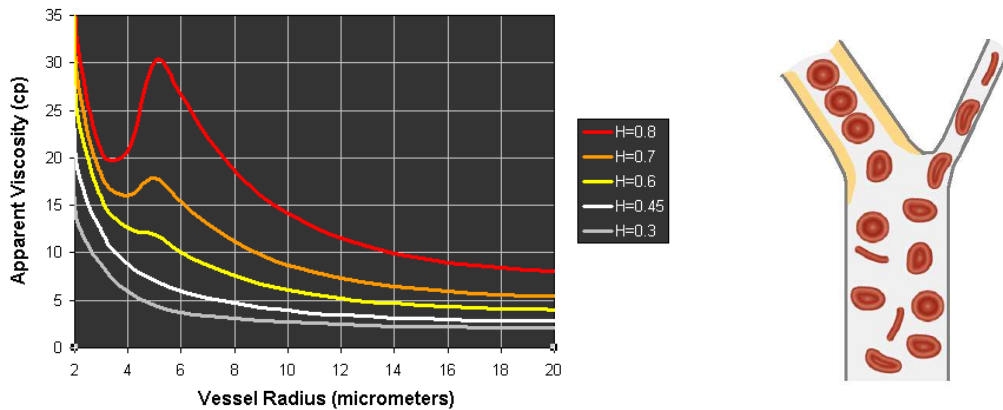


FIGURE 3.8: A gauche, l'évolution de la viscosité apparente en fonction du rayon des vaisseaux pour plusieurs valeurs de l'hématocrite. A droite une illustration de l'effet Fåhræus-Linqvist, où la viscosité diminue au lieu d'augmenter lorsque le diamètre du vaisseau est proche de la taille des globules rouges. Le frottement qui doit normalement s'intensifier, est en fait pratiquement annulé par la formation d'une chaîne de globules rouges qui expulsent le plasma en périphérie. Le plasma joue alors un rôle de lubrifiant. Lorsque le diamètre continue de décroître, les globules rouges obstruent les vaisseaux et doivent se déformer pour avancer ce qui conduit à une forte viscosité.

Modélisation du flux dans un réseau

Le flux sanguin Q peut être classiquement décrit dans chaque vaisseau, de rayon R et de longueur L , à partir de la loi de Poiseuille donnée par :

$$Q = \frac{\pi R^4 \Delta P}{8\mu_{app}(R, H_D)L} \quad (3.11)$$

où ΔP représente la différence de pression entre les deux extrémités du vaisseau et $\mu_{app}(R, H_D)$ la viscosité apparente du sang (comme définie précédemment).

Pour modéliser le flux sanguin dans le réseau vasculaire, on applique la loi de conservation du flux en chaque noeud du réseau, qui exprime le fait que la somme des flux qui entrent et sortent en un point du réseau (i, j) est nulle, soit :

$$\sum_{k=1}^4 Q_{(i,j),k} = 0 \quad (3.12)$$

L'application de cette procédure conduit à une série d'équations linéaires pour les pressions P_i qui peut être résolue par différents types d'algorithmes (méthode *SOR*, méthode de Choleski, etc.) Une fois les pressions aux noeuds déterminées, il est aisé de calculer le flux dans chaque segment du réseau.

L'adaptation vasculaire

Les propriétés rhéologiques du sang et le remodelage du réseau microvasculaire sont interdépendants. En effet, le flux sanguin crée des tensions sur la paroi des vaisseaux (cisaillement, pression, etc.) qui conduisent à l'adaptation du diamètre vasculaire par vasodilatation ou vasoconstriction. En retour, la rhéologie sanguine (viscosité, hématicrite, etc.) est affectée par l'architecture du réseau. Pour modéliser le remodelage vasculaire dans un réseau pré-existant, nous utilisons le modèle théorique proposé par Pries et col. 1998 . Le modèle considère un certain nombre de stimuli qui prennent en compte l'influence de la tension de cisaillement (S_{wss} , wall shear stress), la pression intravasculaire (S_p) et un mécanisme métabolique qui dépend de l'hématocrite (S_m). Ces trois stimuli de base permettent de générer des réseaux vasculaires stables avec des distributions de diamètres des vaisseaux et de valeurs de flux sanguins conformes à la réalité. Le modèle théorique fait l'hypothèse que pour chaque segment du réseau considéré, l'adaptation du rayon (ΔR) sur un intervalle de temps Δt est proportionnel au stimulus résultant (S_{tot}) qui s'applique sur le segment, soit :

$$\Delta R = \underbrace{(S_{wss} + S_p + S_m)}_{S_{tot}} R \Delta t. \quad (3.13)$$

Tension de cisaillement

L'architecture d'un réseau vasculaire obéit à un certain nombre de principes physiques [42]. Le premier a été proposé par Murray (1926). Ce principe se fonde sur la minimisation du coût énergétique dans la maintenance du flux sanguin à travers le réseau. Une

conséquence de ce principe implique que la tension de cisaillement doit rester constante dans chaque vaisseau. Ceci est réalisé à travers les mécanismes d'adaptation du rayon des vaisseaux. Ainsi, le rayon tend à augmenter lorsque la tension de cisaillement augmente et inversement. Le stimulus S_{wss} lié à la tension de cisaillement τ_{wss} peut alors s'exprimer par :

$$S_{wss} = \log\left(\underbrace{\frac{4\mu(R, H_D)}{\pi R^3}}_{\tau_{wss}} |Q| + \tau_{ref}\right) \quad (3.14)$$

où τ_{ref} est une valeur typique constante introduite pour prévenir l'occurrence de comportement numérique singulier lorsque τ_{wss} est très faible [43]. L'adaptation en réponse à la tension de cisaillement tend à renforcer un nombre réduit de branche formant la "colonne vertébrale" du réseau et à éliminer les vaisseaux à faible cisaillement. Le réseau résultant est instable car aucun équilibre ne peut ainsi s'instaurer. Il est donc nécessaire d'ajouter d'autres contraintes (stimuli).

Pression intravasculaire

La pression intravasculaire est un autre stimulus clé de l'adaptation vasculaire. Pries et col. [41] ont observés expérimentalement sur le mésentère de rat, la dépendance entre l'intensité de la tension de cisaillement et cette pression (P). Ils ont proposé une description paramétrique de leurs données expérimentales qui exprime la croissance sigmoïdale de la tension de cisaillement avec la pression de la façon suivante :

$$\tau_e(P) = 100 - 86 \exp\left[-5000 \cdot [\log(\log P)]^{5.4}\right] \quad (3.15)$$

La sensibilité du stimulus à la pression intravasculaire est alors décrite par :

$$S_p = -\log \tau_e(P) \quad (3.16)$$

Stimulus métabolique

L'introduction d'un stimulus métabolique permet de stabiliser le réseau en stimulant la dilatation des vaisseaux ayant une tension de cisaillement faible. Le stimulus s'exprime une fois de plus par une loi logarithmique donnée par :

$$S_m = k_m \log\left(\frac{Q_{ref}}{QH_D} + 1\right) \quad (3.17)$$

où Q_{ref} est un flux de référence supposé plus grand que la plupart des flux existants dans le réseau. Dans nos simulations, Q_{ref} a pour valeur celle du flux dans le vaisseau parent. H_D représente l'hématocrite et Q le flux dans le segment considéré. k_m est une constante qui caractérise l'intensité relative du stimulus métabolique.

L'évolution du rayon vasculaire s'exprime alors par l'équation résultante :

$$\Delta R = \left[\underbrace{\log(\tau_w + \tau_{ref})}_{S_{wss}} - \underbrace{k_p \log \tau_e(P)}_{S_p} + \underbrace{k_m \log \left(\frac{Q_{ref}}{QH_D} + 1 \right)}_{S_m} - k_s \right] R \Delta t \quad (3.18)$$

Le terme additionnel k_s représente la contraction naturelle du vaisseau induite par la lame basal formée de la couverture en péricytes qui assure l'étanchéité et l'intégrité du vaisseau. Cette contractilité s'oppose à la dilatation disproportionnée des vaisseaux. L'intégration de l'ensemble de ces mécanismes dans le modèle computationnel permet de fonctionnaliser les vaisseaux du réseau vasculaire et d'avoir un potentiel pour rendre compte des effets des traitements avec beaucoup plus de réalisme.

3.3.3 Simulation numérique

Nous présentons tout d'abord la simulation du processus de croissance des vaisseaux, puis dans un second temps la simulation qui permet de le fonctionnaliser par les mécanismes d'adaptation vasculaire décrits.

Croissance vasculaire

La simulation numérique du processus d'angiogenèse tumorale est réalisée en initiant un certain nombre de germes sur un vaisseau parent. Ces germes vont alors croître en accord avec les lois de probabilités précédemment définies qui dépendent des conditions environnementales et plus précisément des densités locales en VEGF et fibronectine. Dans la simulation présentée figure 3.9, 5 germes équidistants sont initiés sur le vaisseau parent dont la longueur est de 2 mm. Le réseau se développe par phases successives. Tout d'abord, les vaisseaux croissent à partir des 5 germes en suivant des trajectoires globalement parallèles. A environ 1/3 du parcours, les premières branches se forment, puis les arbres s'interconnectent et deviennent plus denses en s'approchant de la tumeur. La figure 3.10 montre la production de métallo protéases par les cellules endothéliales formant les germes. Ces enzymes participent à la dégradation des fibres de la matrice et rendent l'environnement plus inhomogène ce qui perturbe d'avantage la migration des germes situés en amont en rendant les trajectoires plus erratiques.

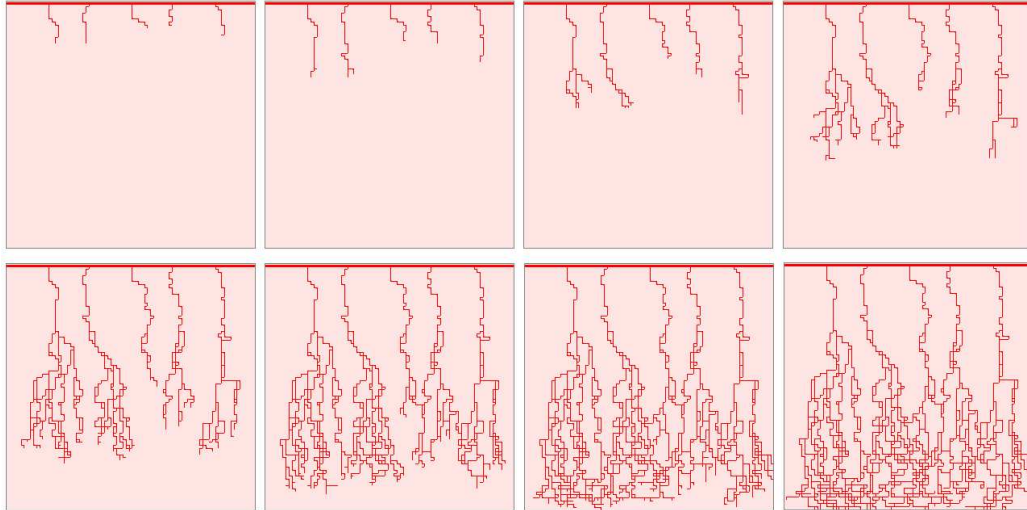


FIGURE 3.9: Croissance vasculaire à partir de l'initiation de 5 germes sur le vaisseau parent.

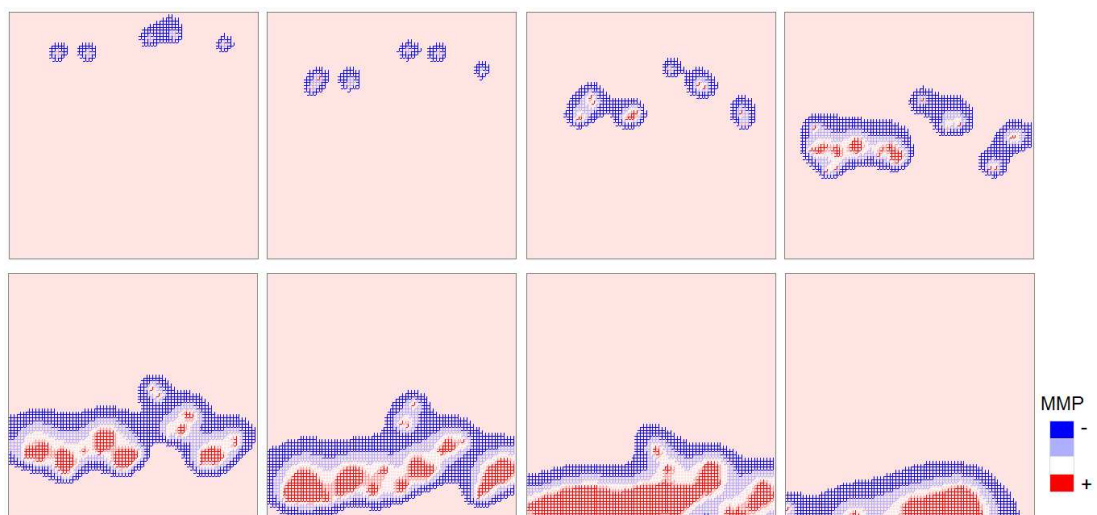


FIGURE 3.10: Production des métallo protéases (MMPs) par les cellules endothéliales situées aux extrémités des germes.

La structure vasculaire résultante est assez proche de structures observées à partir d'un dispositif de fenêtre dorsale sur la souris (Fig. 3.11). A l'exception des rayons des vaisseaux. Dans notre simulation ceux-ci sont tous de même rayon alors que les images expérimentales montrent une forte hétérogénéité. La prise en compte des effets de la rhéologie sanguine sur l'adaptation vasculaire permet de rendre compte de cette hétérogénéité (Fig. 3.13).

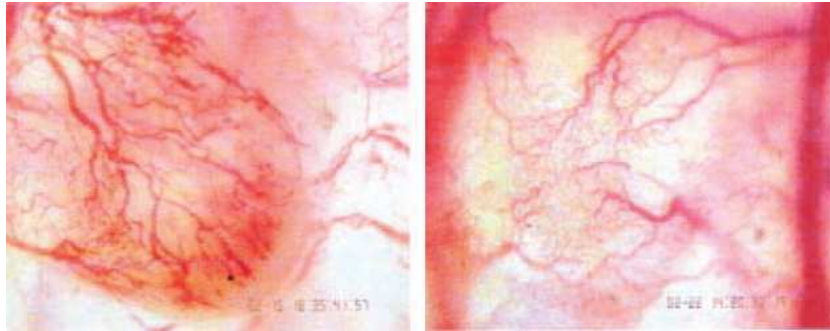


FIGURE 3.11: Réseaux angiogéniques observés à partir d'une fenêtre dorsale sur la souris. *Duda et col. British Journal of Cancer, 2002*

Adaptation vasculaire

La simulation numérique du remodelage du réseau vasculaire a été effectuée à partir du réseau homogène (rayons vasculaires égaux) que nous venons de faire croître. Le sang est perfusé dans le vaisseau parent et les globules rouges sont distribués à travers les différentes branches du réseau vasculaire. Les simulations conduisent ainsi à une distribution de l'hématocrite qui varie localement d'un vaisseau à l'autre (Fig. 3.12), ce qui produit des variations locales de la viscosité lesquelles induisent les changements de rayons. Ceux-ci se répercutent alors sur l'hématocrite et ainsi de suite. Le processus est itéré jusqu'à l'obtention d'un état d'équilibre. Les réseaux "adaptés" résultants montrent cette fois une hétérogénéité conforme à l'observation expérimentale (Fig. 3.13).

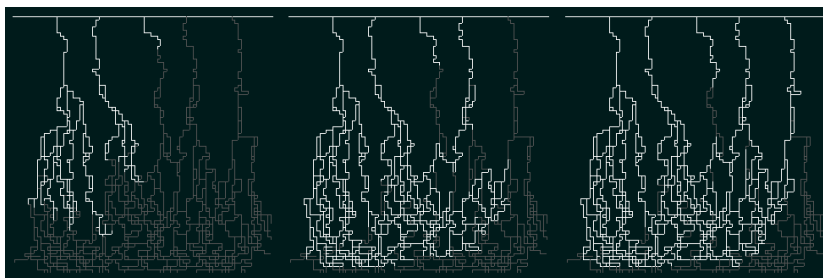


FIGURE 3.12: Cartographie de la dispersion temporelle des globules rouges (hématocrite) dans le réseau vasculaire (trame grise).

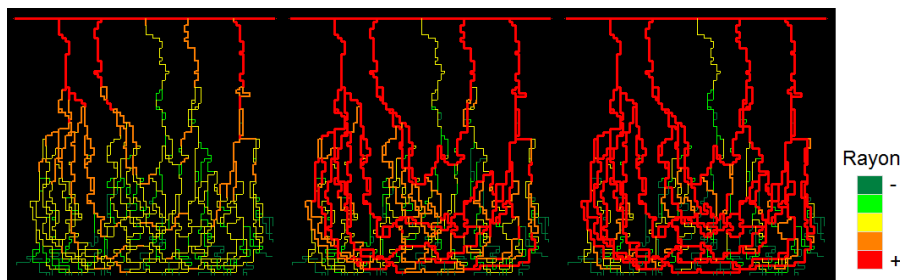


FIGURE 3.13: Phases de l'adaptation des rayons des vaisseaux à partir des contraintes hémodynamiques induites par la perfusion du réseau.

3.4 Conclusion

L'angiogenèse est un mécanisme extrêmement complexe régulé chimiquement et mécaniquement. Nous avons considéré ces deux aspects séparément dans deux contextes différents et avec deux approches également différentes. Nous sommes cependant conscients que chimie et mécanique interviennent effectivement dans ces deux contextes que sont la formation de cordons *in vitro* et l'angiogenèse tumorale.

Le premier aspect et plus précisément les phénomènes de régulation moléculaire qui sous-tendent l'auto-organisation vasculaire restent intensément étudiés au sein de l'équipe DyCTiM par Philippe Tracqui, à la fois sur le plan expérimental à partir du modèle *in vitro* et sur le plan théorique.

Le second aspect mécanique est actuellement en cours d'investigation, dans le contexte de l'angiogenèse tumoral, avec Simon Le Floc'h, mécanicien et postdoctorant de l'équipe. Ce travail porte sur l'intégration des forces mécaniques de traction exercées par les cellules sur les fibres de la matrice extra cellulaire. La déformation du réseau de fibres par les cellules génère une anisotropie dans le réseau qui influence la formation et la migration de nouveaux germes vasculaires. Le phénomène d'anastomose, initialement fondé sur l'artifice d'une probabilité de rencontre, s'explique à présent de façon déterministe par la genèse de chemins privilégiés qu'empruntent les cellules pour se rencontrer et former les boucles d'anastomose du réseau. La modulation de la rigidité de la matrice due à la dégradation des fibres par les MMPs est également prise en compte dans la nouvelle version du modèle actuellement en cours de développement et non présentée dans le cadre de ce mémoire.



Computer-Assisted Therapeutic Strategies

4

Perspectives : Le projet CATS

4.1 Le Contexte

De nombreuses avancées ont été réalisées en cancérologie au cours de ces dix dernières années. Les découvertes nous montrent cependant à quel point le processus du développement tumoral se complexifie lorsqu'une thérapie est mise en jeu. Les capacités d'adaptation cellulaire se sont révélées particulièrement surprenantes et les mécanismes de compensation qui se mettent en place au niveau de la cellule tumorale pour échapper au traitement ont largement été sous évalués et restent encore majoritairement incompris [44, 45]. Parmi ces mécanismes, notons par exemple la dissémination rapide de cellules tumorales au sein du parenchyme cérébral suite à un traitement antiangiogénique des gliomes. Les cellules tumorales en état d'hypoxie cherchent à recruter un réseau vasculaire en libérant des facteurs de croissance angiogénique tel le VEGF, mais si cet état hypoxique vient à se reproduire, les cellules tumorales affectées pour la seconde fois, adoptent une nouvelle stratégie et se mettent massivement à migrer pour échapper au stress de l'environnement [46]. Cette observation montre à quel point la grande instabilité génétique des cellules tumorales, permet sous la pression des contraintes environnementales, une sélection rapide de phénotypes résistants ou plus invasifs. Ce qui conduit à l'utilisation de traitements de plus en plus agressifs voir à l'échec des thérapies, particulièrement dans le cas des gliomes [47].

Afin de lutter contre ces phénomènes de résistance, la thérapie adaptative mettant en oeuvre plusieurs modes thérapeutiques pourrait se révéler un outil efficace dans le traitement des tumeurs les plus récalcitrantes telles que les gliomes [48]. Cependant, de part la complexité du processus global, il est difficile de trouver comment combiner les thérapies. La gestion de tels processus complexes est rendue possible grâce aux outils de modélisation, de simulation numérique et de commandes optimales et prédictives.

La modélisation théorique est un outil qui permet une vision intégrée d'un phénomène (système). La simulation numérique du phénomène décrit par le modèle permet d'effectuer un grand nombre d'expériences virtuelles afin de tester des hypothèses pour mieux comprendre ce système, mais également pour optimiser et prédire ses réactions (Fig. 4.1).

Dans le cadre de ce projet, nous formulons l'hypothèse qu'un système vivant peut être décrit de façon suffisamment satisfaisante par un modèle théorique de façon à pouvoir anticiper ses réactions sous l'effet d'une thérapie [49, 50]. Dans ce contexte, nous souhaitons faire la preuve du concept de Stratégie Thérapeutique Assistée par Ordinateur (STAO). Le but est de piloter la thérapie à partir de tests systématiques sur le *système virtuel* pour déterminer la meilleure solution à mettre en oeuvre sur le *système réel*. Cette thérapie assistée est adaptative dans le sens où les effets sur le système réel sont contrôlés régulièrement pour effectuer les corrections et les ajustements nécessaires afin d'amener progressivement le système réel jusqu'à l'état cible recherché, c'est à dire l'éradication de la population tumorale (guérison) [51]. Cette façon de procéder est courante dans la gestion de systèmes complexes tels que la régulation des trafics routier et aérien, dans les domaines financier et militaire, en robotique dont la robotique médicale. Dans tous ces domaines, il est nécessaire d'analyser rapidement une situation et de déterminer la réponse ou la stratégie optimale pour y faire face. Les notions de danger et de risque sont perpétuellement présentes sous forme de contraintes à gérer.

La réalisation de ce projet nécessite de disposer d'un modèle expérimental *in vivo* facilement contrôlable c'est à dire où il est relativement facile d'observer une évolution sous les effets d'une action et de la quantifier. Le modèle doit être simple sans pour autant s'affranchir des mécanismes clés de la croissance tumorale comprenant la prolifération cellulaire, l'invasion, l'angiogenèse et l'occurrence des mécanismes de résistance au traitement. L'introduction de cellules tumorales de type gliome humain U373 sous un dispositif de fenêtre dorsale implanté sur la souris répond à ces exigences [52, 53].

Les partenaires du projets

Ce projet associe des médecins, des biologistes, des mathématiciens et des automaticiens et regroupe ainsi *in situ* toutes les compétences nécessaires à sa réalisation :

- TIMC-IMAG (Techniques de l'Imagerie Médicale et de la Complexité – Informatique, Mathématiques et Application de Grenoble) regroupe des compétences en modélisation mathématique et computationnelle des systèmes biologiques complexes ainsi qu'en imagerie microscopique des marqueurs tumoraux.
- GIN (Grenoble Institut des Neurosciences) regroupe des spécialistes en oncologie et en imagerie dont l'imagerie optique intravitale.
- GIPSA (Laboratoire Grenoblois de l'Image, de la Parole, du Signal et de l'Automatique), spécialisé dans les problématiques d'optimisation et de commande prédictive.

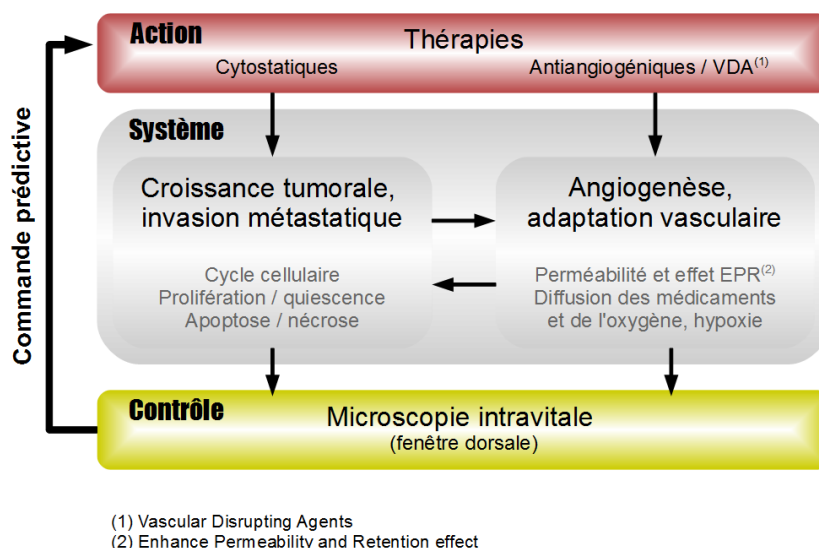


FIGURE 4.1: Diagramme décrivant l'intégration des différentes composantes du modèle théorique et leurs interactions. Le modèle intègre l'action de deux types de thérapies sur l'évolution du système tumoral. La commande prédictive a alors pour but d'adapter l'action (c'est à dire la thérapie) sur le système tumoral en fonction des résultats du contrôle réalisé par microscopie intravitale.

- Ecrins Therapeutics est une société de biotechnologies, spécialisée dans la recherche et le développement de médicaments innovants pour le traitement du cancer, en particulier les agents de destruction vasculaire (VDA) qui ont déjà fait leur preuve *in vivo* et qui ont été brevetés par l'Université Joseph Fourier.

Les financements

Ce projet a été initié en 2009. Il a bénéficié de plusieurs soutiens de la part du Réseau National des Systèmes Complexes (Appel à idées 2009), du CNRS (Appel PEPS-INS2I 2010-2011) et de l'Institut Rhône-Alpin des Systèmes Complexes (Appel IXXI printemps 2011). Ces soutiens ont permis de tester et de valider les différents aspects du modèle expérimental et de faire la preuve de faisabilité.

Les étudiants

Deux étudiantes de master 2 se sont succédées (2008-2009 et 2009-2010) sur le développement du modèle computationnel et ont permis d'en poser les éléments fondamentaux. Le modèle couple à présent les mécanismes de croissance tumorale et le remodelage vasculaire et permet l'intégration des effets des thérapies impliquant des molécules cytostatiques et des agents de destruction vasculaire. Ce travail est présenté en section 4.5. Le modèle computationnel a également été adapté pour décrire spécifiquement les événements observés à partir du dispositif de fenêtre dorsale. L'étudiante poursuit actuellement ce travail grâce à l'obtention d'une bourse de thèse (2010-2013) en co-encadrement avec François Estève du GIN. Ce travail est présenté

en section 4.6.

4.2 Le système réel

4.2.1 Microscopie intravitale et fenêtre dorsale

La pose d'une fenêtre dorsale sur la souris, permet l'implantation de cellules tumorales afin de suivre, par un système de macro fluorescence, le développement de la tumeur et de sa vascularisation [52]. La chambre dorsale est greffée chirurgicalement sur le pli formé par l'étirement de la peau du dos de la souris. Un bout de peau de la taille de la fenêtre est retiré et recouvert par une plaque de verre qui laisse apparaître la zone sous cutanée dans laquelle sont implantées les cellules tumorales qui peuvent être rendues fluorescentes par transfection (GFP). Un cathéter est implanté dans la jugulaire externe ou dans la veine de la queue afin de pouvoir injecter les produits fluorescents nécessaires à l'étude par microscopie intravitale (Fig. 4.2). Ce dispositif unique donne ainsi accès à un grand nombre de paramètres relatifs au flux sanguin, à la perméabilité des vaisseaux, à la diffusion et à la localisation des médicaments, à la migration des cellules en particulier lors de l'invasion tumorale.

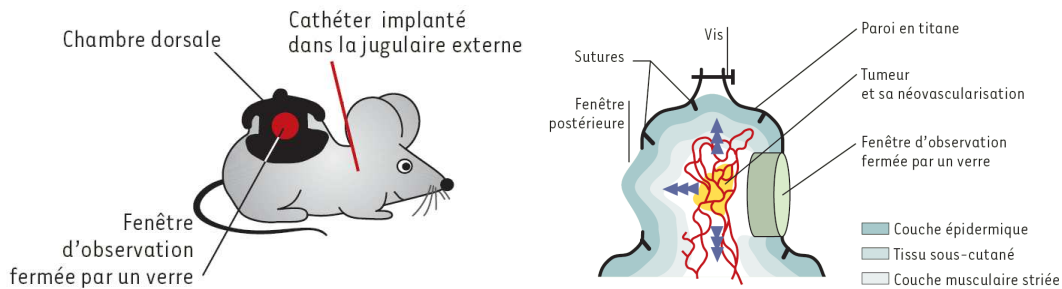


FIGURE 4.2: Le dispositif de la fenêtre dorsale. *Lavigne et col. 2002*

4.2.2 Visualisation de l'angiogenèse tumorale

L'angiogenèse tumorale est induite par le VEGF que produisent les cellules tumorales lorsqu'elles se trouvent en état d'hypoxie. Elle peut également être induite directement sous la fenêtre dorsale par l'introduction d'une source de VEGF, sous la forme d'une éponge chirurgicale imprégnée avec une concentration déterminée. Dans l'expérience présentée (Fig. 4.3) une concentration de $10 \mu\text{g/ml}$ permet d'induire une réponse vasculaire avec d'une part la croissance de nouveaux vaisseaux périphériques et d'autre part la dilatation des vaisseaux déjà présents.

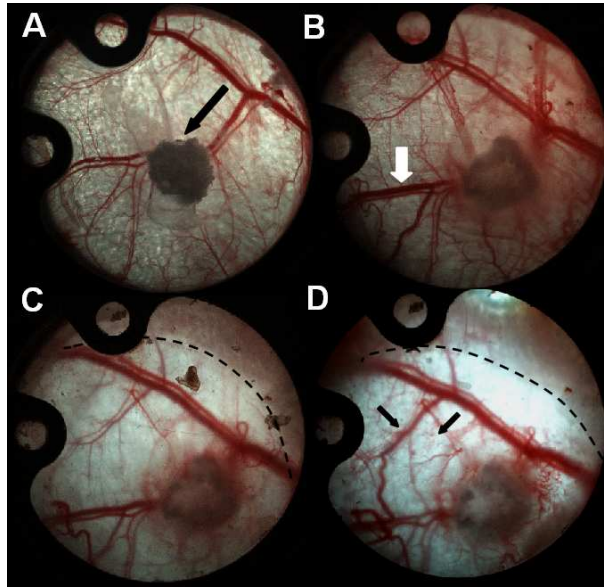


FIGURE 4.3: Angiogenèse induite par une éponge imbibée de VEGF introduite dans la fenêtre dorsale. (A) Jour 0, l'éponge est initialement opaque en raison de sa forte imprégnation en VEGF. (B) Jour 7, le VEGF diffuse rapidement dans la chambre et touche l'ensemble des vaisseaux en commençant par induire leur dilatation. (C) Jour 14, de nouveaux capillaires apparaissent autour de la source. (D) Jour 21, le remodelage vasculaire se poursuit avec la dilatation de nouveaux vaisseaux. Expériences réalisées par L. Hamard et B. van der Sanden, Grenoble Institut des Neurosciences.

4.2.3 Visualisation des effets de médicaments

L'imagerie bi-photon de la vascularisation d'une tumeur de type carcinome de poumon humain (HTB177) introduite dans la fenêtre dorsale sur la souris permet d'observer les effets d'agents de destructions vasculaires, dont nous reparlerons plus en détail par la suite. Un agent fluorescent est injecté par intraveineuse pour imager le réseau vasculaire (Fig. 4.4A) [54]. 30 minutes après l'injection, une augmentation de la perméabilité vasculaire est visible par la dissémination de la fluorescence à travers l'endothélium et dans le tissu (Fig. 4.4B). Peu de temps après, la circulation sanguine s'interrompt dans les vaisseaux intratumoraux mais pas dans ceux situés à la périphérie de la tumeur (Fig. 4.4C). Une heure après le traitement, une seconde injection de l'agent fluorescent montre un arrêt presque total de la circulation sanguine dans la zone tumorale (Fig. 4.4D).

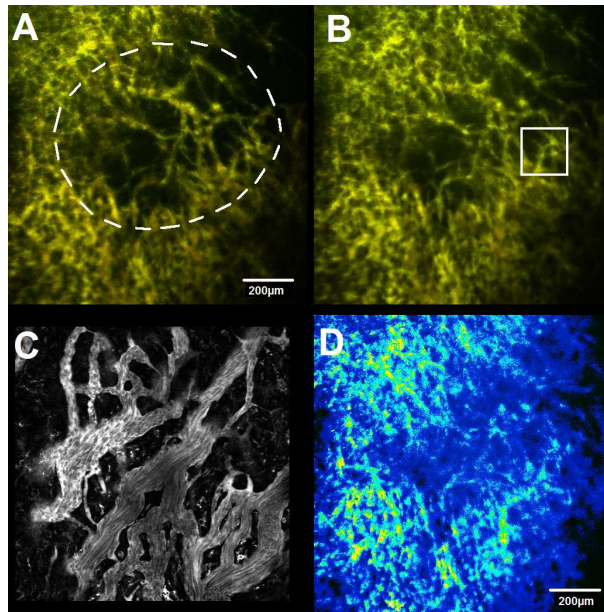


FIGURE 4.4: Visualisation de l'effet des VDAs en imagerie microscopique bi-photon. (A) Réseau vasculaire avant traitement, la tumeur est localisée par la ligne en pointillés. (B) Réseau vasculaire 30 minutes après l'injection des VDAs. La plus forte fluorescence de l'arrière plan révèle une augmentation de la perméabilité vasculaire induite par les VDAs. (C) détail de (B) où les globules rouges sont visibles sous forme de points sombres dans le plasma là où le flux sanguin a été stoppé, tandis que les globules rouges circulants apparaissent sous la forme de lignes sombres dans les vaisseaux toujours perfusés. Les images (A,B) représentent la projection verticale d'intensité de fluorescence de 33 coupes inter distantes de 5 microns. L'image (D) représente la projection verticale de l'écart type de l'intensité de fluorescence. Seules les régions perfusées autour de la tumeur sont visibles. Expériences réalisées par B. van der Sanden, Grenoble Institut des Neurosciences en collaboration avec Andrei Popov, Aurélie Juhem et Cyril Zenga de la société Ecrins Therapeutics.

4.3 Le système virtuel

4.3.1 Le module "Croissance tumorale"

Ce module a pour but de modéliser l'évolution de la croissance de la tumeur telle que nous l'observons avec le dispositif de fenêtre dorsale. Nous avons fait le choix de représenter la tumeur par un automate cellulaire afin d'être en mesure de rendre compte des caractéristiques propres de différents types cellulaires. Nous nous sommes pour cela inspiré de l'automate proposé par Alarcon et col. (2003). L'automate est défini sur une grille G , où chaque élément noté e peut être vide ($e = 0$) ou peut contenir une cellule tumorale de type prolifératif, quiescent, apoptotique ou nécrotique, soit $e = 1, 2, 3$ ou 4 respectivement (Fig. 4.5).

Les transitions entre les différents états cellulaires tumoraux sont conditionnés par le cycle cellulaire et les conditions environnementales. Plus spécifiquement, le niveau d'oxygène, la place disponible et la concentration locale en médicaments déterminent si

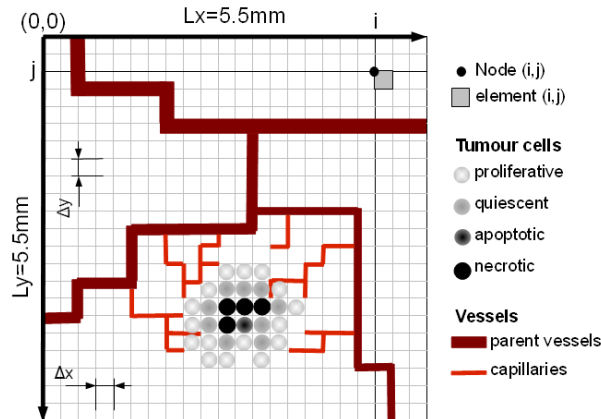


FIGURE 4.5: Le domaine de simulation est un carré de $2mm$ de long ($L_x = L_y$), discrétisé par une grille régulière (i, j) telle que chaque élément $e(i, j)$ de la grille ait un côté $\Delta x = \Delta y = 25\mu m$ soit approximativement la taille d'une cellule. Les vaisseaux occupent les arêtes de la grille c'est à dire une portion de segment entre deux noeuds $N(i, j)$.

la cellule prolifère ou meurt. Ces conditions sont décrites dans le diagramme d'inférence de la figure 4.6.

Cycle cellulaire

Le cycle de division cellulaire est la séquence d'évènements par laquelle une cellule réplique ses composants et les répartit dans deux cellules filles. Le cycle est divisé en 4 phases : $G1$, S , $G2$ et M [55]. Sa prise en compte est nécessaire car il peut se trouver affecté par le manque d'oxygène (hypoxie) mais les effets diffèrent selon s'il s'agit d'une cellule normale ou tumorale. Les deux types cellulaires sont sensibles à l'hypoxie, mais alors que les cellules normales meurent, les cellules tumorales sont capables de réduire leur métabolisme en entrant dans la phase de quiescence $G0$ (Fig. 4.7). Les cellules quiescentes ont ainsi la capacité de survivre à l'état d'hypoxie sur une longue durée et de revenir à l'état prolifératif lorsque le niveau d'oxygène redevient suffisant. Les cellules tumorales peuvent donc résister à l'hypoxie, mais dans une certaine limite. Si l'hypoxie est très sévère ou si l'hypoxie se prolonge pendant trop longtemps alors les cellules tumorales finissent par mourir [55, 56]. Il y a un manque de connaissance concernant la durée de survie cellulaire d'une cellule en phase quiescente. Nous faisons ici l'hypothèse à titre d'exemple que la cellule ne peut survivre qu'une dizaine d'heures avant de mourir par nécrose, bien que des durées de survie considérablement plus importantes aient été reportées (jusqu'à plusieurs mois). Concernant la durée du cycle cellulaire, elle varie d'un type cellulaire à l'autre mais également entre cellules de même type (dans une moindre mesure). Les variations portent essentiellement sur la durée de la phase $G1$. Nous adapterons cette durée selon les cas considérés.

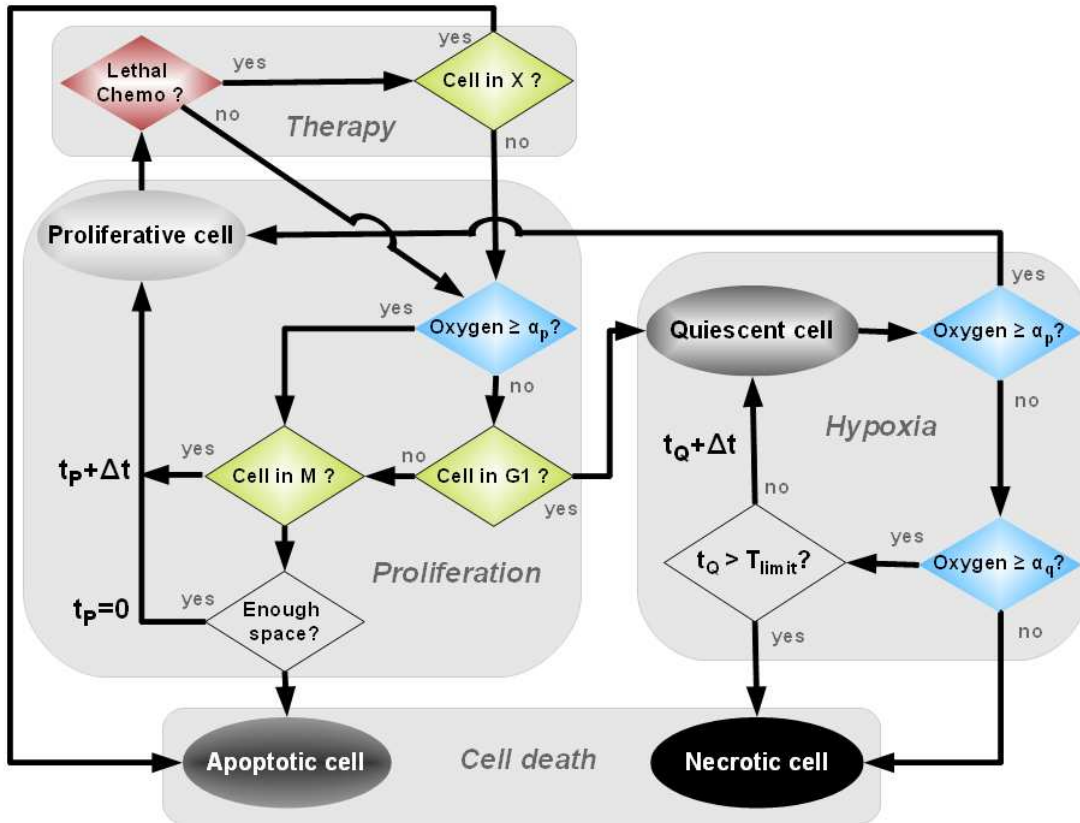


FIGURE 4.6: Diagramme d'inférence pour décrire les transitions entre états cellulaires tumoraux. Un timer $t_P = 0$ est assigné à chaque nouvelle cellule proliférative. Dans des conditions normales d'oxygénation, le timer est incrémenté du pas de temps Δt . Lorsqu'une cellule entre en état d'hypoxie, c'est à dire en phase G_0 , son timer t_P est arrêté et remplacé par $t_Q = 0$. Tant que la cellule reste quiescente, son timer t_Q est incrémenté de Δt . Si l'oxygène redevient disponible, la cellule retourne dans son état prolifératif et son timer retourne à sa valeur t_P à laquelle il s'était arrêté. Si $t_Q > T_{limit}$ alors la cellule devient nécrotique et son nouveau timer t_N s'incrémente à l'infini. La cellule peut également mourir par apoptose (mort cellulaire programmée). Cela se produit dans l'automate si l'espace manque pour la division cellulaire. Dans ce cas, un nouveau timer t_A est assigné à la cellule, de façon cette fois irréversible. Il est incrémenté de Δt jusqu'à atteindre sa limite $t_A = t_{apop}$ au delà de laquelle la cellule est tuée et nettoyée de l'espace de simulation.

Métabolisme cellulaire

Toutes les cellules consomment des nutriments et de l'oxygène pour vivre et accomplir leurs tâches. La consommation de base en oxygène des cellules prolifératives (tumoraux), notée α_p est d'environ $2.3 \times 10^{-16} \text{ mol.cells}^{-1} \text{ s}^{-1}$. Le fait que les cellules quiescentes consomment moins d'oxygène que les cellules "actives" est pris en compte en divisant le taux de base par un facteur 5 [57]. Une cellule proliférative devient quiescente si le niveau en oxygène devient inférieur à α_p . Inversement, si la concentration en oxygène revient à ce niveau, une cellule quiescente redevient proliférative. Si la concentration en oxygène s'abaisse sous le seuil $\alpha_q = \alpha_p/5$, la cellule meurt par hypoxie en

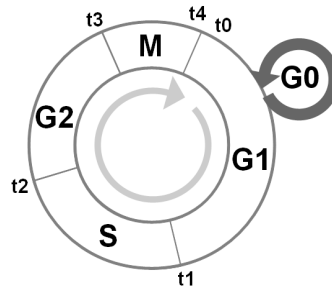


FIGURE 4.7: Représentation schématique des différentes phases du cycle cellulaire. $t_1 - t_0$, $t_2 - t_1$, $t_3 - t_2$, $t_4 - t_3$ représentent respectivement les durées des phases $G1$, S , $G2$, M . Lorsque l'oxygène vient à manquer, les cellules entrent dans la phase $G0$ qui correspond à l'état de quiescence. Cet état est réversible, c'est à dire que la cellule peut redevenir proliférative si le niveau d'oxygène redevient normal.

devenant nécrotique.

Les nutriments ne sont pas pris en compte dans le modèle. Il s'agit de macromolécules dont la taille est bien supérieure à celle des molécules d'oxygène (si l'on exclut les sels minéraux). Leur transport depuis les vaisseaux sanguins jusqu'aux cellules est donc nécessairement plus difficile qu'elle ne l'est pour les petites molécules d'oxygène qui diffusent très facilement à travers la paroi des vaisseaux puis dans les tissus. Nous faisons donc l'hypothèse que l'oxygène est le facteur limitant car s'il n'y a plus d'oxygène, il n'y a forcément plus de nutriments. L'approximation réside essentiellement dans le fait que la réciproque n'est pas vraie et qu'elle peut introduire une modulation du métabolisme de la cellule que nous négligeons.

Notons également que les cellules tumorales ont la capacité de changer de mode de production énergétique en passant en mode anaérobie basé sur la glycolyse. A ce stade, nous avons également négligé cet aspect, mais il sera nécessaire de le prendre en compte à l'avenir en raison de son importance avérée et non négligeable dans l'évolution tumorale.

Mort cellulaire

La mort des cellules cancéreuses peut se produire selon deux mécanismes : l'apoptose et la nécrose. L'apoptose correspond à la mort cellulaire dite "programmée" dans le sens où la cellule déclenche elle-même le processus de destruction qui s'accompagne d'une signalisation en direction des macrophages afin que ceux-ci interviennent pour nettoyer les débris cellulaires. Dans le cas de la mort par nécrose, que l'on caractérise de mort violente, la cellule est détruite par un facteur externe non déclencheur d'apoptose. Dans ce cas là, bien que les fluides aient été expulsés de la cellule, le cadavre nécrosé demeure. L'agrégation des corps cellulaires nécrosés peut atteindre une taille non négligeable (jusqu'à plusieurs millimètres).

Les cellules prolifératives meurent en général par apoptose si elles sont exposées à des molécules cytotoxiques en quantité suffisante. Les cellules quiescentes en revanche sont insensibles à ces molécules puisque les molécules cytotoxiques agissent sur les microtubules pour bloquer le processus de division, or les cellules quiescentes ne se divisent pas. Elles meurent donc essentiellement par nécrose si l'hypoxie devient trop sévère ou se prolonge trop longtemps.

4.3.2 Le module "Angiogenèse"

Le processus angiogénique se déclenche lorsque les cellules tumorales manquent d'oxygène et de nutriments pour continuer de proliférer. Les cellules réduisent alors drastiquement leur métabolisme en stoppant la prolifération et en réduisant ainsi leur besoin en oxygène. Il s'agit de l'état quiescent où phase G_0 qui peut durer jusqu'à plusieurs mois selon les types cellulaires. Les cellules en état de quiescence cherchent à recruter un réseau vasculaire pour être à nouveau alimentées et retourner à leur état prolifératif. Pour cela, les cellules hypoxiques libèrent massivement des facteurs de croissance angiogénique dont le VEGF (Vascular Endothelial Growth Factor) qui induit l'angiogenèse des vaisseaux proches. La modélisation du processus d'angiogenèse tumorale est décrite en section 3.3 du chapitre précédent.

Evolution du VEGF

La concentration en VEGF au point (x, y) et au temps t est notée $V(x, y, t)$. $\tilde{V}(e)$ représente le taux de production du VEGF pour chaque élément e de la grille computationnelle. Le VEGF est supposé émis par les cellules quiescentes uniquement ($e = 2$) avec un taux constant \tilde{V}_{emit} , d'où $\tilde{V}(2) = \tilde{V}_{emit}$ et $\tilde{V}(e \neq 2) = 0$. L'évolution de la concentration en VEGF dans le tissu est décrite par l'équation aux dérivées partielles suivante inspirée et adaptée de [58] :

$$\frac{\partial V}{\partial t} = \tilde{V}(e) + D_V \nabla^2 V - \lambda V - \eta V_{uptake} \quad (4.1)$$

où D_v est le coefficient de diffusion du VEGF, λ son taux de dissociation et η son taux de consommation par les cellules endothéliales qui dépend de la concentration locale V de VEGF de la façon suivante :

$$V_{uptake}(e) = \begin{cases} V_{max} & , \text{ si } V_{max} \leq V \text{ et } e \text{ est une cellule endothéliale} \\ V & , \text{ si } V < V_{max} \text{ et } e \text{ est une cellule endothéliale} \\ 0 & , \text{ sinon} \end{cases} \quad (4.2)$$

Germination des vaisseaux

Comme nous l'avons précédemment décrit (section 3.3), nous supposons que la capacité de germination des vaisseaux augmente avec la concentration locale en VEGF [59, 60]. Nous supposons de plus qu'un germe en formation inhibe toute germination sur une distance de $50 \mu m$ en amont et en aval du vaisseau [61]. La germination des

vaisseaux situés dans la tumeur est différente de celle des vaisseaux situés dans le tissu normal. Le tissu tumoral, par sa nature beaucoup plus dense et acide, offre un environnement moins favorable à la germination [60].

Effondrement vasculaire

Les vaisseaux intratumoraux n'arrivent jamais à maturation en raison de leur incapacité à recruter des péricytes, qui sont les cellules qui tapissent leur paroi extérieure pour renforcer leur imperméabilité et assurer leur intégrité. Ils restent ainsi instables et vulnérables. En particulier, si le flux sanguin diminue et que la tension de cisaillement τ_w exercée par ce flux sur la paroi interne des vaisseaux devient trop faible, alors ceux-ci s'effondrent sur eux-mêmes. Dans le modèle, nous introduisons une valeur seuil pour cette tension de cisaillement, en dessous de laquelle le vaisseau s'effondre et disparaît.

Transport d'oxygène des vaisseaux au tissu

L'oxygène diffuse à travers la paroi des vaisseaux en direction du tissu et est consommé par les cellules. Le profil spatial de la concentration d'oxygène est donné par l'équation aux dérivées partielles suivante :

$$\frac{\partial C}{\partial t} = D_{O_2} \nabla^2 P + \tilde{C}_{emit} - \tilde{C}_{cons} \quad (4.3)$$

où C est la concentration en oxygène dans le tissu, et D_{O_2} son coefficient de diffusion [62]. \tilde{C}_{emit} est le taux avec lequel l'oxygène passe à travers la paroi des vaisseaux (taux d'émission de l'oxygène dans le tissu) et \tilde{C}_{cons} est le taux de consommation de l'oxygène par les cellules du tissu où chaque cellule occupe un élément e de la grille de simulation. \tilde{C}_{emit} dépend de la différence de concentration en oxygène ente les milieux intra et extra vasculaire, H_v et $C(e)$ respectivement. La concentration à l'intérieur des vaisseaux est supposée proportionnelle à l'hématocrite [42]. Le taux d'émission dépend également de la perméabilité γ des parois. Pour chaque élément e de la grille de simulation, les contributions en oxygène de chacun des n vaisseaux v adjacents à chaque élément e sont additionnées ($n = 4$). $\tilde{C}_{emit}(e)$ est alors calculé à partir de :

$$\tilde{C}_{emit}(e) = \gamma \sum_{v=1}^n (H_v - C(e)) \quad (4.4)$$

Bien que la concentration en oxygène tend à décroître à l'intérieur des vaisseaux lorsque l'oxygène diffuse à travers le tissu, nous supposons ici que cette décroissance est négligeable. Nous faisons pour cela l'hypothèse que la quantité d'oxygène véhiculée dans les vaisseaux n'est pas limitée. Le taux de consommation en oxygène des cellules du tissu dépend du type et de l'état cellulaire et est supposé linéairement dépendant de la concentration en oxygène, de même que dans le modèle d'Alarcon et col. 2003.

$$\tilde{C}_{cons} = \begin{cases} k_N C & \text{cellule normale} \\ k_P C & \text{cellule proliférative} \\ k_Q C & \text{cellule quiescente} \\ 0 & \text{cellules nécrotique ou apoptotique} \end{cases} \quad (4.5)$$

avec $k_P > k_N > k_Q$.

4.4 Les deux modes thérapeutiques

Le traitement classique des tumeurs solides consiste à administrer une molécule capable de cibler les cellules tumorales et d'épargner les cellules saines. Les cellules tumorales se distinguent par leur capacité à proliférer. C'est cette différence qui est exploitée dans le choix des molécules utilisées dans les chimiothérapies. Ces molécules agissent pour bloquer le processus de division cellulaire et sont sélectionnées pour leur capacité dites cytostatiques ou cytotoxiques, qui bloque l'action des microtubules qui sont à l'oeuvre dans la division. Le ciblage de la prolifération s'il touche essentiellement les cellules tumorales, n'épargne cependant pas totalement les autres cellules et les dommages collatéraux (effets secondaires) restent souvent importants et impliquent une prise en charge associée spécifique.

Les cytotoxiques sont en général administrés par voie vasculaire. Cependant, contrairement aux vaisseaux sanguins normaux, le réseau vasculaire tumoral est structurellement et fonctionnellement altéré. L'angiogenèse tumorale conduit en effet à un réseau tortueux, dilaté et sacculaire de vaisseaux peu organisés et hyperperméables. Ces défauts, ainsi que la compression des vaisseaux sous l'effet de la prolifération des cellules tumorales augmentent la résistance à la circulation du flux sanguin et altèrent l'alimentation de la tumeur par rapport au réseau dit "normal". Les molécules cytotoxiques véhiculées par le sang peuvent dans certains cas contourner la tumeur sans parvenir à l'atteindre ou en quantité ou concentration trop faibles pour être efficaces. Nous avons pu démontrer ce phénomène grâce à la simulation numérique [49].

La thérapie anti-vasculaire, suggérée pour la première fois par Folkman en 1971, consiste à couper l'acheminement en sang de la tumeur afin d'affamer et d'asphyxier les cellules tumorales dans le but de les éradiquer. Cette thérapie a révélé que ce traitement rendait transitoirement "normal" le réseau tumoral (par nature tortueux et peu efficace)(Fig. 4.8), c'est à dire capable d'alimenter à nouveau très efficacement la tumeur produisant temporairement l'effet inverse à celui désiré [64]. Cette normalisation limitée dans le temps offre cependant une "fenêtre thérapeutique" durant laquelle l'acheminement de la chimiothérapie se trouve optimisé.

La tendance actuelle dans le traitement des tumeurs solides, est de développer l'usage des anti-vasculaires et d'envisager des combinaisons thérapeutiques entre diverses molécules. Dans ce projet, nous nous focalisons sur la mise en oeuvre de molécules cytotoxiques et de molécules anti-vasculaires particulières que sont les VDAs (Vascular Disrupting Agents), dont les caractéristiques sont décrites ci-après.

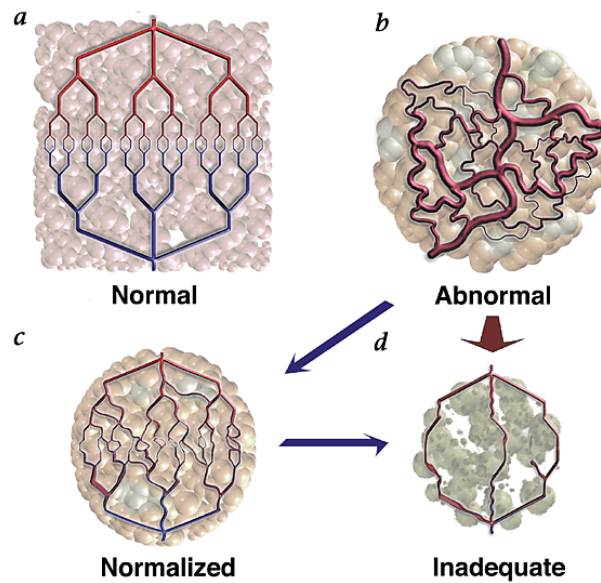


FIGURE 4.8: Réseau vasculaire normal, qui devient anormal lorsqu’il vascularise une tumeur. Le réseau peut-être ”normalisé” par un traitement anti-vasculaire correctement dosé, ou rendu inopérant [65].

4.4.1 Molécules cytotoxiques

Les molécules cytotoxiques bloquent le processus de division cellulaire et n’affectent par conséquent que les cellules prolifératives. La plupart de ces molécules agissent sur des mécanismes propres à une phase spécifique du cycle cellulaire. Ce qui signifie que seules les cellules se trouvant dans la phase appropriée sont sensibles à son action, c’est à dire au traitement. Dans ce modèle, nous proposons de tester deux molécules : l’une affectant la longue phase G2M et l’autre affectant la courte phase S. La méthode d’administration du traitement la plus commune est l’injection intraveineuse qui permet aux molécules une entrée rapide dans le circuit vasculaire. Les doses peuvent être administrées en bolus (c’est à dire de façon quasi instantanée) ou peuvent être perfusées sur plusieurs heures à plusieurs jours. En général, le traitement est administré par cycles. Dans notre modèle, les molécules sont perfusées dans le vaisseau parent du réseau vasculaire. Les perfusions durent de 5 à 10 heures et sont administrées par cycle de 1 à 2 semaines.

4.4.2 Agents de destruction vasculaire

Les VDAs (Vascular Disrupting Agents) opèrent en perturbant spécifiquement l’intégrité de la paroi des vaisseaux du réseau vasculaire tumoral. Cette perturbation conduit à l’asphyxie de la tumeur dont les cellules ne sont plus correctement alimentées et meurent. La tumeur se nécrose alors massivement [66]. L’effondrement des vaisseaux et du flux sanguin s’opère en quelques minutes après l’administration des VDAs et les effets se prolongent au delà de l’heure [67]. Dans certains tests cliniques, les paramètres vasculaires retournent à leur valeur normale, 24 heures après traitement [67].

Dans notre modèle, nous faisons l'hypothèse que les VDAs détruisent les vaisseaux perfusés par une forte concentration de médicaments et ayant de plus un flux sanguin de faible intensité, c'est à dire avec une tension de cisaillement du flux sur les parois vasculaires faible. Ces deux conditions sont considérées comme les plus favorables pour que les VDAs agissent sur l'endothélium. La perturbation des vaisseaux conduit alors à une ré-adaptation par un remodelage vasculaire et une recirculation du flux sanguin dans le réseau modifié. Les VDAs sont administrés dans nos simulations par une injection en bolus d'une durée de 5 secondes dans le vaisseau parent du réseau.

4.5 Premier pas vers la thérapie

Les résultats préliminaires sur la thérapie sont issus du travail de Margarita Pons-Salort, étudiante du Master 2 Modèles Images et Instruments en Médecine et Biologie (2008-2009). Dans ce travail, qui se focalise sur les effets de la thérapie, les croissances vasculaires et tumorales sont découplées. Nous sommes partis d'un réseau vasculaire précédemment généré par notre modèle d'angiogenèse et que nous avons juxtaposé à une tumeur "idéalisée" semi-circulaire et constituée de cellules initialement toutes prolifératives. Nous nous sommes donc placé dans le cas a priori le plus agressif, en ayant en tête l'adage "qui peut le plus, peut le moins!". L'adaptation vasculaire, c'est à dire la dilatation ou la constriction des vaisseaux suite au traitement est prise en compte puisque les effets induits sont immédiats. Par contre la néo-angiogenèse qui se produit sur des échelles de temps plus longues n'a pas été considérée à ce stade encore préliminaire.

Ces résultats ont été soumis au journal Mathematical Modelling of Natural Phenomena sous le titre : "A Computational Framework to Assess the Efficacy of Cytotoxic Molecules and Vascular Disrupting Agents against Solid Tumours", M. Pons-Salort, B. van der Sanden and A. Stéphanou.

4.5.1 Croissance tumorale sans traitement

Une première simulation (Fig. 4.9) présente le cas de la croissance tumorale sans traitement. L'état de la tumeur est représenté toutes les 3 à 4 semaines et montre une croissance régulière sur 18 semaines. La masse tumorale se développe essentiellement le long des vaisseaux où la concentration en oxygène est la plus forte et favorise la prolifération des cellules. La mort cellulaire par apoptose qui se produit lorsque les cellules prolifératives n'ont pas suffisamment d'espace pour se diviser, ce qui est le cas au coeur de la masse tumorale, permet un renouvellement des cellules et le maintient d'un pool prolifératif. Dans cette version du modèle, il est important de noter que les vaisseaux ne sont pas détruits par la croissance tumorale. Ce qui signifie que les conditions d'oxygénation du coeur tumoral ne sont pas altérées et que la concentration en oxygène permet aux cellules de continuer à proliférer. Des zones nécrotiques isolées apparaissent cependant pour les régions tumorales les plus distantes des vaisseaux

principaux. Ces occurrences nécrotiques, sont principalement due à la condition initiale "artificielle" imposée où une tumeur semi-circulaire et proliférative est juxtaposée à un réseau vasculaire pré-existant alors que ces deux entités se développent simultanément et de façon synergique.

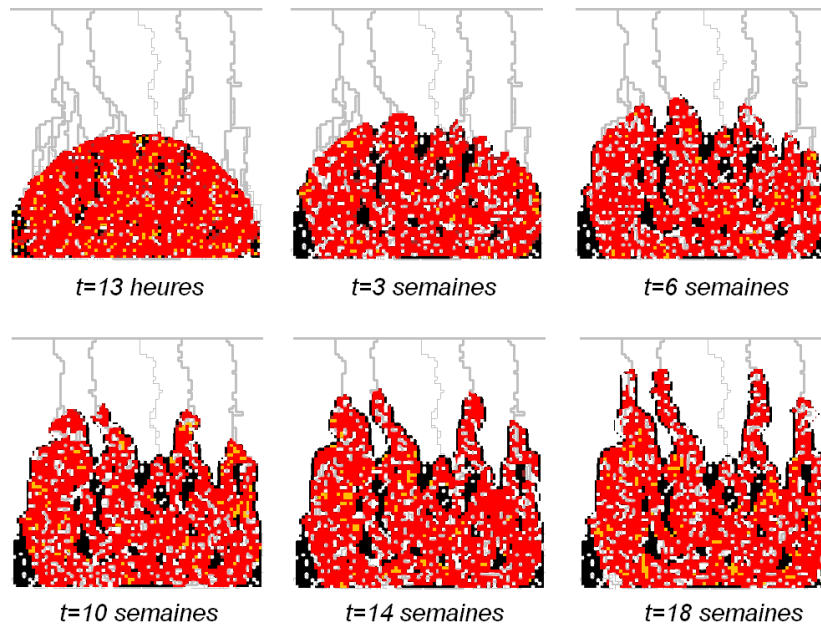


FIGURE 4.9: Croissance tumorale sur plusieurs semaines avec le développement d'excroissances le long des vaisseaux principaux sources d'oxygène (cellules prolifératives en rouge et cellules nécrotiques en noir).

4.5.2 Effet des molécules cytotoxiques

Les effets de molécules cytotoxiques agissant sur une phase spécifique du cycle cellulaire sont simulés. Trois protocoles thérapeutiques sont considérés à partir de la variation de deux paramètres : la durée de perfusion des médicaments et la fréquence des cycles de perfusion.

Les protocoles mis en application sont les suivants :

- protocole A : 5 heures de perfusion une fois par semaine,
- protocole B : 10 heures de perfusion deux fois par semaine,
- protocole C : 10 heures de perfusion une fois toutes les deux semaines.

La figure 4.10 présente l'évolution de la population de cellules prolifératives dans chaque cas simulé. Pour chaque protocole, deux molécules différentes ont été testées : l'une cible la phase G2M de 30 heures et la seconde la phase S de 12 heures (les durées des phases paramétrisent la tumeur et sont prises à titre d'exemple, ces durées étant largement variables d'un type cellulaire à l'autre). La molécule qui agit sur la phase

la plus longue est évidemment plus efficace puisque la probabilité pour que la cellule se trouve dans la phase adéquate du cycle cellulaire est plus grande. La tumeur est en effet éliminée à l'issue de 3 cycles de perfusion pour la molécule agissant sur la phase G2M tandis qu'elle ne peut pas l'être pour la molécule agissant sur la phase S. Dans ce cas, nous observons que la quantité de cellules détruites après chaque cycle de perfusion correspond à la quantité qui re-croît durant la période du cycle. La quantité de cellules tumorales reste néanmoins sous contrôle tant que la thérapie est maintenue. Cependant, si celle-ci est interrompue, la tumeur va poursuivre sa croissance.

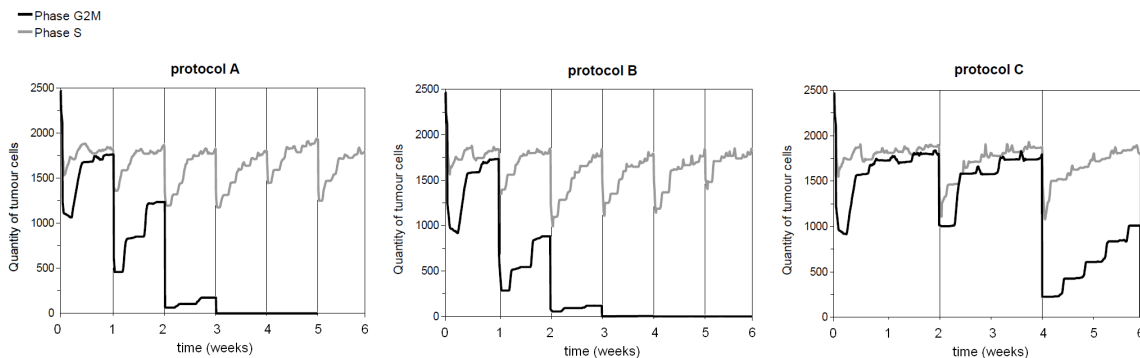


FIGURE 4.10: Comparasion de l'évolution de la population de cellules prolifératives pour les 2 molécules cytotoxiques (l'une agissant sur la phase G2M, l'autre sur la phase S). Chaque graphes représente la mise en application d'un protocole A, B et C (de gauche à droite).

Les figures 4.11, 4.12 et 4.13 montrent l'évolution tumorale pour 3 cas thérapeutiques différents. La figure 4.11 montre l'effet de la molécule qui cible la phase G2M et perfusée selon le protocole A (Fig. 4.10). La quantité de cellules prolifératives décroît massivement en 3 semaines et tombe sous le seuil de détection. Dans le cas de la molécule qui cible la phase S et selon le même protocole, la régénération de la population des cellules prolifératives est observée (Fig. 4.12). La figure 4.13 montre l'effet de la molécule qui cible la phase G2M, perfusée selon le protocole C. Dans ce cas, un temps plus long est nécessaire (10 semaines) pour que la population de cellules prolifératives tombe en dessous du seuil de détection.

La figure 4.14 compare les 3 protocoles thérapeutiques mis en oeuvre avec la molécule agissant sur la phase G2M. Dans les 3 cas, la quantité de cellules prolifératives diminue en dessous du seuil de détection. Cependant, ce résultat est atteint sur un temps plus long pour le protocole C puisque la perfusion des molécules cytotoxiques est effectuée avec une fréquence deux fois plus faible que pour les protocoles A et B. La période plus longue entre deux cycles de traitement permet à la tumeur de recroître. Les protocoles A et B qui diffèrent par la durée d'injection des médicaments (deux fois plus longue dans le protocole B, ce qui signifie que la quantité de médicaments délivrée est deux fois plus grandes) donnent des résultats identiques. Dans les deux cas, la tumeur est éliminée en 3 cycles. Ce résultat nous permet d'éliminer le protocole B puisque le doublement de la quantité de médicament n'améliore pas l'issue du traitement. En

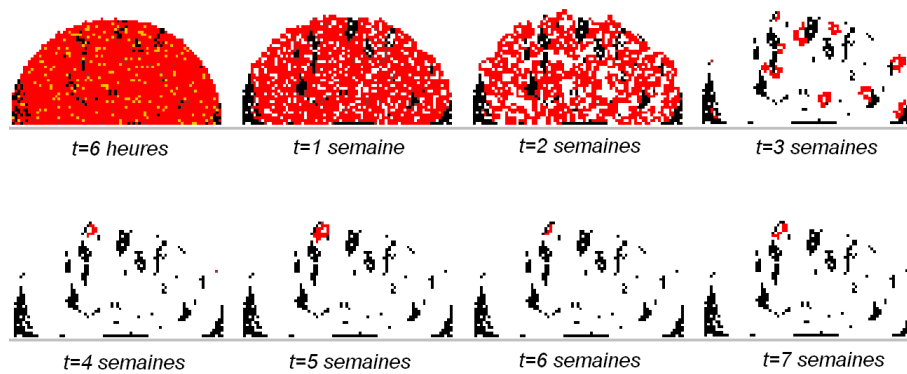


FIGURE 4.11: Effet de la molécule agissant sur la phase G2M, administrée selon le protocole A (cellules prolifératives en rouge et cellules nécrotiques en noir).

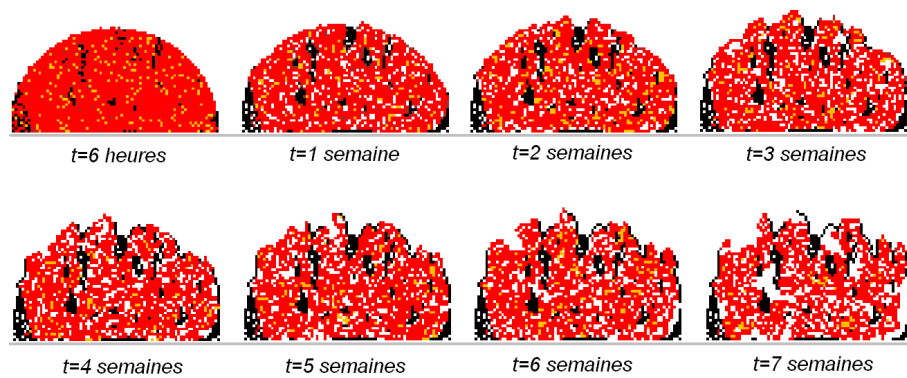


FIGURE 4.12: Effet de la molécule agissant sur la phase S, administrée selon le protocole A (cellules prolifératives en rouge et cellules nécrotiques en noir).

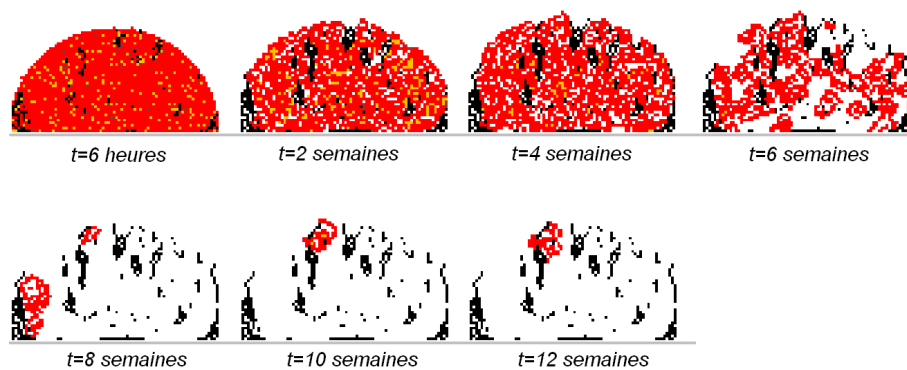


FIGURE 4.13: Effet de la molécule agissant sur la phase G2M, administrée selon le protocole C (cellules prolifératives en rouge et cellules nécrotiques en noir).

revanche, il peut être utile de retenir le protocole C car bien que le traitement soit plus long, il autorise au patient un temps de récupération plus important qui peut s'avérer nécessaire en cas d'effets secondaires particulièrement éprouvants.

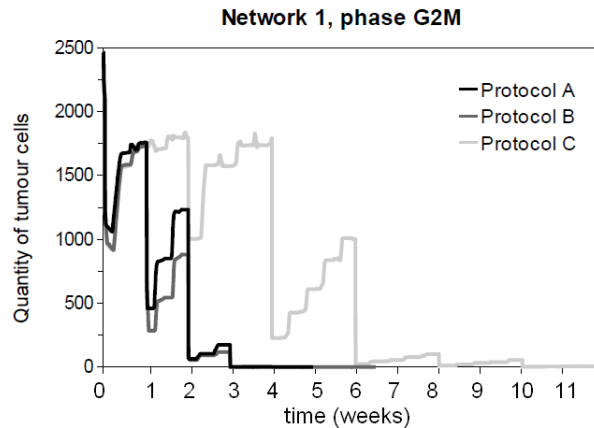


FIGURE 4.14: Comparaison des 3 protocoles thérapeutiques A, B et C, mettant en oeuvre la molécule cytotoxique qui cible la phase G2M.

4.5.3 Effet des agents de destruction vasculaire

Les VDAs sont administrés en injection bolus sur 5 secondes. La figure 4.15 montre les effets du traitement simulé. Le réseau vasculaire, la cartographie d'oxygénation du tissu et l'état de la tumeur sont comparés avant et après traitement. Certains vaisseaux sont détruits et bloquent le flux sanguin. Il s'ensuit l'apparition de larges zones hypoxiques dans le tissu tumoral. Les cellules soumises à l'hypoxie deviennent rapidement nécrotiques. La tumeur, bien que substantiellement affectée par le traitement, ne l'est que partiellement. Ce résultat de simulation montre une issue typique du traitement par VDA, où bien que la masse tumorale soit réduite, elle n'est pas éliminée et peut poursuivre sa croissance. C'est pourquoi le traitement par VDA est en général envisagé en combinaison avec d'autres traitements.

4.5.4 Combinaisons thérapeutiques

Comme nous venons de le voir, l'utilisation des VDAs seuls ne permet pas en général d'éradiquer la tumeur. La combinaison de l'action des VDAs avec des molécules cytotoxiques est à présent envisagée. La figure 4.16 présente deux cas où les VDAs sont utilisés soit avant (Fig. 4.16A) soit après (Fig. 4.16B) les cycles de traitement par cytotoxiques.

Une dose plus faible de cytotoxique est considérée ici avec une perfusion réduite à 3 heures au lieu de 5. Dans les deux cas, une chute initiale de 65% de la quantité de cellule proliférative est mesurée. La recroissance tumorale est légèrement réduite lorsque

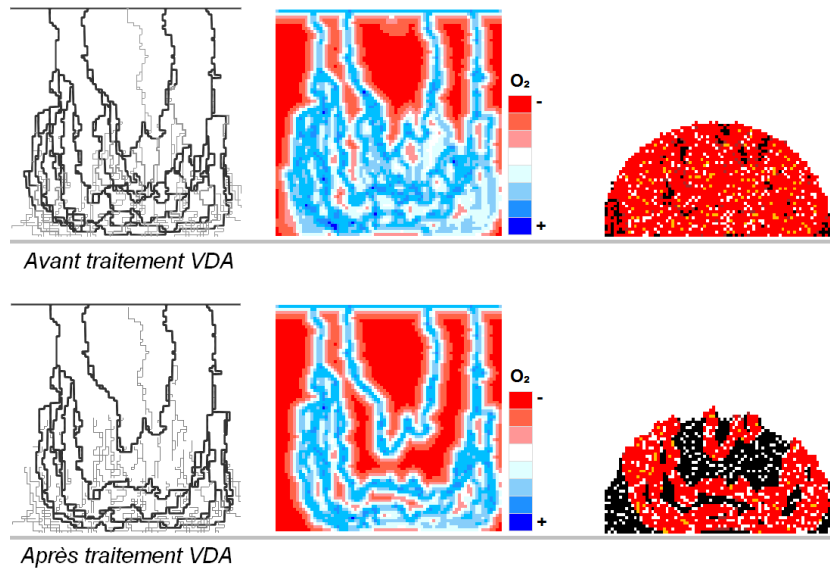


FIGURE 4.15: Simulation de l'effet des VDAs. Comparaison du réseau vasculaire, de l'oxygénation du tissu et de l'état tumoral avant (images du haut) et après (images du bas) traitement (cellules prolifératives en rouge et cellules nécrotiques en noir).

le réseau vasculaire est préalablement traité par le traitement VDA (Fig.4.16A). Les altérations des vaisseaux ne sont cependant pas suffisantes pour éliminer la tumeur par hypoxie et ne permettent pas non plus une amélioration de la délivrance des cytotoxiques à l'ensemble des cellules tumorales puisque les amas de cellules prolifératives recroissent constamment. D'un autre côté, si les VDAs sont utilisés lorsqu'une portion significative de cellules tumorales a été retirée, alors l'élimination des cellules prolifératives restantes peut être réalisée. L'utilisation des VDAs dans ce cas précis permet d'accélérer l'issue positive du traitement en épargnant aux patients les derniers cycles de perfusion de cytotoxiques.

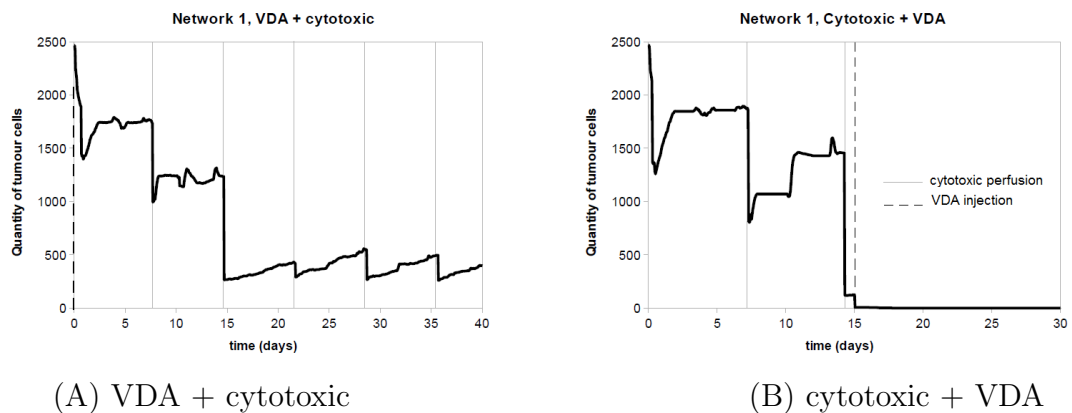


FIGURE 4.16: Effets combinés de la mise en application de molécules cytotoxiques et de VDA.

4.5.5 Bilan

Ce travail préliminaire a eu pour but de donner, par la simulation numérique, des éléments de compréhension sur la complexité de l'évolution tumorale lorsqu'une thérapie est mise en oeuvre. Notre objectif à ce stade fut purement illustratif sans l'ambition de définition de dosage optimum.

Nous avons tout d'abord testé des molécules cytotoxiques qui agissent sur des phases spécifiques du cycle cellulaire. De façon attendue, les molécules qui agissent sur la phase la plus longue sont plus efficaces dans la mesure où la probabilité pour que la cellule se trouve dans la phase sensible est plus grande. La variation des paramètres qui définissent un protocole thérapeutique, c'est à dire la durée et la fréquence d'injection des médicaments, permet de définir la meilleure option pour un cas donné. Par exemple, nos simulations permettent de définir le protocole le plus efficace, c'est à dire celui qui utilise la plus petite dose de médicaments ou alternativement celui qui autorise au patient une plus longue période de récupération si nécessaire.

Les effets des VDAs ont ensuite été testés à leur tour. Leur action a été limitée aux vaisseaux de plus petits débits sanguins, c'est à dire subissant une faible tension de cisaillement, condition pour laquelle la probabilité d'action des VDAs est supposée plus importante. Les simulations montrent que les vaisseaux détruits induisent une forte hypoxie qui conduit à la nécrose tumorale. Bien que l'effet soit important, il n'est cependant pas suffisant pour éliminer l'intégralité de la masse tumorale. La combinaison de l'action des VDAs avec les molécules cytotoxiques a alors été considérée et montre qu'un post-traitement par VDAs peut dans certain cas permettre d'isoler les amas prolifératifs ayant survécus aux cytotoxiques pour les asphyxier.

4.6 Premier pas vers un modèle intégré et validé

Ce travail est l'objet de la thèse d'Anne-Cécile Lesart co-encadrée par François Estève (GIN). L'objectif est d'intégrer l'ensemble des éléments du modèle computationnel et de le valider dans le contexte des expériences réalisées sur le modèle expérimental, c'est à dire à partir des observations réalisées sur la fenêtre dorsale implantée sur la souris. A l'issue de ce travail, nous disposerons d'un système virtuel que nous espérons suffisamment fiable pour se substituer au système réel afin d'aborder, pleinement cette fois, la question complexe de la thérapie.

Ce modèle computationnel a été présenté sous forme de poster intitulé "Un modèle computationnel de croissance tumorale telle qu'observée en microscopie intravitale par une fenêtre dorsale sur la souris" (A.C. Lesart, B. van der Sanden, F. Estève, A. Stéphanou) à l'European Conference of Mathematical and Theoretical Biology (Cra-covie, Juillet 2011) et au XXIème congrès de la Société Francophone de Biologie Théorique (Autrans, Mai 2011) où il a été distingué par le prix de présentation.

Ce travail est l'objet de l'article "On the Importance of the Submicrovascular Network in a Computational Model of Tumour Growth as Observed in a Dorsal Skinfold Chamber on the Mouse". A.C. Lesart, B. van der Sanden, L. Hamard, F. Estève, A. Stéphanou, soumis pour publication en septembre 2011.

4.6.1 Convergence avec les données expérimentales

Données d'entrée du modèle (input)

Dans un premier temps, le dispositif de fenêtre dorsale permet de fournir des images de réseaux vasculaires. Les images acquises jusqu'à présent montre des réseaux parfois très différents les uns par rapport aux autres en densité vasculaire et/ou en connectivité. Ces images sont binarisées afin d'extraire la trame de l'architecture vasculaire qui sera utilisée comme condition initiale à nos simulations. Le but étant de reproduire virtuellement des situations réellement observées.

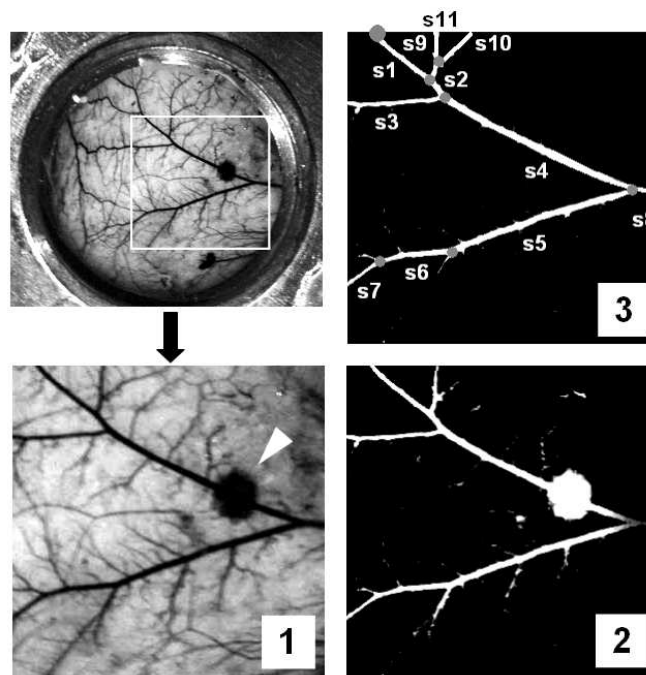


FIGURE 4.17: L'extraction du squelette vasculaire s'effectue en 3 étapes. (1) Une région d'intérêt de 5.5mm de côté est sélectionnée dans l'image; (2) l'image est inversée et un seuil d'intensité est appliqué; (3) les noeuds du réseau vasculaire sont détectés et utilisés pour définir chaque segment s_i que l'on caractérise par leur longueur L_i , leur diamètre D_i et leur inclinaison par rapport à l'horizontale I_i . Remarque : la tâche indiquée par la flèche est due à épanchement sanguin qui s'est produit lors de l'implantation de la fenêtre. Il disparaît ensuite, comme le montre la figure 4.18 qui correspond à un stade plus avancée après l'introduction de la tumeur.

Données de sortie du modèle (output)

Une fois le réseau vasculaire imagé et numérisé, la tumeur peut être introduite dans la fenêtre dorsale. Dans cette expérience, un morceau de tumeur contenant des cellules de gliomes humains de type U373 sont utilisées. Ce type tumoral particulièrement agressif est connu pour produire une forte réponse angiogénique.

Trois jours après l'introduction du morceau de tumeur, on observe effectivement des changements spectaculaires de la structure vasculaire (Fig. 4.18). Tout d'abord, les vaisseaux principaux se sont fortement dilatés et leur tortuosité s'est accentuée. La réponse angiogénique est nettement visible au bout de 3 jours avec la zone sombre qui apparaît autour de la masse tumorale. Cette zone sombre correspond à un épanchement sanguin dû à la formation de germes sur les vaisseaux les plus proches de la tumeur. Dans cet exemple, le suivi de l'évolution tumorale n'a pas pu l'être sur un temps long. Lorsque l'animal est en souffrance, il est euthanasié. La croissance tumorale peut cependant être observée par ce dispositif sur des durées allant jusqu'à 3 semaines.

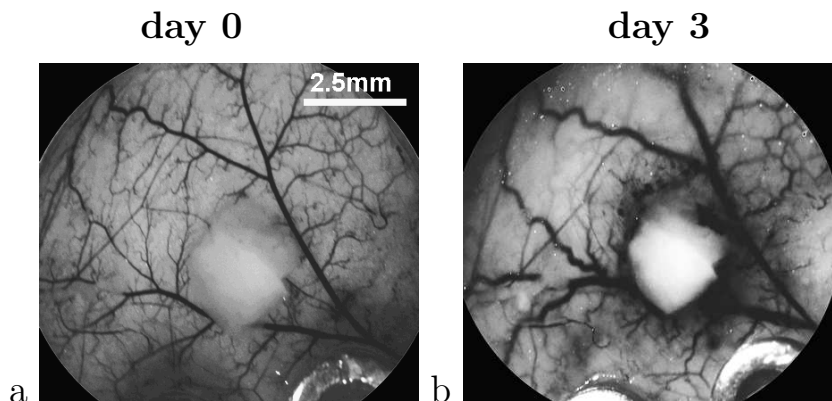


FIGURE 4.18: Images par microscopie de l'évolution vasculaire suite à l'introduction d'un morceau de tumeur. Images réalisées par B. van der Sanden, Grenoble Institut des Neurosciences.

4.6.2 Simulation numérique

A ce stade, le modèle computationnel n'intègre que partiellement les mécanismes d'adaptation vasculaire pour des raisons numériques liées au temps de calcul. Le travail d'optimisation lié à cette question est en cours.

La simulation présentée (Fig. 4.19), intègre la structure vasculaire extraite des images expérimentales (selon la procédure précédemment décrite). La tumeur est positionnée en accord avec sa localisation expérimentale. Les évolutions vasculaires et tumorales ainsi que la cartographie d'oxygénation du tissu associée sont simulées sur une durée de 70 jours.

A l'issue des 10 premiers jours, la tumeur est vascularisée. Le vaisseau le plus proche de la tumeur est concerné en premier par l'angiogenèse. Il est en effet le premier touché par le front de VEGF produit par les cellules quiescentes de la tumeur. Celles-ci apparaissent peu de temps après l'implantation dans le tissu.

Au bout de 30 jours, un corps nécrotique est visible au centre de la tumeur. Il est entouré par une couronne de cellules quiescentes et prolifératives. Ces dernières contribuent fortement à la déplétion en oxygène.

Au bout de 70 jours, certains vaisseaux intratumoraux s'effondrent en raison de la diminution du flux sanguin dans la tumeur. Cela conduit à l'apparition de nouveaux amas nécrotiques isolés et à une distribution inhomogène de l'oxygène dans la tumeur.

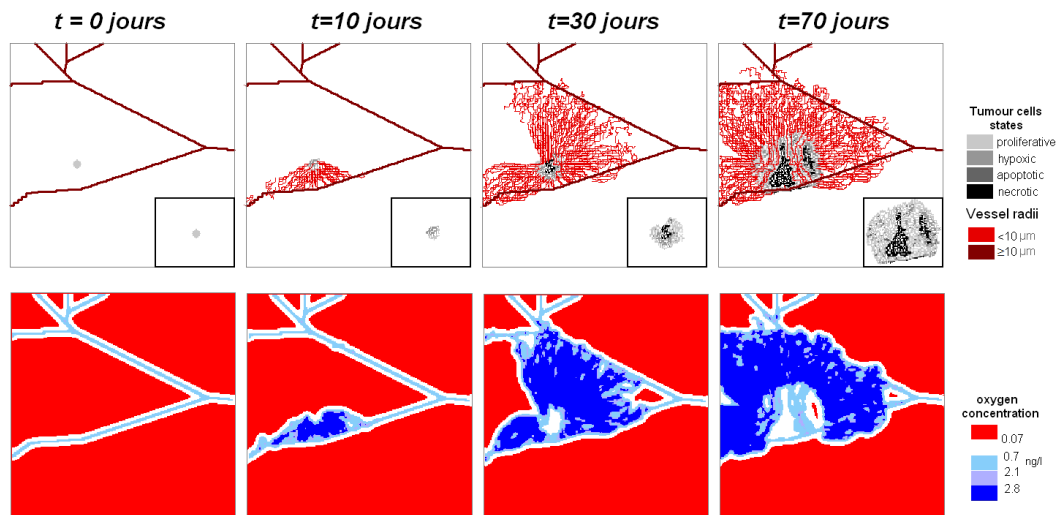


FIGURE 4.19: Simulation numérique de l'évolution du système tumoral et cartographie associée de l'oxygénation du tissu.

4.6.3 Bilan

Sur un plan qualitatif, le modèle computationnel reproduit assez fidèlement les différentes étapes de l'évolution tumorale. Sur le plan temporel, il apparaît que le modèle décrit une situation d'évolution tumorale beaucoup plus lente qu'elle ne l'est en réalité. Cette différence provient du choix probablement inapproprié du paramètre qui régit la durée du cycle cellulaire. Nous avons fixé cette durée à environ 24 heures par défaut de données expérimentales dans le contexte de la fenêtre dorsale. Une mesure effective de cette durée à partir de marqueurs spécifiques des phases du cycle est envisagée. D'autre part, des mécanismes de rétro contrôle de l'angiogenèse sont à l'oeuvre. Ils sont principalement régulés par l'adaptation vasculaire qui n'est pour l'instant pas considérée pleinement dans cette simulation en raison de contraintes numériques précédemment évoquées et en cours de résolution.

4.7 Conclusion

Ce projet a été initié à l'issue d'un séminaire que j'ai donné en Septembre 2009 à l'Institut des Neurosciences de Grenoble sur l'invitation de François Estève. Dans la salle étaient présents B. van der Sanden, A. Popov et E. Barbier qui ont par la suite fortement contribué à l'émergence et à la maturation de ce projet.

Rappelons que la validation du concept de Stratégie Thérapeutique Assistée par Ordinateur (STAO, soit CATS en anglais) dans l'aide à la mise en place de la stratégie optimale pour un cas pathologique donné, autorise le grand espoir d'améliorer l'efficacité des traitements donc la survie des patients. D'autre part, cette stratégie optimisée a pour but de contribuer à améliorer de façon significative le confort du patient en limitant les effets secondaires (surdosage) et en excluant les traitements non adaptés. La mise en application de la STAO en milieu médical pour le traitement des cancers serait ainsi une première en permettant :

- au minimum de rationaliser l'utilisation des modes thérapeutiques existants en assistant le clinicien dans sa prise de décision en fournissant le cadre optimal d'applicabilité de thérapies couramment utilisées,
- au maximum de piloter intégralement la thérapie pour aboutir à la meilleure issue possible pour le patient (éradication de la tumeur ou à défaut prolongement significatif de la durée de vie)

Les premiers jalons pour l'accomplissement de la STAO ont été posés. La faisabilité des divers aspects expérimentaux ont été démontrés, l'intégration des principaux éléments du modèle est réalisée. Cependant le chemin à parcourir pour valider le concept à partir de notre modèle expérimental sur la souris est encore long et devrait occuper pleinement les 2 à 3 prochaines années.

A l'issue, les perspectives sont immenses. Tout d'abord sur le plan de la valorisation, nous pourrions envisager de développer ce concept sous forme d'un outil logiciel à destinations des biologistes qui aura l'ambition de se substituer (au moins partiellement !) à la souris. Cet outil permettra de réaliser des expériences "virtuelles" fiables apportant un gain considérable en temps et en coût. Sur le plan médical, une transposition du concept sur l'homme pourra être envisagée. Une multitude d'étapes intermédiaires seront pour cela nécessaires. Il faudra par exemple confirmer ce concept de STAO sur un modèle "moins accessible" c'est à dire avec une tumeur *in situ* sans la facilité d'observation offerte par la fenêtre dorsale. Nous pensons en particulier au cas d'un modèle de gliome sur le rat (Fig. 4.20). Des améliorations significatives devront être apportées au modèle, pour décrire cette fois l'architecture 3D d'un organe (cerveau) et ses spécificités (matière grise et matière blanche, réseau de fibres anisotropes, etc.) Les moyens d'imagerie seront cette fois indirects et essentiellement fondés sur les capacités de l'imagerie par résonance magnétique (IRM).

Ces nouveaux aspects sont déjà en cours de gestation à travers une collaboration mise en place avec Emmanuel Barbier (GIN) responsable du projet IMOXY financé

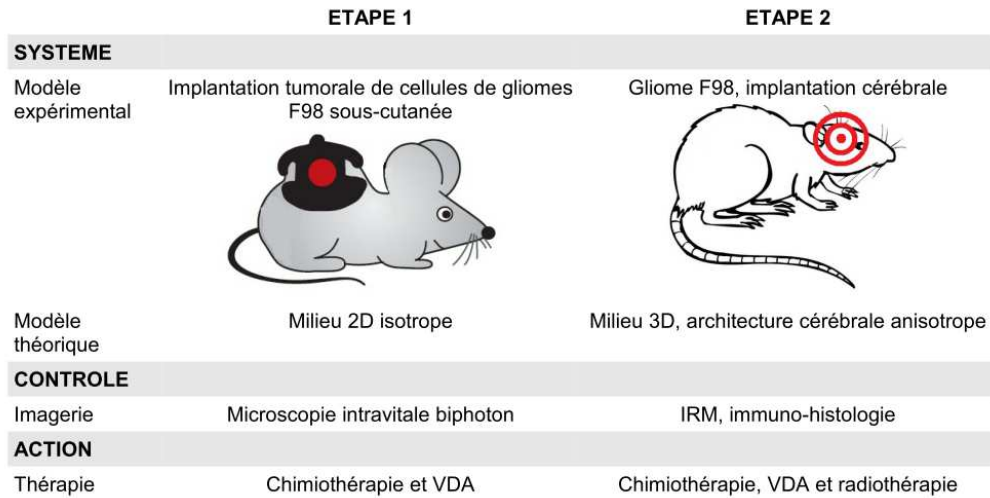


FIGURE 4.20: Les étapes du projet CATS ;-)

par l'ANR. Le projet qui démarre cette année (2011-2013) porte sur l'imagerie et la modélisation de l'oxygénation cérébrale associée à des pathologies telles que l'ischémie et la croissance tumorale.

La question de l'oxygénation des tissus et plus particulièrement des phénomènes hypoxiques seront au coeur de mes recherches. Des études récentes ont en effet démontré que la sélection des phénotypes tumoraux invasifs s'effectuait *via* la répétition d'épisodes hypoxiques sur des intervalles de temps très courts (inférieurs à la minute). Boude-wijn van der Sanden (GIN) étudie actuellement cet aspect à partir de deux modèles expérimentaux *in vitro* et *in vivo* utilisant le dispositif de fenêtre dorsal. Ces travaux expérimentaux offrent une opportunité unique de développer dans le modèle computationnel l'aspect lié à l'oxygénation des tissus en passant par une description fine du métabolisme des cellules et des voies d'activation et de sélection des gènes qui s'opèrent dans la division.

La conception du modèle sous forme de modules permet d'aborder chaque mécanisme de façon individuelle avec un niveau de détail indépendant de ceux des autres modules. Le tout est intégré pour fonctionner de manière synergique. Il est ainsi possible d'affiner progressivement la prise en compte des différents mécanismes dans le modèle computationnel en fonction du contexte, c'est à dire de la finalité que l'on recherche ou de la question que l'on se pose et que l'on veut élucider. Bien que le fondement de notre questionnement se situe sur le plan de la biologie, il sera nécessaire de progresser en parallèle sur les aspects computationnels pour gérer en particulier les temps de calcul qui explosent avec le nombre d'éléments intégrés. Parmi les voies de recherche et de développement, l'adaptation de nos codes numériques aux structures parallélisées multiprocesseurs ainsi qu'un effort sur les représentations graphiques nécessaires à l'appréhension du milieu virtuel seront indispensables dans et pour l'évolution de mes travaux.

Mes travaux de recherche, bien qu'ils aient un peu bifurqué de la thématique des processus morphogénétiques, restent cependant résolument pluridisciplinaires en s'inscrivant toujours dans cette discipline nouvelle qu'est la "Biologie Computationnelle".

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Curriculum Vitae

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Formation et Parcours

- depuis 2004 : CR CNRS, Laboratoire TIMC-IMAG
- 2003–2004 : ATER à l'UFR IMA (Informatique et Mathématiques Appliquées),
Université Joseph Fourier
- 2003 : Qualification aux fonctions de Maître de Conférences en section 26
"Mathématiques Appliquées et Application des Mathématiques"
- 2002–2003 : Postdoctorat à l'Université Heriot-Watt d'Edimbourg, Ecosse
- 1999-2002 : Thèse de doctorat spécialité "Modèles et Instruments en Médecine et
Biologie" de l'Université Joseph Fourier en cotutelle avec l'Université
de Dundee, Ecosse
- 1998-1999 : DEA "Modèles et Instruments en Médecine et Biologie", Université
Joseph Fourier
- 1997-1998 : Maîtrise de Physique Recherche, Université Joseph Fourier

Thèmes de Recherche

(1) Modélisation mathématique de processus morphogénétiques depuis la motilité cellulaire à la croissance vasculaire et tumorale. (2) Etude de l'impact des conditions environnementales mécaniques et topographiques sur ces processus. (3) Investigation par la simulation numérique de protocole thérapeutiques contre les cancers à tumeurs solide (porteuse de projets sur ce thème depuis 2009).

Publications, conférences et encadrements

12 publications dans des revues internationales avec comité de lecture
2 chapitres de livres
1 livre
6 conférences internationales avec comité de lecture (depuis 2005)
3 prix de présentation de poster en conférence internationale
1 prix de présentation de poster en conférence nationale
4 encadrements de Master II (2006 à 2010)
1 co-encadrement de thèse en cours (2010-2013)

Financements obtenus

- 2005 - ATIP Jeunes Chercheurs (15keuro)*
- 2008 - BQR INPG (4keuro)
- 2008 - RTRA Nanosciences (4keuro)
- 2009 - RNCS (Réseau National des Systèmes Complexes) (4keuro)*
- 2010 - IXXI (Institut Rhône-Alpin des Systèmes Complexes) (5keuro)*
- 2010-2011 - PEPS-INS2I (8kEuro)*
- 2011-2013 - ANR (6keuro + bourse postdoctorale d'1 an)

* porteuse du projet

Travaux d'expertise et activité éditoriale

- Expertise de deux projets pour l'ANR (2007 et 2011)
- Examinatrice dans un jury de thèse (2008)
- Referee de journaux internationaux : Acta Biotheoretica, Biophysical Journal, Bulletin of Mathematical Biology, Journal of Mathematical Biology, Cytometry, European Journal of Medical Physics, Journal of Theoretical Biology, Mathematical Modelling of Natural Phenomena
- Co-éditrice du numéro spécial "Cell Migration" du journal "Mathematical Modelling of Natural Phenomena" (Février 2010)

B

Les étudiants encadrés

Encadrements de Master 2 - Ingénierie pour la Santé et le Médicament Option "Modèles, Images et Instruments en Médecine et Biologie"

1. [Ghizlane meskaoui](#)
Année : 2006-2007
Sujet : Caractérisation de l'influence de la rigidité matricielle sur la dynamique de formation des cordons vasculaires in vitro
Travail publié dans *Microvascular Research* (2007)
2. [Céline Franco](#)
Année : 2008-2009
Sujet : Modélisation mathématique de la morphodynamique cellulaire induite par un environnement adhésif discret
Travail publié dans *Mathematical Modelling of Natural Phenomena* (2010)
3. [Margarita Pons-Salort](#)
Année : 2008-2009
Sujet : Modélisation mathématique et optimisation des thérapie contre les tumeurs solides
4. [Anne-Cécile Lesart](#)
Année : 2009-2010
Sujet : Modélisation et optimisation de la thérapie contre les tumeurs solides de type gliome à partir d'un modèle expérimental de fenêtre dorsale sur la souris

Encadrement d'une thèse de l'Ecole Doctorale Ingénierie pour la Santé,
la Cognition et l'Environnement (EDISCE)

1. [Anne-Cécile Lesart](#)

Thèse en cours (2010-2013)

Sujet : Investigation par la simulation numérique de nouvelles pistes thérapeutiques contre les tumeurs cérébrales de type glioblastome multiforme et mise en confrontation expérimentale à partir d'un dispositif de fenêtre dorsale sur la souris

C

Les collaborations

Seules les collaborations en cours et concrétisées par des financements ou des publications sont listées.

Collaborations locales

Une forte collaboration a été engagée depuis 2009 avec des membres du GIPSA⁽¹⁾, du GIN⁽²⁾ et de la société Ecrins Therapeutics dans le cadre du projet CATS "Computer-Assisted Therapeutic Strategy" (voir chapitre 4).



1. **GIPSA** - Grenoble Images Parole Signal Automatique
 - Mazen Alamir
 - Sophie Chareyron-Poydenot



2. **GIN** - Grenoble Institut des Neurosciences
 - Emmanuel Barbier (Projet ANR IMOXY)
 - Boudewijn van der Sanden
 - François Estève (co-encadrement de thèse)



3. **Ecrins Therapeutics**
 - Aurélie Juhem
 - Andrei Popov

Collaborations internationales

Département de Génie Mécanique de l'Université d'Ottawa - Depuis 2010, une collaboration est engagée avec Marianne Fenech et porte sur la mesure des paramètres rhéologiques du sang dans des micro-canaux. Cette collaboration bénéficie du soutien financier de l'université d'Ottawa pour la réalisation des expériences de mesure et de l'Ambassade de France au Canada pour l'échange d'étudiants.

Mathematics Department of Sussex University - Depuis 2007, une collaboration est engagée avec Anotida Madzvamuse et porte sur la modélisation des déformations cellulaires et sur les méthodes numériques pour la gestion des frontières libres. Une publication vient d'être soumise (Août 2011) "Mathematical Modelling and Numerical Simulations of Actin Dynamics in an Eukaryotic cell" U.Z. George, A. Stéphanou, A. Madzvamuse.

D

Liste des publications

Publications dans des revues internationales avec comité de lecture

1. McDougall S.R., M.A.J. Chaplain, A. Stéphanou and A.R.A. Anderson. Modelling the impact of pericyte migration and coverage of vessels and the efficacy of vascular disrupting agents. *Mathematical Modelling of Natural Phenomena, Cell Migration* (2010), 5(1) : 163-202. doi :[10.1051/mmnp/20105108](https://doi.org/10.1051/mmnp/20105108)
2. Franco C., T. Tzvetkova-Chevolleau and A. Stéphanou. On the influence of discrete adhesive patterns for cell shape and motility : a computational approach. *Mathematical Modelling of Natural Phenomena, Cell Migration* (2010), 5(1) : 56-83. doi :[10.1051/mmnp/20105103](https://doi.org/10.1051/mmnp/20105103)
3. Stéphanou A., E. Mylona, M.A.J. Chaplain and P. Tracqui. A computational model of cell migration coupling the growth of focal adhesions with oscillatory cell protrusions. *Journal of Theoretical Biology* (2008), 253 : 701-716. doi :[10.1016/j.jtbi.2008.04.035](https://doi.org/10.1016/j.jtbi.2008.04.035)
4. Tzvetkova-Chevolleau T., A. Stéphanou, D. Fuard, J. Ohayon, P. Schiavone and P. Tracqui. The motility of normal and cancer cells in response to the combined influence of the substrate rigidity and anisotropic microstructure. *Biomaterials* (2008), 29 : 1541-1551. doi :[10.1016/j.biomaterials.2007.12.016](https://doi.org/10.1016/j.biomaterials.2007.12.016)
5. Stéphanou A., G. Meskaoui, B. Vailhé and P. Tracqui. The rigidity in fibrin gels as a contributing factor to the dynamics of in vitro vascular cord formation. *Microvascular Research* (2007), 73(3) : 182-190. doi :[10.1016/j.mvr.2006.12.002](https://doi.org/10.1016/j.mvr.2006.12.002)
6. Stéphanou A., S.R. McDougall, A.R.A. Anderson and M.A.J. Chaplain. Mathematical modelling of the influence of blood rheological properties upon adaptative tumour-induced angiogenesis. *Mathematical and Computer Modelling* (2006), 44 : 96-123. doi :[10.1016/j.mcm.2004.07.021](https://doi.org/10.1016/j.mcm.2004.07.021)

7. Collin O., P. Tracqui, A. Stéphanou, Y. Usson, J. Clément-Lacroix, E. Planus. Spatiotemporal dynamics of actin-rich adhesion microdomains : influence of substrate flexibility. *Journal of Cell Science* (2006), 119 : 1914-1925. doi :10.1242/jcs.02838
8. Stéphanou A., McDougall S.R., Anderson A.R.A. and Chaplain M.A.J. Mathematical modelling of flow in 2D and 3D vascular networks : applications to anti-angiogenic and chemotherapeutic drug strategies. *Mathematical and Computer Modelling* (2005), 41 : 1137-1156. doi :10.1016/j.mcm.2005.05.008
9. Stéphanou A., Chaplain M.A.J. and Tracqui P. A mathematical model for the dynamics of large membrane deformations of isolated fibroblasts. *Bulletin of Mathematical Biology* (2004), 66 : 1119-1154. doi :10.1016/j.bulm.2003.11.004
10. Stéphanou A. and Tracqui P. Cytomechanics of cell deformations and migration : from models to experiments. *Comptes Rendus Biologies (Académie des Sciences)* (2002), 325(4) : 295-308
11. Divet F., Biben T., Cantat I., Stéphanou A., Fourcade B. and Misbah C. Fluctuations of a membrane interacting with a diffusion field. *Europhysics Letters* (2002), 60(5) : 795-801.

Publication dans une revue nationale avec comité de lecture

12. Tracqui P., A. Stéphanou. Description intégrée de processus cellulaires spatio-temporels basée sur un modèle géométrique de transformation. *Technique et Science Informatiques* (2007), 26(10) : 1305-1326.

Livre

13. Stéphanou A. Spatio-temporal Dynamics of the Cell - Characterization from Images and Computer Simulations. Lambert Academic Publishing AG and Co KG, (parution Juin 2010) ISBN 9783838337920

Chapitres de livres

14. Stéphanou A. A computational framework integrating cytoskeletal and adhesion dynamics for modelling cell motility. *Cell Mechanics, From Single Scale-Based Models to Multiscale Modeling*. Ed. Arnaud Chauvière, Luigi Preziosi and Claude Verdier, Chapman and Hall, CRC Press, (2009).
15. Stéphanou A., Ronot X. and Tracqui P. Analysis of Cell motility combining cytomechanical model simulations and an optical flow method. *Polymers and Cell Dynamics - Multiscale Modelling and Numerical Simulations*, pp 91-112 ed. by W. Alt, M. Chaplain, M. Griebel and J. Lenz (2003)

Conférences internationales avec comité de lecture

16. Conference on Computational and Mathematical Population Dynamics, Bordeaux, Juin 2010.

17. European Conference of Mathematical and Theoretical Biology, Edimbourg, Juin 2008 ([prix de présentation de poster](#))
18. International Conference on Statistics, Combinatorics Mathematics and Applications, Auburn, Décembre 2005.
19. European Conference of Mathematical and Theoretical Biology, Dresde, Juin 2005

Invitations (Séminaires et Workshops)

20. Journée Modélisation hybride en biologie et médecine, Grenoble, Février 2011.
21. Séminaire du laboratoire LAPV-CEA, Grenoble, Décembre 2010.
22. Colloque Modèles mathématiques et Imagerie en Cancérologie organisé par le GDR Stic-Santé, Paris, Décembre 2010.
23. Colloque du cancéropôle CLARA "Peut-on envisager un ciblage actif pour des particules de 2 à 100 nm?", Grenoble, Octobre 2010.
24. First International workshop on Differential and Integral Equations with Applications in Biology and Medicine Aegean University, Karlovassi, Samos island, Greece, Septembre 2010.
25. Workshop "A l'interface des sciences du vivant, de l'informatique et des mathématiques" organisé par les fondations Finovi, Neurodis et Synergie Lyon Cancer et l'INRIA Lyon, Juin 2010.
26. Séminaire du groupe Neuro-Oncology, Grenoble Institut des Neurosciences, Septembre 2009.
27. Séminaire Rhône-Alpin de Modélisation du Vivant (Semovi), IXXI Lyon, Avril 2009.
28. Workshop on Cell Biomechanics, FORTH, Heraklion, Crète, Février 2009.
29. Séminaire du Laboratoire de Mathématiques (LAMA), Chambéry, Janvier 2009.
30. Workshop on Multiscale approaches in cell mechanics, Autrans, Janvier 2008.
31. Seminaire du département de Mathématiques, University of Sussex, UK, Novembre 2007.
32. Workshop on Cell Imaging and Deriving Information from Cell Images, Heraklion, Crète, Octobre 2007.
33. Séminaire du Groupe de Recherche en Sciences et Technologies Biomédicales, Ecole Polytechnique, Montréal, Octobre 2006.
34. Séminaire du laboratoire MCS (Matière et Systèmes Complexes), Université Paris 7, Juin 2006.
35. Ecole Interdisciplinaire d'échanges et de formation en biologie - Le temps en biologie. Berder, Mars 2006.

Mémoires

36. Mémoire de Thèse de Doctorat (Février 2002), Université Joseph Fourier et University of Dundee "La Dynamique Spatio-temporelle des Déformations Membranaires et de la Migration Cellulaire : Caractérisation à partir de l'Analyse de Séquences d'Images et Modélisation Théorique"
37. Mémoire de DEA "Modèles et Instruments en Médecine et Biologie" (Septembre 1998), Université Joseph Fourier. "Caractérisation et modélisation des déformations cellulaires spontanées à partir de l'analyse de séquences d'images"
38. Mémoire de stage de Maitrise de Physique (Juin 1997), Université Joseph Fourier "Dynamique et instabilité d'une membrane biologique"



Les publications jointes

Cette annexe regroupe les publications les plus significatives pour chacun des thèmes de recherche présentés.

Thème Motilité

Modèle général des déformations cellulaires

- Stéphanou A., Chaplain M.A.J. and Tracqui P. A mathematical model for the dynamics of large membrane deformations of isolated fibroblasts. *Bulletin of Mathematical Biology* (2004), 66 : 1119-1154. doi :[10.1016/j.bulm.2003.11.004](https://doi.org/10.1016/j.bulm.2003.11.004)

Modèle de la migration cellulaire spontanée

- Stéphanou A., E. Mylona, M.A.J. Chaplain and P. Tracqui. A computational model of cell migration coupling the growth of focal adhesions with oscillatory cell protrusions. *Journal of Theoretical Biology* (2008), 253 : 701-716. doi :[10.1016/j.jtbi.2008.04.035](https://doi.org/10.1016/j.jtbi.2008.04.035)

Modèle des formes cellulaires sur substrats patternés

- Franco C., T. Tzvetkova-Chevolleau and A. Stéphanou. On the influence of discrete adhesive patterns for cell shape and motility : a computational approach. *Mathematical Modelling of Natural Phenomena, Cell Migration* (2010), 5(1) : 56-83. doi :[10.1051/mmnp/20105103](https://doi.org/10.1051/mmnp/20105103)

Thème Croissance Vasculaire

Angiogenèse in vitro

- Stéphanou A., G. Meskaoui, B. Vailhé and P. Tracqui. The rigidity in fibrin gels as a contributing factor to the dynamics of in vitro vascular cord formation. *Microvascular Research* (2007), 73(3) : 182-190. doi :[10.1016/j.mvr.2006.12.002](https://doi.org/10.1016/j.mvr.2006.12.002)

Angiogenèse tumorale

- Stéphanou A., S.R. McDougall, A.R.A. Anderson and M.A.J Chaplain. Mathematical modelling of the influence of blood rheological properties upon adaptative tumour-induced angiogenesis. *Mathematical and Computer Modelling* (2006), 44 : 96-123. doi :[10.1016/j.mcm.2004.07.021](https://doi.org/10.1016/j.mcm.2004.07.021)



A Mathematical Model for the Dynamics of Large Membrane Deformations of Isolated Fibroblasts

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In this paper we develop and extend a previous model of cell deformations, initially proposed to describe the dynamical behaviour of round-shaped cells such as keratinocytes or leukocytes, in order to take into account cell pseudopodial dynamics with large amplitude membrane deformations such as those observed in fibroblasts. Beyond the simulation (from a quantitative, parametrized model) of the experimentally observed oscillatory cell deformations, a final goal of this work is to underline that a set of common assumptions regarding intracellular actin dynamics and associated cell membrane local motion allows us to describe a wide variety of cell morphologies and protrusive activity.

The model proposed describes cell membrane deformations as a consequence of the endogenous cortical actin dynamics where the driving force for large-amplitude

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cell protrusion is provided by the coupling between F-actin polymerization and contractility of the cortical actomyosin network. Cell membrane movements then result of two competing forces acting on the membrane, namely an intracellular hydrostatic protrusive force counterbalanced by a retraction force exerted by the actin filaments of the cell cortex. Protrusion and retraction forces are moreover modulated by an additional membrane curvature stress.

As a first approximation, we start by considering a heterogeneous but stationary distribution of actin along the cell periphery in order to evaluate the possible morphologies that an individual cell might adopt. Then non-stationary actin distributions are considered. The simulated dynamic behaviour of this cytomechanical model not only reproduces the small amplitude rotating waves of deformations of round-shaped cells such as keratinocytes [as proposed in the original model of Alt and Tranquillo (1995, *J. Biol. Syst.* **3**, 905–916)] but is furthermore in very good agreement with the protrusive activity of cells such as fibroblasts, where large amplitude contracting/retracting pseudopods are more or less periodically extended in opposite directions. In addition, the biophysical and biochemical processes taken into account by the cytomechanical model are characterized by well-defined parameters which (for the majority) can be discussed with regard to experimental data obtained in various experimental situations.

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1. INTRODUCTION

As repeatedly emphasised in the current literature, understanding cell motility (namely the ability of a cell to deform and migrate) and the mechanisms governing motility is a vital and essential task as it occurs in many important biological events such as embryogenesis, wound healing or the formation of primary solid tumours and metastases (secondary tumours). The fundamental challenge is to understand the complete scheme in which cell motion occurs in close relationship with the mechanisms of perception of the cell's extracellular medium (e.g., tissue) and the integration of the signals from the local environment. A cornerstone of this dynamic integration scheme is the spontaneous dynamic state of the cell, as revealed by the *in vitro* observations of cyclic changes of cell morphologies. In this context, one of the major propositions regarding cell behaviour, and the one on which this study is based, is the demonstration at the beginning of the 1990s of a certain self-organization of spontaneous cell deformation dynamics, which was until then widely considered as random. The existence of recurring patterns of deformation, such as the appearance of rotating waves of deformation around the cell body, was indeed demonstrated for various cell types such as keratinocytes (Alt *et al.*, 1995), leukocytes (Alt, 1990) and the dictyostelium amoebae (Killich *et al.*, 1993, 1994). These observations have driven the development of several theoretical models (Killich *et al.*, 1994; Alt and Tranquillo, 1995; Le Guyader and Hyver, 1997) which have tried to integrate molecular, chemical

and mechanical elements in order to determine and test the relative importance of the various elements and structure of the cell biophysical and biochemical processes responsible for this self-organization. The existence of what appears to be a 'spontaneous cell dynamic', where 'spontaneous' is taken in the sense of 'outwith any clearly identified and significant stimulations from the environment' (other than small amplitude environmental fluctuations), is a fundamental element to consider as it will affect the cell response to an external stimulation. Despite the relevance of the spontaneous cell dynamic, relatively few studies have been interested in this and remain, for the most part, focused on the migratory behaviour of cells. One reason for this is that the spatio-temporal analysis of cell deformations is a complicated and involved task, even if recent optical flow approaches avoid the need of cell contour segmentation [usually a limiting step for temporal analyses based on polarity maps (Stephanou *et al.*, 2003)]. It has now become clear that the extension/retraction motion of the cell membrane is directly related to the remodelling of the cytoskeleton and more particularly to the dynamic polymerization/depolymerization of actin (Condeelis, 1993; Carlier and Pantaloni, 1997; Borisy and Svitkina, 2000). Actin is a polymer which forms a dense and highly dynamic network of filaments located in the most flattened area found at the periphery (the cell cortex) of a cell cultured *in vitro* on a two-dimensional substrate.

Many different hypotheses have been proposed to explain how cell deformations occur and most of them have mainly focus on the mechanism of cell membrane extension or protrusion.

An early first hypothesis considered a sol/gel transition of actin regulated by local calcium concentration (Oster, 1984). Solation of actin is assumed to occur when a given threshold of calcium is reached. Solation thus triggers the expansion of the actin gel which pushes on the membrane. When the level/concentration of calcium falls below the threshold, re-gelation of actin occurs and the network is able to contract again. This hypothesis, however, requires the necessity of an initial ionic leak across the membrane in order to activate the solation/gelation process, which is therefore not spontaneous (self-consistent).

A second hypothesis suggests that actin polymerization in the neighbourhood of the membrane is the direct cause of protrusion (Theriot and Mitchison, 1991; Carlier and Pantaloni, 1997; Abraham *et al.*, 1999; Borisy and Svitkina, 2000). In this case, a Brownian ratchet mechanism has been proposed (Peskin *et al.*, 1993; Mogilner and Oster, 1996) to explain the intercalation of actin monomers (G-actin) between the growing end of actin filaments and the cell membrane. According to this mechanism, random thermal fluctuations, either of the cell membrane or of the actin fibres, are able to create the gap required for polymerization.

A third hypothesis proposes the involvement of certain molecular motors, with an active role for myosin I which, once coupled to the actin filaments, is able to propulse the filaments towards the membrane through various sliding mechanisms (Lee *et al.*, 1993; Small *et al.*, 1993).

However, the most often referred to hypothesis is the assumption of pressure-driven protrusion (Bereiter-Hahn and Luers, 1998). It is known that actin associated with myosin forms a contractile network. It is then assumed that the contraction of the network creates cytoplasmic flows throughout the cell which lead to an increasing pressure which is able to push the membrane outwards at any location where the membrane finds itself less strongly linked to the actin network. An alternative view proposes that the existence of a constant hydrostatic pressure is sufficient to produce the same effect (Alt and Tranquillo, 1995).

Our aim in this paper is to describe large membrane deformations as observed in resting fibroblasts cells. We thus use as a basis a cytomechanical model formulated initially proposed by Alt and Tranquillo (1995) which put forward the pressure-driven protrusion hypothesis.

The model assumes that the movements of the membrane depend on the interaction between an internal hydrostatic pressure pushing on the membrane and a counteracting stress due the actin filaments through their link with the membrane. The intensity of this retraction stress is assumed to be locally and linearly dependent on the local amount of actin available. Therefore the higher the actin density, the more the stress applied on the membrane is important. Moreover these two opposing forces are assumed to be modulated by an additional stress due to the membrane tension which depends on the intensity and sign of the local curvature of this membrane. As the cell deforms on a 2D substrate there is an additional friction stress characterizing the level of adhesiveness of the cell. Since actin is a polymer, the model obviously considers the actin polymerization kinetics regulated around a chemical equilibrium concentration. The contractile activity of the actomyosin complex (actin coupled to myosin) is also considered in the model. The actomyosin complex present in the cell cortex is described as a viscoelastic and contractile material linked to the membrane.

The model which describes the actin dynamics [by monitoring, for each instant, the actin density $a(\theta, t)$ and its tangential velocity in the cell cortex $v(\theta, t)$] in relation to the membrane extension width $L(\theta, t)$ has been shown to be capable of simulating, in a very realistic way, rotating and pulsating deformations of cell membranes (Alt and Tranquillo, 1995; Stéphanou and Tracqui, 2002) such as those observed in keratinocytes and leukocytes (which are characterized, relatively speaking, by rounded shapes).

The above model, as it is conceived in its present formulation, remains restricted to being able to describe small membrane deformations such as those observed on certain types of cells where the width of the cell cortex $L(\theta, t)$ remains small in front of the radius of the cell body R_0 (see Fig. 1). Therefore, in this paper we would like to generalize this model and extend it in order to describe the dynamics of large membrane deformations, such as those observed on fibroblast cells (Fig. 2).

Indeed fibroblast cells present a different organization of their actin cytoskeleton, where actin filaments tend to form bundles which are radially oriented in the

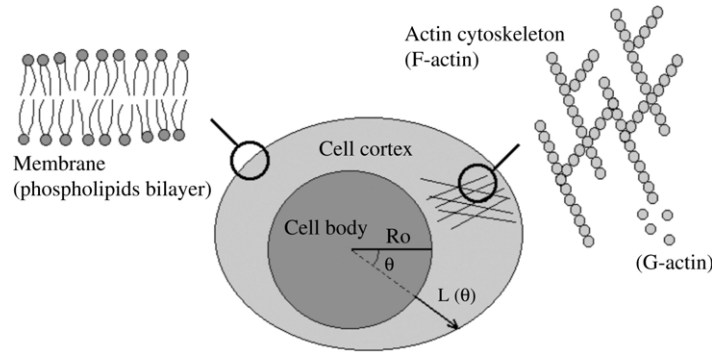


Figure 1. Schematic representation of the cell which illustrates the phospholipid bilayer, the cell membrane and the interconnected network of F-actin filaments which forms the actin cytoskeleton. The cell body is assumed to be confined in a circular area with radius R_0 . The cell cortex where remodelling of the actin cytoskeleton mainly occurs corresponds to the area bounded at one side by the outer boundary of the cell body and at the other side by the cell membrane. The width of the cell cortex in any angular direction θ is given by $L(\theta)$.

protrusive areas of the cell. These bundles stabilize the cell shape without depriving the cell of its motile abilities. Fibroblasts usually exhibit from 2 to 4 stable protrusions in the form of long and narrow membrane extensions homogeneously distributed around the cell body. In contrast to the deformations in round-shaped cells (mainly identified as rotating waves), the nature of the deformations observed in fibroblasts corresponds more to standing waves, where all the protrusions of the cell pulsate in a synchronized way according to various patterns. Despite their morphological and dynamical differences, it is commonly acknowledged that the same underlying mechanisms governing actin dynamics must apply.

In this paper, we first propose to investigate a very simple model for cell membrane movements inspired by the model of [Alt and Tranquillo \(1995\)](#). As we have noted the major limitation of Alt and Tranquillo's model is that it is only valid when dealing with small membrane extensions. This condition is imposed by the derivation of the curvature tension term. Our aim here is to propose a new derivation of that term in order to take into account large membrane extensions. For simplicity at this stage we will consider a time-invariant distribution of actin in the cell cortex, which is spatially varying in the tangential direction, i.e., along the cell cortex. We therefore consider static cell membrane deformations with the aim to evaluate the possible cell shapes that might thus be obtained and to estimate the influence of the new curvature tension term.

As a next step, we will restore the explicit coupling with the actin dynamics. For that, we will replace the (imposed) stationary spatial variation for F-actin concentration by self-generated variations induced by the coupling between F-actin polymerization and cell contractility controlled by the cortical actomyosin network (the latter depending non-linearly on the local amount of F-actin). We will then investigate how this coupling allows us to describe the various dynamical behaviours

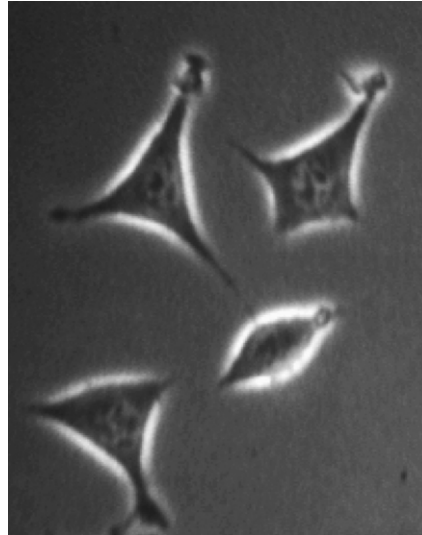


Figure 2. Videomicrograph of non-migrating L929 fibroblasts observed with phase contrast microscopy. This videomicrograph shows the most typical morphologies exhibited by this type of cell at their resting state (namely a non-migrating state). Fibroblasts typically present 'starry' morphologies involving from 2 to 4 thin membrane extensions which are more often homogeneously distributed around the cell body.

which exist, from rotating waves of deformations of round-shaped cells to large membrane extensions in the dynamical form of standing, pulsating waves (such as observed in fibroblasts).

A brief review for the characterization of the cell mechanical properties (more specifically the viscoelastic properties), will then be presented in order to provide a range of parameters from which a quantitative validation of the model will be discussed.

In the final section, we will present how the initial model of Alt and Tranquillo could be extended to deal with cell migration and how such an extension can be realized in the framework of our new model, in order to describe the main morphological features of fibroblast-type individual cell migration.

2. A MODEL FOR CELL MEMBRANE DEFORMATIONS

Experimentally, information on cell morphologies can be obtained from polarity maps (Killich *et al.*, 1993; Alt *et al.*, 1995; Stéphanou and Tracqui, 2002). These maps are obtained by extracting the coordinates of the points of the cell boundary and then reconstructing this boundary in a polar system of coordinates from the choice of a point of reference inside the cell as the origin of the polar coordinates. The point usually chosen as a reference point is the centre of the cell nucleus whose displacement in resting cells is very small in front of the cell membrane

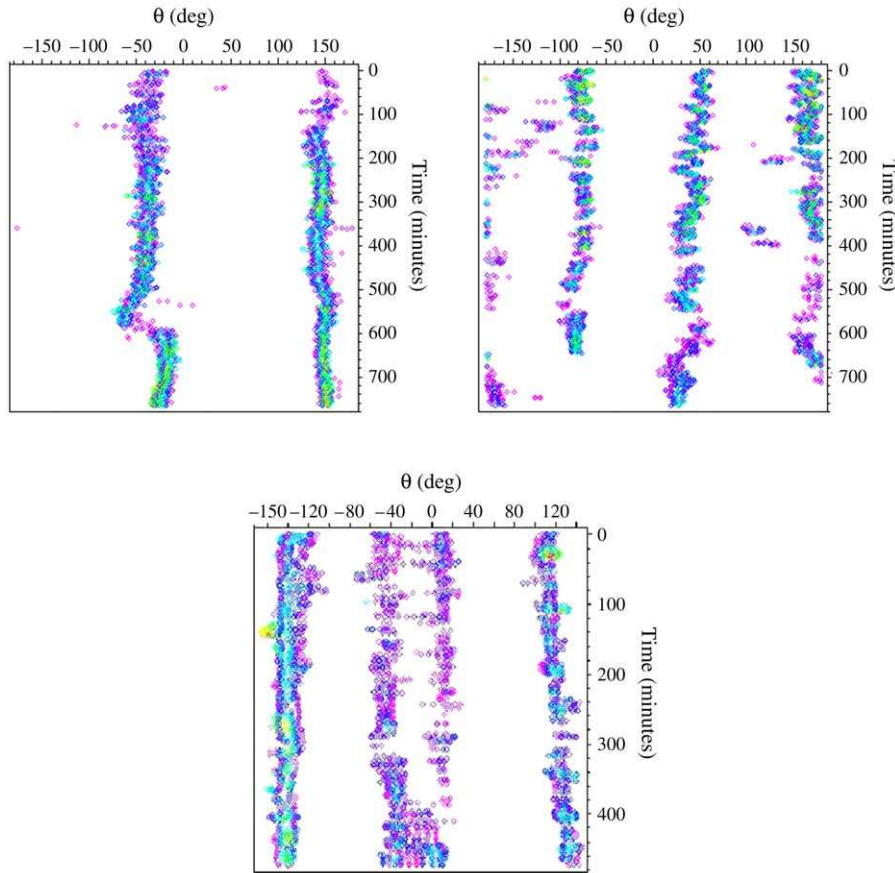


Figure 3. Spatio-temporal representations of the cells (cell polarity maps) which illustrates a variety of typical cell morphologies observed experimentally, with cells presenting, respectively, 2, 3 and 4 simultaneous protrusions each; the protrusive directions usually remain located along one axis for significantly long time periods (up to 12 h).

extensions. The polarity maps thus obtained for each instant are superposed with time to provide a spatio-temporal representation of the cell deformation dynamics. Fig. 3 presents a set of such spatio-temporal maps obtained from L929 fibroblasts and which exhibits typical cell morphologies of such resting cells. Fibroblasts exhibit from 2 to 4 stable protrusions. An example of each of these configurations is given in Fig. 3. One interesting feature of these cells is that the cell membrane protrusions are usually homogeneously distributed around the cell body, which gives symmetrical shapes. As we will see in what follows from our simulation results, spatio-temporal representations also provide an ideal tool to investigate cell membrane dynamics.

2.1. The cytomechanical model. The model we consider for the actin dynamics extends and develops an existing mechano-chemical model which describes an actin cytoskeleton (Lewis and Murray, 1991, 1992).

The model consists of two coupled equations, one describing the chemical dynamics of the material and the other describing its mechanical properties. We assume that the actomyosin network of our model retains the same mechanical properties as the actomyosin cytogel. The stress tensor thus consists of viscous σ_v , elastic σ_e , contractile σ_c and osmotic stress σ_p components. Whereas in the cytogel model sol/gel transition kinetics were considered, in our case we focus on the polymerization kinetics of actin as in the initial model of [Alt and Tranquillo \(1995\)](#). The system of equations describing the actin dynamics is thus written as:

$$\nabla \cdot (\sigma_v + \sigma_e + \sigma_c + \sigma_p) = 0, \quad (1)$$

$$\frac{\partial a}{\partial t} - D \Delta a + \nabla \cdot \left(a \frac{\partial \mathbf{u}}{\partial t} \right) = k_a (a_c - a), \quad (2)$$

where a represents the F-actin concentration, a_c the F-actin concentration at the chemical equilibrium which differentiates the states of polymerization and depolymerization whose rate of polymerization is controlled by the coefficient k_a ; D is the diffusion coefficient for F-actin; $\mathbf{u} = (u, v)$ is a vector denoting the displacement of the elements of the actomyosin network from their original unstrained position, with u and v denoting the radial and tangential component respectively; $\sigma_v, \sigma_e, \sigma_c, \sigma_p$ are the (viscous, elastic, contractile and pressure induced) stress tensors respectively given by

$$\sigma_v = \mu_1 \dot{\epsilon} + \mu_2 \dot{\phi} \mathbf{I}, \quad (3)$$

$$\sigma_e = E' [\epsilon + \nu' \phi \mathbf{I}], \quad (4)$$

$$\sigma_c = \sigma(a) \mathbf{I} = \psi a^2 e^{-a/a_{\text{sat}}} \mathbf{I}, \quad (5)$$

$$\sigma_p = -p(\phi) \mathbf{I} = \frac{P}{1 + \phi} \mathbf{I}, \quad (6)$$

where $\epsilon = \frac{1}{2}(\nabla \mathbf{u} + \nabla \mathbf{u}^T)$ is the strain tensor, \mathbf{I} is the identity tensor, $\phi = \nabla \cdot \mathbf{u}$ the dilation, and μ_1 and μ_2 are the shear and bulk viscosities of the actin network respectively. Finally $E' = E/(1 + \nu)$ and $\nu' = \nu/(1 - 2\nu)$ where E is the Young's modulus and ν the Poisson's ratio. The function $\sigma(a)$ represents the contractile activity of the actomyosin network. This function models the fact that the contractility increases according to a parabolic law with the actin concentration until a saturation concentration $2a_{\text{sat}}$ from which an effect of compaction of the network occurs and leads the contractility to decrease exponentially. $p(\phi)$ represents the osmotic stress which depends on the dilation ϕ .

Membrane deformations are modelled on the basis of the equation proposed by [Alt and Tranquillo \(1995\)](#), but we consider here a new derivation of this equation in order to remove the constraint of small deformations imposed in the original derivation of this term. The mechanical forces acting on the cell membrane are:

- a constant protrusive force P due to the hydrostatic pressure existing inside the cell,
- an active force $\sigma(a)$ which depends on the local concentration of actin,
- a membrane curvature-dependent force τK_L , where τ is a constant coefficient characterizing the membrane tension,
- a friction force between the membrane and the substrate $\Phi V = \Phi \partial L / \partial t$, where Φ is the friction coefficient characterizing the adhesiveness of the cell with the substrate.

Whereas in the model of [Alt and Tranquillo \(1995\)](#), a linear relation for the retraction force exerted by the network on the membrane was considered (γLa), here we have replaced this linear dependency by the non-linear function $\sigma(a)$. This function thus models an active contraction of the network rather than a passive restoring force. The deformation of the membrane is thus given by

$$\Phi \frac{\partial L}{\partial t} = P - \sigma(a)L - \tau K_L \quad (7)$$

where $L(\theta)$ denotes the radial extension of the cell cortex.

The full functional form of the membrane curvature K_L in polar coordinates and without any restriction to small deformations is given by the following expression (see [Appendix A](#)):

$$K_L = \frac{2\left(\frac{\partial L}{\partial \theta}\right)^2 - [L(\theta) + R_0]\frac{\partial^2 L}{\partial \theta^2} + [L(\theta) + R_0]^2}{\left[\left(\frac{\partial L}{\partial \theta}\right)^2 + [L(\theta) + R_0]^2\right]^{\frac{3}{2}}}, \quad (8)$$

where R_0 represents the radius of the cell body ([Fig. 1](#)).

In the initial model of [Alt and Tranquillo \(1995\)](#), the membrane curvature-induced force was depending on the amount of actin in order to represent the membrane-cortex force, here we chose to consider a pure curvature-induced force independent of the actin density.

2.2. Model simplification. In order to simplify the model equations, and more especially to avoid the problem of a free moving cell boundary, we propose to restrict the description of the actin dynamics in a one-dimensional circular active layer of radius r_* . Therefore we do not consider any radial movements of actin in the cell cortex but we assume tangential displacements which can lead to local increase or decrease in density on the circle (i.e., in the tangential direction θ) which affect the intensity of the retraction force. With such an approximation, the two components of the mechanical equilibrium equation can be reduced to a unique equation whose derivation in polar coordinates leads to the following expression (see [Appendix B](#) for details on the derivation):

$$\frac{\partial}{\partial \theta} \left[\frac{\mu}{r_*} \frac{\partial v}{\partial \theta} + \frac{\hat{E}}{r_*} \frac{\partial v}{\partial \theta} + \sigma(a) \right] = \frac{\alpha v}{2 r_*}, \quad (9)$$

where

$$\mu = \mu_1 + \mu_2, \quad \hat{E} = E'(1 + v'), \quad \text{and} \quad \alpha = E' \left[1 - \frac{\frac{3}{2} + v'}{\frac{3}{2} + \frac{\mu_2}{\mu_1}} \right].$$

In this paper we will restrict our study of the model equations by assuming that the parameter α (which represents a viscoelastic coefficient) is always positive (details for the justification of this choice can be found in the [Appendix C](#)). This reasonable hypothesis thus assumes that $v' < \frac{\mu_2}{\mu_1}$. Membrane and actin network dynamics are coupled by means of the following equation, adapted from equation (2) and which describes the conservation of the amount of actin, $Q(\theta, t) = L(\theta, t)a(\theta, t)$, where $Q(\theta, t)$ represents the peripheral actin mass distribution inside the cell cortex, distributed along the cell periphery:

$$\frac{\partial Q}{\partial t} - \frac{D}{r_*^2} \frac{\partial^2 Q}{\partial \theta^2} + \frac{1}{r_*} \frac{\partial}{\partial \theta} (Q\dot{v}) = k_a(Q_c - Q) \quad (10)$$

with $Q_c(\theta, t) = L(\theta, t)a_c$.

The equation for the membrane deformations is still given by

$$\Phi \frac{\partial L}{\partial t} = P - \sigma(a)L - \tau K_L. \quad (11)$$

3. NONDIMENSIONALIZATION AND LINEAR STABILITY ANALYSIS

3.1. Nondimensionalization. We nondimensionalize the equations by making the following substitutions:

$$\begin{aligned} \tilde{t} &= tk_a, & \tilde{a} &= \frac{a}{a_c}, & \tilde{L} &= \frac{L}{R_0}, & \tilde{v} &= \frac{\dot{v}}{k_a R_0}, & \tilde{D} &= \frac{D}{k_a R_0^2}, \\ \tilde{E} &= \frac{\hat{E}}{k_a \mu}, & \tilde{P} &= \frac{P}{k_a R_0 \Phi}, & \tilde{\tau} &= \frac{\tau}{k_a R_0^2 \Phi}, & \tilde{\alpha} &= \frac{\alpha}{k_a \mu}, \\ \tilde{\psi} &= \frac{\psi a_c^2}{k_a \mu}, & \tilde{a}_{\text{sat}} &= \frac{a_{\text{sat}}}{a_c}, \end{aligned}$$

where R_0 represents a typical length in the cell, and μ and Φ are assumed to be equivalent coefficients. Dropping the tildes for notational convenience, the equations are written as:

$$\frac{\partial}{\partial \theta} \left[\frac{1}{r_*} \frac{\partial \dot{v}}{\partial \theta} + \frac{E}{r_*} \frac{\partial v}{\partial \theta} + \sigma(a) \right] = \frac{\alpha}{2} \frac{v}{r_*}, \quad (12)$$

$$\frac{\partial}{\partial t} (La) - \frac{D}{r_*^2} \frac{\partial^2}{\partial \theta^2} (La) + \frac{1}{r_*} \frac{\partial}{\partial \theta} (La\dot{v}) = L(1 - a), \quad (13)$$

$$\frac{\partial L}{\partial t} = P - \sigma(a)L - \tau K_L \quad (14)$$

with

$$\sigma(a) = \psi a^2 e^{-a/a_{\text{sat}}}.$$

3.2. Linear stability analysis. The linear stability analysis is performed in order to define the conditions required for the model parameters to generate self-sustained oscillations of the membrane corresponding to the destabilization of the uniform steady state for the variable L , occurring through a Hopf bifurcation ($\text{Re}[\lambda(k)] = 0$). The steady state is given by

$$a_0 = 1, \quad L_0 = \frac{P}{\sigma(1)}, \quad v_0 = 0. \tag{15}$$

All the equations are linearized about this steady state. The solutions of the linearized system are proportional to $e^{\lambda t + ik\theta}$. By substitution of this expression in the linearized equations we obtain the system

$$\begin{pmatrix} \lambda + \sigma(1) & L_0\sigma'(1) & 0 \\ \lambda + \frac{D}{r_*^2}k^2 & L_0(\lambda + \frac{D}{r_*^2}k^2 + 1) & ik\frac{L_0}{r_*}\lambda \\ 0 & ikr_*\sigma'(1) & -(\lambda + \Omega)k^2 - \frac{\alpha}{2} \end{pmatrix} \begin{pmatrix} L - L_0 \\ a - a_0 \\ v - v_0 \end{pmatrix} = \vec{0}, \tag{16}$$

with $\Omega = E$ and $\sigma'(1) = \partial\sigma(a)/\partial a|_{a=1}$. The dispersion equation associated with this system is given by $\det(M) = 0$ (where M is the matrix above), i.e.:

$$k^2\lambda^3 + a(k^2)\lambda^2 + b(k^2)\lambda + c(k^2) = 0, \tag{17}$$

where

$$\begin{aligned} a(k^2) &= \frac{D}{r_*^2}k^4 + [\Omega + 1 + \sigma(1) - 2\sigma'(1)]k^2 + \frac{\alpha}{2}, \\ b(k^2) &= \frac{D}{r_*^2}[\Omega + \sigma(1) - \sigma'(1)]k^4 \\ &\quad + \left[\frac{\alpha D}{2 r_*^2} + \Omega(1 + \sigma(1) - \sigma'(1)) + \sigma(1)(1 - \sigma'(1)) \right] k^2 \\ &\quad + \frac{\alpha}{2}[1 + \sigma(1) - \sigma'(1)], \\ c(k^2) &= \frac{D}{r_*^2}\Omega[\sigma(1) - \sigma'(1)]k^4 + \left[\frac{\alpha D}{2 r_*^2}(\sigma(1) - \sigma'(1)) + \sigma(1)\Omega \right] k^2 + \frac{\alpha}{2}\sigma(1). \end{aligned}$$

According to the Routh–Hurwitz criteria the condition for the roots of the dispersion equation to have a negative real part ($\text{Re}(\lambda) < 0$) is

$$a(k^2) > 0, \quad c(k^2) > 0 \quad \text{and} \quad a(k^2)b(k^2) - c(k^2) > 0. \tag{18}$$

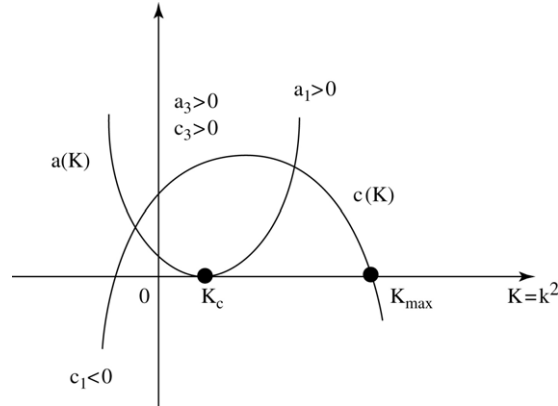


Figure 4. Conditions required to satisfy the Routh–Hurwitz criteria.

For simplicity, we represent the functions $a(k^2)$, $b(k^2)$ and $c(k^2)$ by

$$a(k^2) = a_1k^4 + a_2k^2 + a_3$$

$$b(k^2) = b_1k^4 + b_2k^2 + b_3$$

$$c(k^2) = c_1k^4 + c_2k^2 + c_3$$

and we have $a_1 > 0$, $a_3 > 0$, $c_3 > 0$ and $c_1 < 0$. a_2 , b_1 , b_2 , b_3 and c_2 are not well defined, i.e., they can be either positive or negative. As c_1 is negative, this means that the number of wavenumbers k for which $c(k^2)$ is positive, is limited, i.e., $c(k^2) > 0$ only for $k^2 \in [0, k_{\max}^2]$. As $a(k^2)$ is required to be positive at least for $k^2 < k_{\max}^2$, this means that $b(k^2)$ must also be positive to fulfil the condition $a(k^2)b(k^2) - c(k^2) > 0$.

3.3. Conditions for a bifurcation. The stability analysis is thus performed as follows: first we determined the value of k_{\max}^2 for the condition $c(k^2) > 0$, then as a_1 and a_3 are positive but a_2 can be negative, this means that a bifurcation occurs when $a(k_c^2) = 0$ (see Fig. 4). The wavenumber k_c^2 is the point for which the function $a(k^2)$ has its minimum at 0. We then have to make sure that the function $b(k^2)$ remains positive for $k^2 < k_{\max}^2$ according to the conditions obtained for the choice of the parameters. Details of each of these calculation steps are given in the Appendix C. The expressions obtained for k_{\max}^2 and k_c^2 are as follows:

$$k_{\max}^2 = \frac{2\sigma(1)}{\frac{D}{r_*^2}[\sigma'(1) - \sigma(1)]}, \tag{19}$$

$$k_c^2 = \frac{2\sigma'(1) - \sigma(1) - \Omega - 1}{2\frac{D}{r_*^2}} > 0. \tag{20}$$

Moreover the condition that the parameters must obey at the bifurcation point is given by

$$2\sigma'(1) - \sigma(1) - \Omega - 1 = \frac{\sqrt{2\alpha D}}{r_*}. \quad (21)$$

4. MECHANICAL CHARACTERIZATION OF THE CELL

The model we chose to investigate is based on a mechanical (rather than molecular) description of the cell. Therefore, before we go further in the presentation and analysis of the simulation performed on the model, we present here a brief review of the experimental methods used to mechanically characterize the cell. This stage is essential for defining the range of parameters required for our simulation and at a later stage for the quantitative validation of the model. The mechanical characterization of the cell essentially consists in the determination of the viscoelastic properties of the cytoplasm (and the network of filaments) and the determination of the membrane elastic properties. Two different types of characterization methods can be distinguished: active methods and passive methods.

The first ‘active methods’ are methods where the determination of the viscoelastic parameters requires a direct mechanical interaction with the cell. Such mechanical interactions are usually performed with a micropipette (measurements of aspiration force) (Merkel *et al.*, 2000), microplates (deformations applied on the cell at varying frequencies) (Thoumine and Ott, 1997), or optical (Bar-Ziv *et al.*, 1998; Yanai *et al.*, 1999) and magnetic (Bausch *et al.*, 1998, 1999; Alenghat *et al.*, 2000) tweezers (mechanical stress applied in specific locations in the cell). The atomic force microscopy (Rotsch *et al.*, 1999; Rotsch and Radmacher, 2000) is another active method widely used, which consists in force mapping, i.e., recording force curves by scanning the sample cell with the tip of the device, from which local elastic moduli are measured.

Passive methods, on the other hand, are non-invasive for the cell, which gives them an advantage since the results obtained are far less method dependent than the active ones. Laser tracking micro-rheology (Xu *et al.*, 1998; Yamada *et al.*, 2000) for example, is a method used to estimate the mechanical properties of the cell by tracking the Brownian motion of individual particles naturally present in the cytoplasm of the cell. A closely related techniques is the diffusive wave spectroscopy. Rather than monitoring a single particle, this method monitors the relative motion of many thousands of particles simultaneously. In both methods the viscoelastic moduli are then evaluated from the motions of the particles. Scanning acoustic microscopy (Zoller *et al.*, 1997; Bereiter-Hahn and Luers, 1998) is another passive method which allows one to characterize the stiffness of a material from the reflection of sound waves. This is based on the measurement of the velocity of longitudinal sound waves which is proportional to the elasticity and density of the structure under observation. High sound velocity means high tension in

Table 1. Viscoelastic parameters from extracellular measurements or whole cell measurements.

Technique (Ref)	Cell type	Elasticity (N m ⁻²)	Viscosity (N s m ⁻²)
Magnetic twist (Zaner and Valberg, 1989)	Macrophage	n.a.	2500
Magnetic twist (Wang <i>et al.</i> , 1993)	Endothelial	2	n.a.
Magnetic twist (Pourati <i>et al.</i> , 1998)	Endothelial	10–12.5	n.a.
Magnetic twist (Laurent <i>et al.</i> , 2002, 2003)	Epithelial	34–85	5–14
Micropipette (Sung <i>et al.</i> , 1988)	Leucocyte	0.75 23.8	33
Mech. rheometer (Eichinger <i>et al.</i> , 1996)	<i>Dictyostelium</i>	55	25
Micropipette (Merkel <i>et al.</i> , 2000)	<i>Dictyostelium</i>	200 ± 10 (front)	
Micropipette (Merkel <i>et al.</i> , 2000)		330 ± 20 (rear)	
Cell poker (Zahalak <i>et al.</i> , 1990)	Neutrophil	118	n.a.
AFM (Radmacher <i>et al.</i> , 1996)	Platelet	100–5000	n.a.
Microplates (Thoumine and Ott, 1997)	Fibroblast	1000	100–10000
Spont. retraction (Ragsdale <i>et al.</i> , 1997)	Fibroblast	1700	4 × 10 ⁵
Magnetic tweezers (Bausch <i>et al.</i> , 1998)	Fibroblast	30000	2000
Deformable substrat (Dembo and Wang, 1999)	Fibroblast	2000	n.a.
Deformable substrat (Dembo and Wang, 1999)		6000 (front)	n.a.

fibrillar elements of the cytoskeleton. The use of deformable substrates is another non-invasive technique which allows one to measure traction stresses exerted by migrating cells through the deformations they create on a flexible substrate. This approach can yield direct quantitative information about the detailed magnitude, direction and location of interfacial stresses. Wrinkles created by the cell tractions at the surface of the film are interpreted as the result of compressive forces exerted by migrating fibroblasts (Harris *et al.*, 1980). Other methods, however, use non-wrinkling silicon rubber film, and measure instead the displacement of polystyrene latex beads embedded in the film (Oliver *et al.*, 1999). Computational techniques are then used to convert the displacement information into an image of the traction stress distribution (Dembo *et al.*, 1996; Dembo and Wang, 1999).

4.1. Mechanical parameters. The results obtained from the various methods of characterization are summarized and completed by other measurements in order to provide a ‘global’ source of reference of the cell mechanical parameters presented in Tables 1–4.

5. SIMULATION RESULTS

5.1. Static membrane deformations. Before we perform the analysis of the full model, we first focus in this section on the equation governing the membrane deformations [equation (11)]. Our aim is to evaluate the ability of the simple membrane model to account for typical ‘steady-state’ deformations of fibroblast cells, such

Table 2. Viscoelastic parameters from intracellular measurements.

Technique (Ref)	Cell type	Elasticity (N m ⁻²)	Viscosity (N s m ⁻²)
Magnetic twist (Valberg and Albertini, 1985)	Macrophage	15	2000
Magnetic tweezers (Bausch <i>et al.</i> , 1999)	Macrophage	20–735	210
Optical tweezers (Yanai <i>et al.</i> , 1999)	Neutrophil	1.1 (body)	0.35
Optical tweezers (Yanai <i>et al.</i> , 1999)		0.01 (front)	0.1
Optical tweezers (Yanai <i>et al.</i> , 1999)		0.75 (rear)	0.35
Laser tracking (Yamada <i>et al.</i> , 2000)	Epithelial	72.1	38.2

Table 3. Traction force measurements.

Technique (Ref)	Cell type	Traction force (nN)
Deformable substrate (Oliver <i>et al.</i> , 1995)	Keratocyte	45 (ventral)
Deformable substrate (Oliver <i>et al.</i> , 1995)	Keratocyte	10
Micromachined (Galbraith and Sheetz, 1999)	Keratocyte	13 (max.)
Micromachined (Galbraith and Sheetz, 1999)		4.5 (ventral)
Micromachined (Galbraith and Sheetz, 1999)		0.158 (dorsal)
Deformable substrate (Burton <i>et al.</i> , 1999)	Keratocyte	100–200 (rear)
Deformable substrate (Burton <i>et al.</i> , 1999)		600–700 (body)
Deformable substrate (Burton <i>et al.</i> , 1999)		120–150 (flank)
Deformable substrate (Burton <i>et al.</i> , 1999)	Fibroblast	300–800
Deformable substrate (Dembo and Wang, 1999)	Fibroblast	2000
Microplate (Thoumine and Ott, 1997)	Fibroblast	40
Magnetic tweezers (Bausch <i>et al.</i> , 1999)	Macrophage	0.05–0.9
Magnetic tweezers (Guilford <i>et al.</i> , 1995)	Macrophage	2–10
Micropipette aspiration (Merkel <i>et al.</i> , 2000)	<i>Dictyostelium</i>	13 (retraction)

as exhibited in Fig. 2. For this, we replace here the retraction force $\sigma(a)$ by the function $\gamma(\theta)$ modulated along the angular position θ (see Fig. 1) and that we used to reflect a static state of the actin distribution. The membrane movements are thus described by

$$\Phi \frac{\partial L}{\partial t} = P - \gamma(\theta)L - \tau K_L. \quad (22)$$

The function for the retraction force $\gamma(\theta)$ is then given by

$$\gamma(\theta) = \gamma_0[\alpha + \sin(m\theta)]$$

where α and m represent the coefficients which control the amplitude of the deformation and the mode of deformation respectively.

The analytical solution of equation (11), for the parameter $\tau = 0$ (i.e., no membrane tension) and for an initial condition $L(t = 0) = L_0$ (i.e., a circular shape), is given by

$$L(\theta, t) = \frac{P}{\gamma(\theta)} + \left(L_0 - \frac{P}{\gamma(\theta)} \right) e^{-\frac{\gamma(\theta)}{\Phi} t}. \quad (23)$$

Table 4. Membrane tension (k_B is the Boltzman's constant).

Parameter (Ref)	Value
Membrane tension (Simson <i>et al.</i> , 1998)	$3.1 \pm 1.4 \mu\text{N m}^{-1}$
Membrane tension (Cevc and Marsh, 1987)	$35 \mu\text{N m}^{-1}$
Membrane tension (Raucher and Sheetz, 2000)	$7.0 \pm 0.5 \text{ pN}$
Bending modulus (Simson <i>et al.</i> , 1998)	$391 \pm 156 k_B T$
Bending modulus (Cevc and Marsh, 1987)	$5\text{--}50 k_B T$
Adhesion energy (Simson <i>et al.</i> , 1998)	$22.0 \pm 12.2 \times 10^6 \text{ J m}^{-2}$

Table 5. Maximum and minimum extension of the membrane depending on the membrane tension coefficient τ .

τ	L_{\min}	L_{\max}	ΔL_{\max}
0	0.40	2	1.60
0.05	0.41	1.63	1.21
0.1	0.43	1.42	0.99

The asymptotic solutions of this equation are presented in Fig. 5 (external curves in each case) for a range of modes from $m = 1$ (round-shaped cell) to $m = 10$ ('starry' shape). Fibroblasts usually display from 2 to 4 stable protrusions (Fig. 2). Although morphologies with more than four protrusions exist, they are more rarely observed as they are unstable. In each case the minimum and maximum deformation of the membrane, for the asymptotic state, is given by the following range:

$$\frac{P}{\gamma_0(\alpha + 1)} \leq L(\theta) \leq \frac{P}{\gamma_0(\alpha - 1)}. \quad (24)$$

The maximum amplitude of deformation ΔL_{\max} is thus

$$\Delta L_{\max} = \frac{2P}{\gamma_0(\alpha + 1)(\alpha - 1)}. \quad (25)$$

In order to evaluate the influence of the curvature term on these morphologies, equation (11) is now solved for two different values of the membrane tension coefficient τ (Table 5). The exact analytic resolution of this equation is this time non-trivial, and therefore the equation is solved numerically using a central finite difference scheme which leads to a tridiagonal matrix system solved by the Thomas algorithm (Strikverda, 1989).

The two solutions (for $\tau = 0.05$ and $\tau = 0.1$) calculated for each mode of cell deformation appear for each case in Fig. 5 as the two internal curves. We observe that the initial cell morphologies (those corresponding to $\tau = 0$) are smoothed by the additional membrane tension term. As this term depends linearly on the intensity of the curvature, the more the curvature is sharp (such as in mode $m = 5$ and $m = 10$) the more the effect becomes important in smoothing the cell shapes.

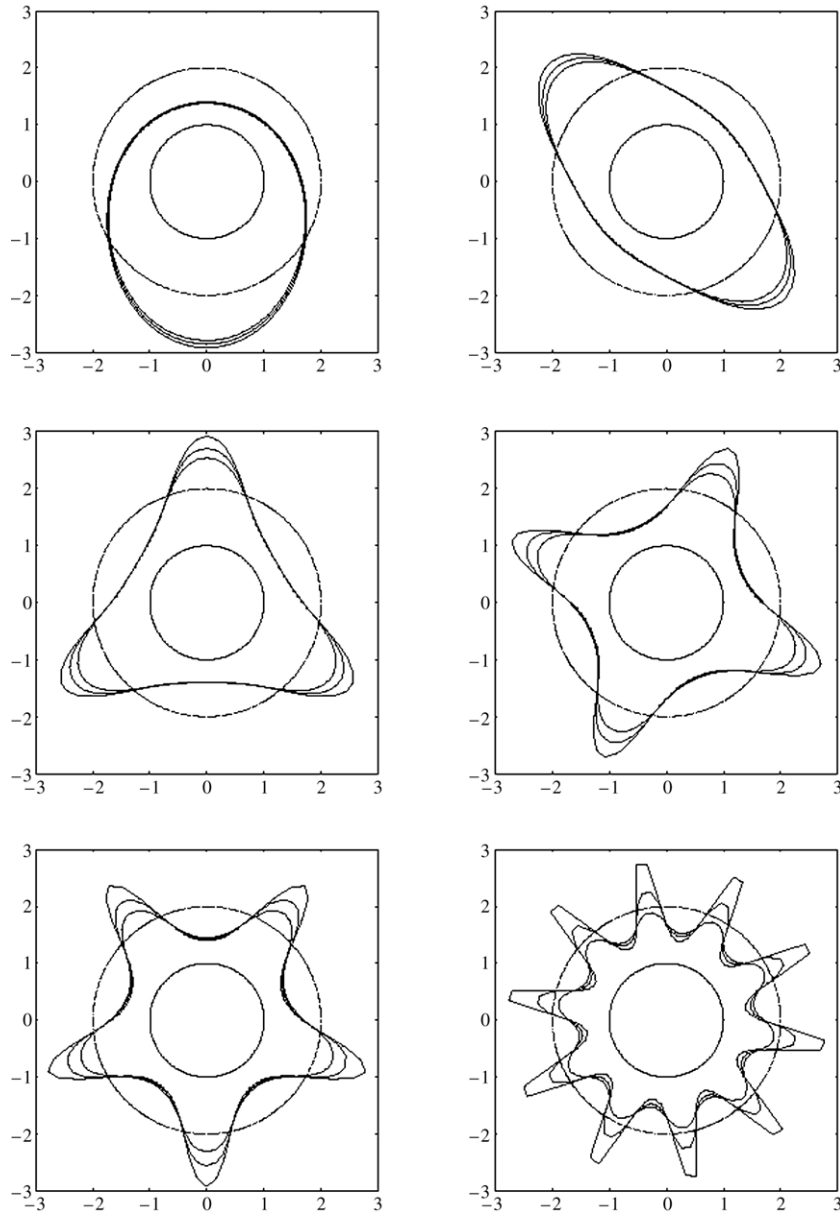


Figure 5. Potential cell morphologies obtained for various modes of deformation ($m = 1, 2, 3, 4, 5, 10$) of the function $\gamma(\theta)$ representing a spatial modulation of the F-actin filament stiffness. In each graph, the dotted curve represents the initial cell shape [circular shape $L(\theta, t = 0) = L_0$] and the most external curve the analytical solution of the equation for the membrane deformations [equation (11)] taken for $\tau = 0$ (passive membrane). The two internal curves correspond to the numerical solutions of that same equation for two different values of the membrane stiffness coefficient, namely $\tau = 0.05$ and $\tau = 0.1$.

5.2. The dynamical model. In this section, we restore the explicit coupling between the membrane and the actin dynamics which is responsible for its

Table 6. Parameters of the simulations.

Figures	D	τ	α	ψ	a_{sat}	P	E
6(a)–6(b)	0.134	0	22	9	1.1	4	2
6(c)–6(d)	0.075	0	39	8	1.1	4	2
6(e)–6(f)	0.00962	0.1	304	10	1.1	4	2

movements. We thus solve now the full system of PDEs involving a hyperbolic [equation (10)], a parabolic [equation (11)] and an elliptic [equation (9)] equation. The hyperbolic and parabolic equations are evolution equations we solved numerically using a Crank–Nicholson finite differences scheme with appropriate discretisation. The elliptic equation is solved through a relaxation scheme. The associated matrix inversions were solved using the Thomas algorithm (Strikverda, 1989) adapted to incorporate the periodic boundary conditions. In all the simulations, the initial conditions are random perturbations of the F-actin concentration around the homogeneous steady-state where the cell has a circular morphology.

The results of the simulations carried out are presented in Fig. 6. In each case, the spatio-temporal maps for the membrane extension $L(\theta, t)$ are displayed together with the associated actin distributions $a(\theta, t)$. The parameters used for each of the simulations are given in Table 6. Parameters, τ , a_{sat} , ψ , p , P and E are arbitrarily defined. D is calculated from equation (20) for a given mode of deformation k_c . The parameter α is then calculated from equation (21). The condition which imposes that $b(k^2)$ is a positive function for the selected set of parameters is verified *a posteriori* (see Appendix C for details on the stability analysis).

In the first simulation (Fig. 6, top graphs) the parameters have been chosen in order to select a lower mode of deformation corresponding to round-shaped cells. We observe, after an oscillating transition phase (from $t = 2$ to $t = 10$ normalized time units), the slow emergence of a rotating wave of deformation around the cell body. The sequence of the asymptotic dynamical state of the cell membrane deformations is displayed in Fig. 7. The wave of deformation rotates in a counter-clockwise direction with a measured periodicity of about $T = 2.8$ normalized time units. This dynamical behaviour is typical of round-shaped cells such as keratinocytes and leukocytes.

In the second simulation (Fig. 6, middle graphs), a symmetrical pulsating state rapidly emerges. The pulsation is characterized by the extension of the membrane along one direction and its simultaneous retraction in the other perpendicular direction. The associated sequence of deformations is displayed in Fig. 8. This time the periodicity of the pulsation is shorter with $T = 2.2$ normalized time units. This pulsating behaviour is characteristic of fibroblast cells.

For the third simulation (Fig. 6, bottom graph) the parameters were defined according to the stability analysis of the previous section so that higher modes of deformation would be selected. The sequence showing the associated cell

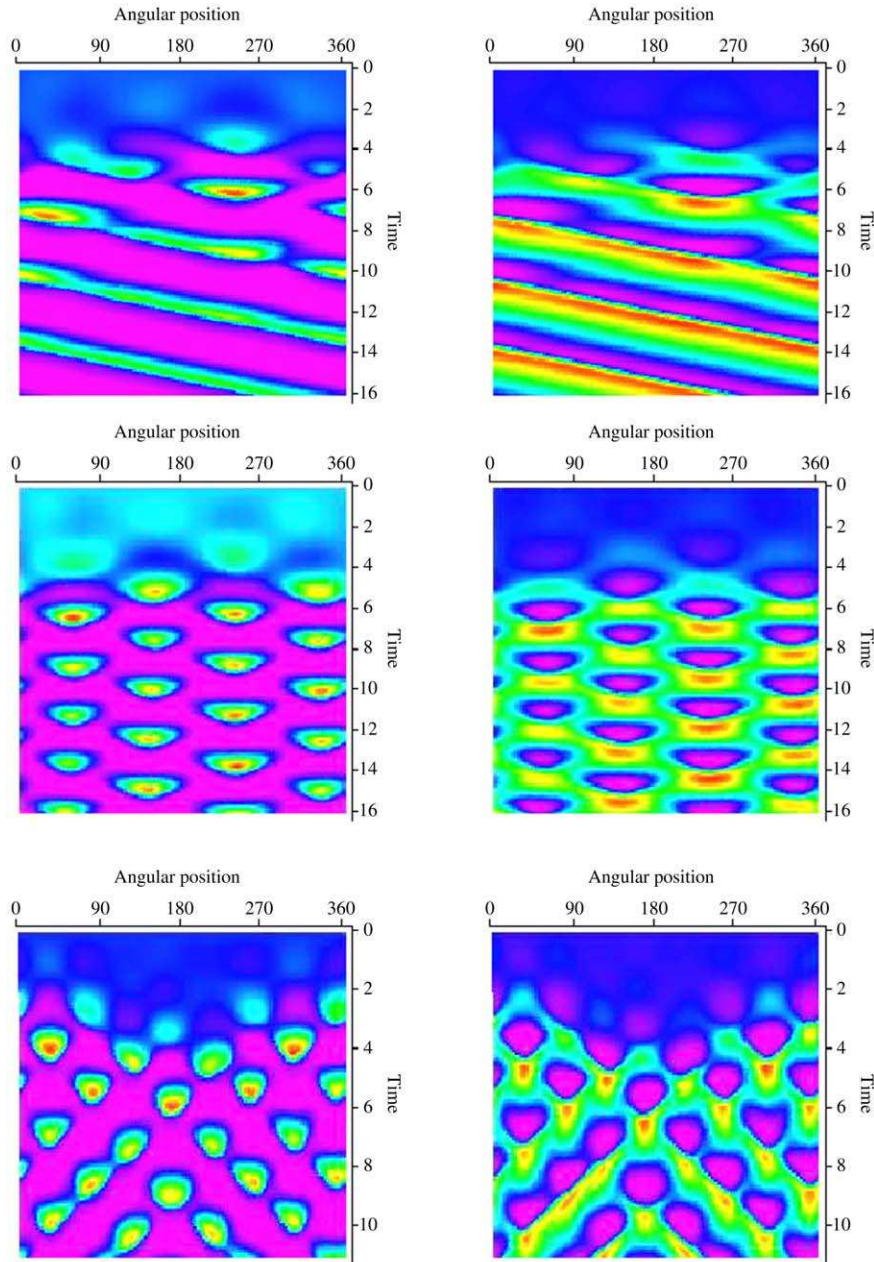


Figure 6. Simulation results of the spatio-temporal evolution of the cell membrane deformations (left side), together with the corresponding actin distributions (right side). Top graphs: rotating wave of deformation; middle graphs: symmetrical pulsation, bottom graphs: asymmetrical (or alternating) pulsation.

movements is displayed in Fig. 9. Snapshots of the simulated cell are taken to cover a full period of deformations where $T = 2.8$ normalized time units, as for the rotating wave case. The simulated cell exhibits coordinated movements

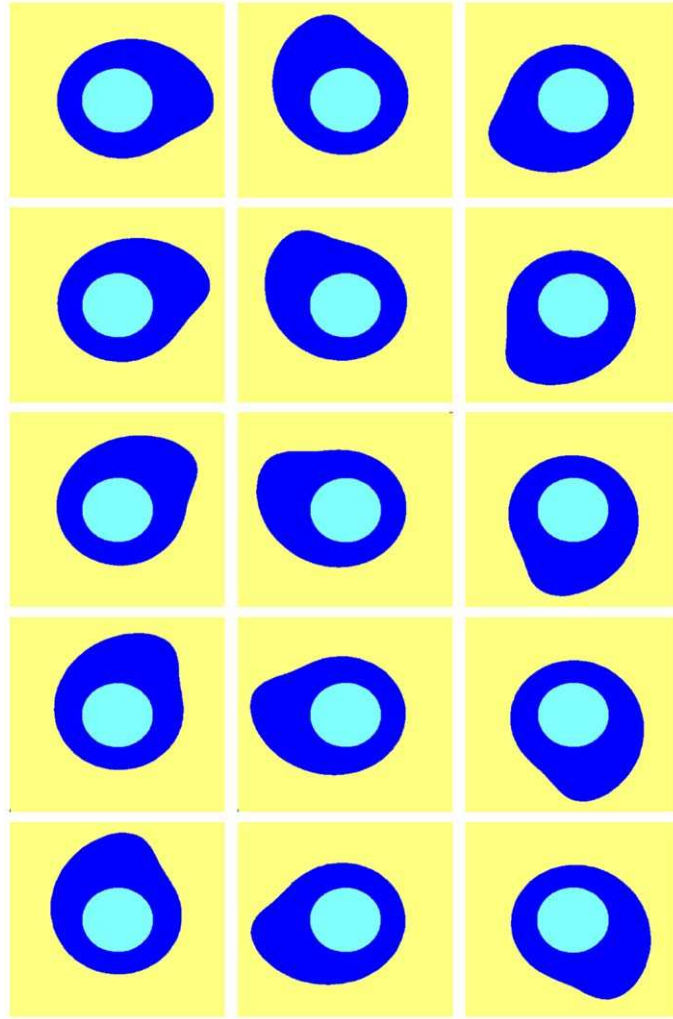


Figure 7. Simulated cell membrane deformations (asymptotic state associated with the top graph of Fig. 6). Snapshots are taken every 200 iterations ($\Delta t = 0.2$). The counterclockwise wave of deformation has a periodicity of about 2.8 normalized time units (sequence to be read from top to bottom).

of extension/retraction of the membrane involving up to four protrusions simultaneously which occur in perpendicular directions from each other. This simulated dynamics of cell deformations is consistent with the movements observed in fibroblasts. A typical example of fibroblast deformations is shown in the videomicroscopy sequence presented in Fig. 10.

In the simulated sequence we observe that a developing protrusion presents a wide front (under the effect of the pressure) whereas during the retraction process the pseudopods become thinner (the actomyosin network pulls on the membrane). Experimentally this difference is not as obvious. However the developing

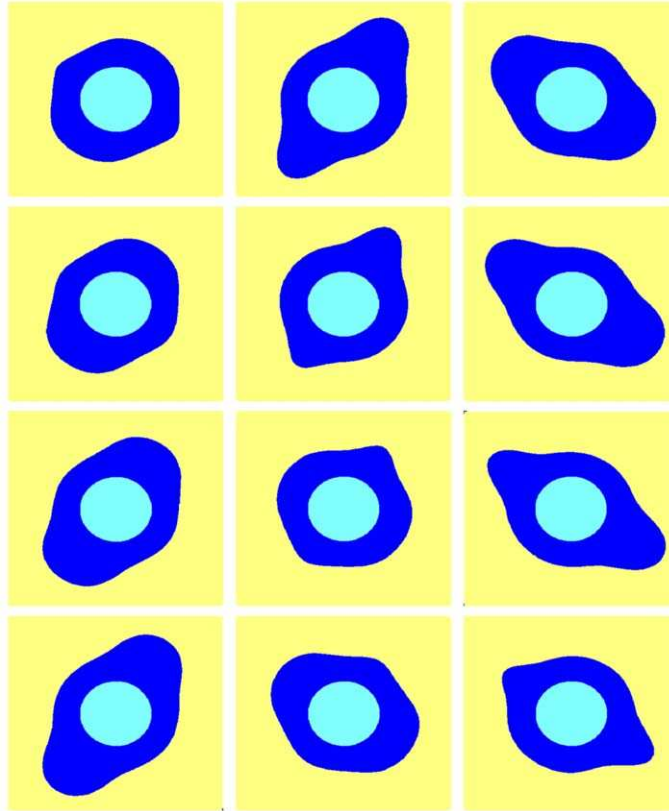


Figure 8. Simulated cell membrane deformations (asymptotical state associated with the middle graph of Fig. 6). Snapshots are taken every 200 iterations ($\Delta t = 0.2$). The pulsation of the cell deformation has a periodicity of about 2.2 normalized time units (sequence to be read from top to bottom).

protrusions exhibit at their tips some kind of ‘blobs’ which disappear as soon as the pseudopods start to retract.

For a more detailed analysis, Fig. 11 simultaneously follows the evolution of the actin distribution as well as its tangential displacement. The 4 graphs presented correspond respectively to the snapshots 1, 3, 4 and 5 of Fig. 9 (associated simulation times are $t = 5; 5.4; 5.6$ and 5.8 normalized units). In the protrusive areas (or pseudopods) the actin density is low and starts to polymerize (and reversely depolymerize in the areas where the critical density is reached, $a_c = 1$). Simultaneously, tangential displacements of actin are observed from zones of low density to zones of higher density which tend to the homogenization of the actin distribution in the cell (Fig. 11, upper left graph). Actin progressively increases at the neighbourhood of the pseudopods. The intensification of the retraction force thus leads to the narrowing of the protrusions (Fig. 11, upper right graph). The actin then enters almost instantaneously in the remaining pseudopods (Fig. 11, bottom left graph) and the distributions of actin in the cell are reversed.

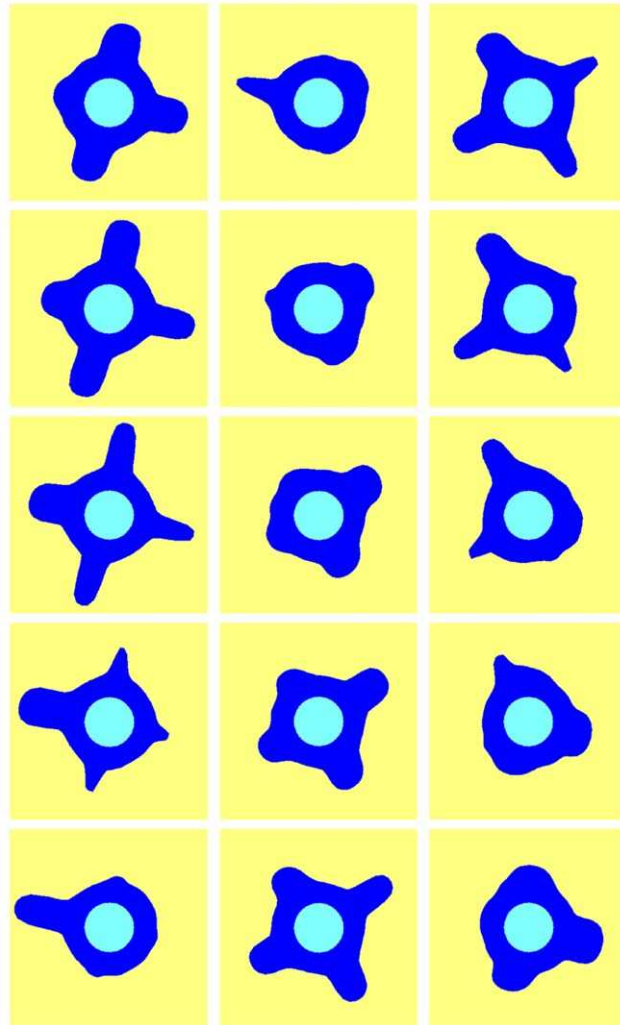


Figure 9. Simulated cell membrane deformations (asymptotic state associated with the bottom graph of Fig. 6). Snapshots are taken every 200 iterations ($\Delta t = 0.2$). The alternating pulsation of the cell deformation has a periodicity of about 2.8 normalized time units (sequence to be read from top to bottom).

The retraction at the tips of the pseudopods is thus suddenly increased and leads to their total retraction (Fig. 11, bottom right graph). New pseudopods then appear in zones of low actin density and a new cycle can start.

Fig. 12 shows the oscillating dynamics on a longer time scale (up to $t = 20$ normalized time units). The upper graph shows the simultaneous evolution of the amplitude of the cell membrane deformation associated with the actin concentration for a given direction corresponding to a pseudopod, and the lower graph shows the simultaneous evolution of the amplitude of two protrusive directions distant from each other with a 45° angle. Oscillations of the cell shape are exhibited with alternated directions of deformation from one cycle to the next one.

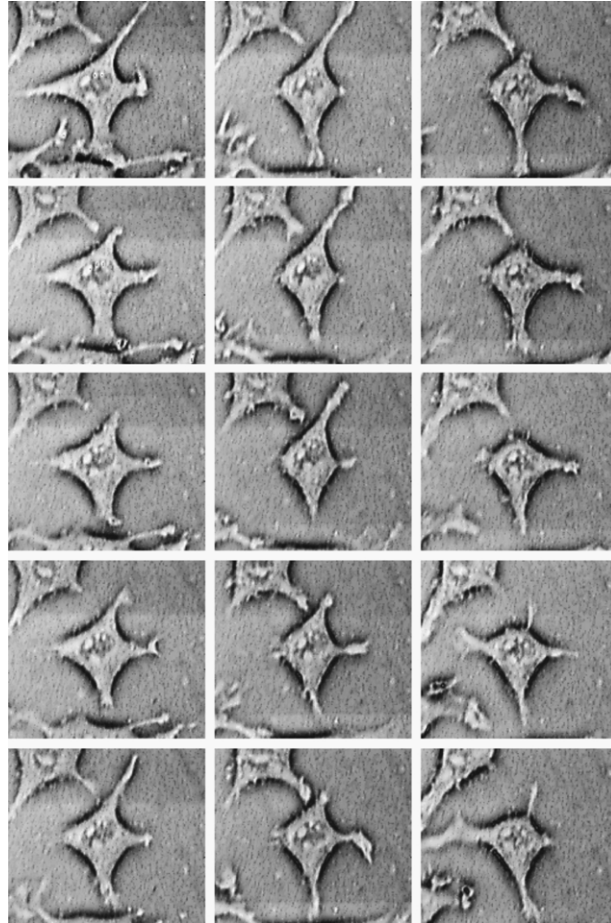


Figure 10. Videomicroscopy sequence of a L929 pulsating fibroblast. The time interval between two consecutive pictures is about 2 min (sequence to be read from top to bottom).

In the simulations performed, it has been possible to switch from one dynamical behaviour to another by changing mainly two key parameters, the diffusion coefficient D for actin in the cytoplasm and the coefficient α which characterizes the cell viscoelastic properties [see equation (9)]. According to the normalization of the parameters (see Section 4.1), this last parameter can be evaluated from the values of k_a and μ which are respectively the actin polymerization rate and the cytoplasmic viscosity, as follows:

$$\alpha = k_a \mu \tilde{\alpha} > 0. \quad (26)$$

The polymerization rate k_a can be estimated as

$$k_a = \frac{T_{\text{simul}}}{T_{\text{real}}} \quad (27)$$

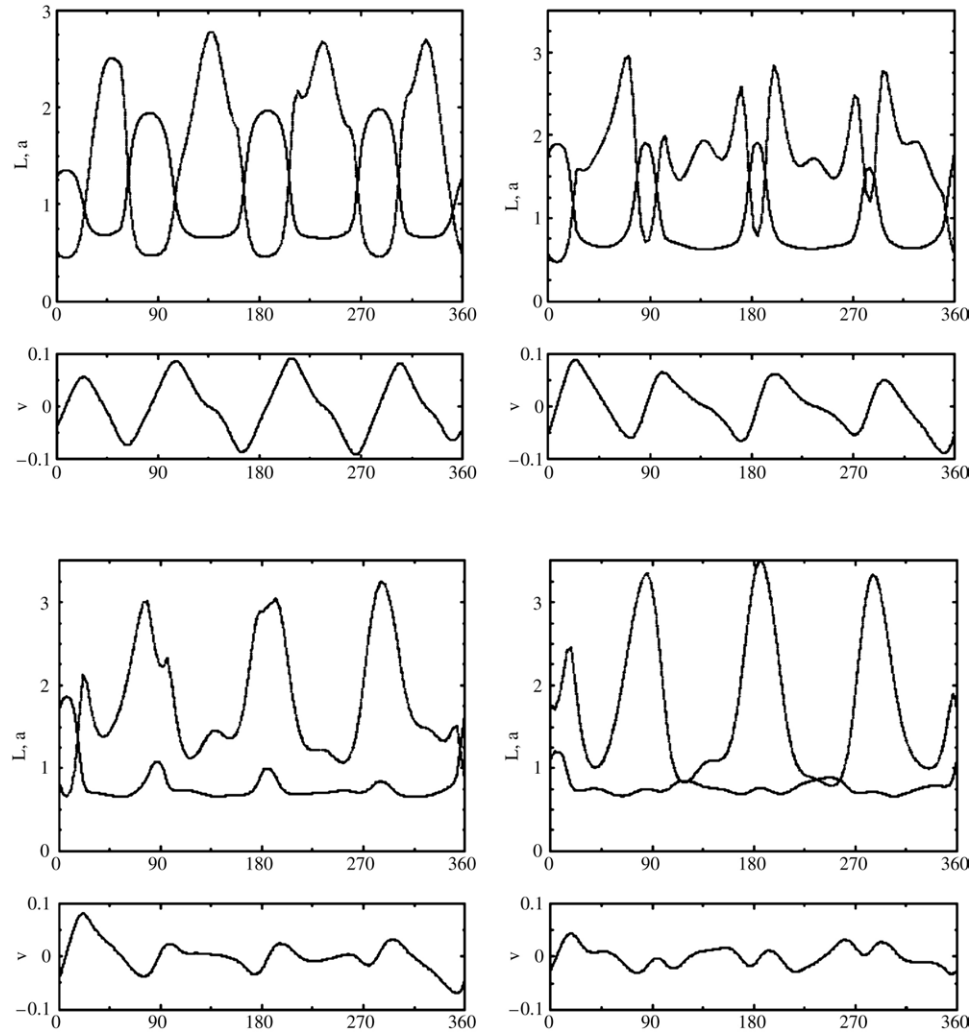


Figure 11. Simultaneous plots of actin distribution and corresponding membrane deformations in upper graphs. In the lower rectangular graphs, the associated tangential displacements of actin are displayed. These four graphs correspond to the snapshots 1, 3, 4 and 5 of the sequence of Fig. 7 associated with the normalized times 5, 5.4, 5.6 and 5.8 respectively.

where T_{simul} is the adimensional periodicity of the simulated cell deformations and T_{real} is the periodicity of the deformations measured on real cells. Then k_a has the dimension of 1/time i.e., s^{-1} . This periodicity can be measured by the extraction of temporal signals for each protrusive direction detected on the experimental polarity maps (Fig. 3). Previous work with this method has allowed us to establish an existing periodicity for L929 fibroblasts of about 30 min (Stéphanou *et al.*, 2003).

Concerning the cytoplasmic viscosity μ , the experimental data collected in Tables 1 and 2 obtained from different studies and using different techniques, lead

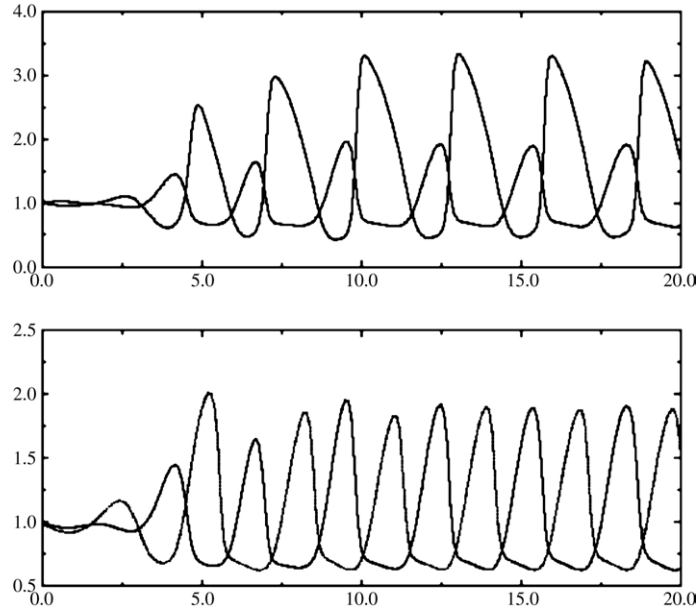


Figure 12. Upper graph: evolution over time of the cell membrane deformations and of the associated actin distribution (higher amplitude curve) in normalized units, for a given protrusive direction. Lower graph: simultaneous evolution of the membrane deformations for two protrusive directions at 45° from each other.

Table 7. Estimated cell elasticity.

Figure	T_{simul}	$\tilde{\alpha}$	$\alpha_{\text{estim}} (\text{N m}^{-2})$
6(a)–6(b)	2.8	22	68
6(c)–6(d)	2.2	39	95
6(e)–6(f)	2.8	304	945

us to take an estimated value around 2000 N s m^{-2} (Valberg and Albertini, 1985; Zaner and Valberg, 1989; Bausch *et al.*, 1998). On this basis, an estimated value of the α coefficient can be obtained for each of the three simulations performed. The values calculated are display in Table 7.

In Tables 1 and 2, we observe that higher values of the cell elasticity are found for fibroblasts cells, namely cells with large deformation amplitudes, whereas smaller values are rather associated with rounded cells (such as endothelial cells, leukocytes or neutrophils). Similarly in our simulations, we managed to obtain fibroblast-type morphologies by increasing significantly the coefficient α . This parameter is a complex one as it depends on the coefficients E' , ν' , μ_1 and μ_2 . It can therefore be considered as a coefficient which reflects the global viscoelastic properties of the cell. We note that simulations performed with smaller values of this coefficient lead at the opposite to rounded-cell type deformation.

6. DISCUSSION AND CONCLUSION

A large variety of behaviour, from rotating waves of deformations to pulsating waves, have been observed, all in a single type of cell, the amoeba *Dictyostelium* (Killich *et al.*, 1993, 1994). A purely geometrical model based on the interaction of superposed waves was used to describe the dynamical behaviours observed. It was shown that the interference patterns of two interacting waves were sufficient to describe the overall diversity of the oscillating states observed in the amoebae. It was then proposed that actin dynamics might account for these oscillations. This affirmation has here been confirmed on the basis of the cytomechanical model initially formulated by Alt and Tranquillo (1995). We have indeed been able to simulate rotating waves of deformations for the selection of low modes of deformation associated with round-shaped cells such as keratinocytes, and to simulate standing pulsating waves of deformation for the selection of higher modes of deformations associated with star-shaped cell morphologies involving large membrane extensions such as for fibroblasts. In this latter case, the model is especially in good agreement with the experimentally observed cell dynamical behaviours as it is able to catch the main features of the fibroblast cells. This is achieved through a fine tuning of the two key concentrations for actin:—the concentration at the chemical equilibrium a_c which determines the polymerization and depolymerization state of actin and—the saturation concentration $2a_{\text{sat}}$ which regulates the intensity of the retraction force.

Considering our relatively good agreement with experimental observations, the assumption that the actin redistribution essentially occurs in the tangential direction appears to be reasonable when dealing with fibroblast deformations. Indeed actin filaments in the cell are preferentially organized radially in the cortex and remain stable in that direction and thus tends to stabilize the cell shape (Cramer *et al.*, 1997). This stabilizing effect, probably linked to the development of new adhesion sites in coordination with the formation of bundles of filaments, is not taken into account by the model and leads to a very motile cell with protrusions occurring in many different spatial directions. However, our globally satisfactory simulation results tend to confirm the formulated mechano-chemical hypotheses, especially the controversial hypothesis of pressure-driven protrusion. They also tend to confirm the idea that the same basic mechanisms might apply and account for the all diversities of different cell behaviours (from rotating to standing waves) and their associated shapes (from round-shaped leukocytes to the large pseudopods of fibroblasts).

From now, a logical extension of the model would be to take into account cell migration in order to provide a complete model for (individual) cell motility.

Such a model extension has already been carried out on the basis of the initial model proposed by Alt and Tranquillo (1995). Realistic migratory behaviour of round-shaped cells (such as leukocytes) could be generated (Stephanou and Tracqui, 2002), but once again because of the limitation of the model which only deals

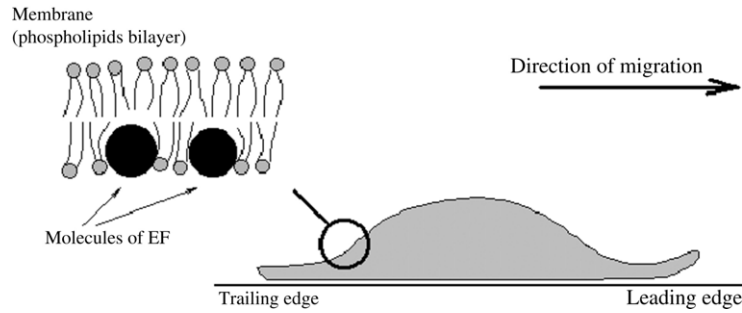


Figure 13. Schematic representation of a migrating cell exhibiting a characteristic dome-like shape where the thickest part represents the cell body. From the mechanical point of view, intercalation of molecules in the membrane is responsible for cell morphological instabilities.

with small membrane extensions, cell lamellipodial extension remained very small. However, now with our new model formulation, it becomes possible to generate fibroblast-type migration, which means to take into account the large lamellipodial extension, the salient feature of fibroblast migration preceding cell translocation.

In the case of chemotaxis, the cell responds to a gradient of chemoattractant diffusing in the medium. Therefore, in order to switch from the spontaneous pulsating state to the migrating state, the model has to incorporate the perception of an extracellular factor by the cell. Rather than consider a molecular point of view, which would involve the cascade of chemical events triggered by the molecules binding to the membrane and leading to actin polymerization and then cell migration, we have chosen to consider a mechanical point of view. This means that we do not consider the chemical properties of the chemoattractant molecules but instead their mechanical interactions with the cell membrane as solid objects (Fig. 13).

Indeed the presence of particle in the membrane leads to a release of the membrane tension as has been demonstrated in several studies (Kim *et al.*, 1998; Nielsen *et al.*, 1998; Soares and Maghelly, 1999). This effect is taken into account in the model by considering that the membrane tension coefficient τ depends linearly on the local concentration C of the extracellular factor at the membrane, namely,

$$\tau(C) = \tau - \Lambda(C) \quad (28)$$

where $\Lambda(C)$ is a function which characterizes the sensitivity of the cell to the extracellular factor. This function can be assumed to first increase with an increased concentration of factor and to decrease when a threshold concentration value is reached. This for example can model the fact that all the membrane receptors become saturated with the factor. However, for simplification, we assume here a linear dependency with the concentration i.e., $\Lambda(C) = \Lambda C$, where the coefficient Λ is switched to 0, above the concentration threshold C_{\max} .

Therefore in the presence of an extracellular gradient of molecules, the membrane tension becomes weaker at the front of the cell (which faces the gradient)

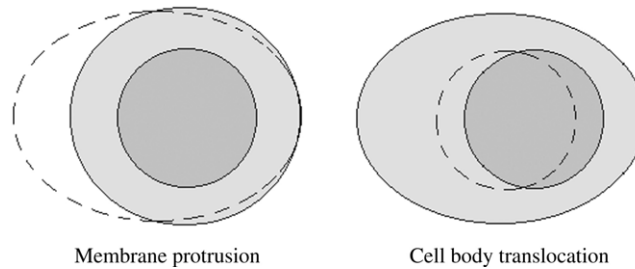


Figure 14. Schematic diagram exhibiting the two-step mechanism of migration, with first the membrane extension along the migration direction and second the cell body translocation, i.e., the displacement of the cell body at the new position of the cell geometrical barycentre. This second step occurs when the adhesion force becomes able to overcome the tension force of the actomyosin fibres in the cortex.

and allows a morphological instability to develop under the form of a lamellipodial extension. Assuming that the membrane receptors (e.g., integrins) are homogeneously distributed on the membrane, the developing lamellipod provides a bigger surface of contact of the cell with its substrate at the front than at its trailing edge. The adhesion force then becomes able to support the traction force exerted by the actomyosin complex whose contraction pulls the cell forward in a simplified two-step mechanism as displayed in Fig. 14 and which summarizes the five-step migration process described by Sheetz *et al.* (1999) i.e., (1) membrane extension, (2) attachment to the substrate, (3) cell contraction, (4) release of the attachment at the trailing edge and (5) recycling of the receptors.

Various simulations describing a range of experimental situations of cell migration have been performed from the extension of the initial model of Alt and Tranquillo (1995) (Stéphanou and Tracqui, 2002). In these simulations, a chemoattractant diffusing from a point source has been used. Here we show the results obtained for a front of chemoattractant. This corresponds to the case where the cell is initially very close to the source (see Fig. 15). The initial state is a circular cell which rapidly deforms by extending a lamellipod at the front. However in this case no real migration of the cell can be achieved as the limitation of small membrane extension imposed by the initial model formulation is reached. This simulation however suggests that large lamellipodial extension can be described and migration achieved from the new model.

Another perspective of this work would also be to further develop the model in order to take into account higher levels of actin organization such as the formation of bundles and the description of the inhomogeneous radial actin distribution in the cell cortex through a real two-dimensional formulation of the mechanochemical system of equations.

The ultimate aim of cell motility modelling would be to be able to propose a more complete and reliable model of cell movements which once dynamically calibrated by experimental data would help to drive new experiments by defining optimum conditions required in order to obtain a given cell behaviour. Knowing which

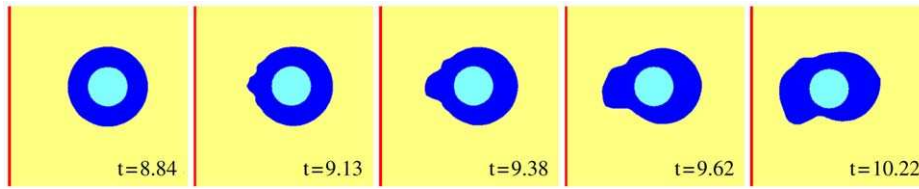


Figure 15. Migration of the cell towards a linear front of chemoattractant, which shows limited lamellipodial extension due to the small deformation limitation of the initial model.

parameters of the model to act on to achieve a desired behaviour (such as increasing the migration rate to favour wound healing or decreasing the migration rate to inhibit cell membrane deformations and migration and subsequently to prevent the invasive spread of cancer cells) would be a major achievement in the field of cell biology.

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APPENDIX A: DERIVATION OF THE CURVATURE TERM IN THE MODEL

The expression for the curvature K in the 2D plan (R^2) defined parametrically by $\mathbf{C}(s) = (x(s), y(s))$ is given by [Stoker \(1969\)](#)

$$K = \frac{x'y'' - x''y'}{(x'^2 + y'^2)^{\frac{3}{2}}}$$

where $x' = \frac{dx}{ds}$, $y' = \frac{dy}{ds}$.

To determine the curvature in polar coordinates (r, θ) , we apply the above formula to

$$\begin{cases} x = r(\theta) \cos \theta, \\ y = r(\theta) \sin \theta. \end{cases}$$

With $r' = dr/d\theta$ and $r'' = d^2r/d\theta^2$, the expression of the curvature obtained for the polar system of coordinates (r, θ) is given by

$$K = \frac{2r'^2 - rr'' + r^2}{(r'^2 + r^2)^{\frac{3}{2}}}.$$

We can thus deduce from this expression the curvature of the cell membrane in the geometry of our model where R_0 is the internal radius of the cell delimiting

the cell body and $L(\theta)$ the extension of the membrane taken from the surface of the cell body. The curvature is

$$K = \frac{2\left(\frac{\partial L}{\partial \theta}\right)^2 - (L(\theta) + R_0)\frac{\partial^2 L}{\partial \theta^2} + (L(\theta) + R_0)^2}{\left[\left(\frac{\partial L}{\partial \theta}\right)^2 + (L(\theta) + R_0)^2\right]^{\frac{3}{2}}}. \quad (\text{A.1})$$

APPENDIX B: DERIVATION OF THE MECHANICAL EQUILIBRIUM EQUATION IN POLAR COORDINATES

The mechanical equilibrium equation is given by

$$\nabla \cdot [\mu_1 \dot{\epsilon} + \mu_2 \dot{\phi} I + E'(\epsilon + v' \phi I) + \sigma(a)I - p(\phi)I] = 0. \quad (\text{B.1})$$

For the sake of simplicity, we propose a 1D approximation where actin dynamics are restricted to the circle of radius r_* . No displacement of actin is allowed in the radial direction u and no contraction of the cytogel can occur in that direction. Therefore $\frac{\partial a}{\partial r} = 0$, $\frac{\partial u}{\partial r} = 0$, $\frac{\partial v}{\partial r} = 0$ and $u = 0$. Although this is very schematic, this hypothesis leads to a qualitative agreement with experimental data. Of course, an explicit description of the structural links between the actin cytoskeleton and the membrane will require the consideration of a full 2D model derivation with both tangential and radial displacements.

The strain tensor in polar coordinates is thus given by

$$\epsilon = \begin{pmatrix} \frac{\partial u}{\partial r} & \frac{1}{2} \left(\frac{1}{r} \frac{\partial u}{\partial \theta} + \frac{\partial v}{\partial r} - \frac{v}{r} \right) \\ \frac{1}{2} \left(\frac{1}{r} \frac{\partial u}{\partial \theta} + \frac{\partial v}{\partial r} - \frac{v}{r} \right) & \frac{1}{r} \frac{\partial v}{\partial \theta} + \frac{u}{r} \end{pmatrix} = \begin{pmatrix} 0 & -\frac{v}{2r} \\ -\frac{v}{2r} & \phi \end{pmatrix},$$

with the dilation $\phi = \nabla \cdot \mathbf{u} = \text{tr}(\epsilon) = \frac{\partial u}{\partial r} + \frac{1}{r} \frac{\partial v}{\partial \theta} + \frac{u}{r} = \frac{1}{r} \frac{\partial v}{\partial \theta}$.

The condition for zero divergence of a stress tensor in polar coordinates is

$$\nabla \cdot \sigma_{\text{total}} = \begin{cases} \frac{\partial}{\partial r} \sigma_{rr} + \frac{1}{r} \frac{\partial}{\partial \theta} \sigma_{r\theta} + \frac{\sigma_{rr} - \sigma_{\theta\theta}}{r} = 0 \\ \left(\frac{\partial}{\partial r} + \frac{2}{r} \right) \sigma_{r\theta} + \frac{1}{r} \frac{\partial}{\partial \theta} \sigma_{\theta\theta} = 0. \end{cases}$$

We apply this formula to equation (1) of our model, where the total tensor is the sum of the individual viscous, elastic, contractile and pressure induced stress tensors given by the equations (3)–(6) respectively. The components of the global stress tensor σ are then given by

$$\begin{cases} \sigma_{rr} = \mu_2 \dot{\phi} + E' v' \phi + \sigma(a) - p(\phi) \\ \sigma_{r\theta} = -\frac{1}{2r} (\mu_1 \dot{v} + E' v) \\ \sigma_{\theta\theta} = (\mu_1 + \mu_2) \dot{\phi} + E' (1 + v') \phi + \sigma(a) - p(\phi) \\ \sigma_{rr} - \sigma_{\theta\theta} = -(\mu_1 \dot{\phi} + E' \phi). \end{cases}$$

The equations for the mechanical equilibrium in polar coordinates, with our conditions $u = 0$ and $\frac{\partial a}{\partial r} = 0, \frac{\partial u}{\partial r} = 0, \frac{\partial v}{\partial r} = 0$, are thus:

$$\begin{aligned} (\frac{3}{2}\mu_1 + \mu_2)\dot{\phi} + E'(v' + \frac{3}{2})\phi &= -r\frac{\partial p(\Phi)}{\partial r}, \\ \frac{\partial}{\partial \theta}[(\mu_1 + \mu_2)\dot{\phi} + E'(1 + v')\phi + \sigma(a)] &= \frac{1}{2r_*}(\mu_1\dot{v} + E'v). \end{aligned}$$

Integration of the first equation $p = 0$ gives the following expression for \dot{v} :

$$\dot{v} = -sv + C_0 \quad \text{with } s = \frac{E'(v' + \frac{3}{2})}{\frac{3}{2}\mu_1 + \mu_2}.$$

C_0 is a free constant, which is set to zero as actin displacement is null at the stationary state. Replacement of this expression in the second equation gives

$$\frac{\partial}{\partial \theta}[(\mu_1 + \mu_2)\dot{\phi} + E'(1 + v')\phi + \sigma(a)] = \frac{v}{2r_*}(E' - \mu_1s).$$

The resulting equation can thus be re-written as follows:

$$\frac{\partial}{\partial \theta}[\mu\dot{\phi} + \hat{E}\phi + \sigma(a)] = \frac{\alpha}{2} \frac{v}{r_*}, \tag{B.2}$$

or

$$\frac{\partial}{\partial \theta} \left[\frac{\mu}{r_*} \frac{\partial \dot{v}}{\partial \theta} + \frac{\hat{E}}{r_*} \frac{\partial v}{\partial \theta} + \sigma(a) \right] = \frac{\alpha}{2} \frac{v}{r_*}, \tag{B.3}$$

with

$$\mu = \mu_1 + \mu_2, \quad \hat{E} = E'(1 + v'), \quad \alpha = E' - \mu_1s.$$

APPENDIX C: LINEAR STABILITY ANALYSIS

(i) **Determination of k_{\max}^2 .** As c_1 is negative, $c(k^2)$ can be positive only for a finite number of wave numbers k^2 . $c(0) = c_3$ being positive, the range of k^2 for which the function $c(k^2)$ is positive is thus given by $k \in [0, k_{\max}^2]$, where k_{\max}^2 is solution of $c(k^2) = 0$, i.e.,

$$c_1k^4 + c_2k^2 + c_3 = 0, \quad \text{with the solution: } k_{\max}^2 = \frac{2\sigma(1)}{\frac{D}{r_*^2}[\sigma'(1) - \sigma(1)]}.$$

(ii) **Determining the conditions for a bifurcation.** As a_1 and a_3 are positive, a bifurcation occurs for the wavenumber k_c^2 where $a(k_c^2) = 0$, k_c^2 being the

wavenumber for which the function $a(k^2)$ is minimum, i.e.,

$$\frac{da(k_c^2)}{dk^2} = 2a_1k_c^2 + a_2 = 0 \quad \text{and} \quad k_c^2 = -\frac{a_2}{2a_1} > 0,$$

$$k_c^2 = \frac{2\sigma'(1) - \sigma(1) - \Omega - 1}{2\frac{D}{r_*^2}} > 0.$$

The condition for k_c^2 to exist is that: $2\sigma'(1) > \sigma(1) + \Omega + 1$. The bifurcation occurs for $a(k_c^2) = 0$, and thus if we replace the expression found for k_c^2 in this equation we obtain the condition that the parameters must obey at the bifurcation point, i.e.,:

$$2\sigma'(1) - \sigma(1) - \Omega - 1 = \frac{\sqrt{2\alpha D}}{r_*}. \quad (\text{C.1})$$

Moreover, we must have $k_c^2 < k_{\max}^2$.

Note: We assume for the study of the equations of the model that the parameter α is positive. However α can obviously be also negative. If α is negative then the coefficients c_3 and a_3 are negative. In that case, no bifurcation can be observed as the value of k^2 for which $a(k^2) = 0$ cannot correspond at the same time to the minimum point of the function. This means that the selection of a given unstable mode is this time not obvious. This justifies our choice to restrict our study to the case where α is assumed to be positive.

(iii) Verification that $b(k^2)$ is positive. The most favourable situation would be to have $b(k^2) > 0$ for any $k^2 \in [0, k_{\max}^2]$. For such a situation, b_1 and b_3 must be positive. Therefore,

$$\begin{aligned} \text{if } b_1 > 0 & \quad \text{then } \Omega + \sigma(1) - \sigma'(1) > 0, \\ \text{if } b_3 > 0 & \quad \text{then } 1 + \sigma(1) - \sigma'(1) > 0, \\ \text{if } \Omega > 1 & \quad \text{then } b_3 > 0 \text{ therefore } b_1 > 0. \end{aligned}$$

Thus $b(k^2) > 0$ for any k if the minimum of the function $b(k^2)$ is positive, i.e.,

$$\frac{db(k_0^2)}{dk^2} = 0 \quad \text{and} \quad b(k_0^2) > 0 \text{ lead to } k_0^2 = -\frac{b_2}{2b_1} \text{ then } b_2 < 0.$$

So we have the condition

$$\frac{\alpha D}{2r_*^2} + \Omega[1 + \sigma(1) - \sigma'(1)] < \sigma(1)[1 - \sigma'(1)]. \quad (\text{C.2})$$

The condition $b(k^2) > 0$ is true if $b(k_0^2) > 0$, i.e.

$$b(k_0^2) = b_1k_0^4 + b_2k_0^2 + b_3 > 0 \quad \text{therefore we must have} \quad 4b_1b_3 > b_2^2.$$

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A computational model of cell migration coupling the growth of focal adhesions with oscillatory cell protrusions

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ABSTRACT

Cell migration is a highly integrated process where actin turnover, actomyosin contractility, and adhesion dynamics are all closely linked. In this paper, we propose a computational model investigating the coupling of these fundamental processes within the context of spontaneous (i.e. unstimulated) cell migration. In the unstimulated cell, membrane oscillations originating from the interaction between passive hydrostatic pressure and contractility are sufficient to lead to the formation of adhesion spots. Cell contractility then leads to the maturation of these adhesion spots into focal adhesions. Due to active actin polymerization, which reinforces protrusion at the leading edge, the traction force required for cell translocation can be generated. Computational simulations first show that the model hypotheses allow one to reproduce the main features of fibroblast cell migration and established results on the biphasic aspect of the cell speed as a function of adhesion strength. The model also demonstrates that certain temporal parameters, such as the adhesion proteins recycling time and adhesion lifetimes, influence cell motion patterns, particularly cell speed and persistence of the direction of migration. This study provides some elements, which allow a better understanding of spontaneous cell migration and enables a first glance at how an individual cell would potentially react once exposed to a stimulus.

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1. Introduction

Cell migration is a crucial process for many physiological and pathological events, such as embryogenesis and cancer metastasis. It is a highly integrated multistep mechanism comprising three well-defined steps, namely membrane protrusion, cell–substrate adhesion and finally contraction leading to cell translocation. Translocation comprises the forward displacement of the cell centre of mass and the detachment of the cell from the substrate. The molecular events orchestrating cell migration are still largely unknown. Nonetheless, some key regulatory molecules involved in each of these essential steps have now been identified (Giannone et al., 2007; Mylona et al., 2006; Li et al., 2005; Firtel and Chung, 2000). Such knowledge has allowed for cell motility events to be both experimentally and mathematically reconstructed and further elucidated.

The process of formation of membrane protrusions has been the source of active debate since the eighties (see review by

Mogilner, 2006). The solation/gelation cycle of the actin gel, mediated by calcium release, was first evoked to explain the protrusions (Oster, 1984). Subsequently, advances in cell imaging revealed that a high density of actin coincided spatio-temporally with the formation of membrane protrusions in migrating cells, indicating that actin polymerization is directly responsible for the protrusions formation (Borisov and Svitkina, 2000; Carlier and Pantaloni, 1997; Theriot and Mitchison, 1991). A Brownian ratchet mechanism (Mogilner and Oster, 1996; Peskin et al., 1993) was proposed to explain how monomers of actin could intercalate between the growing ends of actin filaments and the cell membrane. A treadmilling process, whereby actin filaments depolymerize at the rear of the lamella and are recycled into new filaments assembled at the front, explained how the cell is able to sustain forward protrusion (Carlier and Pantaloni, 1997). This mechanism, which has now been validated both experimentally and theoretically (Bindschadler et al., 2004; Mogilner and Edelstein-Keshet, 2002) is essential for the formation of membrane protrusions in polarized and directionally migrating cells, but does not seem to apply for unstimulated cells. Protrusions developing in unstimulated cells appear via a different mechanism. Specifically, in unstimulated fibroblasts,

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the highly spatio-temporally organized nature of the cell membrane protrusion dynamics was exhibited. Spatial organization was revealed by the observation that cells assume an isotropic shape. In addition, the measure of periodicities in the membrane oscillations confirmed the temporally organized nature of the spontaneous protrusion dynamics (Stéphanou et al., 2004). Membrane blebs forming at the tip of the protrusions suggest that the internal pressure, rather than a Brownian mechanism, may be responsible for initiating the protrusions (Charras et al., 2005; Abraham et al., 1999; Bereiter-Hahn and Luers, 1998). Under this mechanism, contractions generated by the actomyosin complex induce a cytoplasmic flux, which then pushes on the membrane and leads to protrusions at sites where the links between membrane and cytoskeleton are too weak to prevent membrane extension (Paluch et al., 2005, 2006). Simulations based on this hypothesis qualitatively reproduce the spatio-temporal membrane deformations observed in unstimulated keratinocytes (Alt et al., 1995; Alt and Dembo, 1999) and fibroblasts (Stéphanou et al., 2004).

Actin-driven protrusion is the result of a cascade of molecular events initiated by the small-GTPases, in particular Rho-A, which are proteins involved in signal detection (Ridley, 2006; Hall, 2005; Marée et al., 2006). Such activation does not occur in unstimulated cells. Consequently it can be hypothesized that, even though both protrusion mechanisms dynamically coexist in a cell, the functional state of the cell dictates which one will predominate. For example, directional cell migration induced by either chemical (molecular gradients) or mechanical cues (alterations in substrate rigidity or topography) can be guided primarily by polymerization-driven protrusion. On the contrary, protrusions in an unstimulated cell can largely derive from passive hydrostatic pressure. This hypothesis, which aims to distinguish the unstimulated from the directionally polarized cell awaits further experimental and theoretical investigation.

In vivo cell migration, although physiologically imperative, is also energetically demanding and catastrophic if uncontrolled. For that, it is closely guided and fine tuned by both chemical and physical cues. Thus, little attention has been given to spontaneous or random cell migration. However, spontaneous cell migration is of major interest since it allows for isolation and study of cellular mechanisms, such as the coordination of actin turnover with the focal adhesion dynamics both spatially and temporally (Dunn and Zicha, 1995). In this paper, our approach is to develop a mathematical model where spontaneous membrane protrusions are induced by the hydrostatic pressure (Alt and Tranquillo, 1995). Cell membrane oscillations thus result from the interplay between this protrusive force and the counteracting stress from the actin filaments linked to the membrane. The actin fibre tension and actomyosin contractility depend on the local amount of actin, where actin turnover is regulated around a chemical equilibrium density. Various spontaneous pulsating cell behaviours can be generated depending on the choice of the cytomolecular parameters (cytoplasm viscosity, filaments and membrane elasticities, actomyosin contraction tonicity, etc.) (Stéphanou and Tracqui, 2002; Stéphanou et al., 2004).

The model is then further developed to take into account the formation of adhesions with the substrate. Once an adhesion is formed, the model aims to describe how it can mature into a more stable adhesion structure able to support cell traction thus allowing for cell translocation (Chen et al., 2003; Sastry and Burridge, 2000). With the present model, we aim to incorporate the key processes, rather than the detailed molecular events, of adhesion maturation during cell migration. Consequently, we will simply assume the existence of three successive types of adhesions (adhesion spot, A; focal complex, FX and focal adhesion, FA) (Kaverina et al., 2002;

Zaidel-Bar et al., 2004), each being characterized by its lifetime and resistance to traction (Galbraith et al., 2002).

In the subsequent sections of this paper, we initially present the details of the computational model followed by the results, including experimental data presenting key features of fibroblast migration. These experimental data serve as the basis to scale our simulation results *a posteriori*. We then investigate the influence of two important parameters of the model on cell speed: (i) the adhesion strength, which is known to influence cell speed biphasically, thus serving as a test for the model and (ii) the adhesion proteins recycling time and adhesion lifetimes, which demonstrate how actin dynamics and adhesions dynamics are temporally interrelated.

2. Model formulation

As noted previously, cell migration is a multistep process, primarily comprising three distinct steps namely membrane protrusion, adhesion to the substratum, and translocation of the cell via application of traction forces by the contracting cytoskeleton. The cyclical repeat of these three steps allows the cell to migrate in its microenvironment. The model proposed herein comprehensively addresses each of these distinct processes. Since random migration is our main interest, we assume the substrate to be isotropic in all aspects (chemically, mechanically, etc.). Therefore, we do not describe its properties explicitly.

2.1. A model for spontaneous cell membrane protrusions

Many different hypotheses have been proposed to explain the spontaneous oscillatory movement of the cell membrane and the role of actin turnover (i.e. polymerization/depolymerization cycles) in the recurring protrusion and retraction dynamic of the lamellipod. Among the currently published models, we chose the model by Alt and Tranquillo (1995) as the basis. The main reason being that this model considers both the molecular events of actin turnover dynamics and the related mechanical properties of the cell membrane and cytoskeleton. This model has successfully described the appearance of rotating deformation waves around the cell body of keratinocytes (Alt et al., 1995) and leukocytes (Alt, 1990), as well as more complex periodic pulsating patterns such as those observed in L929 fibroblasts (Stéphanou et al., 2004).

The model considers that cell protrusion dynamics depend on the biophysical properties (i.e. viscoelasticity and contractility) of the cortical network of actin and myosin filaments, which underlies the cell membrane and surrounds the cell body. Moreover this highly dynamic network is able to: (i) disassemble at locations where it becomes condensed and (ii) reassemble where it is weaker (i.e. in newly formed cell protrusions, such as lamellipodia). Thus, cell protrusions form as a response initially to a mechanical non-equilibrium state involving peripheral forces on the cell membrane and then the actin turnover induced by intracellular spatial variation. The model thus addresses cortical actin dynamics, which are mainly responsible for cell shape reorganization, by focusing on the ability of actin to:

- polymerize into F-actin and depolymerize into G-actin,
- interact with myosin to generate the contractile activity in the cell,
- move through the cytoplasm via convection.

The local amount of F-actin also determines the intensity of the resistive stress applied on the membrane as the result of cross-links between the actin cytoskeleton and the membrane proteins.

This resistive stress together with the stress induced by the cortex–membrane curvature balance the intra-cellular hydrostatic pressure. This pressure acts as the “driving force” for membrane protrusion in the model. This pressure is reinforced by active polymerization of actin filaments, when adhesions have formed on the expanding membrane, thus clustering actin polymerization to form stress fibres.

2.1.1. Model equations

The model considers the two-dimensional annular domain bounded by the cell body and the cortex–membrane complex at the lamellar tip. Specifically, the cell body is represented with a fixed circular shape to withstand the tension/pressure in the annular cytoplasm ring, whereas the cortex–membrane complex at the lamellar tip defines a free radially moving boundary. We denote by $L(\theta, t)$ the width of the annular domain along any radial direction located by the angle θ ($0 \leq \theta \leq 2\pi$) (Fig. 1).

For simplicity, we transform the two-dimensional problem into a one-dimensional version. We assume, as in Alt and Tranquillo (1995), that the F-actin density as well as its convective tangential velocity, are constant in the radial direction. The second assumption implies that the cortical network can slide with respect to the membrane, which is partly justified considering that membrane proteins involved in the network–membrane connections are mobile within the membrane. Thus the three variables considered in the spontaneous cell oscillations model are:

1. the F-actin concentration in the cortex $a(\theta, t)$,
2. the F-actin tangential velocity $v(\theta, t)$,
3. the width of the cell cortex annulus $L(\theta, t)$, given by the membrane position as measured from the surface of the cell body.

The spatio-temporal evolution of these three variables is given in the following set of partial differential equations (PDEs) (full details are given in Stéphanou et al., 2004). The first equation represents the conservation of F-actin in the cortex. The rate of actin turnover (i.e. polymerization/depolymerization), character-

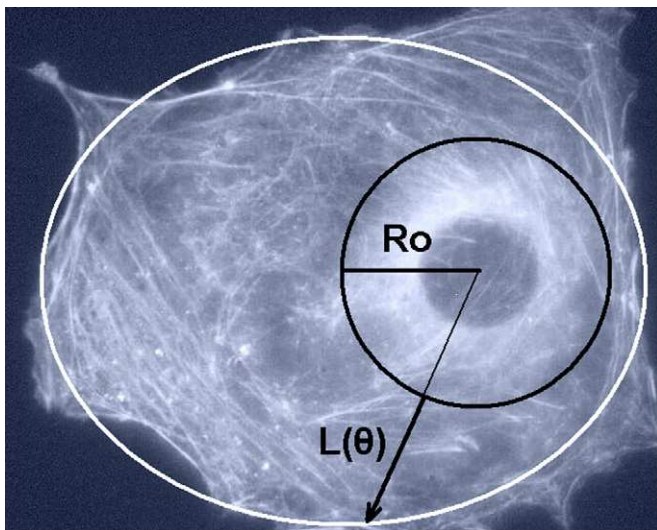


Fig. 1. Schematic representation of the cell in the theoretical model. Two zones are considered: (i) the cell body, which includes the nucleus surrounded by a dense network of filaments confined by a circular area with radius R_0 and (ii) the cell cortex where remodelling of the actin cytoskeleton mainly occurs and corresponds to the area bounded at one side by the outer boundary of the cell body and at the other side by the cell membrane. The width of the cortex in any angular direction θ is given by $L(\theta)$.

ized by η , depends on the local value of F-actin concentration relative to the stationary concentration at the chemical equilibrium a_0 . The equation is thus

$$\frac{\partial}{\partial t}(La) = -\frac{\partial}{\partial \theta}(Lav) + \eta L(a_0 - a) \tag{1}$$

The second equation describes the balance of forces applied at the lamellar tip on the cortex–membrane complex in the radial direction. The model takes into account:

- the viscous friction due to the sliding movement of the cortex–membrane over the substratum, linearly depending upon the actin density and modulated by the coefficient Φ_1 ,
- the intra-cellular hydrostatic pressure β_1 ,
- the resistive elastic stress due to the cortical network–membrane attachment which intensity linearly depends upon the actin density, with γ_1 the elasticity coefficient for the actin network,
- a curvature-dependent stress due to the surface tension of the cortex–membrane complex characterized by the coefficient τ_1 .

The equation is given by

$$\Phi_1 a \frac{\partial L}{\partial t} = \beta_1 - \gamma_1 La + \frac{\partial}{\partial \theta} \left(\tau_1 a \frac{\partial L}{\partial \theta} \right) \tag{2}$$

Finally, the third equation concerns the balance of forces applied on the cortical network in the tangential direction. It includes:

- the frictional force on the network moving within the cytosol, with magnitude controlled by a drag coefficient Φ_0 (related to the attachment of the network with the membrane and other types of filaments),
- a viscous stress with viscosity coefficient for the cytoplasm μ_0 ,
- the membrane curvature-induced stress with coefficient τ_0 .

This equation is given by

$$\Phi_0 av = \frac{\partial}{\partial \theta} \left[\mu_0 a \frac{\partial v}{\partial \theta} + \sigma_0(a) - \frac{\partial}{\partial \theta} \left(\tau_0 a \frac{\partial L}{\partial \theta} \right) \right] \tag{3}$$

The contractile activity of the actomyosin network is modelled by the non-linear function $\sigma_0(a)$ (Fig. 2). Two mechanical states

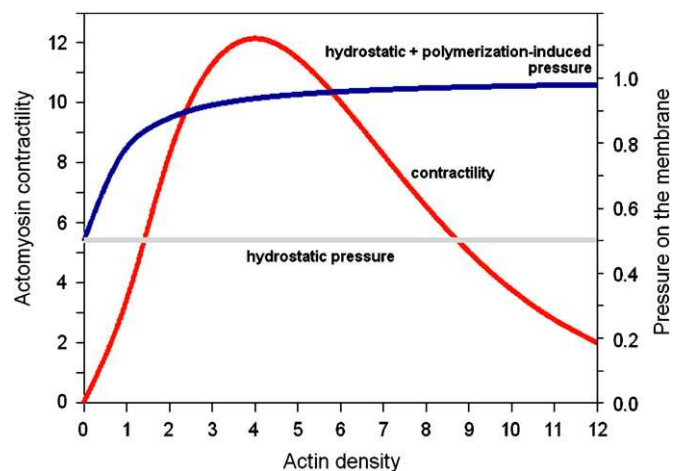


Fig. 2. Profile of the actomyosin contractility as a function of the actin density $\sigma(a)$ (red curve). The contractility first increases with the actin density until it reaches a saturation density ($a_{sat} = 4$). Above this point, contractility decreases due to a compaction effect of the network of filaments. Active pressure on the cell membrane due to the combination of fluid hydrostatic pressure β and actin polymerization-induced pressure $\beta(a)$ (blue curve).

have to be distinguished according to the value of the F-actin concentration $a(\theta, t)$ with respect to a saturation concentration a_{sat} . Specifically, if $a(\theta, t)$ is lower than a_{sat} , the contractile stress increases according to a parabolic law. However, above the saturation threshold a_{sat} , the stress decreases exponentially as a consequence of the network compaction. The non-linear function proposed by Alt and Tranquillo (1995) is the following:

$$\sigma_0(a) = \psi_0 a^2 e^{-2a/a_{sat}} \quad (4)$$

where the coefficient ψ_0 controls the magnitude of the contractile stress.

The dimensionless system of equations is given by

$$\frac{\partial}{\partial t}(La) = -\frac{\partial}{\partial \theta}(Lav) + L(1-a) \quad (5)$$

$$a \frac{\partial L}{\partial t} = \beta - \gamma La + \delta \frac{\partial}{\partial \theta} \left(\tau a \frac{\partial L}{\partial \theta} \right) \quad (6)$$

$$av = \frac{\partial}{\partial \theta} \left[\mu a \frac{\partial v}{\partial \theta} + \sigma(a) - \frac{\partial}{\partial \theta} \left(\tau a \frac{\partial L}{\partial \theta} \right) \right] \quad (7)$$

$$\text{with } \sigma(a) = \psi a^2 e^{-2a/a_{sat}} \quad (8)$$

The non-dimensionalization and the stability analysis of the system of equations are detailed in Appendix A. The dimensionless parameters taken for the simulations are given in Table 1.

2.1.2. Boundary conditions and numerical methods

The model implies periodic boundary condition for the variables, given by:

$$L(0, t) = L(2\pi, t), \quad a(0, t) = a(2\pi, t), \quad v(0, t) = v(2\pi, t) \quad (9)$$

The model equations are numerically solved with finite difference methods based on the Crank–Nicholson scheme. Hyperbolic and parabolic equations, associated with the conservation of actin $a(\theta, t)$ and the cell membrane position $L(\theta, t)$ respectively, implies matrix inversions which have been performed with the Thomas Algorithm, adapted to periodic boundary conditions. The elliptic equation ruling the tangential displacement of actin $v(\theta, t)$, is solved using a successive over-relaxation (SOR) method (Strikverda, 1989). The time step Δt has been chosen in order to satisfy the accuracy and stability condition of the numerical scheme used ($\Delta t = 0.01$).

2.2. Formation and maturation of the adhesions

Three different types of cell adhesions are considered in the model, the adhesion point (A), the focal complex (FX) and the focal adhesion (FA). Each adhesion type corresponds to a particular level of maturation characterized by its lifetime and resistance to traction (Fig. 3). A is thermodynamically unstable and assumed to have a short lifetime and a weak resistance to traction (Nicolas et al., 2004). Nonetheless, it can mature into a FX if stimulated by a moderate traction force, since too strong a traction breaks it. FX and FA, which are more stable structures, have longer lifetimes. FX

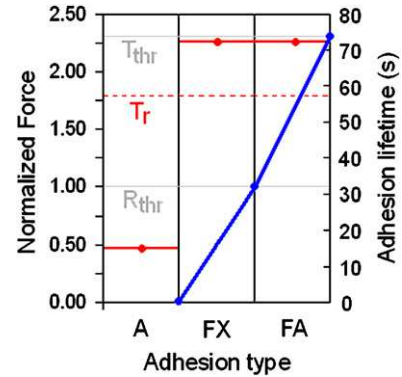


Fig. 3. Diagram showing the adhesion strength (blue curve) and lifetime (red spots) for each adhesion type as used in the coming simulations. Each type of adhesion needs to reach a threshold force to mature to the next level (a positive force for A → FX, R_{thr} for FX → FA). T_{thr} represents the threshold for cell translocation, and T_r the adhesion protein recycling time.

can mature into FA, if actively stimulated, that is if the traction force from the actin filaments is beyond a given threshold force. Finally, only FA are assumed to be able to resist high stress, thus leading to cell translocation.

Proper modelling of cell adhesions thus requires to take into account a gradation in the effects produced by each adhesion on the cell depending on its type. We first assume that whatever its type, an adhesion creates an increased friction between the membrane and the substratum. For a higher level of maturation, we moreover consider the process by which active actin polymerization reinforces protrusion at the leading edge in order to contribute in the generation of the traction force magnitude required for cell translocation. This phenomenon is modelled by introducing a “protrusive switch”, where passive hydrostatic pressure β on the membrane is reinforced by the active polymerization-induced pressure $\beta(a)$.

These friction and pressure effects are modelled by two additional terms in the equation describing the cortex–membrane movements. The modified equation is given by

$$(a + \alpha \delta_{adh}) \frac{\partial L}{\partial t} = \beta + \beta(a) \delta_{FA} - \gamma La + \delta \frac{\partial}{\partial \theta} \left(\tau a \frac{\partial L}{\partial \theta} \right) \quad (10)$$

where α is a friction coefficient and $\beta(a)$ is modelled by the function

$$\beta(a) = \frac{2\beta}{\pi} \arctan(a) \quad (11)$$

This pressure increases with the density of actin and rapidly reaches a plateau corresponding to the maximum of the actin polymerization rate (Fig. 2).

The Dirac functions are used to materialize the adhesion sites and are given by

$$\delta_{adh}(\theta, t) = \delta_A(\theta, t) + \delta_{FX}(\theta, t) + \delta_{FA}(\theta, t) \quad (12)$$

with

$$\delta_X(\theta, t) = \begin{cases} 1 & \text{if } \theta \text{ corresponds to an adhesion} \\ & \text{site of type } X \\ 0 & \text{elsewhere} \end{cases} \quad (13)$$

Note that in this model formulation, the adhesions affect only the membrane movements (Eq. (6)) and do not directly affect the actin dynamics (Eqs. (5) and (7)).

2.2.1. Adhesion spot formation

Since migration is unstimulated, we assume that small and weak adhesion spots A can form spontaneously with

Table 1

Dimensionless parameters which define the cell mechanical and chemical properties

Parameter	Notation	Value
Protrusive hydrostatic pressure	β	0.5
Actin network elasticity	γ	0.5
Cytoplasm viscosity	μ	2.0
Membrane elasticity	τ	1.5
Curvature-related coefficient	δ	0.001
Contractile coefficient	ψ	5.6
Saturation density of actin	a_{sat}	4.0

a minimum of conditions, due to energy fluctuations induced by the chemical reactions within the cell (Nicolas et al., 2004). We thus simply assume that the probability to form an adhesion is higher: (i) where the membrane extension is maximum, that is where the cell offers a greater surface of contact with the substrate and (ii) where the amount of actin is sufficient since the density of integrins, which are the main protein constituents of cell to substrate attachment, depends on it.

In the model, *A* occurs spontaneously at the extremity of the protrusive membrane, where the two aforementioned conditions are fulfilled. That is, where the membrane extension is maximum ($L_{max} > L_{thr}$) and associated with a sufficient local amount of actin ($a > a_{thr}$). The flowchart in Fig. 4 summarizes the different steps of the maturation process.

A is characterized by a short lifetime and weak adhesion strength. It is therefore too weak to support traction forces leading

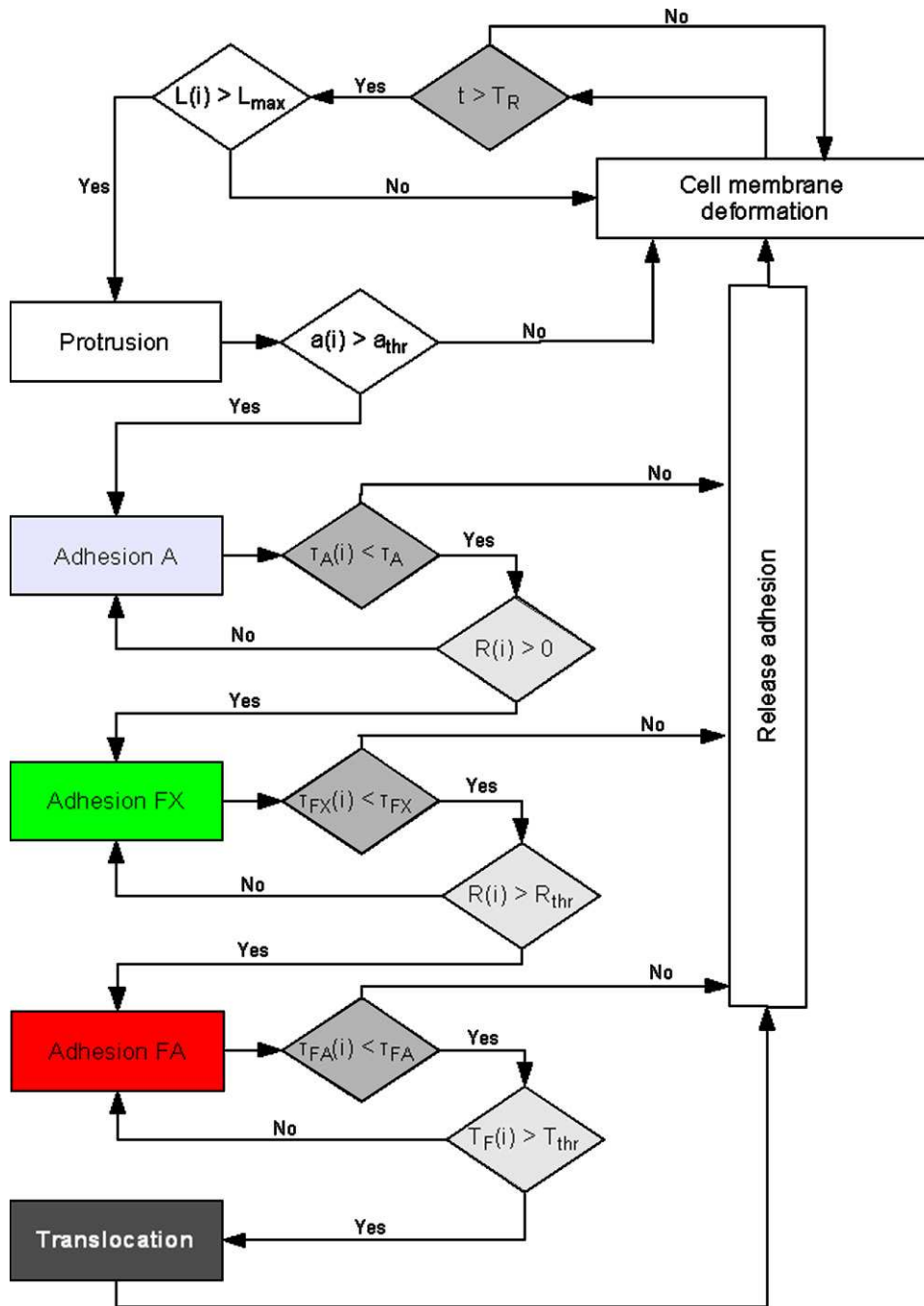


Fig. 4. Flowchart describing the formation and maturation process of the adhesion *i* from the simple adhesion point (*A*) to the focal adhesion (*FA*) via the focal complex (*FX*) and ultimately leading to cell translocation. First, membrane deformations are generated with the model previously described. If the membrane extension is big enough ($L(i) > L_{max}$), then, if the protrusion is accompanied by a sufficient amount of actin ($a(i) > a_{thr}$) the primary adhesion *A* is formed. If the resulting force becomes a traction force ($R(i) > 0$), before the adhesion lifetime expires ($\tau_A(i) < \tau_A$) then *A* becomes *FX*. Similarly, if the traction applied by the actin filaments on *FX* is above a threshold ($R(i) > R_{thr}$), before the *FX* lifetime expires ($\tau_{FX}(i) < \tau_{FX}$) then *FX* becomes *FA*. Finally, if the traction force becomes high enough to support the cell translocation ($T_F(i) > T_{thr}$), before the *FA* lifetime expires ($\tau_{FA}(i) < \tau_{FA}$) then the cell moves forward. In that case all adhesions are released and the cell enters a refractory period T_R during which no adhesions can form until $t > T_R$, where t is the elapse time since translocation.

to cell translocation. It needs to mature first into a more stable structure (Kaverina et al., 2002).

2.2.2. Maturation process

A matures into FX and then into FA through the incorporation of additional molecular components in a hierarchical way (Zaidel-Bar et al., 2004). The first molecules involved are $\alpha_v\beta_3$ -integrin and phosphotyrosine, closely followed by talin and paxillin. As the FX keeps growing, other proteins, such as α -actinin, FAK, VASP, Arp2/3, and vinculin are further recruited. This leads to a close connection with the matrix, where the distance between the membrane and the substrate is significantly reduced as observed with interference reflection microscopy (Izzard, 1988). Whereas FX maturation occurs during cell protrusion, the transformation of FX into FA is mechanically induced by an increase in tension from actin filaments (Galbraith et al., 2002) concomitant with the recruitment of zyxin and tensin.

Back in the model, we assume that maturation of A into FX simply occurs if the adhesion is mechanically solicited through tension forces exerted by the actin filaments of the cell cytoskeleton. In order to decide whether an adhesion should grow or not, we define a force-related criterion, by calculating the resulting force $R_F(\theta_i, t)$ existing at time t for each adhesion i . This resulting force corresponds to the sum of the contributions of all the individual forces $F(\theta_j, t)$ balancing the movements of the cortex–membrane complex for each directions θ_j . These forces are then projected on the θ_i -direction supporting the adhesion i ($\theta_i = 2\pi i/m$ with m the number of points defining the membrane boundary), namely

$$R_F(\theta_i, t) = \sum_{j=0}^m F(\theta_j, t) \cos(\theta_j - \theta_i) \quad (14)$$

with

$$F(\theta_j, t) \text{ such that } a \frac{\partial L}{\partial t} + F(\theta_j, t) = 0 \quad (15)$$

$F(\theta_j, t)$ is derived from Eq. (10), neglecting the curvature-related term since its contribution is small. It thus comprises: (i) the adhesion-related term responsible for an increased tension between the cell body and the adhesion site, if an adhesion has formed, (ii) the passive tension from the actin filaments existing everywhere in the cell and modulated by the local membrane extension and local density of actin, and (iii) the pressure term, which tends to repel the cell body from the adhesion site. $F(\theta_j, t)$ for site j at time t is given by

$$F(\theta_j, t) = \underbrace{\delta_{adh}(\theta_j, t) \gamma_2 [L(\theta_j, t + \Delta t) - L(\theta_j, t)]}_{\text{adhesion-related tension}} + \underbrace{\gamma L(\theta_j, t) a(\theta_j, t)}_{\text{filaments passive tension}} - \underbrace{[\beta + \beta(a) \delta_{FA}(\theta_j, t)]}_{\text{pressure force}} \quad (16)$$

with $\gamma_2 = \alpha/\Delta t$, Δt being the time step of the numerical scheme. Note that $F(\theta_j, t)$ strongly depends on the nature of the site j . For

example, if j is not an adhesion site then only the passive contributions remain, i.e. $F(\theta_j, t) = \gamma L(\theta_j, t) a(\theta_j, t) - \beta$.

If the resulting tension force (R_F) exerted between the adhesion site and the cell body is positive (i.e. if the force is a traction force) then A matures into FX. If this condition is not fulfilled during the lifetime τ_A of A, then the adhesion breaks (Fig. 4). Similarly, the maturation of FX into FA occurs if R_F applied on the adhesion reaches a threshold tension (R_{thr}) during the lifetime τ_{FX} of FX. Once FA is formed, recruitment of actin occurs at the adhesion site to promote the formation of stress fibres. Fig. 5 shows an epifluorescent photomicrograph of an adherent cell (B.2), which exhibits the FA and actin cytoskeleton locations and interconnection in the cell. FA are mainly located at the cell periphery and connect the stress fibres, formed by bundles of actin filaments. The fibres contract and contribute in pulling the cell body forward.

2.3. Cell translocation

The model assumes that stress fibres connect the cell body to FA, hence only this adhesion type is considered able to transmit and sustain adequate traction force for effective cell translocation. In order to determine the direction θ_M for cell translocation (Fig. 6), the resulting traction force T_F existing in each stress fibre i is calculated as follows:

$$T_F(\theta_i, t) = \sum_{j=0}^m \left\{ \underbrace{\gamma_2 [L(\theta_j, t + \Delta t) - L(\theta_j, t)]}_{\text{adhesion-related}} + \underbrace{\gamma L(\theta_j, t) a(\theta_j, t)}_{\text{filament-related}} \right\} \delta_{FA}(\theta_j, t) \cos(\theta_j - \theta_i) \quad (17)$$

The cell translocation then corresponds to the displacement of the cell centroid (i.e. centre of the nucleus) in the θ_M -direction for which the resulting traction force T_F is the greatest. We note that in the translocation process, only the cell centroid is moving while the points forming the membrane do not.

In order to evaluate the amplitude of the displacement of the cell centroid, we simply assume that it is proportional to the resulting calculated force. Such proportionality corresponds to an “elastic release” whereby the stress fibre suddenly breaks once a threshold force T_{thr} is reached. The displacement r of the cell centroid is thus given by

$$T_F(\theta_M, t) = kr \text{ therefore } r = \frac{T_F(\theta_M, t)}{k} \quad (18)$$

where k is a coefficient characterizing the elasticity of the medium (stress fibre immersed in the cytosol) (parameters T_{thr} and k are given in Table 2).

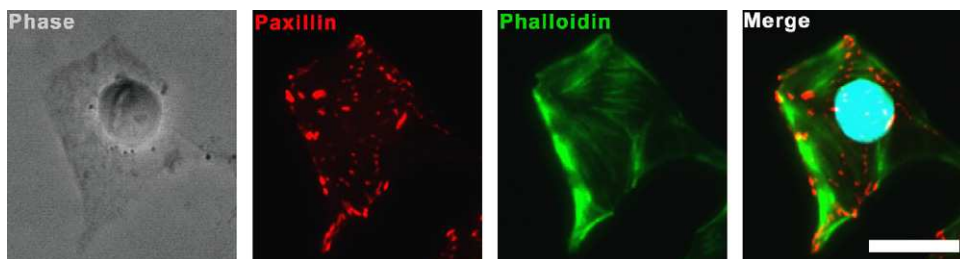


Fig. 5. Epifluorescent photomicrograph of FA and cytoskeleton of an adherent MCF7 cell. FA are revealed by the staining of paxillin, which is one of the early proteins of FA. Phalloidin stains actin cytoskeleton, whereas DAPI (blue) is used as a DNA marker to reveal the nucleus. Magnification bar corresponds to 20 μm .

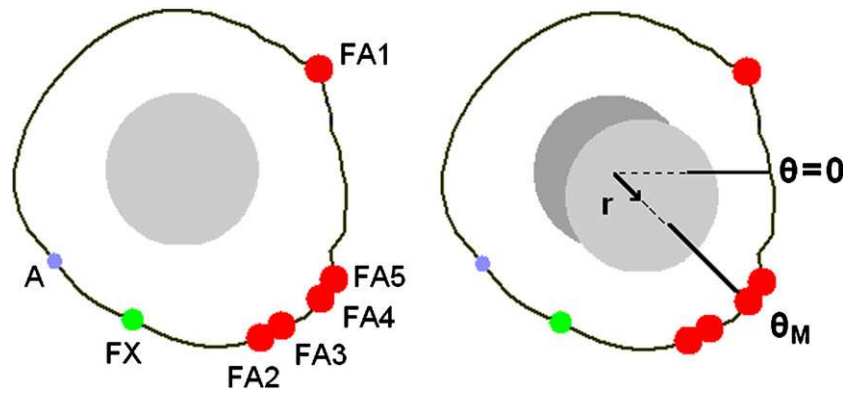


Fig. 6. Snapshot of a simulated cell showing the various adhesion types (A, FX and FA) that have formed at the cell periphery. Only FA contributes in the calculation of the cell displacement (left diagram). The cell centroid is pulled in the θ_M -direction corresponding to the greatest traction force from the filaments anchored to the related focal adhesion. Vector r represents the cell centroid displacement from its initial position (dark grey) to its final position (light grey).

Table 2

Simulation parameters which define the conditions for the formation and maturation of the cell adhesions and the cell translocation

Parameter	Notation	Value
Membrane extension threshold	L_{thr}	1.0
Actin density threshold	a_{thr}	1.1
Tension threshold	R_{thr}	1.0
Friction coefficient for adhesion	γ_2	5.0
Translocation threshold	T_{thr}	2.3
Stress fibre elasticity	k	14

3. Results

3.1. Experimental background

Fibroblast cells were chosen as an experimental model, since we have previously studied and characterized the non-migrating pulsating behaviour of this cell type. In addition, these cells are easily isolated and studied on a single-cell basis. The main focus of this paper is the spontaneous migratory behaviour of fibroblasts. Unlike directional migration, spontaneous migration is neither elicited nor guided by a gradient of chemicals or other factors. In our experimental set-up, spontaneous fibroblast migration was recorded on the uncoated plastic surface of a cell culture dish, which means a mechanically and structurally isotropic substratum (Appendix B.1).

Three different behaviours of spontaneously migrating fibroblasts are depicted in Fig. 7, namely slow (Cell 1), fast (Cell 2) and palindromic (i.e. bi-directionally moving) (Cell 3). The associated cell outlines and migratory tracks are presented in Fig. 8. Details of image acquisition and analyses are presented in Appendix B.3. The classical scenario of fibroblast migration is observed, with a protrusion at the leading edge of the cell and retraction of the uropod in the rear. For the fastest moving cells (2 and 3), a long and thin membrane extension persists at the uropod as the cell body translocates faster than the rate of focal adhesion release at the rear.

To further characterize cell migration, the cell speed and cell area are calculated over time for each type of migratory behaviour (Fig. 9). Specifically, the speed remains relatively constant for the slowly migrating cell with a mean value of $0.19 \pm 0.11 \mu\text{m}/\text{min}$. On the contrary, the speed profile of the fast migrating cell exhibits sharp changes with successive phases of acceleration and deceleration, thus resulting in a motility profile characterized by speeds over a great range ($0.60 \pm 0.43 \mu\text{m}/\text{min}$; Fig. 9A). Similarly,

the palindromic migratory behaviour, where the cell moves bi-directionally, is characterized by an intermediate oscillating profile ($0.46 \pm 0.31 \mu\text{m}/\text{min}$).

Cell area is also altered depending on motility behaviour and the phase of migration cycle (Fig. 9B). Specifically, the slow migrating cell has a mean area of $616 \pm 65 \mu\text{m}^2$, whereas the corresponding cell area of the fast migrating cell is approximately 1.3-fold higher ($807 \pm 94 \mu\text{m}^2$). Apparently, the cell needs to increase its surface area to allow for more adhesion sites to be formed. This increased adhesion with the substratum enables the cell to exert the traction forces required to move forward. However, when the cell apical surface becomes too extended, thus allowing for even more adhesions to form, then cell locomotion becomes inefficient as the cell is strongly tethered to the substratum. This is the case of the palindromic cell, where peak cell area ($1245 \mu\text{m}^2$) is accompanied by minimal translocation speed ($0.18 \mu\text{m}/\text{min}$; Fig. 9C).

3.2. Numerical simulations

In the simulation of cell migration we take as initial condition a small random perturbation ε ($|\varepsilon| \leq 0.05$) of the homogeneous steady state (L_s, a_s, v_s), with

$$L(\theta, 0) = 1, \quad a(\theta, 0) = 1 \pm \varepsilon, \quad v(\theta, 0) = 0 \quad (19)$$

This corresponds to the state of a cell just after mitosis, where its shape is circular and actin density relatively homogeneous. The model parameters are chosen so as to allow selection of an oscillating state according to the stability analysis of the PDEs system presented in Appendix A.2. This choice of parameters thus corresponds to a Hopf bifurcation point. The parameter ψ representing the contractile activity of the actin network, which is directly deduced from ρ , is taken as the bifurcation parameter. In order to destabilize the bifurcation state, the value of ψ is increased, corresponding to an intensification of the actomyosin complex contractility. The parameters β and γ which represent the protrusive hydrostatic pressure and the elastic coefficient of the actin network respectively, are not involved with the mode selection and are chosen independently.

The model parameters used to simulate spontaneous cell oscillations, select a pulsating state with mode 4. This state, which involves a membrane pulsation between two perpendicular protrusive directions, corresponds to the spontaneous pulsating state observed and characterized on isolated fibroblast cells (Stéphanou and Tracqui, 2002). These parameters are:

$$\mu = 2, \quad \tau = 1.5, \quad \delta = 0.001, \quad \beta = \gamma = 0.5, \\ a_{sat} = 4, \quad \psi = 5.6 \quad (20)$$

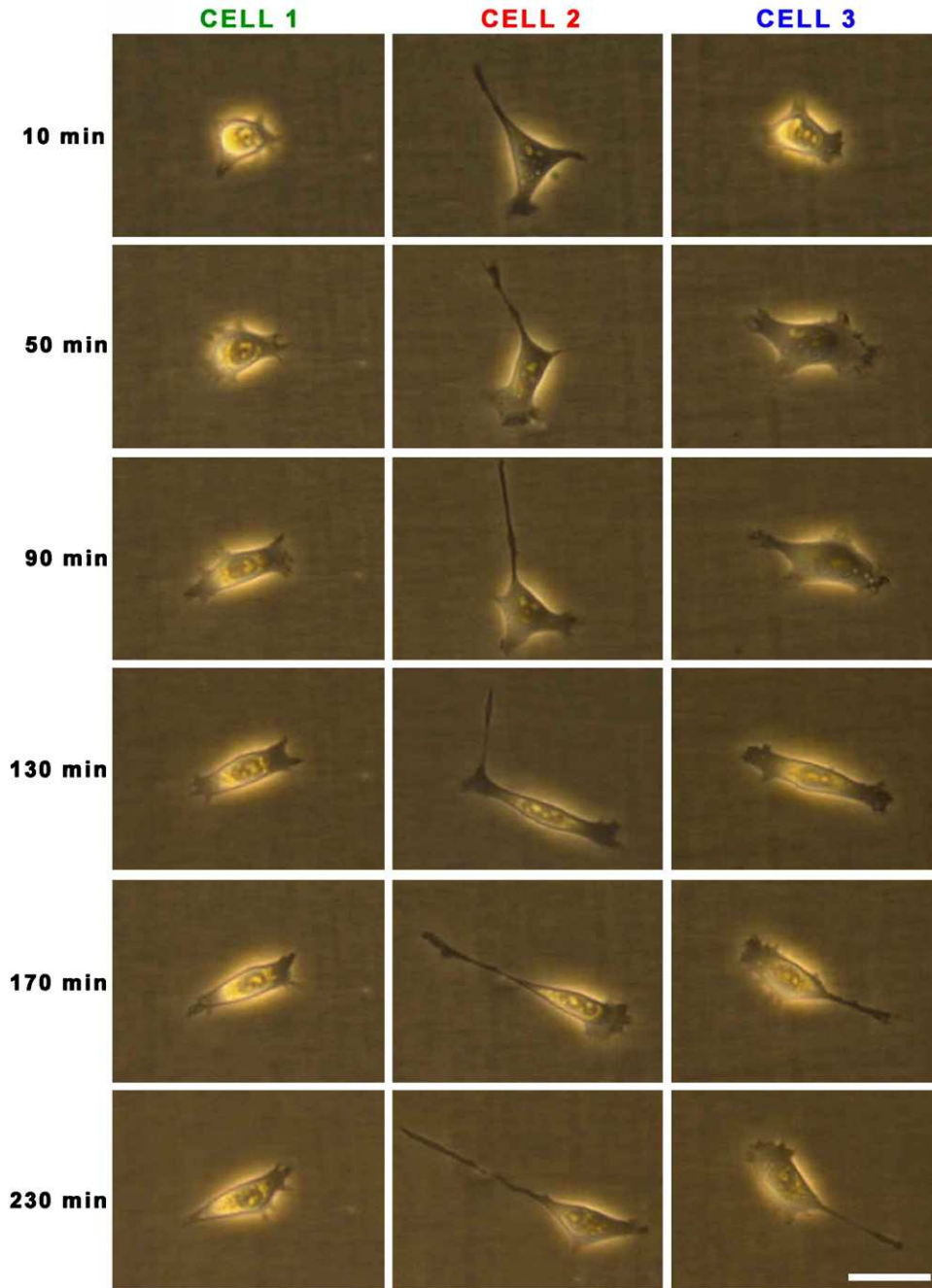


Fig. 7. Time-lapse microscopy of NIH 3T3 fibroblasts. Cells were seeded on an uncoated polystyrene cell culture dish and their spontaneous migratory tracks were captured every 10 min for 4 h. The phenotypic alterations of a slow (Cell 1), a fast (Cell 2) and a palindromic (Cell 3) migrating fibroblast are depicted in 40-min intervals. Magnification bar represents 20 μm .

Cell membrane oscillations and the related actin fluctuations in the cortex can lead locally to the formation of an adhesion spot, if the membrane extension and actin density both reach given thresholds (Table 2). These thresholds reflect the fact that the highest probability to form an adhesion occurs if the membrane offers a greater contact area to the substrate, with a sufficient amount of actin to connect/nucleate the integrins. Then the maturation of the adhesions, according to the rules described in the flowchart (Fig. 4), ultimately leads to cell translocation, that is effective cell migration. All the parameters related to the formation and maturation of the adhesions are given in Tables 2 and 3.

Fig. 10 presents snapshots of migration of the virtual cell. Each snapshot shows: (i) the cell shape, (ii) the cell migratory path

(trajectory) and (iii) cell adhesion spots with their corresponding level of maturation (*A*, *FX* or *FA*). The snapshots are not evenly distributed in time, as the aim is rather to show some representative examples of the distribution of the different types of adhesions along the cell membrane during migration.

Three different phases in the migration process can be identified in the simulation (Fig. 10), which correspond to the three different migrating behaviours experimentally observed. First a bi-directional migrating phase (palindromic migration) from snapshot *A*–*C* then a slow migrating phase from *D* to *E*, where the cell centroid randomly moves with a very small amplitude around an “equilibrium” spot, and finally a fast migrating phase from *F* to *H* characterized by a persistent direction of migration.

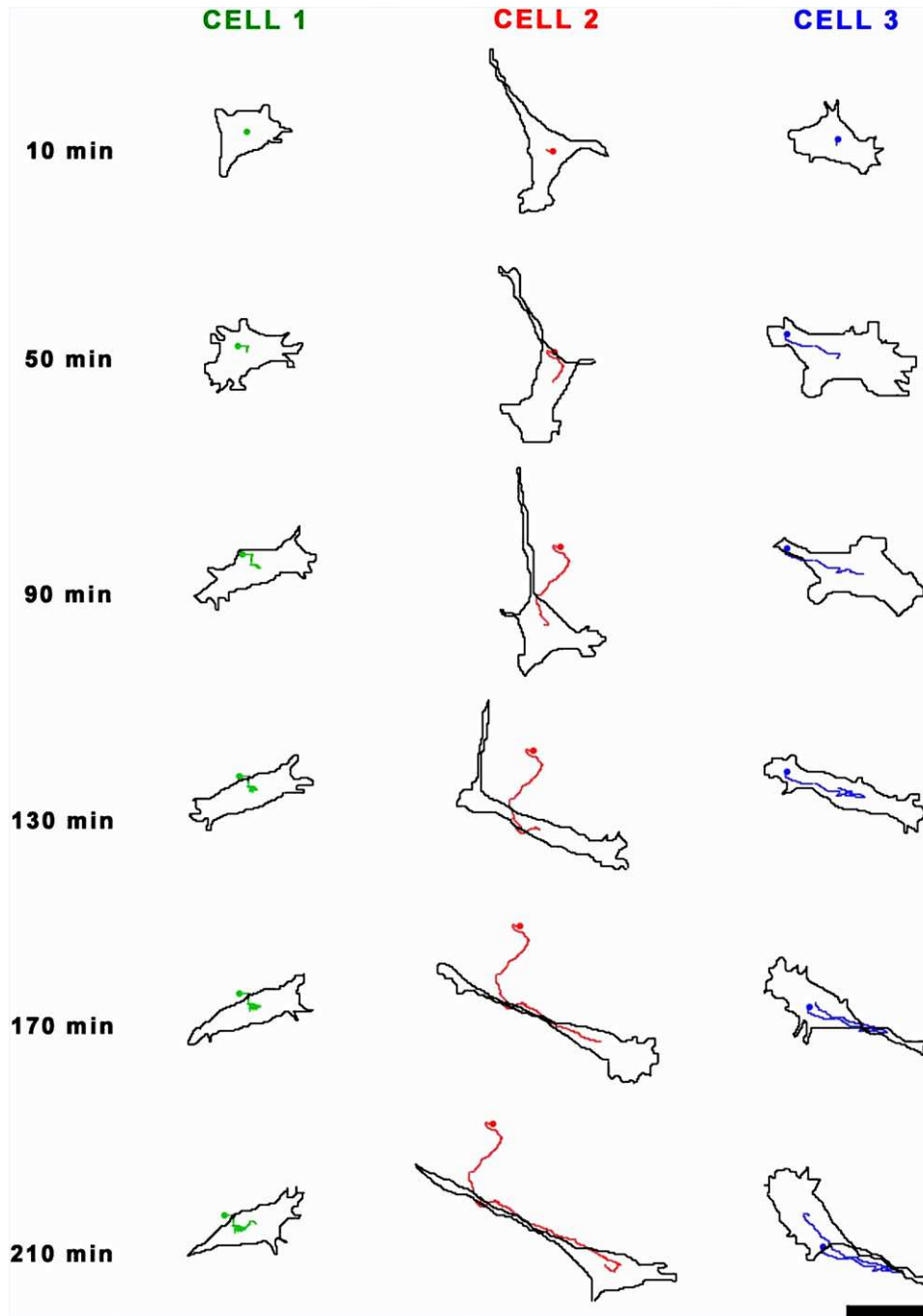


Fig. 8. Cell outlines and migratory tracks of NIH 3T3 fibroblasts. The cell's outline and centroid (nucleus centre) trajectory were manually tracked from phase time-lapse images of fibroblasts over a period of 4 h. Serial images at 40 min intervals demonstrate the cell shape alterations that accompany three motility behaviours, namely slow (Cell 1), fast (Cell 2) and palindromic (Cell 3). Magnification bar represents 20 μm .

Concerning the adhesions, the simulation shows that all the different types often co-exist (Fig. 10D–F). Moreover, several adhesions appear simultaneously since the conditions for adhesion concerns an area of the cell membrane more than a single spot (Fig. 10C–F).

In each case, the formation of focal adhesions indicate the direction of the next step of the cell trajectory.

3.2.1. *A posteriori* scaling of the model with experimental data

Spatial and temporal scaling of the model are based on the measurements previously performed on the 3T3 fibroblasts.

Specifically, spatial scaling is realized from measurements of the radius of the cell nucleus ($R_0 = 7 \mu\text{m}$). Temporal scaling is performed by fitting the time it takes for the fast migrating cell to move along a given distance. Measurements from the experimental data give a distance of about 140 μm covered by the cell in 4 h. During its fast moving phase the virtual cell (Fig. 10E–H) is covering a distance of about 70 μm in 50 000 iterations, that we assimilate to 2 h.

With this calibration, the evolutions of the simulated cell speed and the associated cell area (Fig. 11) can be plotted and compared with the experimental measurements. As the model calibration was performed from the fast migrating phase, the

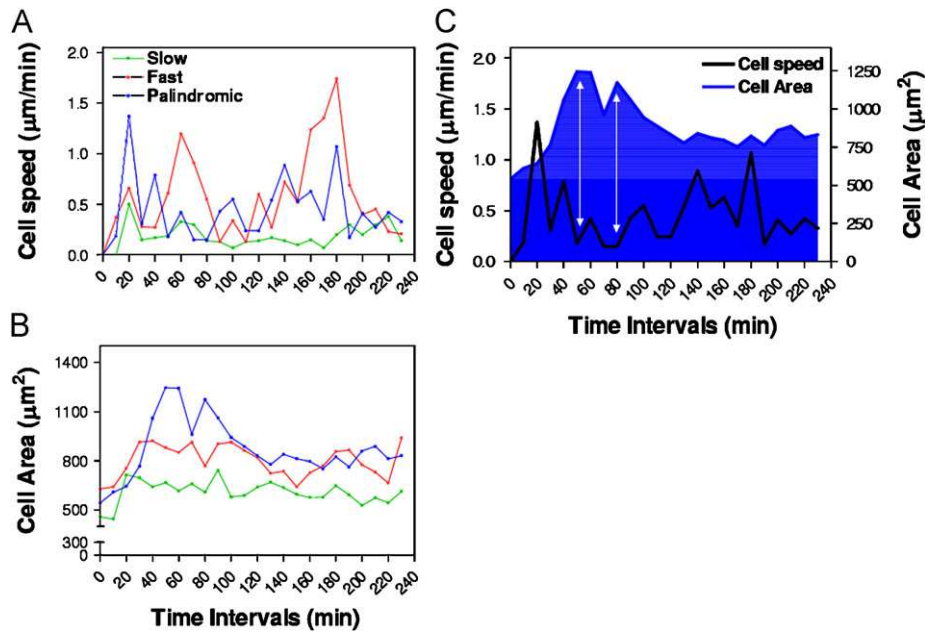


Fig. 9. Experimental data on speed (A) and cell area (B) of migrating NIH 3T3 fibroblasts. Live cells were recorded every 10 min for a total of 4 h via time-lapse microscopy and migratory behaviour was analysed for a slow (Cell 1), a fast (Cell 2) and a palindromic (Cell 3) fibroblast. During migration, cell speed was minimized when cell area was the largest (white double arrows) as shown for the palindromic fibroblast (C).

Table 3

Temporal simulation parameters which govern: (i) the adhesion proteins recycling time, that is the time during which the cell remains unable to form an adhesion and (ii) the lifetimes of the different types of adhesion

Parameter	Notation	Iterations	Time (s)
Adhesion proteins recycling time	T_r	400	58
Adhesion point lifetime	τ_A	100	14
Focal complex lifetime	τ_{FX}	500	72
Focal adhesion lifetime	τ_{FA}	500	72

mean speed measured obviously coincides with a simulated value of $0.60 \pm 0.14 \mu\text{m}/\text{min}$. The mean cell speeds measured for the palindromic and slow phases of the simulated cells are 0.50 ± 0.27 and $0.39 \pm 0.31 \mu\text{m}/\text{min}$, respectively. This is in relatively good agreement with the speed measured on the fibroblast cells. Concerning the mean cell area, the value measured for the simulated cell is $730 \pm 46 \mu\text{m}^2$, which is an intermediate value in the range of the areas experimentally measured (from 616 to $886 \mu\text{m}^2$). As observed experimentally, the minimum cell speed corresponds to the largest cell area. The model is therefore able to illustrate this important phenomenon of cell migration.

3.2.2. Influence of the adhesion strength on cell speed

The parameter γ_2 appears as a friction coefficient in the model equations. Consequently, it can be interpreted as a representation of the strength of the adhesion of the cell to its substrate. The influence of this parameter on the cell speed is evaluated through simulations. Fig. 12 shows that the increase in the adhesion strength from $\gamma_2 = 5$ to 30, first leads to increase in the cell speed from 0.51 up to $0.64 \mu\text{m}/\text{min}$. However, further increase in γ_2 decreases the cell speed to $0.54 \mu\text{m}/\text{min}$. This biphasic dependency of the cell speed on the adhesion strength is in complete agreement with well-established experimental results (Palecek et al., 1997). Palecek and colleagues demonstrated that cell speed increased to fibronectin concentration. However, when fibronectin concentration became too high, the attachment of the cell to the substrate was so great that migration was impeded.

3.2.3. Influence of the recycling time of adhesion proteins on cell speed

The recycling time of adhesion proteins (e.g. integrins) (T_r) corresponds to a refractory period during which the cell remains unable to form new adhesions with the substrate. This event occurs immediately after cell translocation when all adhesions are released.

Fig. 13 shows that the cell speed decreases with increased recycling time. For a short recycling time $T_r = 14$ s, the cell is moving much faster with a speed reaching $0.91 \mu\text{m}/\text{min}$. This is due to the fact that the cell remains partially polarized with higher actin density at the leading edge, where the focal adhesions just broke, therefore the conditions for rapid formation and growth of new adhesions are optimal. On the contrary, when the recycling time ($T_r = 115$ s) lasts longer, the cell is given more time to fully restabilize its shape. This leads to a much slower cell speed of $0.35 \mu\text{m}/\text{min}$. Interestingly, the decrease in the cell speed is not linear to the increased recycling time but rather occurs stepwise.

To better understand how the recycling time of adhesion proteins is influencing cell speed through the formation and maturation of adhesions, the distribution of the different types of adhesions (A, FX, and FA) developed by the cells is presented in Fig. 14 for three different recycling times, namely short ($T_r = 14$ s), long ($T_r = 115$ s) and intermediate ($T_r = 72$ s). Each case corresponds to a migration time of 6 h. The simulation results shows that for the short recycling time, 24% of the cell adhesions matured to FA, 30% of the adhesions remain at stage A and 45% do not mature beyond the FX stage. Increasing the recycling time first leads to a marked decrease in the amount of cell adhesions with the substrate. Specifically, 1.5 and 1.8 times less adhesions are formed during intermediate and long recycling times respectively. The amount of adhesions reaching the FA stage also decreases drastically from 24% to 15% for $T_r = 72$ s and only 9% for $T_r = 115$ s. For the intermediate recycling time, the same amount of adhesions remains at stage A (30%) but less adhesions mature to stage FA (15%), whereas for the longer recycling time the maturation becomes even more difficult at an earlier stage as more adhesions remain at stage A (40%).

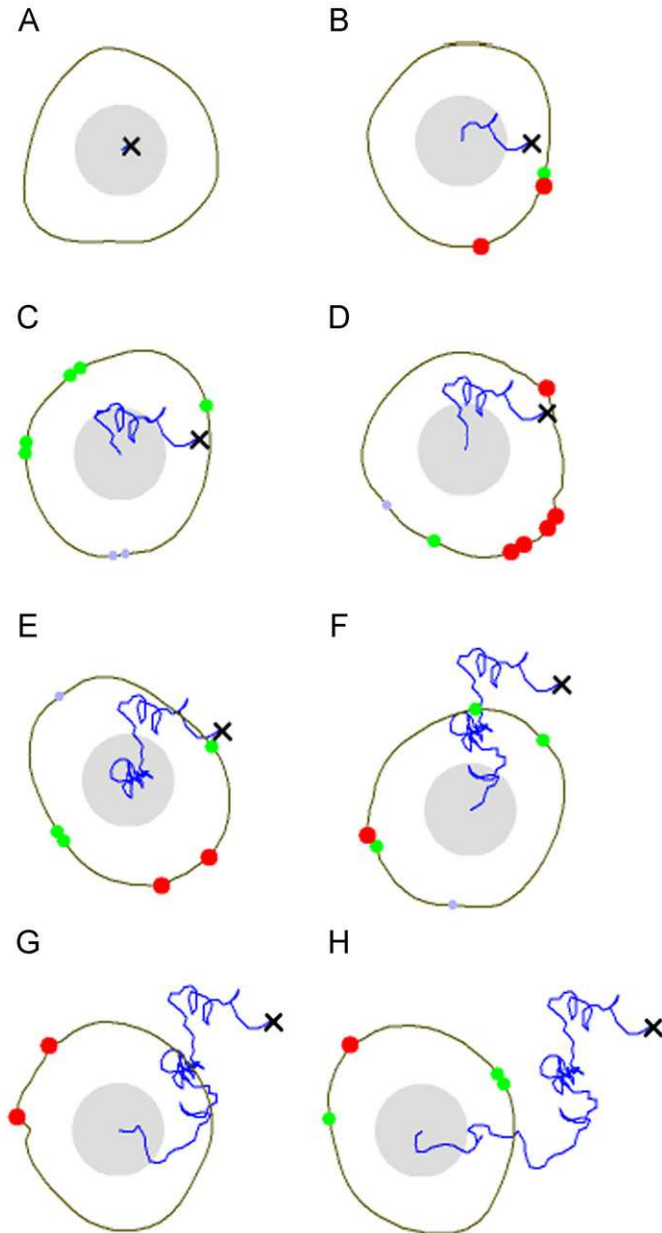


Fig. 10. Snapshots of the simulation of a spontaneously migrating cell. The initial position of the cell centroid is marked by the cross. The cell trajectory appears in blue. The different types of adhesion, i.e. adhesion points (A), focal complexes (FX) and focal adhesions (FA) appear as violet, green and red spots, respectively. (A) $t = 12$ min, (B) $t = 47$ min, (C) $t = 106$ min, (D) $t = 131$ min, (E) $t = 221$ min, (F) $t = 286$ min, (G) $t = 309$ min and (H) $t = 352$ min.

In all three cases, the fraction of adhesions that effectively lead to cell translocation represents only 1% of the total amount of cell-to-substrate adhesions. However, small, this fraction remains coherent with observations on unstimulated fibroblasts, which mainly exhibit membrane fluctuations rather than effective migration.

3.2.4. Influence of the adhesions lifetimes on the cell speed

Each adhesion type is characterized by a limited lifetime. Adhesion lifetime increases with the adhesion maturation level, as adhesions become progressively more stable. This means that $\tau_A < \tau_{FX} \leq \tau_{FA}$. To evaluate the influence of adhesion lifetime on cell speed, simulations were run for a range of values for τ_A with

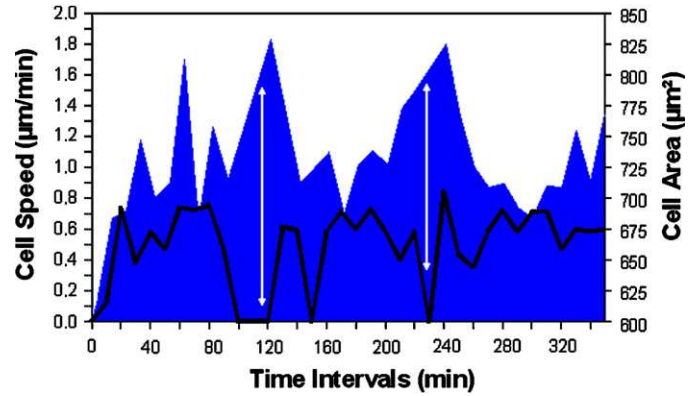


Fig. 11. Evolution of the speed (black line) and associated area (in blue) of the simulated cell. In agreement with experiments, the cell speed is minimized when the cell area is the largest (white double arrows).

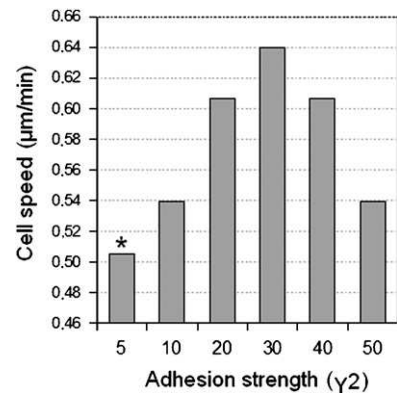


Fig. 12. Cell speed measured for a range of values of the parameter γ_2 simulating the cell adhesion strength to the substrate. The bar with the asterisk, which designates the simulation for $\gamma_2 = 5$, corresponds to the reference simulation presented in Fig. 10.

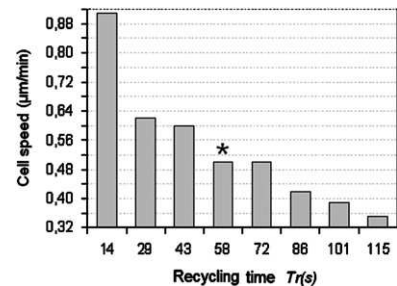


Fig. 13. Influence of the recycling time of adhesion proteins T_r on mean cell speed of simulated cells. The bar marked by an asterisk ($T_r = 58$ s) corresponds to the reference simulation presented in Fig. 10.

increasing magnitude m for $\tau_{FX} = \tau_{FA}$ relative to τ_A . In other words, $\tau_{FX} = \tau_{FA} = m\tau_A$, with $m = 2, 3$ and 5 successively.

We note that in our simulations the same lifetime value is consistently taken for τ_{FX} and τ_{FA} . This stems from the conditions we defined for cell translocation, where a threshold tension needs to be reached. This threshold has to be reached within a few iterations after the formation of a focal adhesion at a site of optimal conditions. Otherwise the threshold is never reached and further maintaining the focal adhesion causes numerical instabilities.

Fig. 15 presents curves obtained for three different magnitude m . It appears that changes between the different magnitudes of

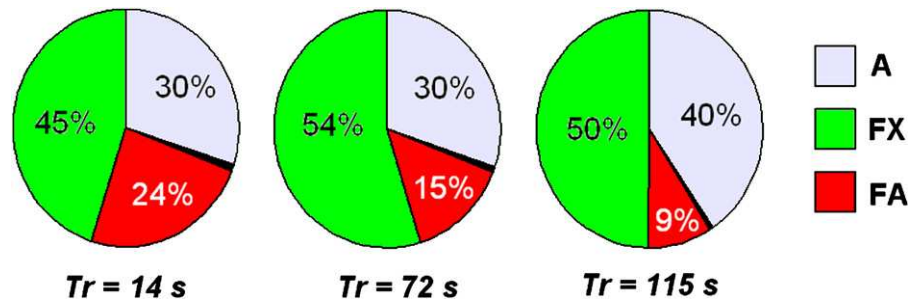


Fig. 14. Percent distribution of the different types of adhesions developed by the simulated cells, as evaluated for three different values of the cell recycling time ($T_r = 14, 72$ and 115 s). The different types of adhesion, i.e. adhesion points, focal complexes and focal adhesions appear in violet, green and red, respectively. The small fraction of focal adhesions, which effectively induce the cell translocation, appears in black.

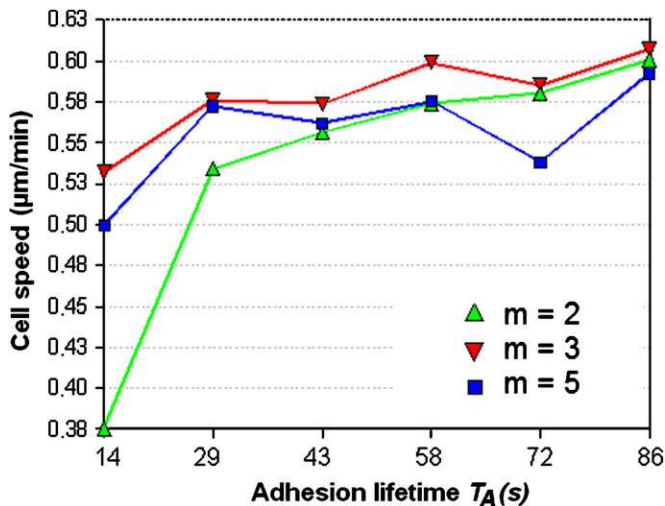


Fig. 15. Cell speed as a function of the adhesion spot lifetime τ_A . The three curves correspond to three different magnitude ($2\times$, $3\times$ and $5\times$) of the focal complex and focal adhesion lifetimes τ_{FX} and τ_{FA} relative to τ_A .

τ_{FX} and τ_{FA} relative to τ_A have more effect on the cell speed, when τ_A is minimal (14 s). Differences between the curves tend to decrease for greater values of τ_A , since the increase in the adhesion lifetimes has a homogenizing effect ($\tau_A = 86$ s). In general, the cell speed increases with increasing values of τ_A . Speed always increases for $m = 2$, whereas the evolution of the speed with τ_A tends to be more irregular with increasing magnitude m .

Fig. 16 shows different cell trajectories corresponding to the three curves in Fig. 15 for $\tau_A = 43, 58$ and 72 s. Although the cell speeds are relatively homogeneous between the cells, from $0.54 \mu\text{m}/\text{min}$ (I) to $0.60 \mu\text{m}/\text{min}$ (E), the trajectories can be very different with more or less persistent directions of migration. In order to evaluate the directional persistence of the cell trajectories, we calculate an “erraticity index” E_{rr} (da Fontoura Costa et al., 2005). This index is given by the ratio between the distance D separating the initial and final positions of the cell and the total length L of the cell trajectory, i.e. $E_{rr} = D/L$. Therefore, if E_{rr} tends to 0, then the trajectory is very erratic, whereas if E_{rr} tends to 1, then the trajectory is very oriented.

We prefer this simple method to obtain a measure of the directional persistence via this “erraticity index” since the trajectories considered are small (23 points only for the experimental data). An autocorrelation analysis (Dunn and Zicha, 1995) or the calculation of the speed and persistence time coefficients of a persistent random walk (PRW) model (DiMilla et al., 1992) are methods requiring much more points to produce a reliable evaluation of the directional persistence.

A preliminary comparison between the erraticity coefficient for the fast migrating cell (Fig. 8) and the associated fast phase of the simulated cell (Fig. 10) shows similar values with $E_{rr} = 0.38$ and 0.32 , respectively.

As shown in Fig. 16, the trajectories B and E are the least erratic with $E_{rr} = 0.20$ and 0.23 , respectively, which correspond to the experimental slow migrating cell where $E_{rr} = 0.23$. In contrast, C, G and H are the most erratic with E_{rr} ranging from 0.05 to 0.07. It appears that short τ_A associated with a low magnitude m , meaning a smaller difference between $\tau_{FX,FA}$ and τ_A , favours the development of a more oriented trajectory.

4. Discussion

Cell migration is a highly regulated process, with membrane protrusion, cell adhesion to the substratum and translocation through cell contraction being its key events. In order to satisfactorily describe such a sequence of events with numerical simulations, it is essential for a mathematical model to integrate elements of: (i) actin dynamics, since actin turnover and contractility are mainly responsible for cell membrane oscillations; (ii) adhesion dynamics, the process by which adhesions form and mature to ensure the support required for cell translocation. Many models have been recently proposed to investigate some of these aspects (Larripa and Mogilner, 2006; Marée et al., 2006; Wolgemuth, 2005; Gracheva and Othmer, 2004). However, because of the complexity of these cell migration-related phenomena at the whole cell scale, these models remain limited to a one-dimensional description of the lamellipodium. The paper by Marée et al. (2006) is the exception, since the model predicts the entire cell shape and exhibits the role of the small GTPases coupled to the actin cytoskeleton in the context of chemotaxis. In this paper, we also propose a model at the whole cell scale, which focuses on the explicit coupling between actin and adhesion dynamics.

Our primary goal here has been to address the problem of migration with a special focus on random cell motility. Most theoretical studies of cell migration investigate directionally, as opposed to randomly, migrating cells. Directional migration implicitly supposes that the cell is “activated” by an external factor, either chemical (e.g. chemoattractive source) or mechanical (e.g. heterogeneous rigidity or topography of the environment). The cells sense these physical and chemical cues in their microenvironment, process the information and respond appropriately by changing their morphology and/or motility pattern. Numerous proteins are actively produced and recruited to generate the cell’s response. In other words, the “cell protrusive machinery” is switched on. Although most migratory phenomena are induced and directionally guided *in vivo*, random migration *in*

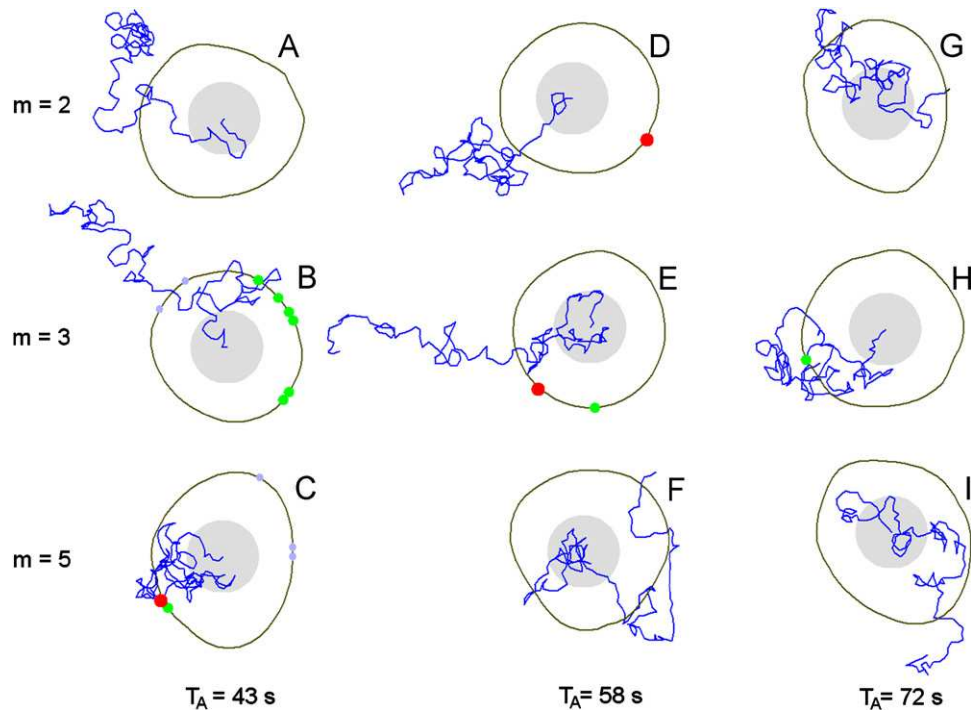


Fig. 16. Simulated cell trajectories observed at the 6th hour for a range of adhesions lifetimes with $\tau_A = 43, 58$ and 72 s (columns) and magnitude $m = 2, 3$ and 5 (rows).

vitro is of great interest to study the cell state, without external stimulation, when the cell is considered unactivated and its protrusive machinery switched off.

Additionally, directional cell movement is orchestrated by different effector molecules than those involved in random migration, thus different mechanisms are activated. For instance, pressure-driven membrane protrusion through cytoplasmic flux (Charras et al., 2005; Abraham et al., 1999; Bereiter-Hahn and Luers, 1998) was for long opposed to actin polymerization-driven protrusion (Borisy and Svitkina, 2000; Carlier and Pantaloni, 1997; Theriot and Mitchison, 1991), when in fact these two processes co-exist. Specifically, pressure-driven protrusion is predominant in resting cells (i.e. non-migrating cells), where spontaneous actomyosin contractility remains the main cell activity. In contrast, actin polymerization-driven protrusion is predominant in activated cells, where a number of actin regulation proteins are recruited to produce efficient actin network turnover (with induced branching, treadmilling, etc.) (Carlier and Pantaloni, 2007; Borisy and Svitkina, 2000). It is thus important to identify the cell dynamic state. Hence, investigation of the unactivated (*unstimulated*) state of a cell is fundamental if one wants to properly describe the cell reaction to an external signal.

Unstimulated cells have been shown to exhibit spontaneous membrane protrusions, oscillating and spatio-temporally organized, with the appearance of specific cell shapes, and periodically repeating patterns (Germain et al., 1999; Stéphanou et al., 2004; Giannone et al., 2004). A simple mathematical model based on actin turnover and contractility, where pressure-driven protrusion competes with actin filaments retraction on the membrane, has allowed us to reproduce typical self-organized features of fibroblasts' spontaneous movements (Stéphanou et al., 2004). In this paper, we proposed to extend this model by coupling the spontaneous membrane deformations with the formation of adhesions, essential for cell migration.

Energy fluctuations can account for the formation of simple adhesion spots (Nicolas et al., 2004). However, to generate the force required for cell translocation, i.e. to pull the cell forward,

actin recruitment at the cell's leading edge is necessary to increase the traction force from the filaments and overcome the adhesion force. Thus our model proposes a “protrusive switch”, whereby passive hydrostatic pressure on the membrane is reinforced by an active actin polymerization-induced pressure. This process occurs at the adhesion site, once the adhesion matures to a focal adhesion. This phenomenon is considered autocatalytic, since cell contractility is responsible for the maturation of the adhesion, which progressively leads to the protrusive switch.

Numerical simulations performed show that the model is able to reveal the main features of fibroblast cell migration. That includes alternating phases where the cell can either explore a very short perimeter (slow migration), move bi-directionally (palindromic migration) or transiently assume a persistent directional migration (fast migration). In agreement with our experimental data, simulations also show that the cell speed is correlated with the maximum cell area, as a consequence of the protrusive switch phase.

To further validate the model, we verified the influence of adhesion strength upon the cell speed. It is well known that cell speed and adhesion strength have a biphasic relationship. Specifically, cell speed increases with the adhesion strength until further increase in cell adhesion strength hinder migratory speed (Palecek et al., 1997). The model managed to reveal this relationship within the authorized numerical limits.

The influence of the temporal parameters of the model, more specifically the adhesion proteins recycling time and the adhesion lifetimes were also investigated. In the model, we made the simplifying assumption that all adhesions are released while the cell translocates (Kirfel et al., 2004). The adhesion proteins recycling time induces a refractory period immediately after translocation. This corresponds to the time during which the virtual cell is not allowed to make new adhesion with the substrate. A longer recycling time means that the cell is given more time to recover its spontaneous pulsating state. Once this state is reached it becomes more difficult to destabilize the cell, that is to recreate the conditions for migration. This implies that

the cell is potentially more reactive, with a quicker migration speed, when in a perturbed state rather than in equilibrium. This happens because actin distribution is more heterogeneous, which favours the formation of focal adhesions, thus promoting migration.

Interestingly, increasing the adhesions lifetime has the opposite effects on migration speed. Specifically, increasing the adhesion lifetime, increases the probability that an adhesion will reach the next level of maturation. Then, tension forces have more time to develop and produce efficient cell translocation, resulting in higher migration speeds. Simulations also suggest that the persistence in the direction of migration can be affected by the choice of the relative adhesion lifetimes.

Actin and adhesion dynamics are interrelated (Gupton and Waterman-Storer, 2006), with complex relationships between intracellular events (actin turnover, maturation of the adhesions, recycling of the proteins, etc.). The model demonstrates that alterations of the temporal parameters can modify the spontaneous cell migrating behaviour, thus providing with insight on how the cell would potentially react to an external stimulation. A future step in this direction is to investigate the explicit coupling between the cytoskeleton elements with the extracellular matrix through its mechanical and topographical properties. Such a model is essential for disciplines such as tissue engineering and the developing nano-patterning technologies, since it will provide the conceptual framework required to interpret the growing body of experimental observations of the individual and collective cell behaviours on micro- and nano-patterned substrates.

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Appendix A. Non-dimensionalization and linear stability analysis

A.1. Non-dimensionalization

The system of PDEs Eqs. (1)–(3) is non-dimensionalized by setting the following dimensionless variables:

$$\tilde{t} = t\eta, \quad \tilde{a} = \frac{a}{a_0}, \quad \tilde{L} = \frac{L}{R_0}$$

The normalized parameters are then

$$\tilde{\beta} = \frac{\beta_1}{\Phi_1 a_0 R_0 \eta}, \quad \tilde{\gamma} = \frac{\gamma_1}{\Phi_1 \eta}, \quad \tilde{\mu} = \frac{\mu_0}{\Phi_0}, \quad \tilde{\psi} = \frac{\psi_0 a_0}{\Phi_0 R_0 \eta}$$

$$\tilde{a}_{sat} = \frac{a_{sat}}{a_0}, \quad \tilde{\tau} = \frac{\tau_0}{\Phi_0 \eta^2}, \quad \tilde{\delta} = \frac{\tau_1 \Phi_0}{\tau_0 \Phi_1}$$

Dropping the tildes for notational simplicity, we obtain the following system of dimensionless PDEs:

$$\frac{\partial}{\partial t}(La) = -\frac{\partial}{\partial \theta}(Lav) + L(1-a) \quad (21)$$

$$a \frac{\partial L}{\partial t} = \beta - \gamma La + \delta \frac{\partial}{\partial \theta} \left(\tau a \frac{\partial L}{\partial \theta} \right) \quad (22)$$

$$av = \frac{\partial}{\partial \theta} \left[\mu a \frac{\partial v}{\partial \theta} + \sigma(a) - \frac{\partial}{\partial \theta} \left(\tau a \frac{\partial L}{\partial \theta} \right) \right] \quad (23)$$

$$\sigma(a) = \psi a^2 e^{-2a/a_{sat}} \quad (24)$$

A.2. Linear stability analysis

The linear stability analysis of the dimensionless system (Eqs. (21)–(23) for periodic boundary conditions) considers the behaviour of small perturbations around the homogeneous steady state, which is given by

$$L_s = \frac{\beta}{\gamma}, \quad a_s = 1, \quad v_s = 0 \quad (25)$$

We then look for each variable L , a , and v , a solution with the general form:

$$w - w_s = e^{\lambda t + im\theta}, \quad (26)$$

where m represents the spatial mode of deformation. By substitution in the linearized system of equations one obtains

$$\begin{pmatrix} \lambda + \gamma + m^2 \delta \tau & \beta & 0 \\ \lambda & \frac{\beta}{\gamma}(1 + \lambda) & im \frac{\beta}{\gamma} \\ -im^3 \tau & -im\rho & 1 + \mu m^2 \end{pmatrix} \begin{pmatrix} L - L_s \\ a - a_s \\ v - v_s \end{pmatrix} = 0 \quad (27)$$

where $\rho = d\sigma/da|_{a=a_s}$. If M denotes the matrix above, the dispersion relation between λ and m which has to be satisfied for the system to have a non-trivial solution is given by

$$\det(M) = (\lambda + \gamma + m^2 \delta \tau) \left[\frac{\beta}{\gamma}(1 + \lambda)(1 + \mu m^2) - m^2 \rho \frac{\beta}{\gamma} \right] - \beta \left[\lambda(1 + \mu m^2) - m^4 \tau \frac{\beta}{\gamma} \right] = 0$$

$$\text{i.e. } \lambda^2 + \lambda \left(m^2 \delta \tau + 1 - \frac{m^2 \rho}{1 + \mu m^2} \right) + \frac{m^4 \beta}{1 + \mu m^2} + (\gamma + m^2 \delta \tau) \left(1 - \frac{m^2 \rho}{1 + \mu m^2} \right) = 0$$

The roots of the dispersion relation above, which give the temporal evolution of perturbations with spatial mode m , are given by the following pair of eigenvalues of the matrix M :

$$\lambda(m) = \frac{1}{2} \left[-1 + \frac{m^2 \rho}{1 + \mu m^2} - m^2 \delta \tau \right] \pm \frac{1}{2} \sqrt{\left(2\gamma - 1 + \frac{m^2 \rho}{1 + \mu m^2} + m^2 \delta \tau \right)^2 - 4\gamma(\gamma + m^2 \delta \tau) - 4 \frac{m^4 \beta \tau}{1 + \mu m^2}} \quad (28)$$

A.2.1. Conditions for the existence of an oscillating state

A simple way to look for self-sustained oscillations of the plasma membrane is to find model parameters which correspond to a destabilization of the uniform steady state of the variable L . If this destabilization occurs through a Hopf bifurcation, then standard theory predicts the existence of limit cycle type solutions. Here, we are interested in spatially non-uniform solutions corresponding to the appearance of extending/retracting protrusions along different axes. These axes will be determined by the existence of unstable spatial modes for the cytomechanical model solutions. A Hopf bifurcation then occurs for complex eigenvalues, i.e. when $\text{Re}[\lambda(m)] = 0$. The value of m which maximizes $\text{Re}[\lambda(m)]$ is given by

$$m_0 = \sqrt{\frac{1}{\mu} \left(\sqrt{\frac{\rho}{\delta \tau}} - 1 \right)} \quad (29)$$

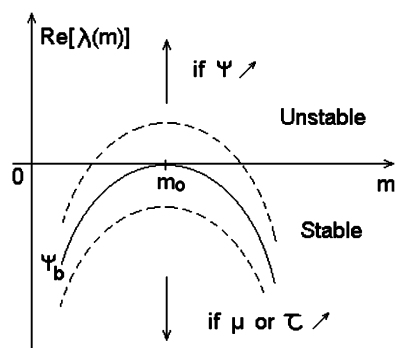


Fig. 17. Schematic stability diagram.

We note that the coefficients β and γ , which represent the protrusive hydrostatic pressure and the elasticity coefficient of the network controlling the retraction of the lamellipod, respectively, have no influence on the modes selection. The key parameters are the cytoplasm viscosity μ and the actin–membrane complex elasticity τ controlling the surface tension. As intuitively expected, large values of τ and μ , which both tend to increase the stiffness of the system correspond to small value of m_0 , i.e. to a small number of potential membrane protrusions. Conversely, large values of the parameter ρ (i.e. ψ), which monitors the contractility of the F-actin network, favour the destabilization of a larger number of spatial modes (Fig. 17).

Appendix B. Cell culture, immunostaining, and image analysis

B.1. Cell culture

NIH 3T3 fibroblasts were maintained in DMEM + 10% FBS + P/S (GM) at 37 °C and 5% CO₂ in a humidified chamber. On the experimental day, cells were trypsinized and re-plated on uncoated polystyrene P35 plates at a density of 1×10^3 cells/P35 in GM. Following 2 h to allow for cell adhesion, GM was replaced by a low serum medium (DMEM + 0.5% FBS) buffered with 25 mM HEPES to completely fill the plate. A seal was created between the plate tip and its inverted lid with silicon grease to prevent medium evaporation. The plate was then placed on a heated stage electronically maintained at 37 °C (eba 100 mc-L, Lastungselektronik JENA GmbH, Germany). Digital images of migrating cells were acquired every 10 min for a total of 4 h using a Leica DM IL microscope equipped with a DFC 480 DCC digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Photoexposure of the cells between recording intervals was minimized to avoid phototoxicity.

B.2. Immunocytochemistry and staining

For immunocytochemistry and staining, cells from a human mammary adenocarcinoma cell line (MCF7) were used. Cells were maintained as described above and seeded (1×10^3 cells/cm²) on glass coverslips coated with 0.15 mg/ml Collagen I. After overnight incubation in a humidified environment at 37 °C and 5% CO₂, cells were fixed with 4% paraformaldehyde at room temperature for 15 min, followed by a 2 min incubation in permeabilization buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 50 mM NaCl, 3 mM MgCl₂, 0.5% Triton X-100, and 300 mM sucrose in PBS). Non-specific staining was blocked with 0.1% Triton X-100 and 10% goat serum for 1 h at room temperature. Following incubation with a mouse monoclonal anti-paxillin antibody

(1:750 in 0.04% Triton X-100 and 1% bovine serum albumin) at 4 °C overnight, cells were incubated with Alexa Fluor 594 goat anti-mouse secondary antibody (1:200) and Alexa Fluor 488 phalloidin (1:200) for 1 h in the dark to reveal focal adhesions and actin cytoskeleton, respectively. Cell nuclei were visualized by the addition of 1 μ g/ml 4',6-diamidino-2-phenylindole (DAPI) in the final wash. Coverslips with cells were mounted with Pro-Long Gold antifade reagent and sealed. Focal adhesions, actin cytoskeleton and cell nuclei were observed under a Leica DMIL microscope equipped with epifluorescence and images acquired with a Leica DFC 480 DCC digital camera and Leica imaging software (LAS; Leica Microsystems). The secondary antibody, phalloidin and Pro-Long antifade reagent were purchased from Molecular Probes (Carlsbad, CA, USA), whereas all other reagents and chemicals were purchased from SIGMA (St. Louis, MO, USA).

B.3. Analyses

Migration analyses were performed on cells that remained in the field of view for the entire recording period. Cells actively proliferating or contacting other cells during imaging were excluded from the analyses. The outline, centroid (i.e. centre of nucleus), and migratory track were manually drawn for each cell for the entire recording period using Adobe Photoshop version 7.0 (Adobe Systems Incorporated, San Jose, CA, USA). Adjustments of image brightness and contrast were performed equally throughout an entire image. Migration distance and cell area were measured using ImageJ software with calibrated settings (NIH, Bethesda, MD, USA). Cell speed (μ m/min) was accordingly calculated. Graphs were generated using GraphPad Prism version 4.0.

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On the Influence of Discrete Adhesive Patterns for Cell Shape and Motility: A Computational Approach

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Abstract. In this paper, we propose a computational model to investigate the coupling between cell's adhesions and actin fibres and how this coupling affects cell shape and stability. To accomplish that, we take into account the successive stages of adhesion maturation from adhesion precursors to focal complexes and ultimately to focal adhesions, as well as the actin fibres evolution from growing filaments, to bundles and finally contractile stress fibres.

We use substrates with discrete patterns of adhesive patches, whose inter-patches distance can be modulated in order to control the location of the adhesions and the resulting fibres architecture. We then investigate the emergence of stable cell morphologies as a function of the inter-patches distance, for two different cell phenotypes generated from the model. Force generated by the stress fibres on the focal adhesions and specifically the influence of the cell contractility are also investigated.

Our results suggest that adhesion lifetime and fibre growing rate are the key parameters in the emergence of stable cell morphologies and the limiting factors for the magnitude of the mean tension force from the fibres on the focal adhesions.

Key words: motility, stress fibres, focal adhesions, micropatterned substrates, integrative modelling.

AMS subject classification: 92B08, 92C17

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List of abbreviations

- AP: Adhesion Precursor;
- CB: Circular Bundle;
- DF: Dorsal Fibre;
- DSF: Dorsal Stress Fibre;
- ECM: Extracellular Matrix;
- FA: Focal Adhesion;
- FX: Focal complex;
- TA: Transverse Arc;
- TF: Transverse Fibre;
- TSF: Transverse Stress Fibre.

1. Introduction

The control of cell motility is one of the great challenges of cell biology since migration in its many forms (*e.g.* chemotaxis or durotaxis) is crucial to many physiological and pathological events. However migration is just one aspect of motility. Indeed this term refers more generally to the ability for the cell to move spontaneously, that is to make movement by itself. Those movements do not necessary lead to migration. There are many circumstances *in vivo* where the non-migratory cell movements are as essential. For example the cell polarization and alignment in muscles or the cell membrane movements to maintain homeostasis in a tissue.

The *in vitro* study of cell membrane deformations and cell shape is essential to understand how the cell regulates its cytoskeletal architecture and the resulting forces to migrate. Intense research in the field has revealed the importance of the cell environment [11, 8, 34, 26]. Recent breakthroughs in the micro/nanotechnology techniques, including the engineering of specific biomimetic matrices and substrates; in molecular biology, with the continuing discovery of new regulatory and transduction pathways, as well as in microscopy, with the development of new dynamic imaging techniques (FRET, FRAP, *etc.*), have on one hand accelerated knowledge acquisition on the mechanisms involved. In the other hand, these advances have further highlighted the complexity and highly integrated nature of cell motility. The up-to-date knowledge points out three essential and interrelated factors for motility: cell adhesion, cell cytoskeleton and cell environment.

Cell adhesions are the foci of cellular attachment [20]. They constitute the physical links between the extracellular matrix (ECM) and the actin cytoskeleton of the cell. They act as signalling

hubs by conveying both biochemical and mechanical signals between the cell interior and the ECM. The actin cytoskeleton is a highly dynamic structure of filaments and fibres which can assemble and disassemble in response to these signals. The cytoskeleton contractile properties, resulting from the association of actin and myosin, allow the cell to generate tension forces and transmit them to the ECM *via* the adhesion sites. This gives the ability for the cell to sense the nature and mechanical properties of its surroundings.

The application of the micro and nano technologies in biological experimentation have broadened our understanding on the adhesion-actin cytoskeleton interactions by providing a powerful means to control the extracellular environment [11], either chemically (*e.g.* substrate functionalization with different ECM proteins), mechanically (*e.g.* substrate or matrix rigidity manipulation) [26], geometrically (*e.g.* shape and pitch length of adhesive pattern) [13] or topographically (3D adhesive structures with pillars or lines) [33, 35].

In parallel, mathematical models have proven to be useful tools for decoding cell motility processes and many have been proposed over the last few years. Mathematical models first aim at capturing cell motility features and subsequently at predicting cell behaviour or its dependence on specific key parameters. Mathematical models also allow to screen, through numerical simulations, a wide range of hypotheses thus sparing cost and time dedicated to laboratory experimentation. Nonetheless, the mere complexity of cell motility as a collective process has forced mathematical modelling studies to isolate and address only one or very few parameters at a time. For instance, many physical models describe the architecture [19, 21] and orientation [29, 17] of a static cell structure, such as the actin stress fibres network. Deshpande *et al.* [5, 6, 23] went further by proposing a bio-chemo-mechanical model for cell contractility, coupling stress fibre and adhesion turnover. However whole-cell models are still rare and lack integration of all concurrent intra and extracellular coupling events involved in motility. For instance, Kuusela and Alt (2009) take into account the actin and adhesion dynamics but neglect the maturation process of stress fibres [18]. Conversely, Coskun *et al.* (2007) consider the stress fibre dynamics to the detriment of adhesion dynamics [4]. The model proposed by Stéphanou *et al.* (2008) [32] and further extended in [30], which provides the basis for this paper, includes adhesion dynamics but neglects the explicit coupling of actin cytoskeleton to adhesions.

In this paper, we propose to improve the above biomechanical model of cell motility [30], by coupling the cell adhesion dynamics to the actin cytoskeleton remodelling. This time, the formation and maturation processes of actin fibres from the initial filament growth and bundling to the resulting mature stress fibres is explicitly taken into account. We note that our model only applies to describe cell motility on 2D substrates since the formation of mature focal adhesions and stress fibres rarely occur in 3D matrices [9]. In order to investigate the coupling between adhesions and actin fibres maturation processes, a discretized extracellular environment (*i.e.* substrate) with adhesive patches is considered. The substrate is rendered adhesive only at specific sites under the form of patches with predetermined geometry, size and spatial organization. A regular hexagonal network of small square patches is considered. The size of the patches ($4 \times 4 \mu\text{m}^2$) is chosen so

as to allow adhesions to cluster and grow while forming isolated adhesive islands [13]. The size of these islands is big enough to promote the formation of stress fibres. A range of inter-patches distance for the network are considered from 10 to $24\mu\text{m}$ so as to modulate the lengths of the fibre architecture. This parameter is essential to decipher force generation in the cell.

In the next section of this paper (section 2), we will present the biological background. We focus on the current knowledge on adhesions and fibres maturation and turnover and describe the key elements and events that serve as the basis to build our computational model. The model hypotheses and its mathematical description are described in section 3. Section 4 presents the simulation results and investigates specifically the importance of the inter-patches distance for cell shape, motility and force generation. Section 5 comments on the main results of this work and presents further possible extensions to elucidate the many remaining questions that need to be addressed to understand the full complexity of cell motility.

2. Biological background

Cell motility relies on the interactions between three main components: the *actin cytoskeleton* whose fibres organisation depends on the formation and distribution of the *adhesions*, which are themselves strongly dependent upon the *extracellular environment* and more specifically on the extracellular matrix mechanical and chemical properties (Fig. 1).

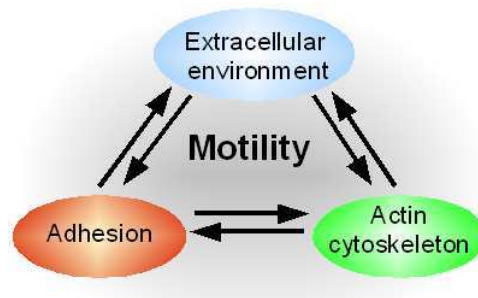


Figure 1: Adhesion, actin cytoskeleton and extracellular environment are three interacting elements controlling cell motility

To properly describe cell motility, it is therefore necessary to account for all three components and to investigate the tight coupling between them. In the subsequent sections we present the contribution of each of these three components from which our computational model is built.

2.1. Formation and maturation of adhesions

Adhesions are transmembrane proteins structures responsible for binding the cell to its environment, including other cells and the extracellular matrix. More than simple physical attachments, adhesions act as signalling hubs that detect, coordinate and transmit signals so that the cell can

adapt its behaviour to the cues of its surroundings. Consequently, abnormal cell behaviours are often related to defects in the signalling cascade. Since we are interested in the motility of isolated cells, we will only consider cell matrix adhesions in this paper.

Adhesions are highly dynamic structures evolving in size and shape. Nearly ninety distinct proteins have been identified to contribute to individual adhesions [41], some being specific to one adhesion type. They can then be classified depending on their molecular composition [39, 25, 20]. Adhesion precursors (AP) form spontaneously. They correspond to the binding of an integrin receptor to ECM proteins such as fibronectin or collagen. APs form preferentially at the cell periphery on glass substrates, that is at the lamellipodium. The lamellipodium is an actively moving cell area where the membrane spreads over the 2D substrate [28]. APs dissociate with the passing movements of the membrane or mature by recruiting new proteins such as talin and paxillin to form a more stable adhesion type called focal complex (FX), which is strong enough to anchor the membrane to the ECM. As FXs grow, other proteins such as α -actinin, FAK, VASP, Arp2/3 and vinculin are further recruited. These signalling and binding proteins allow the adhesion to nucleate actin filaments [36]. Mechanical stimulation on these adhesions, under the form of an increased tension force from the contractile cytoskeleton [10], causes adhesion maturation into focal adhesions (FAs) through the recruitment of zyxin and tensin. However, loss of tension leads to disassembly of these proteins. Although FAs are stable enough to support high stress from the contractile actomyosin complex required for cell translocation, they can also act as anchors restraining or even preventing cell migration.

2.2. Formation and maturation of stress fibres

Stress fibres are contractile actomyosin bundles and are the last stage of the fibre maturation process. They play a key role in cell motility by regulating adhesion turnover and exerting cell mechanical forces on the substrate. The maturation process of the actin cytoskeleton fibres involves the association of actin filaments with a range of specific proteins, such as myosin II, which confers the contractile properties of the actomyosin complex, tropomyosin, which regulates the binding of myosin to actin filaments, and α -actinin which is a cross-linker involved in the attachment of the actin cytoskeleton to the membrane [27].

Different types of actin bundles can be identified in the cell, depending on their location, orientation (radial or transverse), size and thickness, contractile properties, and attachment to the cell structures (other cytoskeleton fibres or adhesions). Four types of bundles have been identified and described so far (see [22] for a review):

- *transverse arcs*, formed at the leading edge of the cell by the assembly of short bundles of actin. The high density of a branched network of actin in the lamellipodium nucleated by the Arp2/3 complex and its rapid turnover involving many actin regulating proteins, generates numerous transverse arcs. Myosin II is essential for the actin arcs structures formation and stabilization in the spreading margins and it is incorporated as the arcs flow backwards through the lamellipodia towards the base of the lamella. Transverse arcs are not attached to

the substrate. Nonetheless, they can connect to substrate-related structure and transmit tension to the environment.

- *circular bundles*, formed by the accumulation of the transverse arcs as they reach the lamellipodia. This is occurring at the early stage of cell spreading, typically one hour after plating the cells [27]. The contractility of circular bundles regulates the formation of focal adhesions in the spreading process.
- *dorsal stress fibres*, resulting from the elongation of actin filaments at focal adhesions. α -actinin is a cross-linker involved in the formation of bundles of actin filaments. A dorsal fibre is therefore attached to a focal adhesion at one end and the other end elongates mostly in the radial direction by rising towards the dorsal surface of the cell [24]. It can ultimately connect to a circular bundle [27]. Despite the term "stress" associated to their name, dorsal stress fibres are not contractile, however they convey contractility once connected to a contractile structure (circular bundle or other contractile fibres).
- *ventral stress fibres*, formed mainly by the adjoining of two dorsal stress fibres. Ventral stress fibres are consequently anchored at both ends by a focal adhesion [14]. These fibres are located at the ventral side of the cell, that is next to the adhesion surface *in vitro*.

Figure 2 presents a diagram which synthesizes the main maturation phases of adhesions and fibres, as they will be implemented in the computational model. We make several simplifications. First, we do not consider the ventral stress fibres since they result from the adjoining of dorsal stress fibres only that latter fibre type is explicitly considered. Second, we strictly reserve the name of dorsal stress fibres (DSF) to contractile fibres. Consequently, unattached, that is non-contractile fibres are then named dorsal fibres (DF). Finally since the model is bidimensional, we do not differentiate the ventral side from the dorsal side of the cell. We assume that the fibre dynamic is all occurring in the same plane.

2.3. Cell environment

The cell environment determines cell fate and function [11]. It has been shown that, stem cells placed on substrates of different stiffness differentiate into distinct cell lineages [8]. Recent advances in micro and nano fabrication techniques and surface chemistry allow to construct artificial cellular microenvironments with precisely controlled mechanical characteristics, topography, and geometry. Specifically, the ability to manipulate the localization and shape of adhesive areas has expanded our ability to study the mechanisms governing cell shape [1]. Furthermore, discrete micropatterned substrates constructed with regularly distributed adhesive pillars or patches and coated with different binding proteins (*e.g.* fibronectin, collagen, etc.) are closer to the *in vivo* context, in which a cell adheres to the extracellular matrix when it encounters a binding protein. Using substrates with discrete adhesion sites, Guillou *et al.* (2008) [13] experimentally revealed the complex nature of membrane protrusion. First, filopodia grow until they reach an adhesion patch. Then filopodia consolidate and actin dynamics trigger membrane protrusion at the tip of the filopodia in contact with an adhesion site. By manipulating the shape and size of adhesive patterns

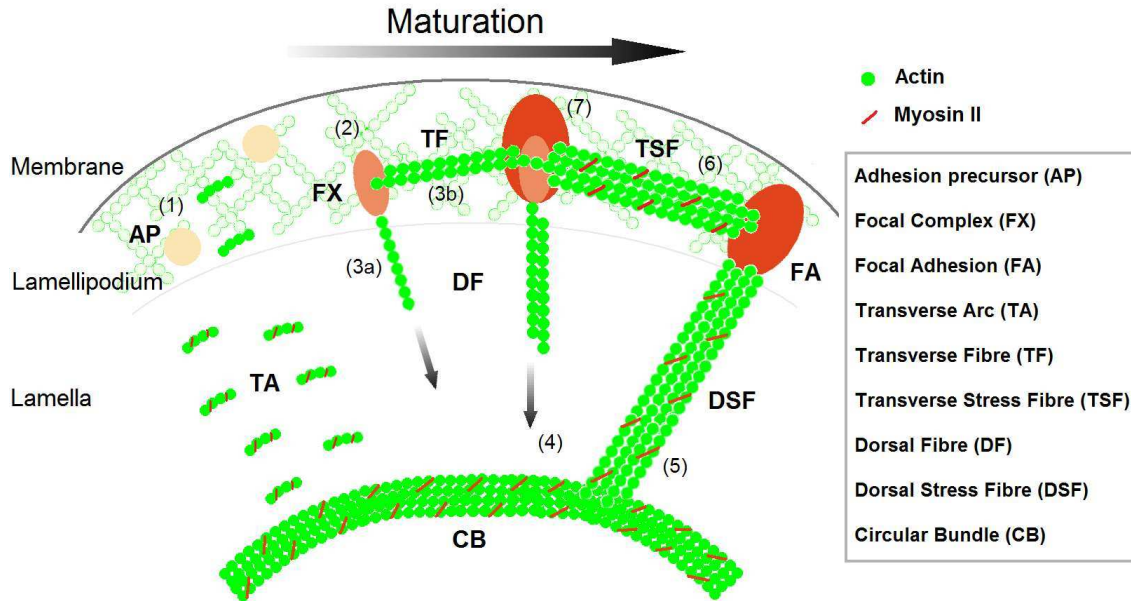


Figure 2: Seven different stages in the adhesion and actin fibre maturation can be clearly distinguished. Adhesion precursors (AP) form at the lamellipodium (1). In parallel, transverse arcs (TA) are formed from the shedding of the branched actin network. Then they flow towards the cell centre, become contractile by recruiting myosin II in the lamella and accumulate to form a circular contractile bundle (CB). An AP grows until it reaches a critical size and then matures into a focal complex (FX) (2). FXs are nucleation points of dorsal fibres (DF)(3a) and transverse fibres (TF)(3b) in the radial and tangential directions respectively. A DF elongates until it connects to the CB (4). This connection makes it become contractile and mature into a dorsal stress fibre (DSF) (5). A TF linked to a FA recruits myosin II to become contractile and matures into a transverse stress fibre (TSF) (6). A TSF is linked at both sides by a FA (7).

(e.g., V-, U-, or T-shape), it is now known that stress fibres work against the membrane tension by forming above non-adhesive areas [34, 15]. The clustering of FAs only at the substrate adhesive patches and their bridging with stress fibres influence cell polarization. Thus, cells geometry of actin cytoskeleton and consequently functional response, including migration, are directly depending upon adhesion site localization.

In order to decipher and understand how the maturation of cell adhesions is dependent upon the maturation of stress fibres and *vice versa*, we are considering a patterned extracellular environment formed by discrete adhesive patches, regularly arranged, in order to control the positioning and size of the adhesion zones of the cell which will in turn restrain the fibre lengths. By varying the inter-patches distance we are able to study how the environment is conditioning the stability of the cell shape and how this is related to cell force regulation, that is the magnitude of the force that the cell is able to develop and sustain.

Our model for adhesion-fibre coupling is essentially based on results from the literature described in this section. However, we performed some preliminary experiments to assess the behaviour of fibroblast cells on the adhesive arrays we implemented and tested in our model. The aim of those experiments is to provide the qualitative baseline for our simulations. Murin 3T3 fibroblast cells are plated on a regular hexagonal network of adhesive patches with varying inter-patches distance (see Appendix A and B for details on cell culture and microfabrication of the adhesive patches respectively).

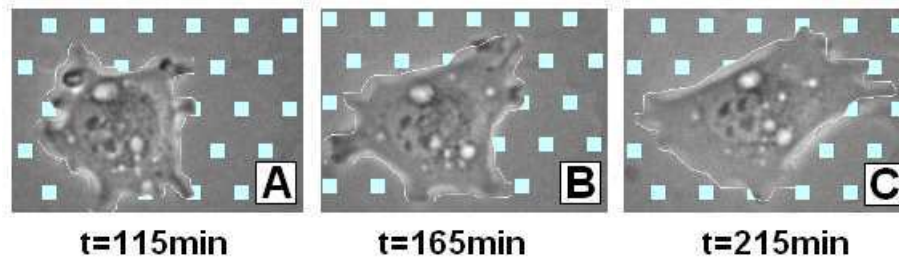


Figure 3: Successive snapshots of a 3T3 fibroblast cell plated on a network of adhesive patches, taken at time $t = 115\text{min}$ (A), $t = 165\text{min}$ (B) and $t = 215\text{min}$ (C). The inter-patches distance is $10\mu\text{m}$ and the patch size is $4 \times 4\mu\text{m}^2$. Adhesive patches are highlighted by graphical post-treatment with PhotoFiltre ©. The reference time ($t = 0$) corresponds to the instant of cell deposition on the substrate.

Figure 3 shows a typical example of the evolution of the cell shape on discrete adhesive patterns. The cell initially exhibits a round shape with several short protruding zones (A). The cell progressively spreads and forms new adhesions with more distant patches (B) which will ultimately constrain its shape to a more polarized and geometrical shape (C).

3. Model

Cell motility and the emergence of stable cell morphologies on discrete adhesive patterns are investigated with a computational model. The model is based on a set of partial differential equations to describe continuous cell membrane deformations resulting from the competition between protrusion and retraction forces, conditioned by actin turnover, with a cellular automaton. The latter integrates the inter-related maturation of focal adhesions and stress fibres which are by nature discrete components in the cell. This work thus further extends successive works by Stéphanou *et al.* (2004, 2008, 2009), where large membrane deformations [31], adhesion maturation [32] and the formation of actin stress fibres [30] were successively addressed.

3.1. Cell membrane deformations

The model considers the two-dimensional annular domain bounded by the cell nucleus on one side and the plasma membrane on the other side, with the latter defining a free-moving boundary. We

denote by $L(\theta, t)$ the width of the annular domain along any radial direction θ ($0 \leq \theta \leq 2\pi$) and time t (Fig. 4), $a(\theta, t)$ is the free actin concentration at location θ and time t in the lamellipodium and $v(\theta, t)$ represents its tangential velocity. Periodic boundary conditions apply to these variables.

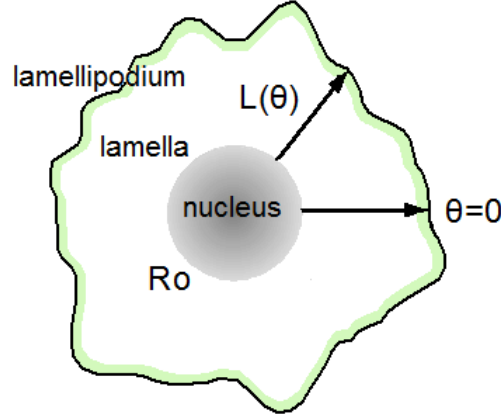


Figure 4: Theoretical representation of the cell. The cell nucleus (in grey) is represented by a circular domain with radius $R_0 = 7\mu m$. Two zones are distinguished (i) the lamellipodium which is the narrow peripheral zone (green area) containing a dense network of interconnected actin filaments, and (ii) the lamella which is the zone located between the lamellipodium and the cell nucleus. Polar coordinates are used. The horizontal axis corresponds to $\theta = 0$. $L(\theta)$ is the distance between the nucleus and the membrane in any angular direction θ .

The normalized system of partial differential equations ruling the movements of the cell membrane is given by:

$$\frac{\partial}{\partial t}(La) = -\frac{\partial}{\partial \theta}(Lav) + L(1 - a) \quad (3.1)$$

$$av = \frac{\partial}{\partial \theta} \left[\mu a \frac{\partial v}{\partial \theta} + \sigma(a) \right] \quad (3.2)$$

$$\delta(a) \frac{\partial L}{\partial t} = F_{out} + F_{in} + \kappa \quad (3.3)$$

where the two first equations describe the actin turnover and its displacement in the lamellipodium, with μ the viscous coefficient for the cytoplasm and $\sigma(a) = \psi a^2 \exp(-a/a_{sat})$ the contractility of the actomyosin network, which locally depends on the actin density and whose magnitude is controlled by the contractility coefficient ψ . The third equation describes the movements of the cell membrane resulting from the force equilibrium, where $\delta(a)$, κ , F_{out} and F_{in} represent the cell adhesive condition, curvature-induced tension of the membrane, the protrusion and the retraction forces respectively. These components are modelled on the basis of the following assumptions:

- the formation of an adhesion is translated by an increase friction between the cell membrane and the substrate, on top of the base friction assumed to be linearly depending upon the

local actin density, that is $\delta(a) = a + \alpha\delta_{AP}$. α is the friction coefficient and $\delta_{AP} = 0$ or 1 whether there is an AP, only adhesion type allowed to slide relatively to the substrate, or not. If the adhesion is a FX or FA then the membrane is tethered to the substrate which means $\partial L/\partial t = 0$;

- the cell membrane and the underlying actin network in the lamellipodium form a membrane-actin cortex. This cortex can be considered as a reticulated structure modelled by a succession of springs between a discrete number of membrane points. The tension of each individual spring i is given by $f_i = -k_m(l_i - l_0)$ with k_m the elasticity coefficient of the membrane, l_i the membrane length between two consecutive points and l_0 the resting length between points of the unstretched membrane (circular cell shape). The resulting curvature-induced tension influencing the cell membrane radial displacement are thus given by $\kappa = \hat{f}_{\text{left}} + \hat{f}_{\text{right}}$, where \hat{f}_{left} and \hat{f}_{right} are the radially projected contributions of the left and right springs at the considered membrane point, with $\hat{f} = f \cos \phi$, ϕ being the projection angle;
- the protrusion of the membrane is primary due to the cell hydrostatic pressure β . This term is reinforced by a repulsive force from the circular bundle, with intensity β_b , when the membrane approaches its vicinity, that is $L(\theta) \leq R_{\text{CB}} + \varepsilon$, R_{CB} being the radius of the circle enclosing the CB and ε the tolerance approach value. The resulting protrusive term is thus given by $F_{\text{out}} = \beta + \beta_b\delta_{\text{CB}}(\theta)$ with $\delta_{\text{CB}}(\theta) = 0$ or 1 whether the vicinity condition of the membrane with the bundle is verified or not at membrane point θ ;
- the retraction is due to the actin network-membrane attachment and linearly depends upon the actin density, that is $-\gamma La$, with γ the elasticity coefficient for the actin network in the lamellipodium. Additionally if a TF is attached to the considered membrane point, it creates a tension force, similar to the membrane spring tension, modelled by $F_{\text{fibre}} = -k_f(l_f - l_{f0})$, with k_f the elasticity of the fibre, l_f its length and l_{f0} the resting length of the fibre corresponding to its unstretched state. Once again, only the radial component of the force which contributes to cell membrane movements is considered, that is $\hat{F}_{\text{fibre}} = F_{\text{fibre}} \cos \phi$, with ϕ the radial projection angle. The resulting retraction force is then given by: $F_{\text{in}} = -\gamma La + \hat{F}_{\text{fibre}}$. If the TF ultimately matures into a TSF, we assume that the tension forces of the fibre underneath the membrane is strong enough at each of its ends so that the membrane spontaneously sticks to the newly created TSF.

3.2. Formation and maturation of adhesions and fibres

The formation, maturation and fate of adhesions and actin fibres are tightly linked. That is why it is difficult to describe them independently. In this section we will describe how their dynamics (outlined in figure 2) is coupled to the model of membrane deformations and integrated into a computational framework (Fig. 5). We retain the same notation as in figure 2 to describe the seven maturation stages.

Three adhesions types are differentiated in the model, depending on their maturation level, from AP to FA *via* FX. It is assumed that APs form spontaneously when the cell is in contact with an adhesive patch (Fig. 5.(1)). Thermal fluctuations can account for the energy required for the formation of this type of transient and weak adhesion type. APs can easily break if the membrane moves too quickly or above a non-adhesive zone. We assume that AP matures into FX through a clustering process. The cluster grows until it reaches a critical size S_{crit} above which it becomes a FX (Fig. 5.(2)).

FXs are stronger adhesions, which adhere the membrane to the substrate locally and therefore prevents membrane movements. Moreover, FX promotes the formation of a DF (Fig. 5.(3a)), that elongates in the radial direction (*i.e.* towards the cell centre) and the formation of a TF that is assumed to appear spontaneously between two consecutive and not too distant FXs (Fig. 5.(3b)), thanks to the readily available underlying actin network in the lamellipodium.

Finally, when mechanically solicited FX can mature into FA. We consider an implicit solicitation, occurring when the DF connect to the contractile CB (Fig. 5.(4)). In that case, traction force is conveyed by the DF from the CB to the parent adhesion FX which makes it mature into a FA, while the DF becomes a DSF (Fig. 5.(5)).

In parallel, when an adhesion matures into a FA, the TF connected to that FA spontaneously matures into a TSF (Fig. 5.(6)). TSFs are contractile and pull on the adhesions at both of their ends, thus allowing the FXs to mature into FAs (Fig. 5.(7)).

Stress fibres either DSF or TSF, are contractile and generate tension forces. If the resulting force R_f from these fibres measured at a FA is below a threshold tension then the FA disassembles triggering the simultaneous disassembly of all fibres connected to it.

Limitations

We note that our model exclusively considers peripheral adhesions since it is widely acknowledged that cell adhesions mainly form at the cell periphery, that is in the lamellipodium [12, 37] if we except podosomes and fibrillar adhesions [2]. Integrins located at a distance less than $0.5\mu\text{m}$ from the cell edge have been shown to have a higher affinity for the extracellular matrix. They consequently lead to more stable integrin-cytoskeleton links and are reinforced under force stimulation [16]. The formation of adhesions with patches located underneath the cell is not considered in the model. We thereby implicitly assume that they play a secondary role that remains to be investigated. We also note that a critical size criterion for integrin clusters is used in the computational model to mark the transition from AP to FX. However the model does not describe the physical growth of FX and FA. The growth of the adhesions allow them to progressively increase their resistance to traction that we take into account in a stepwise manner in the model without explicitly describing any changes in the adhesions size.

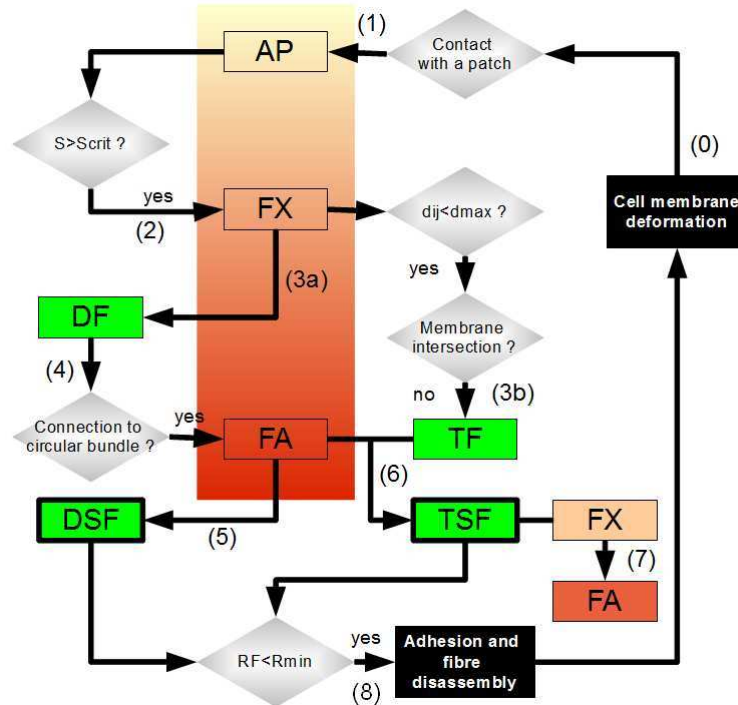


Figure 5: Flowchart describing the formation and maturation of both adhesions and actin cytoskeleton. The membrane bends (0) and APs are recruited as soon as contact with an adhesive patch is established (1). After reaching a critical size S_{crit} , the cluster of APs forms a FX (2). Then two events may occur. In the first case, a DF grows from the FX (3a) until it reaches the circular contractile bundle (4). This contact makes this DF able to exert forces on the FX which matures into FA. This maturation triggers the maturation of the DF into a DSF (5). In the second case, after the formation of an FX (2), if another FX is sufficiently close (closer than d_{max}) and if there is space for a filament to form (that is if the membrane is not an obstacle, this is translated in the model by no intersection between the fibre and the membrane), a TF spontaneously grows between the two neighbouring FXs (3b). If a TF is connected to a FA at one end, the TF matures into a TSM (6) and in turn, the FX at the other end of the TSM matures into FA (7). The resulting force R_F exerted by the stress fibres (DSF and TSM) on the adhesions sites is responsible for their becoming (8). If the force is too weak (below R_{min}) then the adhesion and related fibres disassemble.

3.3. The circular bundle and fibre survival

The formation of the CB is not explicitly described in our framework. It is assumed that while the cell starts to spread on the substrate, transverse arcs generated in the lamellipodium thanks to the turnover of the branched actin network, flow from the cell periphery to the cell centre and spontaneously gather to form the CB according to the scenario proposed in [27]. The CB is therefore quickly appearing during the first few iterations of the simulated cell.

The role of the CB, as demonstrated by Senju and Miyata (2008)[27], is essential for the organisation of the cell stress fibres which will be ultimately responsible for shaping the cell architecture at the stationary state. Based on these experimental observations, we integrate within our computational framework, the key events, which are the maturation of DF into DSF once they connect to the CB. In this scenario the CB, which is a contractile structure, "transmits" its contractile properties to the fibres, which in turn allow the adhesions to mature.

Each time a new fibre connects to the CB, it pulls on it as a spring and induces distortion and rotation. In the model, we choose to describe the bundle as a "deformable" ellipse. At each iteration, the displacement of each bundle point due to the traction of the fibres is calculated. Then the scattered set of displaced points are fitted with an ellipse using a linear least-squares method.

As the CB moves and deforms, some fibres initially linked to it can break whereas other fibres are now close enough to connect to it. In order to decide on the fate of each fibre, a set of criteria is defined as follows:

- we first evaluate the rotation angle of the ellipse. If it is bigger than 45 degrees then we proceed with the next criterion otherwise the connections with the fibres can be broken or created depending on the new distance between the filament ends and the bundle (Fig. 6(1)).
- second, if the DSFs form an angle smaller than 45 degrees with the major axis of the ellipse, then DSFs are maintained otherwise they are disassembled (Fig.6(2)).

3.4. Spatiotemporal scaling of the model

Spatial scaling is based on measurements performed on 3T3 fibroblasts. The radius R_0 of the cell nucleus which remains relatively constant from one cell to another is chosen as the reference length for our model to convert pixels into micrometers. Measurements give $R_0 = 7\mu m$.

Temporal scaling is a more delicate issue. We need to identify a process with the appropriate time scale to account for both fast and quick events in the cell. We choose as the reference time scale the elongation rate of the DF, which has been measured experimentally at $0.34 \pm 0.15 \mu m / min$ [22, 27]. With this value, we identify the time with the number of iterations it takes in the simulation to grow such a fibre on the typical length of $R_0 = 7\mu m$. According to the growth rate this time corresponds to $21min$ for 600 iterations, which means that 1 iteration corresponds to $0.03min$.

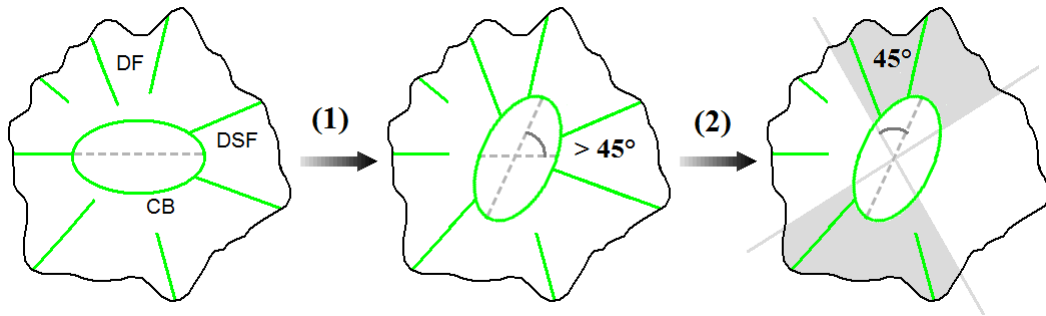


Figure 6: Schematic drawing of the cell to explain the rules managing the assembly or disassembly of DSF. DFs make connections to the circular bundle CB and thus become DSFs which distort it. The new position, shape, and rotation angle of the ellipse major axis are calculated. (1) If the angle of rotation is bigger than ± 45 degrees, we apply another criterion angle which governs DSF disassembly. (2) Following such a rotation of the bundle, if the angle formed between the major axis of the bundle and the DSFs is bigger than ± 45 degrees then the DSFs are disassembled (outside the grey areas).

4. Results

The aim of the simulation results presented in this section is two-fold. First, we focus on the importance of the distance between consecutive adhesive patches on the resulting cell morphologies and their stability. Second, we look in more details how the resulting shape is related to the mean force generated by the fibres on the focal adhesions. The influence of the parameter ψ governing the intensity of cell contractility is investigated.

The simulations are performed by changing the parameters β and γ ruling the balance between membrane protrusion and retraction respectively. This allows to account for a wider range of potential cell phenotypes. For example, a protrusive phenotype characterized by extensive cell spreading with longer and more localised membrane extension can be simulated with $\beta > \gamma$ (protrusion dominates over retraction). In the other hand, less protrusive cells, that is with smaller membrane extensions and more rounded shapes are simulated with $\beta = \gamma$. Fibroblasts are typically good representatives of the protrusive phenotype, since these cells spread extensively with large membrane protrusions when plated *in vitro* on a 2D substrate. In the other hand and in the same experimental 2D *in vitro* conditions, keratinocyte fits well with the weakly protrusive phenotype. We will therefore use these two representative cell types to illustrate and compare the two protrusive phenotypes in our simulation results.

In our simulations of cell deformation, the initial condition corresponds to a small random perturbation ϵ ($|\epsilon| \leq 0.05$) of the homogeneous steady state, with:

$$L(\theta, 0) = 1 \quad a(\theta, 0) = 1 \pm \epsilon \quad v(\theta, 0) = 0$$

This corresponds to the state of a cell freshly seeded on the patterned substrate right after trypsinization from its culture dish. The cell shape is circular and actin density relatively homogeneous. At this time, neither adhesions nor actin structures are formed.

The parameters of the model used in the numerical simulations are given in appendix C.

4.1. Influence of the inter-patches distance on cell morphology

The deposition of a fibroblast-type cell ($\beta > \gamma$) on a patterned substrate with an inter-patches distance of $10\mu m$ is simulated. The simulation presented in Figure 7 shows the initial round cell which spreads on the patterned substrate over time by forming adhesions and fibres to ultimately reach a constrained steady state in about 71 minutes. Initially, the membrane making contact with the substrate as it spreads, induces the formation of APs at adhesive sites (Fig. 7.A). At this early stage of cell spreading, TAs flow towards the cell centre where they coalesce to form the CB (Fig. 7.B). Although this phenomenon is represented graphically in the simulation, it is not described explicitly in the model. APs mature into FXs as soon as they reach a critical size. Concomitantly to APs maturation into FXs, TFs form between consecutive FXs while DFs emerging from the FXs start to grow radially (Fig. 7.B). Once the DFs reach the CB they allow FXs to mature into FAs and consequently become DSFs. As a result, all TFs related to newly formed FAs mature into TSFs (Fig. 7.C). The maturation of adhesions and fibres leads to a fully constrained polygonal cell shape where the membrane is "trapped" by the underlying TSFs (Fig. 7.D).

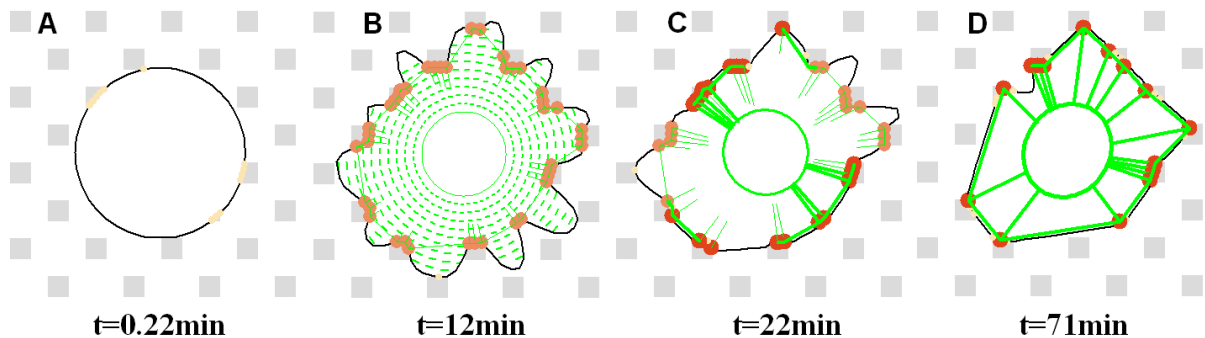


Figure 7: Simulation of the different spreading phases of a cell on an adhesive network with an inter-patches distance $d = 10\mu m$ ($\beta = 0.8$, $\gamma = 0.5$, $\psi = 5.4$). The four main phases are the cell deposition on the substrate (A), the formation of the CB (B), the maturation of the adhesions and fibres (C) and the resulting stationary state (D). The different types of adhesions (APs in yellow, FXs in orange and FAs in red) and actin structures (thin lines: non-contractile fibres; thick lines: contractile fibres) are depicted.

On a qualitative basis, the simulated evolution of the cell shape (Fig. 7) strongly resembles our experimental observation of a cell plated on the same patterned substrate (Fig. 3). Although the process starts much quicker in the simulation, since the cell is assumed to make immediate links

with the substrate. Experimentally, the cell needs first to recover from the trypsinization process.

Figure 8 presents the different resulting cell states obtained depending on the inter-patches distance d of the adhesive pattern from 10 to $24\mu m$. For each case, about ten cells have been simulated by virtually seeding each of them at a random location relative to the adhesive pattern. The resulting shapes are different while keeping the same typical features as shown by the two cells represented in each case. The simulated cells generally tend to adopt polygonal shapes dictated by the distribution of the adhesions on the underlying adhesive pattern. As d increases, the cell shapes are less constrained, part of the membrane remains free to move since no transverse fibres could be generated to constrain it. The increased inter-patches distance makes it more difficult for the cell to create FAs. Consequently the cell membrane is only partially constrained, which means that part of the membrane remains free to move (protrude and retract). The cell thus either quickly reaches a fully constrained stable stationary state for small inter-patches distance up to $14\mu m$ (Fig. 8.A, B, C) or an oscillating state for bigger values of d , where the membrane forms pikes that explore the environment in the search of adhesive zones (Fig. 8.D, E, F, G). Transitory adhesions occur, but none are able to mature since the membrane retracts before a fibre had time to form and stabilize the membrane. Figures 8.H and 8.I show the evolution of the cell spreading area with time for two oscillating cells. The curves show that the membrane exploration process is mostly random for these two cells. However more periodical oscillations can also be observed for more constrained cell states.

Figure 9 compares the evolution of the cell spreading area with time for a range of inter-patches distances from $d = 10$ to $20\mu m$ and for two protrusion/retraction balance rates $\beta = \gamma$ and $\beta > \gamma$ which are used to approach keratinocyte and fibroblast phenotypes respectively.

The mean area of the cells measured at the stationary state is obviously bigger for an increased protrusion/retraction rate. A 60% increase of this rate leads to a 37% increase in the mean cell area from $554 \pm 59\mu m^2$ for $\beta = \gamma = 0.5$ (Fig. 9.A, C, E, G) to $758 \pm 54\mu m^2$ for $\beta > \gamma$ with $\beta = 0.8$ and $\gamma = 0.5$ (Fig. 9.B, D, F, H). However despite this difference in the protrusion/retraction rate, used to account for different cell phenotypes, the cells exhibit stable stationary states for inter-patches distances d up to $12\mu m$ corresponding to fully constrained cell morphologies (Fig. 9.A, B, C, D). For larger d , the cells exhibit oscillating stationary states around a mean surface area (Fig. 9.E, F, G, H). As a consequence, it appears that as long as the cell is able to develop stable adhesions and fibres, it can be fully constrained by its environment whatever its ability to protrude and retract its membrane (that is, its phenotype) provided that the adhesive patches are not too far apart. Indeed, when d is small, the cell can easily find sites to form multiple adhesions in all directions as it spreads. However as d increases, the cell has difficulties to reach multiple adhesion sites in an homogeneous way. Therefore the cell adhesions and fibres mature anisotropically, favouring the direction where the first adhesive sites were encountered. The membrane is thus only partially constrained since the free membrane cannot reach the most distant adhesive sites or if it can, it cannot grow DFs quickly enough, that is before the FXs are disassembled due to their limited lifetime.

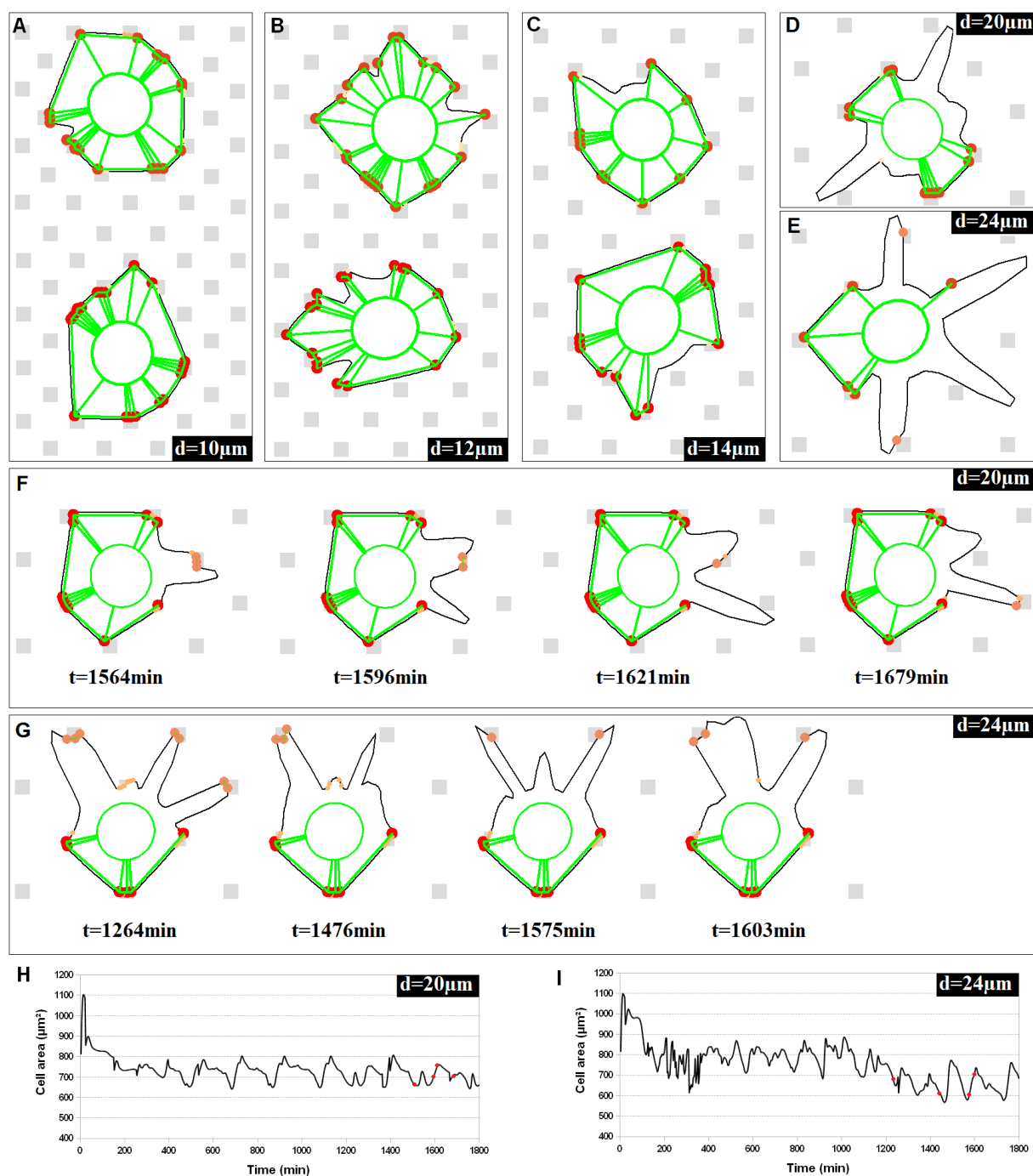


Figure 8: Resulting cell morphologies simulated for different inter-patches distances d ($\beta = 0.8$, $\gamma = 0.5$, $\psi = 5.6$ except for A (top), C (bottom) and F where $\psi = 5.4$). Stationary states are reached at times: $t = 63\text{min}$ (A, top) $t = 59\text{min}$ (A, bottom), $t = 71\text{min}$ (B, top), $t = 126\text{min}$ (B, bottom), $t = 120\text{min}$ (C, top), $t = 101\text{min}$ (C, bottom), $t = 363\text{min}$ (D) and $t = 294\text{min}$ (E). Cells in D, E, F and G are only partially constrained and the free membrane keeps oscillating. H and I show the oscillations associated with cells in F and G respectively. The spots on the curves H and I correspond to the snapshots in sequences F and G.

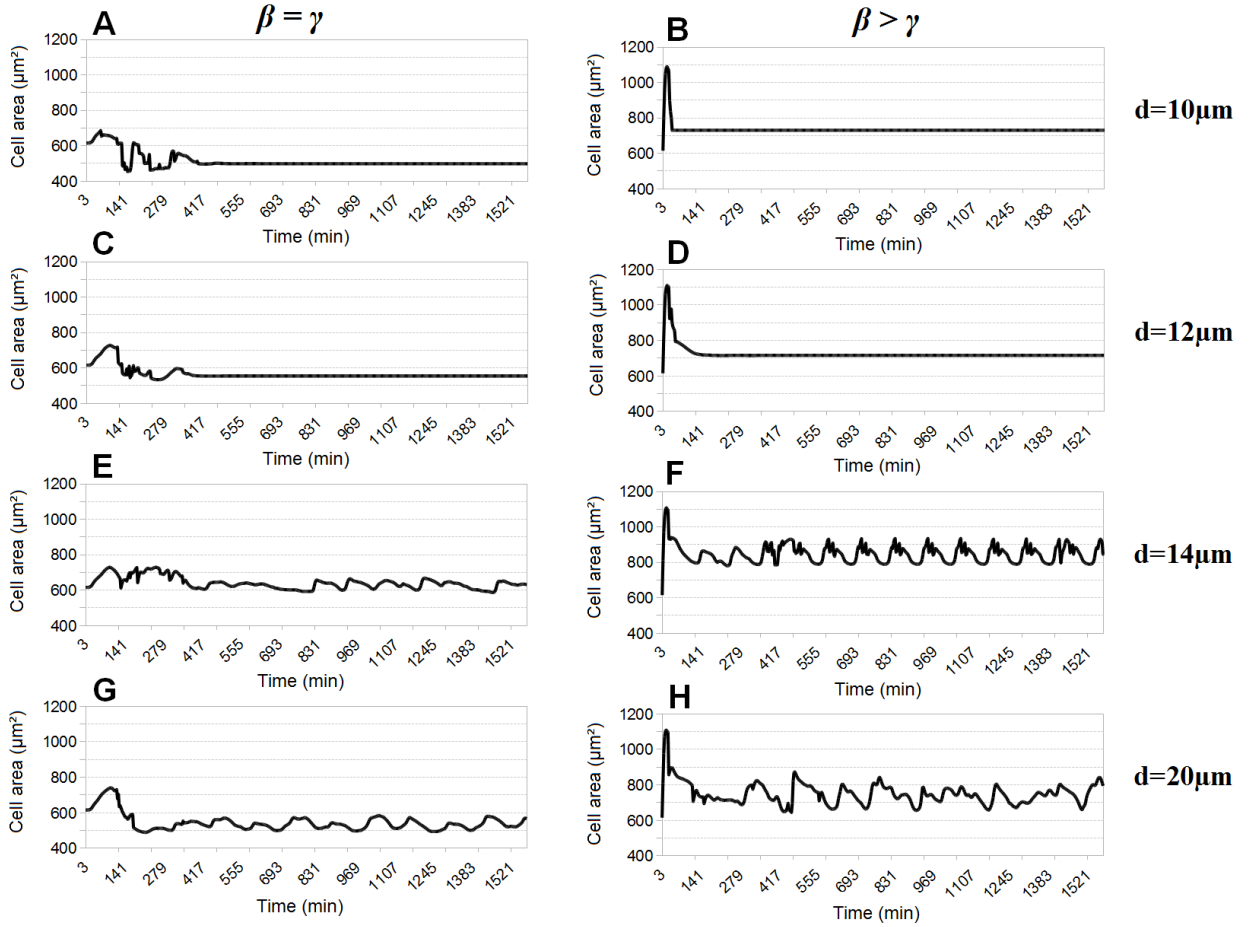


Figure 9: Temporal evolution of the cell area for different inter-patches distances d (with $\psi = 5.6$). Left column: $\beta = \gamma = 0.5$; right column: $\beta > \gamma$ with $\beta = 0.8$ and $\gamma = 0.5$.

4.2. Influence of cell contractility on FA maturation and force generation

FA adhesions survival depends on a mechanical force. If the resulting traction force exerted on a FA is below a threshold force ($R_F < R_{\min}$), then the adhesion disassembles. Thus to investigate the forces at stakes in the cell, it is first necessary to assess the conditions that lead to the maturation of the cell adhesions into FAs.

Figure 10 shows that for small inter-patches distance up to $d = 12\mu\text{m}$ more than 80% of the FXs formed mature into FAs, whatever the protrusion/retraction balance (*i.e.* cell phenotype) and cell contractility. For the protrusive phenotype (*i.e.* fibroblast-like) the maturation rate is even higher, above 90% (Fig. 10.B).

For higher values of d , more heterogenous cases are found with some cells maintaining a high

level of maturation of their FXs into FAs and some other with a significantly lower maturation rate down to 60% for $d = 14\mu m$. This goes further down as d increases to $20\mu m$ with 40% for $\beta = \gamma$ and 20% for $\beta > \gamma$, uncorrelated with the contractility ψ .

It appears that FXs maturation into FAs is high for constrained cell shapes whereas stationary oscillating states are rather correlated with smaller maturation rates. The discrepancies for the FAs maturation rate observed for higher inter-patches distance can be explained as follows:

- if the free cell membrane while oscillating, encounters an adhesive patch then it can form FXs. However these FXs cannot mature into FAs if the time it takes for the DFs to grow exceed the adhesion lifetime. That happens if the adhesion is located too far away from the cell body. In that case cell membrane oscillations lead to a sharp decrease of the FAs maturation rate, since lots of FXs are formed in the course of membrane oscillations but unsuccessful to mature into FAs. This is mainly the case for the protrusive phenotype where the cell membrane explores a wider environment(Fig. 10.B).
- on the other hand, if the free cell membrane oscillates without meeting an adhesive patch, then the maturation rate of FXs into FAs is high since it only corresponds to the quick and successful maturation process related to the constrained side of the cells. It is the case for non-protrusive phenotype where the movements of the membrane are reduced and the cell cannot make adhesions with distant patches. Consequently less adhesions are formed but all of them mature into FA, either quickly for higher contractility through contractile fibre "contamination" or slowly for lower contractility thanks to the reduced movements of the membrane that preserve the forming adhesions. Intermediate contractility can potentially leads to intermediate situations with decreased maturation (Fig. 10.A).

These simulation results tend to suggest that the contractility plays a secondary "modulating" part in the adhesion maturation process. The interpatch distance and the phenotype are more important according to the model.

Figure 11 presents the evolution of the mean traction force exerted by the stress fibres (DSF and TSF) on the FAs as a function of the inter-patches distance d . The curves represent the average values of measurements performed in three simulated cells. Once again, two different balances for the protrusion/retraction (*i.e.* cell phenotypes) are considered as well as three different values for the cell contractility coefficient ψ .

The forces generated by the stress fibres on the FAs are relatively homogeneous since they do not vary by more than 10% around a mean value of $2.15n.u.$

Numerical simulations reveal that, whatever the protrusion/retraction balance and cell contractility, the traction force generally tends first to increase with d , reach a plateau and then decrease as d keeps on increasing. Considering that the forces are generated by the DSFs and TSFs, both modelled as springs whose tension magnitude is proportional to their length, the bell-shape profiles can be explained as follows:

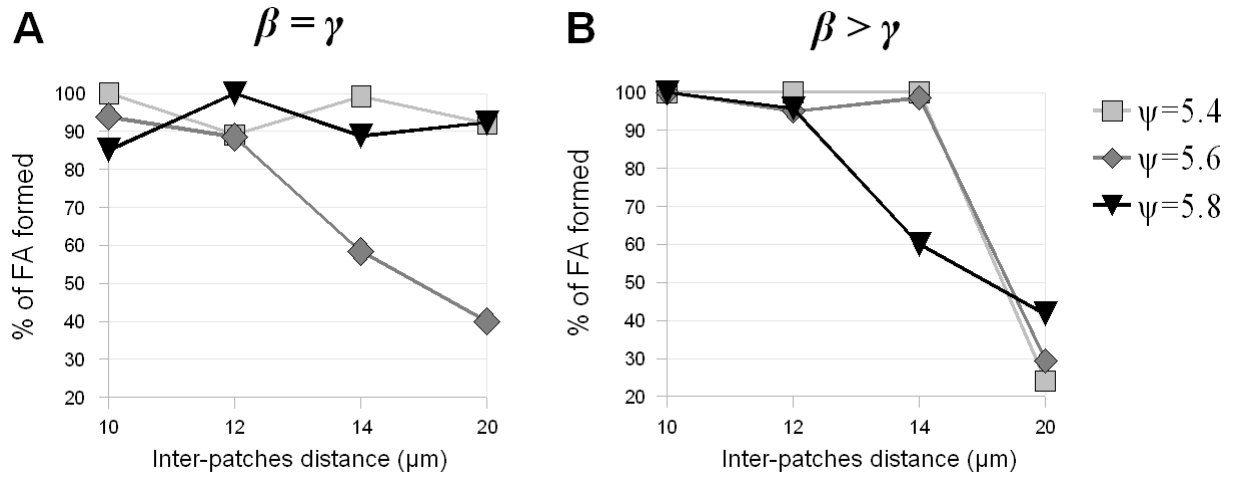


Figure 10: Percentage of FXs which have matured into FAs, evaluated at the stationary state, as a function of the inter-patches distance d . (A) $\beta = \gamma = 0.5$; (B) $\beta > \gamma$ with $\beta = 0.8$ and $\gamma = 0.5$. Three values of the cell contractility coefficient ψ are considered in each case.

- if d is small ($d < 12\mu\text{m}$), the lengths of the DSFs and TSFs are also small and so are the generated tension forces,
- if d is intermediate ($12 \leq d \leq 14\mu\text{m}$), the lengths of the stress fibres increase which induces a higher magnitude of forces,
- finally, if d is big ($d > 14\mu\text{m}$), as seen previously, it becomes more difficult for the cell to establish homogeneously stable adhesions and fibres. Consequently, the cell shape is more anisotropic with a partially constrained area and a partially free membrane. The constrained area is developing next to the CB, which means that many more shorter DSFs are created and very few longer TSFs (Fig. 8.D, E). This results in the decrease of the magnitude for the mean traction force.

Whereas the curves profiles for different values of the contractility ψ remain very close in evolution and magnitude, for the protrusive phenotype (*i.e.* fibroblast-like), they differ quite significantly in magnitude for the other keratinocyte-like phenotype. In that latter case, the maximum of the mean traction forces (corresponding to $d = 12\mu\text{m}$) increases with the cell contractility from 2.10 up to 2.36 *n.u.*, which corresponds to a 0.26 *n.u.* increase between ψ -curves. For the fibroblast case, the maximum force difference is twice smaller with 0.13 *n.u.*

This phenomenon is mostly explained by the fact that the contractility effect is overcome by the predominantly protrusive cell phenotype. Large membrane extensions dominate the cell movements and the contractility only plays a reduced role in the force generation according to the model.

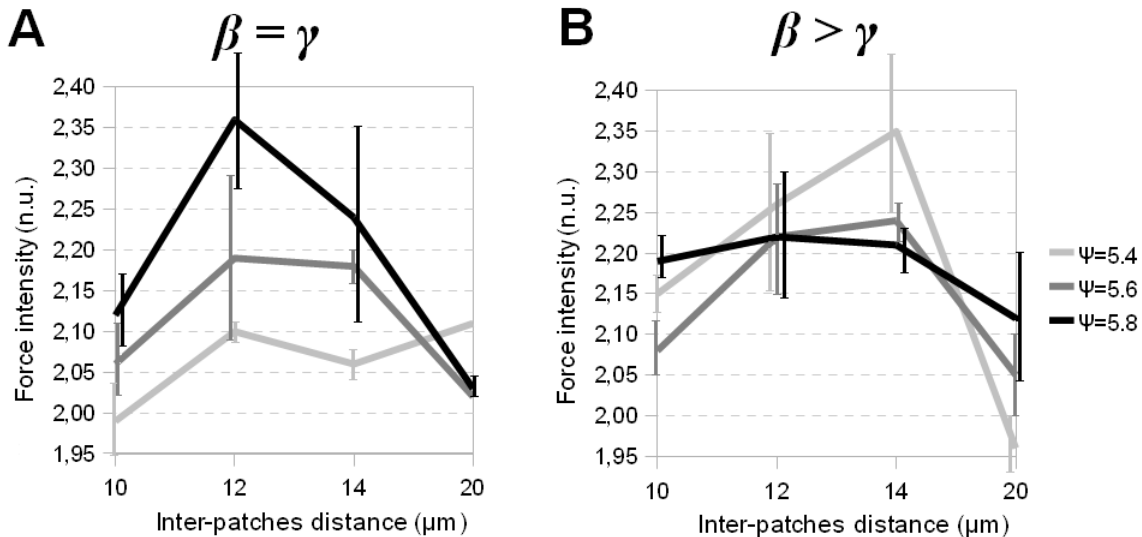


Figure 11: Evolution of the mean traction force (in normalized units: n.u.) from the stress fibres on each FA for different inter-patches distance d and evaluated at the cell stationary state. Measurements are averaged for 3 simulated cells for each point of the curves (vertical bars represent the standard deviations around the mean values). (A) $\beta = \gamma = 0.5$; (B) $\beta > \gamma$ with $\beta = 0.8$ and $\gamma = 0.5$. Three values of the cell contractility coefficient ψ are considered in each case.

5. Conclusions

In this paper, we propose a model that couples the formation, maturation and turnover of cell adhesions and actin fibres. For that we take into account the successive stages of adhesion maturation from adhesion precursors to focal complexes and ultimately to focal adhesions; as well as the actin fibres evolution from the growing filaments, their bundling and the acquisition of contractility to form the stress fibres. The two events are closely interdependent and coordinated as recently highlighted by a number of studies [27, 22] which serve as a basis for the development of our computational model.

Since our aim is to investigate the relationship between adhesions and fibres, a means to control the position of the former and the resulting architecture of the latter is to use specifically engineered substrates to discretize the cell adhesive environment. For that a network of regularly arranged adhesive patches is considered. This allows to isolate and position the different adhesive sites in the cell from which the fibres grow and consequently allows to generate more organized fibre structures. It is therefore easier to decipher the mechanisms ruling the cell shape through the control acquired on them to generate this shape. Moreover, the use of such patterned substrate gives us a mean to investigate the impact of the environment on the cell internal organization, by varying the distance between consecutive adhesive patches.

In a first stage, simulations were realised to calibrate the model parameters in order to qualita-

tively match our experimental observations of cell spreading on adhesive patches. The model was successful to reproduce the successive spreading phases and the resulting constrained cell shape, where the square shape obtained for the virtual cell resembles the real cell for the same environmental conditions.

We then proposed to enlarge our analysis with the systematic comparison between two different cell phenotypes. Those could be approached by tuning the model parameters defining the cell intrinsic mechanical properties. In that specific case, acting on the parameters which govern the protrusion/retraction balance on the membrane, allows to differentiate two major cell types, a protrusive type and a non-protrusive one. The protrusive type characterizes fibroblast cells, which easily spread on 2D substrates with large membrane extensions. The non-protrusive type on the other hand, fits well with keratinocytes which are round-shape cells, with narrow membrane extension on 2D substrates.

The simulations realised revealed that whatever the cell phenotype (which more precisely reflects here the cell protrusive potential), the cell shape is essentially governed by the inter-patches distance of the adhesive pattern. For distances up to $12\mu m$ the simulated cell shapes are fully constrained by the stress fibres and the cell morphologies are mostly geometrical and stable. However for higher inter-patches distances the cell is unable to homogeneously develop stress fibres and the resulting shapes are only partially constrained. Free partial cell membrane movement leads to cell shape oscillations.

One crucial point of this work is the question of force generation in the cell. For that we evaluated the mean traction force generated at each focal adhesions. We investigated in particular the influence of the parameter governing the magnitude of the cell contractility. We found that the level of force remains relatively homogeneous, with only a 10% maximum variation around the mean value. However the force *versus* inter-patches distance profiles, interestingly revealed bell-shaped curves, increasing for small distances and decreasing for large ones with a maximum always found for the critical distance of $12\mu m$. Indeed the size of the stress fibres which generate the tension forces depends on the inter-patches distance. Small distance leads to small fibres with reduced forces whereas larger distances lead to longer fibres and higher forces up to the point where the fibres cannot form homogeneously anymore in the cell. The lifetime of the adhesion supporting their growth being limited, long fibres cannot mature quickly enough, thus limiting the formation of the stress fibres on distant patches and the capacity for the cell to develop higher forces. We moreover observed that the cell contractility modulates the level of force generated by the cell only for the non-protrusive keratinocyte phenotype. This modulation role is not visible for the protrusive fibroblast phenotype, since contractility is most likely dominated by the protrusive effect in that case.

We show by varying the distance between the adhesive patches of the patterned substrate, that the adhesion lifetime and the growing rate of the fibres are the key parameters conditioning the cell morphologies and their stability (either constrained or oscillating). Guillou *et al.* (2008) [13] al-

ready suggested that a critical and limited filament length exists in the cell. We further suggest that this length could also depend on the combination between the adhesion lifetime and the filament growing rate. Future experimental work to specifically investigate this aspect is awaited. On the theoretical side, the model also deserves further refinements. Fibrillar adhesions for example have not been considered since this adhesion type is mainly located out of the cell periphery [3, 7, 40]. Additionally, more cytoskeletal components than actin structures should be incorporated. Indeed, microtubules have been shown to play a key role in the coordination of adhesion disassembly [3, 38].

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Appendix

A Cell culture and videomicroscopy

Swiss mouse 3T3 fibroblasts were cultured in Dulbecco's Modified Eagles Medium (DMEM, Sigma Aldrich) supplemented with 10% foetal calf serum (Invitrogen), 1% L-glutamine and 0.25% penicillin/streptomycin. Cells were seeded on the patterned substrate and allowed to adhere for one hour. They were then placed on the microscope stage for image acquisition. An inverted Axiovert 135 microscope (Carl Zeiss, Jena, Germany) equipped with an incubating chamber was used to maintain the cells in physiological conditions at 37°C and 5% CO_2 in a wet atmosphere. Images were recorded every 5 minutes with a CDD coolsnap camera (Roper Scientific) for 8 hours.

B Microfabrication of the Adhesive Patches

Cleaned glass slides are rendered hyper hydrophilic (water contact angle of 20°) through successive ethanol washing and oxygen plasma treatment. Then, slides were uniformly coated with a $1.5\mu\text{m}$ thick UV-sensitive film and irradiated at specific places to form patches of hydrophilic zones through a chromium mask. The patches were rendered highly hydrophobic by vapour deposition of a commercial fluoro silane product, OPTOOL DSXTM from Daikin. After silane treatment the polymerized resist was dissolved with acetone. The adhesive patches do not require to be functionalized with matrix proteins for the cell to adhere. We therefore used such uncoated (extracellular matrix free) substrates in our experiments.

Figure 12 shows a picture of the patterned substrate we used, before removal of the resist (patches become invisible after removal).

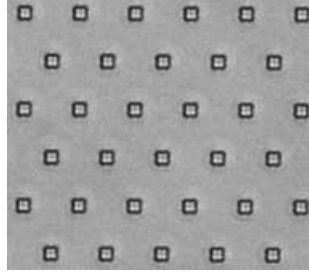


Figure 12: Network of adhesive patches with an inter-patches distance of $12\mu m$ and a patch size of $4 \times 4\mu m^2$.

C Simulation parameters

The parameters appearing in the system of partial differential equations (eqs. 3.3) and defining the cell mechanical properties including viscoelasticity (μ , γ), contractility (ψ), friction (α) and protrusion (β) which govern the cell movements are dimensionless parameters (Table 1). Details on the normalization of the system and choice of these parameter values and dimension can be found in [31, 32]. The coefficients used to describe the tension forces of the membrane and fibres have been adjusted relatively to the other mechanical parameters to correspond to a realistic range of values. For example, the elasticity of the fibres k_f is chosen in the same range as the elasticity of the actin network γ . The elasticity of the membrane being much smaller than the one of the fibres, the value k_m is chosen accordingly.

Parameter	notation	value
actin network elasticity	γ	0.5
cytoplasm viscosity	μ	2.0
protrusive hydrostatic pressure	β	0.5 or 0.8
cell contractility	ψ	5.4 to 5.8
saturation actin density	a_{sat}	2.0
APs friction coefficient	α	0.05
repulsion coefficient from the CB	β_b	2β
membrane elasticity	k_m	0.05
actin fibre elasticity	k_f	0.2
maximum resulting traction force	R_{min}	0.6

Table 1: Dimensionless parameters defining the cell biomechanical properties used in the numerical simulations.

The length parameters have been evaluated from experimental observations (Table 2). The critical size of the AP correspond to $300nm$. We consider that a cluster is formed from four APs to estimate the critical size S_{crit} from which a FX is formed. Finally, one crucial parameter is the

lifetime of the FX. It is admitted that it is about a few minutes [39], whereas the more stable FA can be sustained from 30 to 90 minutes [2]. In the model, since we do not describe the molecular cascade for adhesion regulation, we do not limit the lifetime of the FA however, we set the lifetime for FX to the lower boundary of 30 minutes.

Parameter	notation	value	units
radius of the cell nucleus	R_0	7	μm
membrane segment resting length	l_0	0.9	μm
fibre resting length	l_{0f}	7	μm
maximum distance allowed between FXs	d_{\max}	22	μm
critical size of the APs cluster	S_{crit}	1.2	μm
FX lifetime	τ_{FX}	30	<i>min</i>

Table 2: Spatiotemporal parameters defining the characteristics lengths and time used in the model simulations.

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The rigidity in fibrin gels as a contributing factor to the dynamics of *in vitro* vascular cord formation

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Abstract

While the formation of vascular cords in *in vitro* angiogenesis assay is commonly used to test the angiogenic properties of many molecular or cellular components, an extensive characterisation of the dynamics of this process is still lacking. Up to now, quantitative studies only focused on the resulting capillary structures characterised through static morphometric approaches. We therefore propose in this paper a rather extensive characterisation aiming to identify different stages in the dynamics of this process, through the investigation of the influence of the rigidity of the fibrin extracellular matrix on the growth of the vascular cords. Using time lapse videomicroscopy, the time evolution of relevant morphodynamical parameters has been considered both at the cell level and at the cell population level. At the cell level, a trajectography analysis of individual cells observed in different locations of the growing network has been conducted and analysed using a random walk model. From image sequence analysis and segmentation *i.e.* extraction of the boundaries of the lacunae formed through matrix degradation and cell tractions, the evolution of the lacunae surface has been precisely quantified, revealing different phases and transitions in the growth patterns. Our results indicate that the rigidity of the extracellular fibrin matrix strongly influences the different stages, *i.e.* the dynamics of the angiogenic process. Consequently, optimal rigidity conditions for the formation of stable vascular cord networks could be identified in the context of our experiments.

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Introduction

The formation of new blood capillaries from pre-existing vessels *via* endothelial cell sprouting, proliferation and migration is a process named angiogenesis (Risau, 1997; Conway et al., 2001). Adult endothelial cells are normally quiescent and apart from certain developmental processes (e.g. embryogenesis) and wound healing, angiogenesis is mostly a pathological process. Examples of such processes include arthritis, some eye diseases and solid tumour development. Tumour vascularisation is indeed an aggravating factor of cancer as tumour cells might potentially use the newly formed vascular network to invade the organism, thus promoting the development of metastases (Folkman, 1995; Carmeliet, 2003, 2005).

Angiogenesis is a multifactorial process regulated by various soluble (cytokines, proteases, peptides) and insoluble (matrix proteins) substances (Sottile, 2004), but also by the mechanical properties of the extracellular matrix (Vailhé et al., 1997, 1998, 2001; Deroanne et al., 2001; Lafleur et al., 2002) characterised by its elastic and viscous properties.

In order to investigate the influence of the numerous factors contributing to the formation of new capillaries, several *in vitro* models have been developed (Vernon et al., 1995, Vernon and Sage, 1999; Collen et al., 1998; Vailhé et al., 2001). *In vitro* angiogenesis models are indeed pertinent to describe earlier phases of vascular growth, *i.e.* the formation of vascular cords. In these models, endothelial cells are seeded on biogels such as collagen, fibrin or matrigel. On fibrin gel, the cells spontaneously self-organise by degrading the gel and by exerting traction forces which induce the formation of lacunae *via* the development of dilatation zones (Hall et al., 2001; Vailhé et al.,

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2001). These lacunae then grow in number and size (Fig. 1), and lead to matrix remodelling, thus influencing the final architecture of the capillary-like structures.

Characterisation of the mechanical regulation of angiogenesis allowed assessment of the role of the gel rigidity on the formation of the vascular cords, both experimentally and theoretically (Manoussaki et al., 1996; Namy et al., 2004). The gel rigidity is tuned by changing the concentration of matrix proteins in the gel, such as fibrinogen or collagen. Vailhé et al. (1997, 1998) have shown for the case of human umbilical vein endothelial cells (HUVEC) seeded on fibrin gels, that more rigid gels (*i.e.* with higher fibrinogen concentration) led to a decrease in the number of lacunae formed. They also observed that the vascular cords only formed for a fibrinogen concentration higher than 0.5 mg/ml and that almost no cords were observed within 48 hours for a concentration higher than 2 mg/ml. In that case, cell traction forces were too weak to induce a significant mechanical deformation of the gel, this interpretation being strongly supported by theoretical models (Namy et al., 2004). These original experimental results therefore suggest that there may exist some optimal conditions in terms of biogel rigidity for the generation of vascular cord networks, where the mechanical resistance of the biogel modulates both cell migration and cell traction forces. Indeed it is known that such kind of optimal condition exists for the motility of cells (Pelham and Wang, 1997; Discher et al., 2005), as well as for the development of cell traction forces (Ferrenq et al., 1997; Tranqui and Tracqui, 2000). Thus, the biogel rigidity influences cell migration, *i.e.* the process called durotaxis. Different experiments have shown that cell traction forces are enhanced when the apparent rigidity of

the gel is increased. This is observed in experiments of cell-populated gels by comparing contraction forces generated in fixed and free floating gels (Shreiber et al., 2003).

Several studies have reported morphometric data (such as a capillary network index defined as the ratio between the cell-free area and the total area of the culture) evaluated only at the end of the angiogenic process, *i.e.* once the capillary-like structures are formed (Vailhé et al., 1997, 1998; Guidolin et al., 2004). However a proper quantitative analysis of the different phases of lacuna morphogenesis is still lacking and would be highly valuable in defining possible optimal conditions for the angiogenic process.

The aim of this paper is to propose pertinent morphodynamical criteria that can be monitored with time in order to identify and to discriminate different phases in the *in vitro* angiogenic process, from the appearance of the first lacunae until the formation of a stable vascular structure. We therefore propose an original and rather extensive quantification of the dynamical and multi-scale aspects of a specific *in vitro* angiogenic process using endothelial EAhy926 cells cultured on a fibrin extracellular matrix. The analysis is achieved by performing lacunae segmentation (*i.e.* the extraction of the coordinates of the points forming the lacunae boundaries) and cell tracking (Serini et al., 2003; Ambrosi and Gamba, 2004) from videomicroscopy time lapse sequences in order to extract the pertinent morphodynamical data. By considering individual growth patterns of the lacunae, their shape index, as well as the correlations between lacuna growth and individual cell trajectories close to and far away from the lacunae boundaries, it is possible to

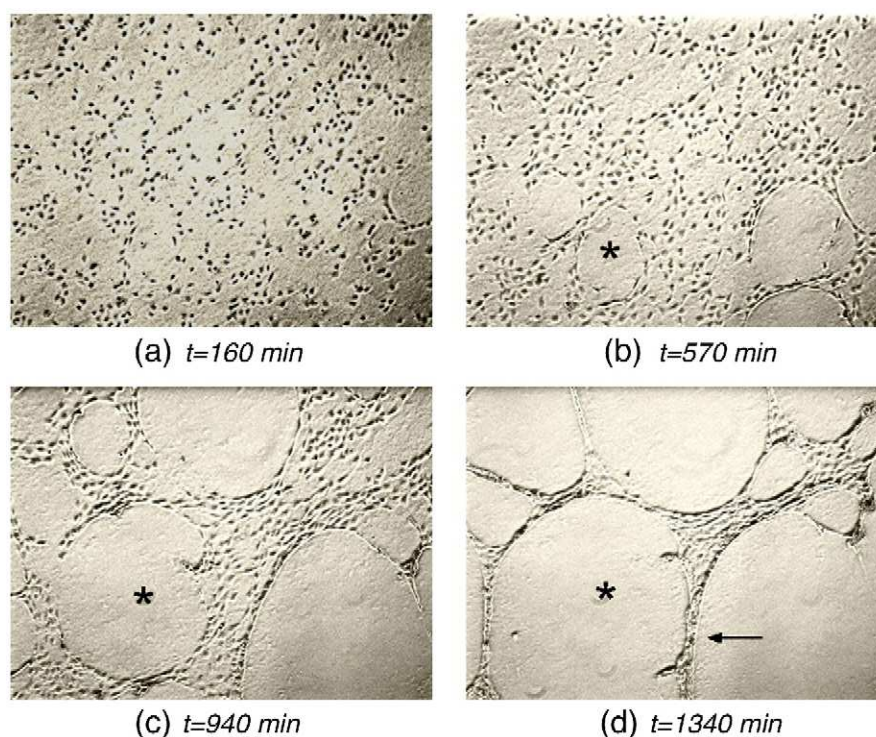


Fig. 1. Phase contrast videomicroscopy images showing four successive stages ($t=160$, 570, 940 and 1340 min) of the *in vitro* angiogenic process for a gel with intermediate rigidity. The asterisks always indicate the same lacuna growing with time and the arrow indicates a vascular cord.

precisely characterise the different stages of the vascular network formation. The influence of the fibrin gel rigidity on the time evolution of these morphodynamical indicators is quantified. The data obtained provide an indirect evaluation of the variations in the mechanical tension within the gel for the different biogel rigidities considered. From these data, an optimum rigidity for the fibrin gel can be identified with regard to the formation of stable vascular cords within 48 hours after seeding the cells.

Materials and methods

Cell culture

Experiments are performed using the human permanent endothelial EAhy926 cell line. This cell line is derived from the fusion of Human Umbilical Vein Endothelial Cells (HUVEC) with the A549 human lung epithelial carcinoma cell line. The choice for this cell line is motivated by the fact that these cells are immortalised and much easier to grow than HUVECs as they do not require any particular extracellular matrix to proliferate. Moreover, the range of sensitivity to substrate rigidity is slightly shifted towards more rigid gels for the EAhy926, *i.e.* for a fibrinogen concentration ranging from 1 to 3 mg/ml, whereas for the HUVECs the concentration range is from 0.5 to 2 mg/ml. Very smooth gels (0.5 mg/ml) are difficult to produce, it is therefore easier to work with more rigid gels.

EAhy926 cells are grown in complete medium composed of Dulbecco's Modified Eagle Medium supplemented with 2-mM glutamine, 10% heat-inactivated foetal calf serum (Invitrogen, Life Technologies) and penicillin-streptomycin (Life Technologies) in a cell culture flask at 37 °C in a humidified CO₂ (5%) atmosphere. When the cells reach a confluent state, they are sub-cultured using a trypsin/EDTA mixed solution to detach adherent cells from the culture flask and are in turn seeded at 14,000 cells/cm² in complete medium.

Fibrin gels

Gels with different fibrinogen concentrations are considered by varying the monomers density from 1 to 3 mg/ml for a constant quantity of thrombin (0.2 units/ml, Sigma) in PBS solution in order to control the gel rigidity. An increased fibrinogen concentration leads to an increased rigidity for the gel (Benkherourou et al., 2000). The term “flexible” is conventionally used to designate a gel with fibrinogen concentration of 1 mg/ml, and the term “rigid” to designate a gel with concentration of 3 mg/ml. Gels with fibrinogen concentration of 2 mg/ml thus correspond to an – “intermediate rigidity”. Solutions of 1 ml are poured in Petri dishes (diameter of 3.5 mm) in order to obtain 1 mm thick fibrin gels.

In vitro angiogenesis

For each experiment, two 3.5 mm Petri dishes are coated with fibrin gels and are left overnight (*i.e.* 18±2 hours) in an incubation chamber before seeding cells. Cells are then seeded at 20,000 cells/cm² on the fibrin gels in a culture medium containing 0.5% foetal calf serum. One of the dishes is then placed in the incubation chamber of a videomicroscopy stage for image acquisition. The surface of the dish is manually scanned until the first lacuna appears. This area is then selected as the first sample zone of the fibrin. The growth of the lacunae successively appearing in this sampling zone is recorded until stable cords are formed or lacunae start to disintegrate. Then, another sampling field is selected for image acquisition either in the same dish or in the second dish. This second group of acquisition fields corresponds to more advanced stages of the morphogenetic process, without the corresponding early phases. The sequences obtained are thus non-homogeneous in time as these are limited by different temporal observation windows. The instant of cell seeding is taken as the time of reference for the *in vitro* angiogenic process.

Time-lapse videomicroscopy

Phase-contrast images of living cells adherent on gels are recorded using an inverted Axiovert 135 microscope (Carl Zeiss, Jena, Germany) equipped with an incubation chamber. Cells are maintained during the whole acquisition time at a temperature of 37 °C with 5% CO₂ in a wet atmosphere. Images are performed with a CDD Coolsnap HQ camera (Roper Scientific). 5 × and 2.5 ×, NA 1.4, Plan-apochromat objectives (Carl Zeiss) are used. The size of the image field is 696×520 pixels, which corresponds to a real size of 1.6×1.2 mm for the 5 × objective and 3.2×2.4 mm for the 2.5 ×. Images are acquired every 10 minutes over a period of time varying from 10 to 50 hours depending on the progress of the angiogenic process under observation.

Image analysis

All image sequences are analysed with a software tool developed in our laboratory that allows for the segmentation of deforming objects and the trajectography of moving objects.

Lacuna segmentation

At each time step, the images are segmented by extracting coordinates from a number of points forming the boundary surface of each lacuna. Their surfaces defined by $S(t)$ are automatically calculated from these boundary points. The growth of the surface of each lacuna is then followed independently. By summing the surface $S_i(t)$ of each lacuna i it is also possible to follow the evolution of the cell occupation rate of the acquisition field under study. The time lapse taken between two consecutive images is 30 minutes for this segmentation analysis sufficient enough to follow the growth process without any loss of information.

Cell trajectography

For the trajectography analysis, individual cell displacements have been tracked down. The sampling time interval between consecutive analysed images is reduced to 10 minutes. The trajectory of the cell is then reconstructed by plotting the successive positions of the cell centroid.

Evaluation of the morphodynamical parameters

Lacuna growth kinetics (LGK) and time sampling

From the curve depicting the time evolution of the lacuna surface, the growth kinetics of the lacuna has been estimated from the slope ($\Delta S(t)/\Delta t$) of the corresponding curve $S(t)$ measured in four distinct time intervals covering the entire duration of all studied sequences. This time sampling into four intervals enables us to distinguish various phases in the angiogenic process and to homogenise the extracted data. The first time interval ranging from 0 to 700 minutes corresponds to the initiation of the angiogenic process, *i.e.* the formation of the first lacuna. The last time interval, from 1700 minutes to the end, corresponds to the growth and extension of well-defined cellular cords. Two intermediate time intervals, each 500 minutes long, correspond to intermediate phases of the angiogenic process, during which lacuna extension and deformation proceed in pace with the fibrin gel reorganisation.

Lacuna shape index

The shape of each lacuna is fitted with an ellipse and the lacuna shape index is then defined as the ratio between the short and long axes of this ellipse.

Cell trajectory analysis

At each time $t_n = n \cdot \Delta t$, where $\Delta t = 10$ minutes is the sampling time interval, the position of each cell centroid $\{x(t_n), y(t_n)\}$ is recorded. A quantitative characterisation of each cell trajectory can be inferred from these data by using a persistent random walk model to describe cell migration. In this modelling approach, the square displacement $d.d(t_n, i\Delta t)$ of each cell is computed over a time period $[i, \Delta t]$ between the successive positions $\{x(t_n), y(t_n)\}$ and $\{x(t_n + i\Delta t), y(t_n + i\Delta t)\}$:

$$d.d(t_n, i\Delta t) = [(x(t_n + i\Delta t) - x(t_n))^2 + (y(t_n + i\Delta t) - y(t_n))^2]$$

Then the mean-square displacement $\langle d.d(t_k) \rangle$ for a given time $t_k = k \cdot \Delta t$ is obtained by averaging all the distances computed from overlapping intervals of width k covering all cell positions, from the initial time t_0 up to the final observation time $t_{\text{end}} = N \cdot \Delta t$. Application of this iterative procedure gives $\langle d.d(t_k) \rangle$ according to the following relationship:

$$\langle d.d(t_k) \rangle = \frac{1}{(N - k + 1)} \sum_{i=0}^{N-k} d.d(t_i, k \Delta t)$$

Mean persistence time

It is defined as the time during which the cell maintains one direction of migration, with an allowable deviation of more or less 30 degrees for the entire trajectory.

Statistics

For each sequence, the different morphological and dynamical parameters at the lacuna level are measured (surface, shape index, growth kinetics) and expressed as mean value plus or minus the associated standard deviation.

Results

Morphodynamical characterisation of lacuna growth

Observation of the *in vitro* angiogenic process shows the progressive formation of lacunae emerging from the organisation of subconfluent cells initially homogeneously distributed on the fibrin gel (Figs. 1a, 1b). The lacunae then grow and fuse (Fig. 1c) until a network of vascular cords is formed at the intersection of the lacunae (Fig. 1d).

The extraction of the boundaries of each lacuna through segmentation allows us to calculate their surfaces and to follow their evolutions with time as shown in Fig. 2. Each curve of Fig. 2 refers to the growth of the surface of a lacuna such as those observed in Fig. 1. Interruption of the curves can be due to (i) the end of the acquisition of the sequence, (ii) the fusion of two neighbouring lacunae, (iii) the exit of the lacuna from the field of observation. In the second case, the fusion phenomenon is marked clearly by the jumps of the surface values observed on some of the curves. In this case, only the larger of the two lacunae just before the fusion, is still followed.

The graphs representing the evolution of the lacunae surfaces (Fig. 2) gives us information about the sequence duration, the starting and ending times of the angiogenic process (see also Table 1), the maximum lacunae surfaces reached and the kinetic profile of the growth of each lacuna.

Lacuna growth kinetics (LGK)

Two types of LGK can be identified from Fig. 2: (i) a slow kinetics where the surfaces of the lacunae remain small and stable (or even decrease) with time; (ii) a high kinetics marked by a significant increase of the surfaces with time (a sharp increase often corresponds to the fusion of two lacunae). Fig. 3 shows the mean LGK. Only lacunae with high kinetics, *i.e.* of interest for morphogenesis are considered. Identical growth kinetics profiles are obtained for the first two time intervals, *i.e.* for the earlier phases of the

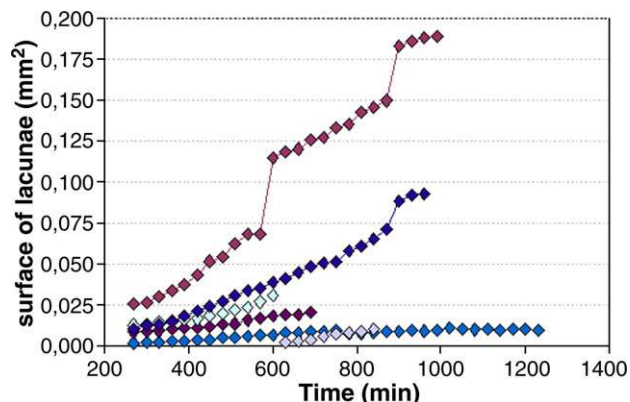


Fig. 2. Graph showing the simultaneous evolution of the surfaces of the lacunae belonging to the same representative sequence. This sequence corresponds to a gel of intermediate rigidity.

angiogenic process, where the maximum growth kinetics occur for intermediate gel rigidity (fibrinogen concentration of 2 mg/ml). For more advanced time intervals, the LGK decreases sharply for the intermediate gel rigidity and no data are available for this type of gel for the last time interval ($t > 1700$ minutes) since the angiogenic process has already ended (see Table 1).

The temporal evolution for each fibrinogen concentration shows that for the intermediate concentration (2 mg/ml) the LGK increases from the first time interval to the second and decreases or remains stable in the third and fourth time intervals, indicative of a decline in angiogenesis (similarly for the high fibrinogen concentration (3 mg/ml)). In the case of low fibrinogen concentration (1 mg/ml) corresponding to a more flexible biogel, the LGK continues to rise, indicative of a faster lacuna growth.

Shape index evolution

Fig. 4 presents the measurements of the shape index of the lacunae evaluated for each fibrinogen concentration at four particular instants (snapshots), with one snapshot per time interval (*i.e.* $t = 540, 1020, 1500$ and 2000 min from cell seeding).

For each snapshot, it is first observed that the mean shape index has the largest value for the intermediate rigidity biogel (fibrinogen concentration of 2 mg/ml). Observation of the shape index for each type of gel shows that it is increasing with time, meaning that the lacunae are progressively deforming from initial circular shapes to more elongated ones. It is worth noticing that the third snapshot ($t = 1500$ min) is an exception, as the shape index for rigid gels (fibrinogen concentration of 3 mg/ml) is sharply decreasing. This is due to the increase of lacunae fusions as the tensions generated by the cells are higher for rigid gel. The fusion therefore leads to a sudden relaxation of the tension around the edge of the lacunae, which are temporarily recovering more circular shapes. On the other hand, for the case of low fibrinogen concentration (1 mg/ml) corresponding to a flexible gel, tensions are never high enough to generate strong lacuna deformations.

Table 1
Summary of the angiogenic starting and ending times for the different fibrin gels considered

Fibrinogen concentration (mg/ml)	1	2	3
Starting time (min)	240	150	180
Ending time (min)	2940	2000	2550

Starting and ending times correspond to the appearance of the first lacuna and to the stabilisation of the vascular cords network (or alternatively the network disruption) respectively.

Correlation of the lacuna shape index with its surface

The lacuna shape index only provides information about the lacuna deformation. It is therefore interesting to look also at the corresponding size of the lacuna to get a deeper understanding on biogel remodelling mechanisms leading to stable vascular cords.

Fig. 5 shows that there are two different profiles of the mean surface as a function of the fibrinogen concentration, for the first two and last two snapshots respectively. In the first two snapshots ($t=540$ min and $t=1020$ min), the mean surfaces are growing with time according to the fibrinogen concentration (or gel rigidity) to reach a maximum for the surfaces corresponding to intermediate gel rigidity. The mean surfaces are decreasing for higher fibrinogen concentrations (3 mg/ml), *i.e.* for a more rigid gel.

For the more advanced times (two last snapshots, $t=1500$ min and $t=2000$ min), these profiles are reversed as the surfaces of the lacunae are smaller for intermediate concentrations (2 mg/ml).

The correlation of the lacuna shape index with the surface reveals that for intermediate fibrinogen concentrations (2 mg/ml), the lacuna surface reaches rapidly a maximum and then decreases while the shape index is still increasing (Fig. 4). This means that the newly formed vascular cords undergo important tension forces and are able to sustain these tensions by deforming without breaking. This suggests that the generated network of vascular cords reaches, at least transiently, a mechanical equilibrium for this intermediate 2 mg/ml fibrinogen

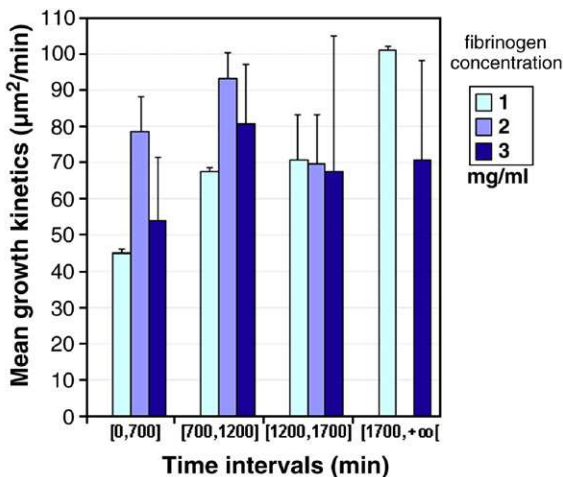


Fig. 3. Representation of the mean lacuna growth kinetics for each fibrinogen concentrations (from 1 to 3 mg/ml) given in four time intervals. [0,700], [700,1200], [1200,1700], [1700, +∞] (in minutes).

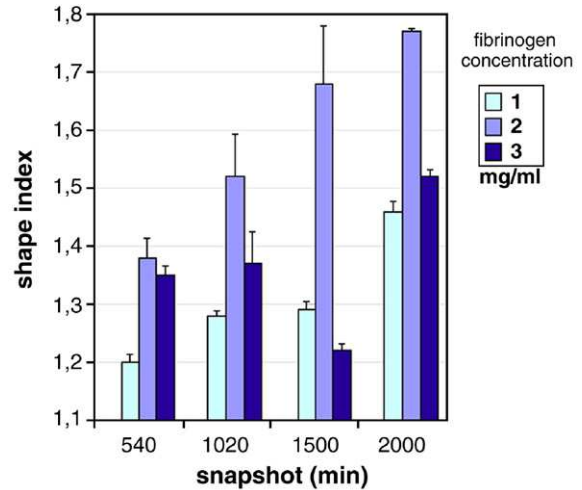


Fig. 4. Representation of the mean shape index for each fibrinogen concentrations (from 1 to 3 mg/ml) given for four snapshots ($t=540$, 1020, 1500 and 2000 minutes from cell seeding).

concentration: the network meshes are not deforming any more through cell tensions, but the network ultimately decays as the cells continue to degrade the gel.

For more flexible fibrin gel (1 mg/ml), observations show that the deformation and the surface of the lacunae are not significantly evolving before a long period of time. This means that there is some delay before the angiogenic process begins (see Table 1). Indeed, under our specific experimental conditions, the fibrin gel might be too flexible to transmit the tension forces between neighbouring cells, which trigger cell organisation. In this case, no stable vascular cord networks can be obtained.

For higher fibrinogen concentration (3 mg/ml), *i.e.* for a more rigid gel, the lacuna deformation is much smaller than for intermediate fibrinogen gel (2 mg/ml), whereas the lacunae

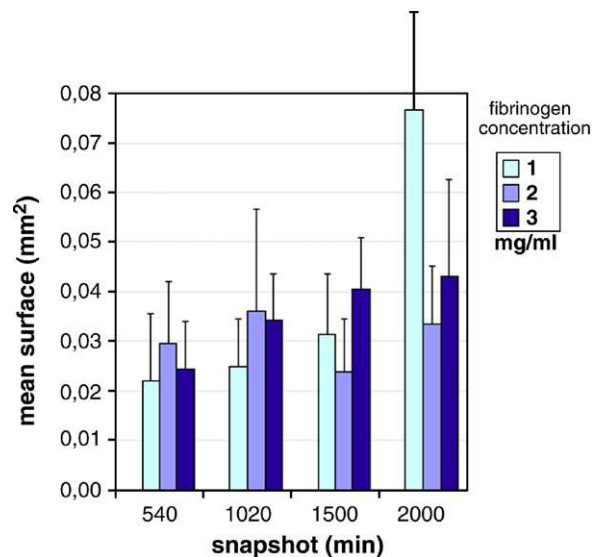


Fig. 5. Representation of the mean surface of the lacuna for each fibrinogen concentrations (from 1 to 3 mg/ml) given for four snapshots ($t=540$, 1020, 1500 and 2000 minutes from cell seeding).

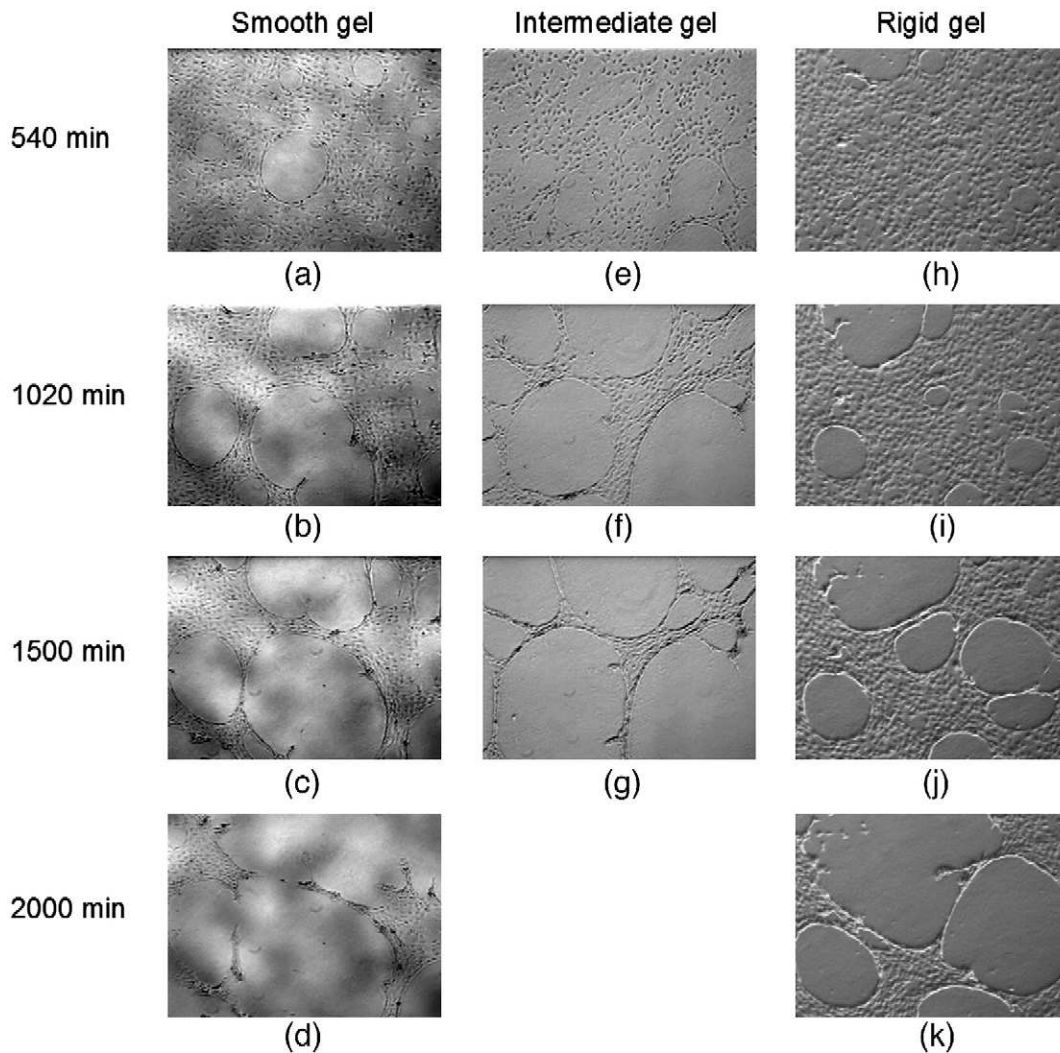


Fig. 6. Phase contrast videomicroscopy images comparing the major phases of the vascular cord formation process for each type of gel rigidity. (a–d) smooth gel, (e–g) intermediate rigidity gel, (h–k) rigid gel. Note that the process is generally faster for intermediate rigidity gels.

surfaces are relatively similar in both cases. This indicates that the gel is too stiff to be deformed by the cells.

Fig. 6 presents the simultaneous evolution of the formation process of the vascular cords for the three different types of gel rigidity, smooth, intermediate and rigid.

Cell trajectory analysis

The tension forces generated by the cells on the biogel lead to the formation of macroscopic structures, the vascular cord networks. Since it is acknowledged that cellular traction is coupled to cell migration during this morphogenetic process (Namy et al., 2004), the aim of the trajectory analysis is to determine reciprocally at a lower scale, how the tensions on the biogel influence the individual cell migration, *i.e.* the state of the system at the microscopic cell scale. For this purpose, three different cell localisations, corresponding to different levels of cell tensions, are considered: (i) far from the lacunae (Fig. 7a), where the tensions are assumed to be lower, (ii) at the intersection of two lacunae (Fig. 7b) and (iii)

near the lacunae (Fig. 7c) where the tensions are supposed to be much higher.

The results show that the endothelial cell trajectories depend on cell localisation: when the cells are located far from the lacunae, they migrate with a high random component. When they are located between two lacunae, the cells follow straight trajectories, while when they are nearby a lacuna their trajectories follow the edge of the lacuna. In these last two cases, the cells are in fact mainly following the orientation of the tension lines in the fibrin gel.

Fig. 8a presents the representative trajectory of a cell located far away from the lacunae. The calculation of the mean-square displacement of the trajectory (Fig. 8b) allows the characterisation of the trajectory as a persistent random walk. The evaluation of the mean persistence time for this trajectory corresponds to 80 minutes, which is relatively small compared to the duration of the sequences.

Evaluation of the speed of migration of the cells is made from six trajectories for each fibrinogen concentration of the gel. A slight increase of the mean cell speed with the fibrinogen

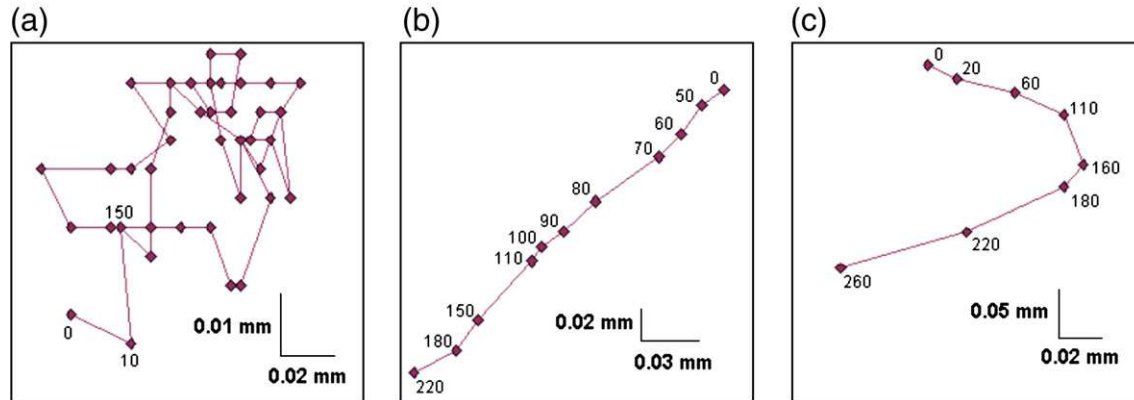


Fig. 7. Three typical cell trajectories, (a) random far from the lacunae, (b) straight line at the intersection of two lacunae, (c) curved trajectory along the lacuna boundary. The numbers associated to the points of the trajectories in each case designate the time in minutes. In the first trajectory (a) the points are mostly 10 minutes apart in the remaining part of this trajectory.

concentration from 1 to 2 mg/ml is obtained, with $16 \mu\text{m}/h \pm 5 \mu\text{m}/h$ and $19 \mu\text{m}/h \pm 5 \mu\text{m}/h$ respectively. On 3 mg/ml fibrin gel, a similar speed of $18 \mu\text{m}/h \pm 5 \mu\text{m}/h$ is measured. Although the evolution of the cell speed as a function of the fibrinogen concentration of the gel is not significantly different, a slight increase of the cell speed is however observed for more rigid

gels that tend to decrease slightly. This result agrees with previously published results, where migration *versus* substrate rigidity exhibits bell-shaped profiles (Pelham and Wang, 1997; Discher et al., 2005).

Discussion

The characterisation of the dynamics of *in vitro* vascular cord formation has been realised from an original approach coupling two methods of analysis dealing with two different spatial scales. The extraction of the lacunae boundaries through segmentation provides data at the network scale (lacunae and cords) and the trajectography analysis allows description of phenomena occurring at the cell scale, by following individual cell movements. In a first part, we have considered the influence of the fibrinogen concentration (and thus of the rigidity of the fibrin gel) on generating tension forces and on the resulting cell organisation into a network of vascular cords. In a second part, we have investigated the influence of the tension lines, generated within the gel, on the cell migratory behaviour.

Coupling the above two methods of analysis allowed for the extraction of pertinent morphodynamical parameters characterising this specific *in vitro* angiogenic process on fibrin gels. It was therefore possible to show that the fibrinogen concentration, directly related to the biogel rigidity, influences the multi-phase dynamics of the vascular cord formation.

The influence of the fibrin gel rigidity on the dynamics of the angiogenic process could be demonstrated due to the time sampling method used. Thus, four phases in the lacuna growth process could be discriminated: (i) the appearance of the lacunae, (ii) the lacunae growth and first fusions, (iii) the lacunae deformation, and (iv) the stabilisation and/or disruption of the vascular cord network.

Such time sampling approach has allowed for the consideration of more homogeneous kinetics (within each time interval) than if time had been treated as a continuous variable. The time sampling could be refined *a posteriori* and adapted to the kinetics measured for each gel concentration. Indeed, our study shows that for intermediate fibrinogen concentration (2 mg/l) the angiogenic process proceeds faster.

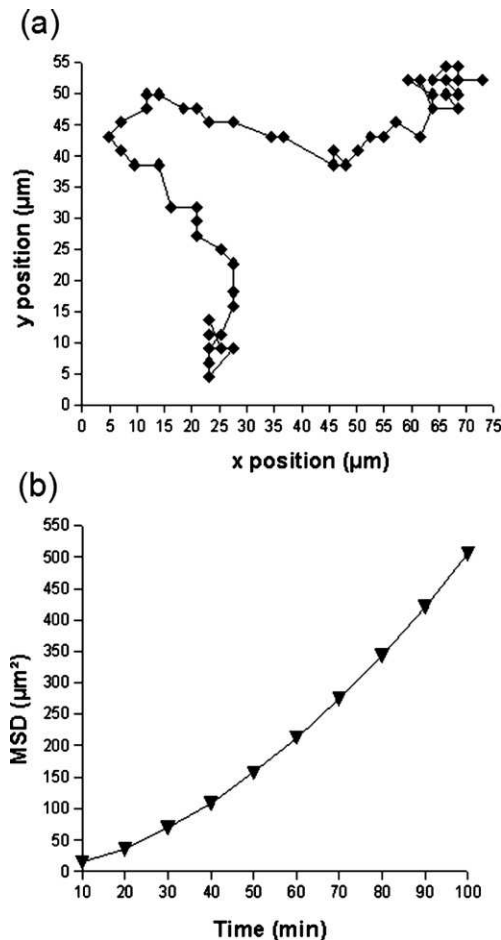


Fig. 8. (a) Trajectory of a cell located far from the lacunae, the points of the trajectory are 10 minutes apart from one another; (b) associated mean-square displacement of the cell.

The time intervals could then be modulated accordingly to fit more closely to the different dynamical phases identified for the lacuna growth process. It is expected that this refined analysis would allow better discrimination between the data obtained for each type of gel.

It is important to notice that in the interpretation of the results presented, variations of the fibrinogen concentrations are considered, which influence the morphodynamics of the angiogenic process both through modifications of the biogel rigidity and also through an increase in the number of adhesion sites for the cells. Previous works have already demonstrated that both factors are relevant in cell/matrix interactions (Shiu et al., 2005). In order to assess the relative importance of these two parameters, experiments were developed in previous works that allowed for the variation of the biogel rigidity without altering the number of adhesion sites. This was possible by changing the biogel thickness while keeping a constant fibrinogen concentration. Very thin gels have an apparent rigidity much higher than thicker gels. Results obtained with HUVEC have shown that whatever the fibrinogen concentration used (from 0.5 to 2 mg/ml) no vascular cords could be formed (Vailhé, 1997). This leads to the conclusion that the rigidity is more dominant than the number of adhesion sites in our *in vitro* angiogenesis model. It is therefore the ability of the gel to deform (*i.e.* its rigidity) that will determine the final vascular structure. This is supported both by experimental (Vernon et al., 1995) and theoretical (Namy et al., 2004) results of *in vitro* models where the cell network formed over a spatial gradient of gel thickness. The number of adhesion sites is however important for cell motility, as the migration velocity *versus* the number of adhesion sites is known to present bell-shaped profiles. Hence this parameter acts as a modulating factor of the *in vitro* angiogenic process.

Our study indicates that there may exist an optimal fibrin gel rigidity leading to stable networks of vascular cords within 48 hours. This fibrin gel rigidity corresponds to the intermediate rigidity in our specific conditions, *i.e.* a fibrinogen concentration close to 2 mg/ml. Indeed, for this particular concentration, the appearance of lacunae and the formation of vascular cords occur more rapidly.

For more flexible (1 mg/ml fibrinogen concentration) or more rigid (3 mg/ml fibrinogen concentration) gels, the angiogenic process cannot fully develop as stable vascular cord networks are more difficult or even impossible to generate within 48 hours. In the first case, tensions in the gel cannot be transmitted between cells, whereas in the second case the limiting aspect might be the difficulty for the cells to deform the matrix associated with the decrease in cell migration, as cells tend to adhere more strongly to their substrate.

All these data characterise in depth the endothelial cell behaviour on fibrin gels in the context of *in vitro* angiogenesis. They are of interest, considering that fibrin is often used as a biomaterial (Christman et al., 2004; Hasegawa et al., 2005; Mol et al., 2005; Urech et al., 2005; Yao et al., 2005; Sreerexha and Krishnan, 2006; Van Lieshout et al., 2006). It is therefore important to take into consideration that depending on the

fibrinogen concentration the dynamics of the formation of the vascular structures can be dramatically modified.

Finally, our results provide information at the cellular level through the trajectography approach coupled to the more global context of cell/matrix organisation. This is of interest in particular with regards to new mathematical modelling approaches based on hybrid mathematical formulations (Kevrekidis and Whitaker, 2005; Stéphanou et al., 2005) that couple multi-scale discrete (such as the individual cell migration) and continuous events (lacuna growth).

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Mathematical modelling of the influence of blood rheological properties upon adaptative tumour-induced angiogenesis[☆]

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Abstract

In this paper, we present a theoretical investigation of the influence of blood flow through a tumour-induced capillary network, whereby the vascular architecture adapts as it grows to the associated haemodynamic forces resulting in what we describe as adaptive tumour-induced angiogenesis (ATIA). The network is generated in response to tumour angiogenic factors (TAFs), which are released from hypoxic cells within a solid tumour. We first describe a refined model for tumour-induced angiogenesis, which aims to describe the capillary growth process at the cellular level by explicitly taking into account the effects of matrix degrading enzymes and the local properties of the host tissue during endothelial cell migration. We then incorporate blood rheological properties into the formulation and investigate the influence of wall shear stress induced by the blood flow during dynamic vascular growth. We then go on to examine a number of feedback mechanisms affecting vascular resistance and network architecture. The mechanisms considered include those proposed by Pries and co-workers [A.R. Pries, T.W. Secomb, P. Gaetgens, Structural adaptation and stability of microvascular networks: theory and simulation, *Am. J. Physiol. Heart Circ. Physiol.* 44 (1998) H349–H360; A.R. Pries, B. Reglin, T.W. Secomb, Structural adaptation of microvascular networks: functional roles of adaptative responses, *Am. J. Physiol. Heart Circ. Physiol.* 281 (2001) H1015–H1025; A.R. Pries, B. Reglin, T.W. Secomb, Structural adaptation of microvascular networks: roles of the pressure response, *Hypertension* 38 (2001) 1476–1479] and both haemodynamic (non-linear viscosity) and metabolic constraints are taken into account. Subsequent simulations of chemotherapeutic drug perfusion through the system show that vascular adaptation leads to a significant benefit in treatment delivery to the tumour. The results clearly demonstrate that the combined effects of network architecture and vessel compliance should be included in future models of angiogenesis if therapy protocols and treatment efficacy are to be adequately assessed.

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1. Introduction

Flow modelling in a tumour-induced capillary network has been considered recently in [4] and [5]. In these papers, flow simulations through vascular networks were performed in order to investigate the efficiency of chemotherapy treatments as they passed from parent blood vessel to tumour surface via an associated capillary bed. The capillary bed

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was generated from an angiogenesis model proposed in [6]. Vascular growth was described by a discrete formulation of the associated system of partial differential equations. This approach and modelling technique permitted the tracking of each individual endothelial cell as it emerged from a parent vessel and migrated through the extracellular matrix. Endothelial cells were assumed to have several different components of migration comprising: (i) random motility, (ii) chemotaxis in response to a generic tumour angiogenic factor (TAF) released by the tumour cells, and (iii) haptotaxis in response to fibronectin gradients emerging from the extracellular matrix and through degradation and production by the endothelial cells themselves. Flow modelling techniques used previously in the context of petroleum engineering to model the flow of water, oil and gas through the interstices of a porous rock [7] were adapted to model blood and drug flow through these microvascular networks. Although blood was rather crudely considered to be a Newtonian fluid in this early work, results from [4] highlighted two important effects that could be responsible for the failure of some therapy regimes. First, it was found that a considerable amount of the drug injected into the parent vessel simply by-passed the tumour by way of the highly interconnected capillary bed. The second effect related to the dilution of the drug as it became dispersed throughout the tumour-induced vasculature: the concentration of any drug reaching the tumour became so dilute as to have little effect on the tumour cells. Simulations were then performed to investigate ways of reducing these two effects and thereby optimise the drug uptake by the tumour. Increasing the mean capillary radius of the capillary bed and/or decreasing the blood viscosity both led to a significant increase in the drug uptake. Although these results were interesting from a qualitative perspective, the model was somewhat naïve, with blood perfusion modelled as the flow of a Newtonian fluid through rigid cylindrical capillaries.

The second paper [5] examined how vascular remodelling affected the distribution of blood flow in the system. Capillary pruning algorithms were designed to reflect how different anti-vascular and anti-angiogenic drugs were thought to operate *in vivo*. Simulations demonstrated that drug uptake could be increased by up to 130% via the random removal of vessels and this suggested the possibility of developing a new cancer treatment strategy, *viz*, coupling the administration of an antiangiogenic drug (to first of all optimise the vasculature) to subsequent chemotherapy treatment (thereby ensuring maximum delivery).

The aim of the current paper is to implement a number of significant improvements in the modelling approach by considering the flow of a non-Newtonian fluid in an adaptive network: a network that evolves both spatially and temporally in response to its associated flow distribution. We present results corresponding to a number of different stages in the formulation of what we call our adaptive tumour-induced angiogenesis (ATIA) model.

Blood is a complex fluid, the rheological properties of which lead to interesting feedback mechanisms during perfusion. For example, shear stresses generated within the capillary bed by the flowing blood strongly influence vessel adaptation and network remodelling [8–12]. These shear stresses are, in turn, affected by blood viscosity, the distribution of which depends upon a non-uniform distribution of haematocrit (the volume fraction of red blood cells in the blood) within the host vasculature (the Fåhræus effect). However, the distribution of haematocrit itself depends upon the spatial architecture of the underlying network and so the feedback is established.

Blood rheology and its influence on the remodelling of microvascular networks has been intensely studied in [1–3] both experimentally and theoretically. From these studies Pries and co-workers have formulated a model for vascular adaptation incorporating a number of feedback mechanisms. They have demonstrated that the basic requirement for the generation of stable vascular structures involves a combination of both haemodynamic and metabolic stimuli. Such vascular adaptation processes have been recently applied to model radii adaptation of a regular network [13]. The aim in [13] was to model the oxygen distribution within a two-dimensional hexagonal network and to determine its influence on the dynamics of a colony of normal and cancerous cells. Subsequent simulations produced inhomogeneous distributions of haematocrit and oxygen tension and highlighted the important role played by hypoxic cells during tumour invasion.

The model of adaptive tumour-induced angiogenesis presented in this paper begins with a discussion of a new formulation of tip cell migration that acknowledges the important function of matrix metalloproteinases (MMPs) [14] during angiogenesis in the absence of flow [15,16]. The aim of this new model is to incorporate mediation in vessel growth via extracellular matrix (ECM) proteolysis by specific enzymes produced by endothelial cells (EC). A number of recent publications have demonstrated the importance of enzymes from the MMP family and their involvement in the regulation of the various stages of the angiogenic process [17–20]. These MMPs are involved in the migration of EC within the ECM, EC proliferation, and the remodelling of the basement membrane of newly formed vessels. Their importance is such that these proteinases and their regulation form new targets for cancer treatment. As our ultimate goal is to propose a global modelling framework within which to further investigate new treatments, it is

important to incorporate the MMP effect into the modelling. Another key aspect of the MMP issue relates to its effect on transmural transport — tumour vascular networks are very leaky and proteolytic activity plays a vital part in the breaching of pre-capillary (non-mature) membranes by ECs during sprout formation. Although we will not take vessel leakiness into account in the present paper, this aspect is currently under investigation in an attempt to describe drug diffusion through the tumour tissue itself.

Having described the extended model of angiogenesis to be used as a foundation for further development, attention is next focused upon some aspects of vascular adaptation. The earlier paper [4] considered angiogenesis and flow as two separate (i.e. non-interacting) issues — a rigid network of capillary elements was generated from the PDE formulation and then the flow of a Newtonian fluid was considered within this static structure. Here, however, we will consider an angiogenesis model that is coupled to perfusion. This involves modelling a dynamic network that interacts with a non-Newtonian fluid. Two different feedback mechanisms are considered separately in order to assess their individual impact upon drug delivery. Their combined effects will be presented at a later time.

We begin by considering the influence of blood flow on the branching process — blood circulation creates a shear stress distribution on the capillary walls and, in some cases, this enhances the sprouting and branching potential for non-mature vessels, i.e. vessels which have not yet been coated and stabilised by the formation of a basal lamina. The resulting vascular structures are compared with structures generated without shear stress effects.

We then go on to consider vascular remodelling, where the radii of vessels within a pre-existing homogeneous network evolve dynamically under the influence of different haematocrit distributions. The implications for cancer therapy are then discussed in relation to the results. The ultimate aim of this work is to produce a model of adaptive tumour-induced angiogenesis with bed architecture evolving under the combined effects of tumour-angiogenic factors, fibronectin, random motility, matrix degrading enzymes and blood perfusion.

2. A mathematical model for tumour-induced capillary growth

2.1. A discrete stochastic description of network growth

The model proposed here describes how endothelial cells emerging from a parent vessel migrate towards a tumour and ultimately form a vascular network that supplies nutrients for continued development. The model is inspired by the tumour-induced angiogenesis model initially proposed in [6]. The model assumes that endothelial cells migrate through (i) random motility, (ii) chemotaxis in response to tumour angiogenic factors (TAF) released by the tumour and (iii) haptotaxis in response to fibronectin (FN) gradients in the extracellular matrix [6,15,21,22]. If we denote by n the endothelial cell density per unit area, then the equation describing endothelial cell conservation is given by:

$$\frac{\partial n}{\partial t} = \overbrace{D\nabla^2 n}^{\text{random}} - \overbrace{\nabla \cdot (\chi(c)n\nabla c)}^{\text{chemotaxis}} - \overbrace{\rho\nabla \cdot (n\nabla f)}^{\text{haptotaxis}}. \quad (1)$$

The chemotactic migration is characterised by the function $\chi(c) = \chi/(1 + \delta c)$ which reflects the decrease in chemotactic sensitivity with increased TAF concentration. The coefficients D , χ and ρ characterise the random, chemotactic and haptotactic cell migration respectively.

Tumour angiogenesis factors (TAFs) and fibronectin (FN) bind to specific membrane receptors on endothelial cells and subsequently trigger molecular cascades inside the ECs, activating cell migratory machinery. One consequence of this activation process is the production by the cells of a matrix degrading enzyme (MDE), which enhances the attachment of the cells to fibronectin contained in the extracellular matrix. The endothelial cells are consequently able to exert the traction forces required to propel themselves during migration.

In the initial model [6], endothelial cell densities and their global influence on TAF and FN concentrations were considered in a continuous formulation. Here, we choose to focus on local effects and consider the influence of each individual cell on its local environment. The model is then given by the following set of equations:

$$\frac{\partial c}{\partial t} = -\eta n_i c, \quad (2)$$

$$\frac{\partial f}{\partial t} = \beta n_i - \gamma m f, \quad (3)$$

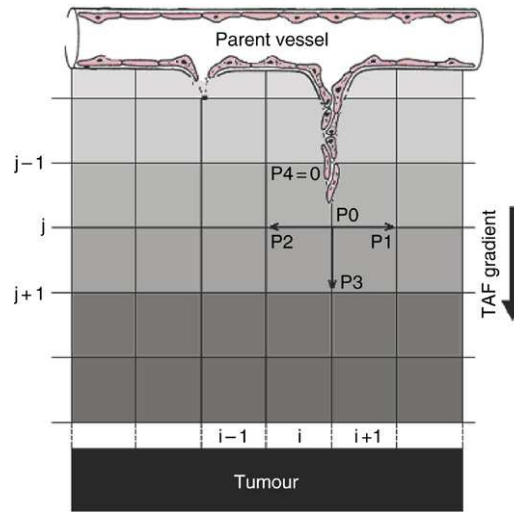


Fig. 1. Schematic diagram of a section of the 2D-grid used in the simulation procedure illustrating how the capillary growth process is taken into account in the simulations. At each node, the sprout can grow in 3 possible directions in 2D (and 5 possible directions in 3D).

$$\frac{\partial m}{\partial t} = \alpha n_i + \varepsilon \nabla^2 m - \nu m \tag{4}$$

where c represents the TAF concentration, f the FN concentration, m the MDE density and n_i a Boolean value (1 or 0) that indicates the presence or absence of an endothelial cell at a given position. The parameters β and α characterise the production rate by an individual endothelial cell on FN and MDE respectively and η its TAF consumption rate. The major difference with the earlier model is that degradation of fibronectin f , characterised by the coefficient γ , no longer depends directly on the endothelial cell density n . This now depends upon the MDE density m produced by each individual endothelial cell n_i at rate α [23,14–16,24]. The MDE once produced, diffuses locally with diffusion coefficient ε , and is spontaneously degraded at a rate ν .

The displacement of each individual endothelial cell, located at the tips of each growing sprout, is given by the discretised form of the endothelial cell mass conservation Eq. (1). The discretisation performed from Euler finite difference approximation [25] leads to:

$$n_{l,m}^{q+1} = n_{l,m}^q P_0 + n_{l+1,m}^q P_1 + n_{l-1,m}^q P_2 + n_{l,m+1}^q P_3 + n_{l,m-1}^q P_4 \tag{5}$$

where l and m are positive parameters which specify the position of the endothelial cell on the 2D grid i.e. $x = l\Delta x$ and $y = m\Delta y$. Time discretisation is represented by $t = q\Delta t$. The migration of the individual cell is determined by the set of coefficients P_i which relate to the likelihood of the cell being stationary (P_0), or moving left (P_1), right (P_2), up (P_3) or down (P_4) (Fig. 1). These coefficients incorporate the effects of random, chemotactic and haptotactic movement and depend upon the local chemical environment within the ECM (i.e. the local FN and TAF concentrations).

2.2. Modified rules for branching and anastomosis

The processes of branching (formation of new sprouts from existing sprout tips) and anastomosis (formation of loops by fusion of two colliding capillary sprouts) are incorporated into the discretised form of the model as in [6]. These rules constitute the basic requirements for the generation of realistic vascular networks. One weakness of the model in [6] for network growth, however, is that the influence of the wall shear stress is not considered (despite the fact that this mechanism plays a leading role in growth and branching). In the current paper, we incorporate this mechanism into the modelling and assess its effect upon network architecture. The rules for branching have been adapted, as both sprouts and vessels can branch under the influence of wall shear. As the shear stress is due to the blood flowing through the capillaries, vessel branching can only occur after some degree of anastomosis has taken place. Additional conditions for vessels branching are as follows: (i) the likelihood of a vessel branching increases

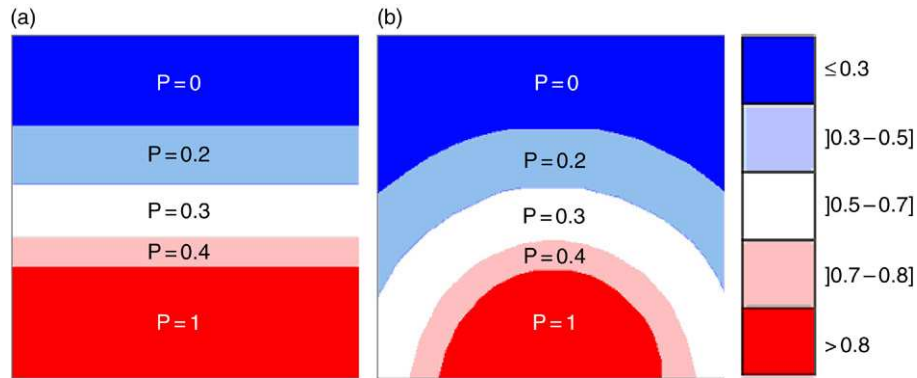


Fig. 2. Initial TAF distribution produced by the tumour located at the lower boundary of the domain. (a) corresponds to a large tumour or alternatively to a row of tumour cells and leads to a linear gradient whereas (b) corresponds to an individual circular tumour located in the middle of the lower boundary and leads to a circular gradient. (Colour code: TAF density as a fraction of the maximum TAF value)

with both the local TAF concentration *and* the magnitude of the shear stress affecting the vessel wall; (ii) the vessel must reach a certain level of maturation before it is able to branch. This is based on experimental observations by laser scanning confocal imaging of fluorescent vessels [26] which reveals that angiogenic sprouts mainly emanate from functional vessels, i.e. vessels which have formed a lumen to carry plasma and blood. Contrasting this, once a basal lamina is formed around a vessel, the endothelial cells become less sensitive to angiogenic factors and branching cannot occur anymore [26,27].

2.3. Boundary and initial conditions

The domain considered is a square of length $L = 2$ mm [28]. The parent vessel from which the vascular network grows is located at the upper edge of the domain and the tumour is located on the opposite edge as indicated in Fig. 1.

We assume that the capillary sprouts remain confined within the boundary of the domain, which means that no-flux boundary conditions are imposed on the boundaries. Initial TAF and FN profiles are shown schematically in Fig. 2. In order to initiate the vascular growth, five initial sprouts are evenly distributed along the parent vessel as shown in Fig. 2(a) and 2(b).

3. Blood rheological properties and vascular adaptation mechanisms

3.1. Non-Newtonian blood properties

Blood is a very complex biphasic medium, composed of many different constituents. The solid phase essentially contains cellular elements: (i) red blood cells (erythrocytes) involved in oxygen and carbon dioxide transport; (ii) white blood cells (leukocytes) involved in the organism's defence against invasion by bacteria and viruses, and (iii) platelets involved in clotting cascades. These solid elements are carried in the plasma, which constitutes the fluid phase. The plasma is a solution containing electrolytes as well as organic molecules such as metabolites, hormones, enzymes, antibodies and other proteins. Solid elements represent approximately 45% of the total blood composition — red cells are predominant. A measure of the solid phase is given by the blood haematocrit, which represents the volume fraction of red blood cells contained in the blood. The average human haematocrit has a value of around 45%. Table 1 shows representative sizes of the red blood cells in comparison with the capillary microvessels.

Owing to its biphasic nature, blood does not behave as a continuum and the viscosity measured while flowing at different rates in microvessels is not constant. At a given flow rate (Q) in a cylindrical capillary of radius R , measured viscosity is really an “apparent viscosity” and is determined from the following expression:

$$\mu_{\text{app}} = \frac{\pi R^4}{8L} \cdot \frac{\Delta P}{Q} \quad (6)$$

where L is the capillary length and ΔP the axial pressure drop. The relative apparent viscosity (a dimensionless parameter) is then defined by dividing the apparent viscosity by the plasma viscosity, i.e.: $\mu_{\text{rel}} = \mu_{\text{app}}/\mu_{\text{plasma}}$.

Table 1
Typical dimensions of blood cells and capillary microvessels [29]

Dimension (μm)	
2	Red blood cell thickness and platelets dimension
2.8	Minimum vessel diameter through which a red cell is able to pass
8	Red blood cell diameter
10	Mean capillary diameter
20	Size of the largest white blood cells

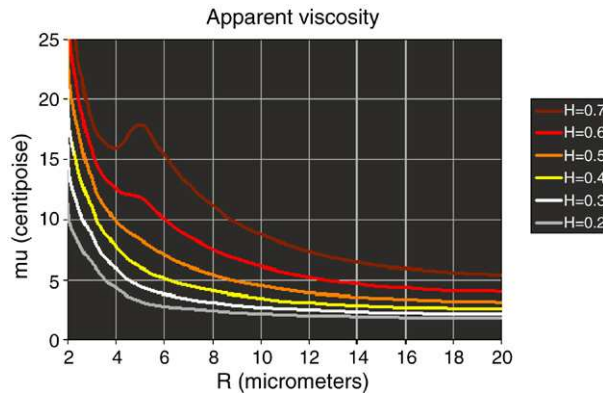


Fig. 3. Apparent viscosity profile as a function of the vessel radius for different values of the haematocrit [1].

The plasma viscosity is typically 1.2 *cp* [30]. Direct measurement of blood viscosity in living microvessels is very difficult to achieve with any degree of accuracy. Pries et al. [31] have proposed an alternative approach, which entails comparisons of the flow distribution in a numerical network (generated by a mathematical model) with similar experimental systems. The relationship which was found to offer the best fit with the experimental data at the microvascular scale is given by:

$$\mu_{rel}(R, H_D) = \left[1 + (\mu_{0.45} - 1)f(H_D) \left(\frac{2R}{2R - 1.1} \right)^2 \right] \left(\frac{2R}{2R - 1.1} \right)^2 \tag{7}$$

where $\mu_{0.45}$ is the viscosity corresponding to the normal average value of the discharge haematocrit ($H_D = 0.45$), R the vessel radius and $f(H_D)$ a function of the haematocrit. These terms are defined as:

$$\mu_{0.45} = 6e^{-0.17R} + 3.2 - 2.44e^{-0.06(2R)^{0.0645}}, \tag{8}$$

$$f(H_D) = \frac{(1 - H_D)^C - 1}{(1 - 0.45)^C - 1}, \tag{9}$$

$$C = (0.8 + e^{-0.15R}) \left(-1 + \frac{1}{1 + 10^{-11}(2R)^{12}} \right) + \left(\frac{1}{1 + 10^{-11}(2R)^{12}} \right). \tag{10}$$

The apparent viscosity corresponding to Eq. (8) is plotted in Fig. 3 for various values of the haematocrit over the range of radii typical of the capillary scale. It can be seen that the apparent blood viscosity generally increases with decreasing capillary radius, although the precise relationship is actually haematocrit-dependent.

3.2. Modelling flow through a network

This area of research has been extensively studied in the context of petroleum engineering, where the main focus has been to investigate the flow of water, oil, and gas through the interstices of a porous rock (see [7] for an overview). A first step towards adaptation of fluid modelling techniques from the oil industry to biomedical applications has previously been performed in [4] and [5]. However in this earlier work, blood viscosity and vessel radii were

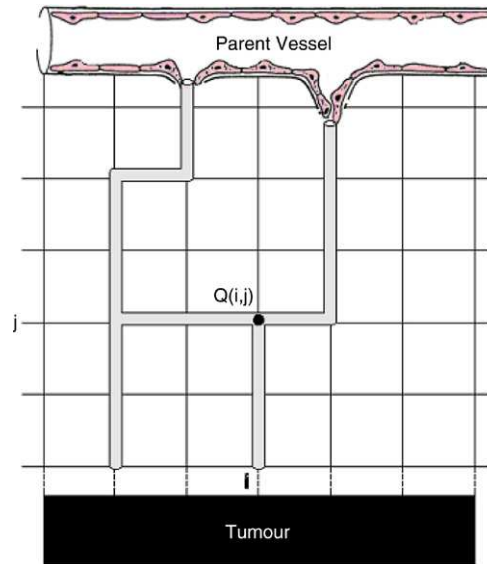


Fig. 4. Schematic representation of the vessels superimposed onto the grid used for flow calculations.

considered invariant (constant blood viscosity and fixed capillary radii), whereas biological structures tend to exhibit some degree of compliance. Here, we extend the earlier formulation to account for variable blood viscosity and evolving capillary radii. Network flow calculations are based upon a local relationship between pressure gradient D and flow Q at the scale of a single capillary element of length L and radius R . Such a relationship in the case of a non-Newtonian fluid can be approximated by the following expression:

$$Q = \frac{\pi R^4 \Delta P}{8\mu_{\text{app}}(R, H_D)L} \quad (11)$$

where $\mu_{\text{app}}(R, H_D)$ is the apparent blood viscosity which depends on the local blood haematocrit and radius of the vessel through Eq. (8). Assuming mass conservation and incompressible flow at each junction (i, j) where capillary elements meet (a node) (Fig. 4), we can write for each node:

$$\sum_{k=1}^N Q_{(i,j),k} = 0 \quad (12)$$

where the index k refers to adjacent nodes and $N = 4$ in $2D$ or 6 in $3D$. This procedure leads to a set of linear equations for the nodal pressures (P_i) which can be solved numerically using any of a number of different algorithms (e.g. Successive Over-Relaxation (SOR), Choleski conjugate gradient method, Lanczos method). Once nodal pressures are known, Eq. (11) can be used to calculate the flow in each capillary element in turn.

3.3. Stimuli influencing vessel diameter adaptation

Blood rheological properties and microvascular network remodelling are interrelated issues. Indeed, blood flow creates stresses on the vascular wall (shear stress, pressure, tensile stress) which lead to adaptation of the vascular diameters via either vasodilatation or constriction. In turn, blood rheology (viscosity, haematocrit, etc.) is affected by the new network architecture. To model vascular remodelling of a pre-existing network, we propose to use the theoretical model developed in [1]. The model considers a number of stimuli on vessel diameter adaptation (S_i) that account for the influence of the wall shear stress (S_{wss}) , the intravascular pressure (S_p) and a metabolic mechanism depending on the blood haematocrit (S_m) . The authors argue that these stimuli $(S_{\text{wss}}, S_p, S_m)$ form a basic set of requirements in order to obtain stable network structures with realistic distributions of vessel diameters and flow velocities. The theoretical model assumes that for each segment of the network considered, the change in its radius

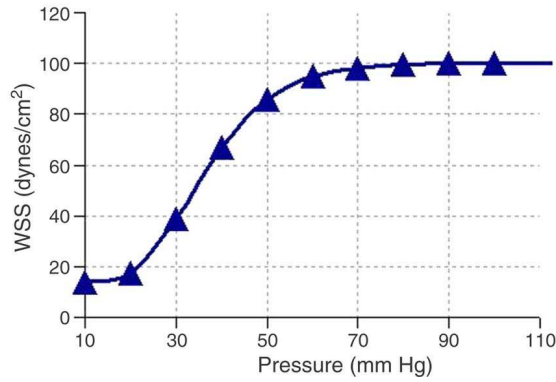


Fig. 5. Function $\tau_e(P)$ showing the magnitude of the wall shear stress as a function of the transmural pressure. The curve is fitted from experimental results obtained by Pries et al. [2] on the rat mesentery.

(ΔR) over a time step Δt is proportional to both the global stimulus (S_{tot}) applied on the segment and to the initial vessel radius R , i.e.

$$\Delta R = S_{\text{tot}} R \Delta t. \quad (13)$$

The global stimulus on the segment under consideration corresponds to the sum of each individual stimulus S_{wss} , S_p , and S_m corresponding to the wall shear stress, the intravascular pressure, and the metabolic stimulus respectively [2,3], hence:

$$S_{\text{tot}} = S_{\text{wss}} + S_p + S_m. \quad (14)$$

Each stimulus will now be briefly discussed.

3.3.1. Wall shear stress

The organisation of vascular networks depends on a number of physical principles [13], the first of which was proposed in [32]. This principle is based on the minimization of energetic costs in order to sustain blood flow throughout the vasculature. A consequence of this principle implies that the wall shear stress should remain constant in each vessel [33]. This is realised via adaptations in vessel radii. Hence vessel radius tends to increase with increasing wall shear stress, whilst wall shear stress decreases with increasing radius. The wall shear stress stimulus is here described as in [1], by the following logarithmic law:

$$S_{\text{wss}} = \log(\tau_w + \tau_{\text{ref}}) \quad (15)$$

where τ_w is the actual wall shear stress in a vessel segment calculated from:

$$\tau_w = \frac{4\mu(R, H_D)}{\pi R^3} |Q| \quad (16)$$

and τ_{ref} is a constant included to avoid singular behaviour at low shear rates [2]. Adaptation in response to the wall shear stress stimulus alone tends to reinforce a single dendritic path in the network corresponding to the main flowing backbone of the vasculature whilst simultaneously eliminating the low-flow paths. In this case, the resulting network is unstable, as no consistent balance for the radius and flow distribution can be achieved if the wall shear stress stimulus is considered in isolation.

3.3.2. Intravascular pressure

Intravascular pressure is another key stimulus for vascular adaptation. Pries et al. [1] have experimentally observed on the rat mesentery the dependence of the magnitude of the wall shear stress with the local intravascular pressure (P). They proposed a parametric description of their experimental data, which exhibits a sigmoidal increase of the wall shear stress with increasing pressure through the following (Fig. 5):

$$\tau_e(P) = 100 - 86 \cdot \exp\left[-5000 \cdot [\log(\log P)]^{5.4}\right]. \quad (17)$$

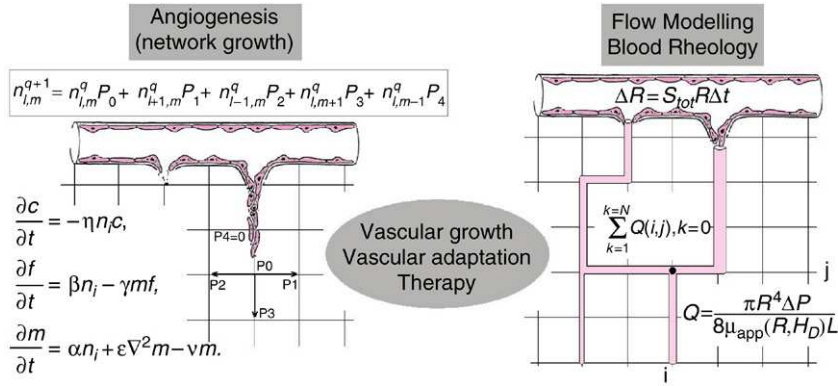


Fig. 6. Mathematical modelling of angiogenesis coupled to flow modelling techniques. Taking into account dynamic remodelling due to blood rheological properties will ultimately allow us to investigate adaptive tumour-induced angiogenesis (ATIA).

The sensitivity of the corresponding stimulus to intravascular pressure is then described by:

$$S_p = -\log \tau_e(P). \tag{18}$$

3.3.3. Metabolic haematocrit-related stimulus

The introduction of a metabolic stimulus stabilizes the network by stimulating vessel growth in segments with low flows. The stimulus is once again described by a logarithmic law given by:

$$S_m = k_m \log \left(\frac{Q_{ref}}{QH_D} + 1 \right) \tag{19}$$

where Q_{ref} is a reference flow assumed to be larger than most of the flows in the network. In our simulations, Q_{ref} corresponds to the flow in the parent vessel. H_D represents the discharge haematocrit in the vessels, Q the flow in the segment and k_m is a constant characterizing the relative intensity of the metabolic stimulus.

3.3.4. Vascular adaptation

In light of the relationships described above, the model for vessel adaptation is given by the following:

$$\Delta R = \left[\underbrace{\log(\tau_w + \tau_{ref})}_{S_{wss}} - \underbrace{k_p \log \tau_e(P)}_{S_p} + \underbrace{k_m \log \left(\frac{Q_{ref}}{QH_D} + 1 \right)}_{S_m} - k_s \right] R \Delta t. \tag{20}$$

The additional term k_s represents the shrinking tendency of a vessel. This term is interpreted in [1] as reflecting a natural reaction of the basal lamina, which acts to counter any increase in vessel diameter.

Incorporation of the aforementioned mechanisms into our modelling framework now allows us to incorporate dynamic remodelling of the growing vasculature under the influence of blood rheological properties. This significant improvement in angiogenesis modelling allows us to describe vascular growth in a far more realistic manner and should lead to improved predictions of treatment efficiency. Fig. 6 shows schematically how the PDE formulation of vessel evolution and the effect of blood perfusion within the developing vascular network are coupled.

4. Simulation results

In the following section, we present results corresponding to a number of different stages in the formulation of our adaptive tumour-induced angiogenesis (ATIA) model. The first series of simulations is undertaken to demonstrate the effect of explicitly incorporating matrix degrading enzyme activity into the formulation. We are particularly interested in assessing its effect upon global bed architecture, i.e. can this extended model produce realistic vascular structures that compare well with experimental observations?

Table 2
Sprout branching probability as a function of the local TAF concentration

TAF concentration	Sprout branching probability
≤ 0.3	0.0
$]0.3-0.5[$	0.2
$]0.5-0.7[$	0.3
$]0.7-0.8[$	0.4
0.8	1.0

Throughout this first set of simulations, only *sprout* branching is considered and this is assumed to be controlled primarily by TAF concentrations in the local sprout environment. The next series of simulations, however, models capillary growth, sprout branching and *vessel* branching under the combined effects of both local TAF concentration and local wall shear stress. The resulting vascular structures are compared with structures generated without shear stress effects.

The final series of simulations examines the influence of blood rheological properties on vascular remodelling. Several stimuli are considered synergistically and results from the ATIA model are compared with the theoretical and experimental treatment in [1–3].

4.1. Simulation of tumour-induced capillary growth

The new model for tumour-induced capillary growth described by the system of equations (1)–(4) was solved numerically on an $80 \times 80(x, y)$ grid using square cells of side $\Delta x = \Delta y$. The system of equations (1)–(4) was solved for each grid point at each time step and the resulting variables c and f were then used to calculate the coefficients P_0 to P_4 appearing in Eq. (5), which define the preferred migratory direction of each endothelial cell, i.e. the direction of growth of each sprout. Unless otherwise indicated, the dimensionless parameter values used for the simulations presented in this paper were as follows [6]:

$$D = 0.00035 \quad \delta = 0.6 \quad \chi = 0.38 \quad \rho = 0.16 \\ \eta = 0.1 \quad \beta = 0.05 \quad \gamma = 0.1 \quad \alpha = 10e^{-6} \quad \varepsilon = 0.01 \quad \nu = 3.$$

The time parameter was normalized as:

$$\tilde{t} = \frac{t}{\tau} \tag{21}$$

with $\tau = L^2/D_c$, where $L = 2$ mm was the length of the domain and $D_c = 2.9 \times 10^{-7} \text{ cm}^2\text{s}^{-1}$ was taken as the diffusion coefficient for TAF [34,35].

At this stage, only sprout branching was considered, with the likelihood of a sprout branching only depending upon the local TAF concentration. The sprout branching probabilities associated with the various TAF concentration ranges have been chosen on a qualitative basis and reflect the well-documented observation of increased branching close to a tumour boundary [36,37]. The values chosen are given in Table 2 and reflect the values in [6].

Figs. 7 and 8 present the simulation results associated with an initial linear gradient of TAF (cf. Fig. 2(a))— Fig. 7 shows the network growth and vascular architecture, whilst Fig. 8 shows the associated MDE concentration in the domain. We observe in Fig. 7 the stochastic nature of each of the five sprout trajectories as they progress towards the tumour (the surface of which occupies the lower boundary of the domain). At time $\tilde{t} = 2$ (corresponding to $t = 3$ days), sprout branching starts to occur for sprouts 2 and 4 (numbered from left to right) and by $\tilde{t} = 3$ (4.5 days) a small degree of local anastomosis has already taken place for three of the five sprouts. Note, however, that no perfusion would occur within the capillary bed at this time, as no large-scale arcades have yet been formed.

Anastomosis increases considerably with increased sprout branching in regions far from the parent vessel and the various vascular trees rapidly connect with one another after $\tilde{t} = 6$ (9 days). This increase in anastomosis is related to an increase in lateral migration due to the appearance of a connected MDE front as shown in Fig. 8 (from $\tilde{t} = 6$). It takes approximately 15 days for the growth process to be completed, i.e. for the vasculature to connect the tumour to the parent vessel and hence to the blood supply. It is assumed that once a sprout reaches the tumour, it continues to

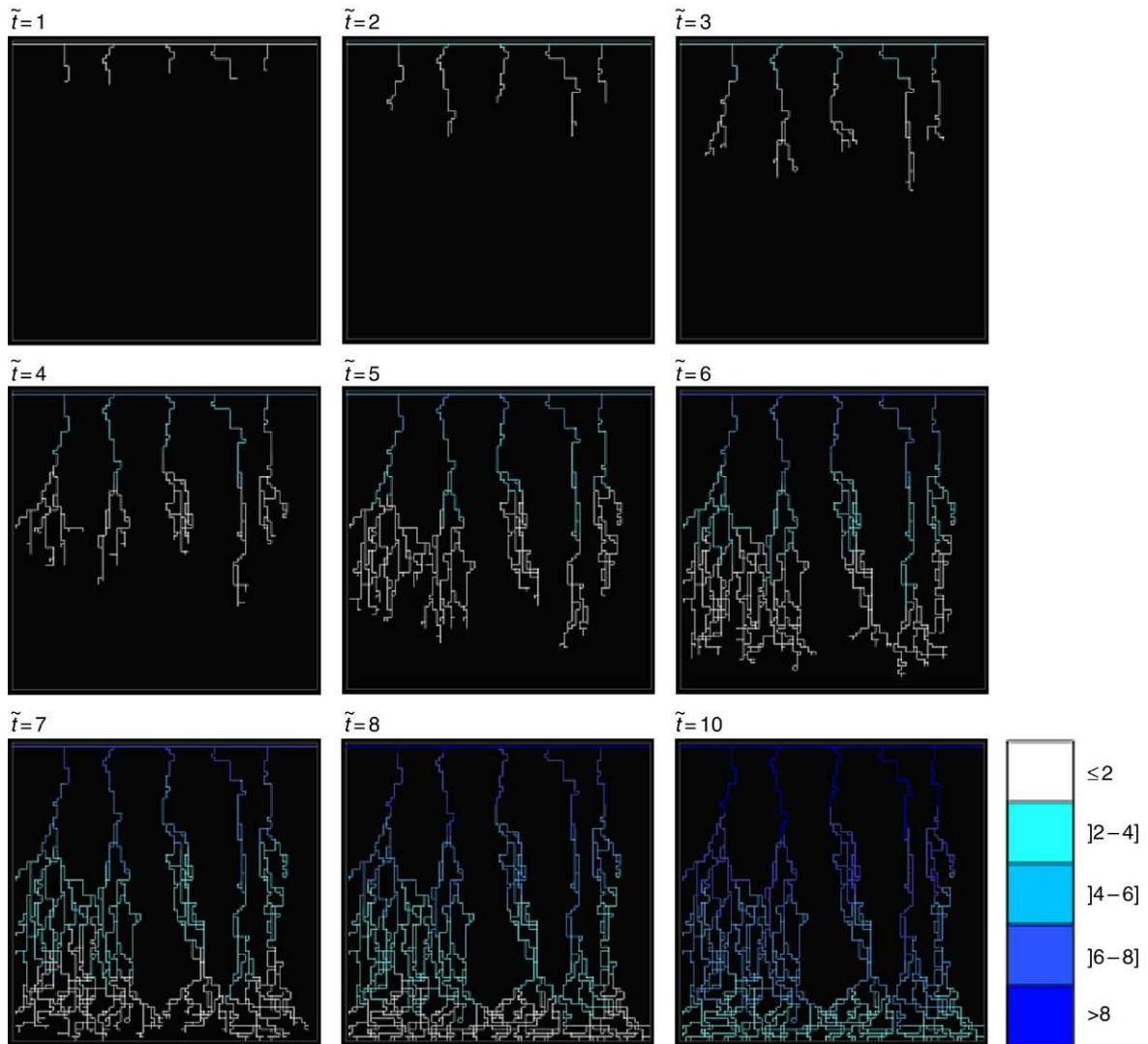


Fig. 7. Capillary network growth in response to an initial linear gradient of TAF. Only sprout branching has been considered for the simulations. The colour code used allows us to identify the age of each vessel from $\tilde{t} \leq 2$ (i.e. $t \leq 3$ days) to $\tilde{t} > 8$ (i.e. $\tilde{t} = 12$ days).

grow into it. In terms of the present model this means that the sprouts disappear from the domain of simulation, and this explains why the MDE is no longer present in the domain at the end of the simulation (Fig. 8).

Figs. 9 and 10 present simulation results associated with an initial circular gradient of TAF (cf. Fig. 2(b)) and show the network growth and associated MDE concentration in the domain. We observe that the five initial sprouts rapidly converge ($\tilde{t} = 3.5$). This consequently leads to the fusion of the individual MDE sources which restricts the capillary growth to a narrow corridor, where the MDE concentration is high. This continues until the sprouts reach the tumour and the sources disappear from view. Once again, the vascularisation process takes approximately 15 days.

4.2. Capillary network growth regulated by the wall shear stress

We now present results from simulations that take into account the influence of wall shear stress upon the branching process — this leads to vessel branching in addition to the existing sprout branching previously considered. Note that sprout branching is still important in the production of the first connections between the growing vascular trees, allowing blood to flow into the evolving capillary bed, thereby creating the shear stresses required for vessel branching. The combined effects of the local wall shear stress and TAF concentration upon vessel branching probability have been

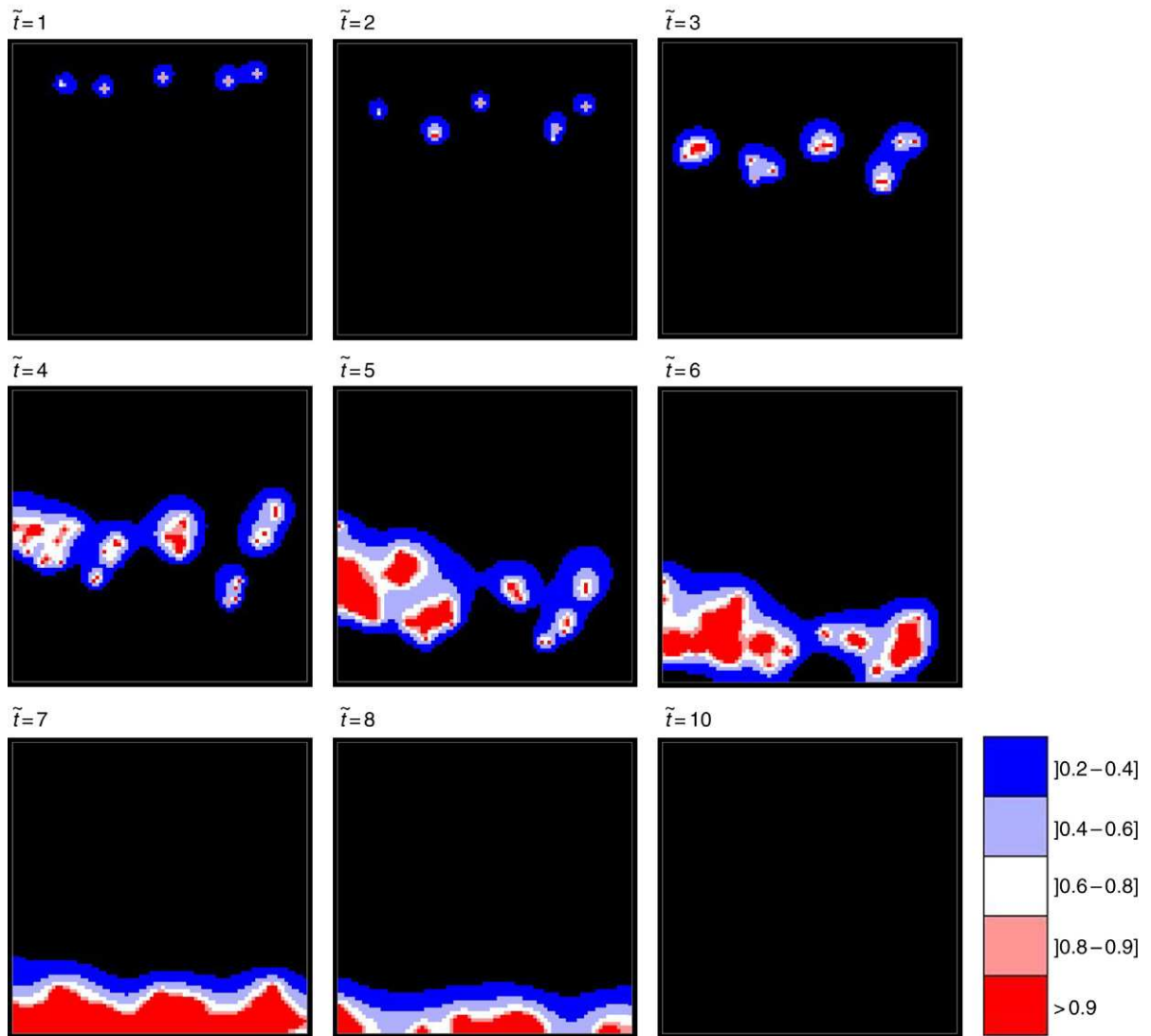


Fig. 8. MDE density produced by the endothelial cells of the growing sprouts. The density profile of MDE is associated with the capillary network growth in response to a linear gradient of TAF. The colour code represents the MDE density as a fraction of its maximum value.

implemented in the model as described in Table 3. The probabilities chosen for the vessel branching process have once again been defined on a qualitative basis in order to reflect the combined influence of the wall shear stress (WSS) and local TAF concentration. High values of WSS in tandem with high local TAF concentrations lead to a higher branching probability, whilst lower values of one or both of WSS and TAF concentration lead to lower branching probability. Note that the process of vessel branching supplements the process of sprout branching. Therefore the probabilities of sprout branching are still the same and given in Table 2; hence, we regain the original results (Figs. 7 and 8) when the shear stress effect is removed.

Another constraint on vessel branching is the age of the vessel. This range has been fixed at [4–8] days in the simulations (i.e. from $\tilde{t} = 2.66$ to $\tilde{t} = 5.33$), and corresponds to a timescale where the vessel is sufficiently mature for branching but has not had sufficient time to develop a basal lamina (which would contribute considerably to the stabilization of the network) [27,38].

Figs. 11 and 13 display the simulation results, which correspond to an initial linear gradient of TAF and an initial circular gradient of TAF, respectively. We note that the model parameters used for these simulations are the same as those used in the previous simulations. The associated MDE density evolution corresponding to each case are given in

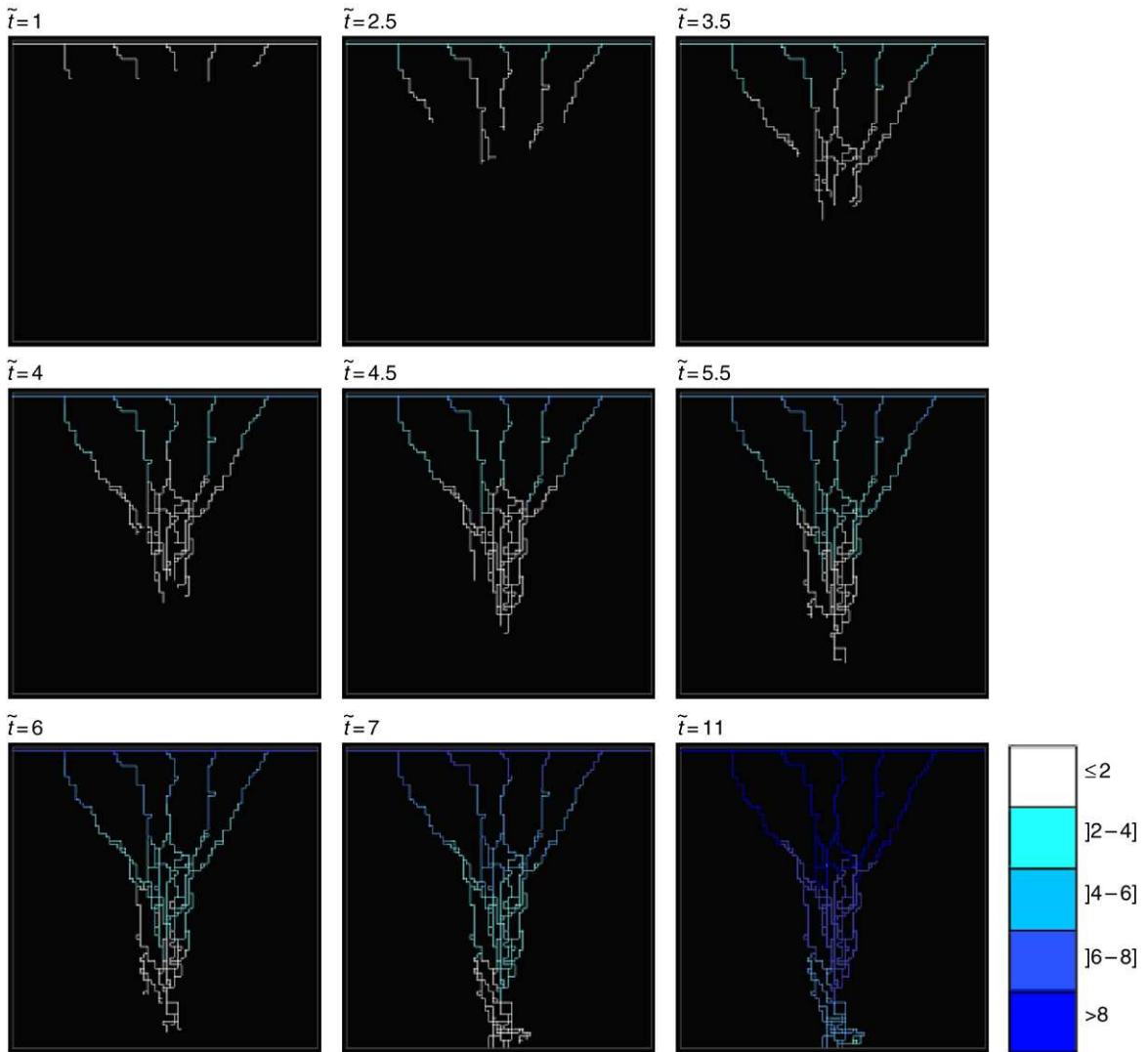


Fig. 9. Capillary network growth in response to an initial circular gradient of TAF. Only sprout branching has been considered for the simulations. The colour code used allows us to identify the age of each vessel from $\tilde{t} \leq 2$ (i.e. $t \leq 3$ days) to $\tilde{t} > 8$ (i.e. $\tilde{t} = 12$ days).

Table 3

Vessel branching probabilities according to the local TAF concentration and to the magnitude of the wall shear stress

[WSS] [TAF]	≤ 0.4	$]0.4-0.6]$	$]0.6-0.8]$	0.8
≤ 0.3	0	0	0	0
$]0.3-0.5[$	0	0	0.1	0.3
$]0.5-0.7[$	0	0	0.3	0.5
$]0.7-0.8[$	0	0.1	0.4	0.6
0.8	0	0.5	0.8	1.0

(The TAF concentrations are normalized by the maximum value $c_0 = 10^{-10} M$ taken for the simulations as in [6], and the WSS values are fractions of the maximum stress τ_{ref} [2].)

Figs. 12 and 14. In both cases, sprout branching is initially the only branching process and the vascular architectures are identical to those emerging from the previous simulations (compare Figs. 11 and 13 with Figs. 7 and 9). Vessel branching only begins after the vascular trees have formed arcades — the capillary bed then becomes perfused with

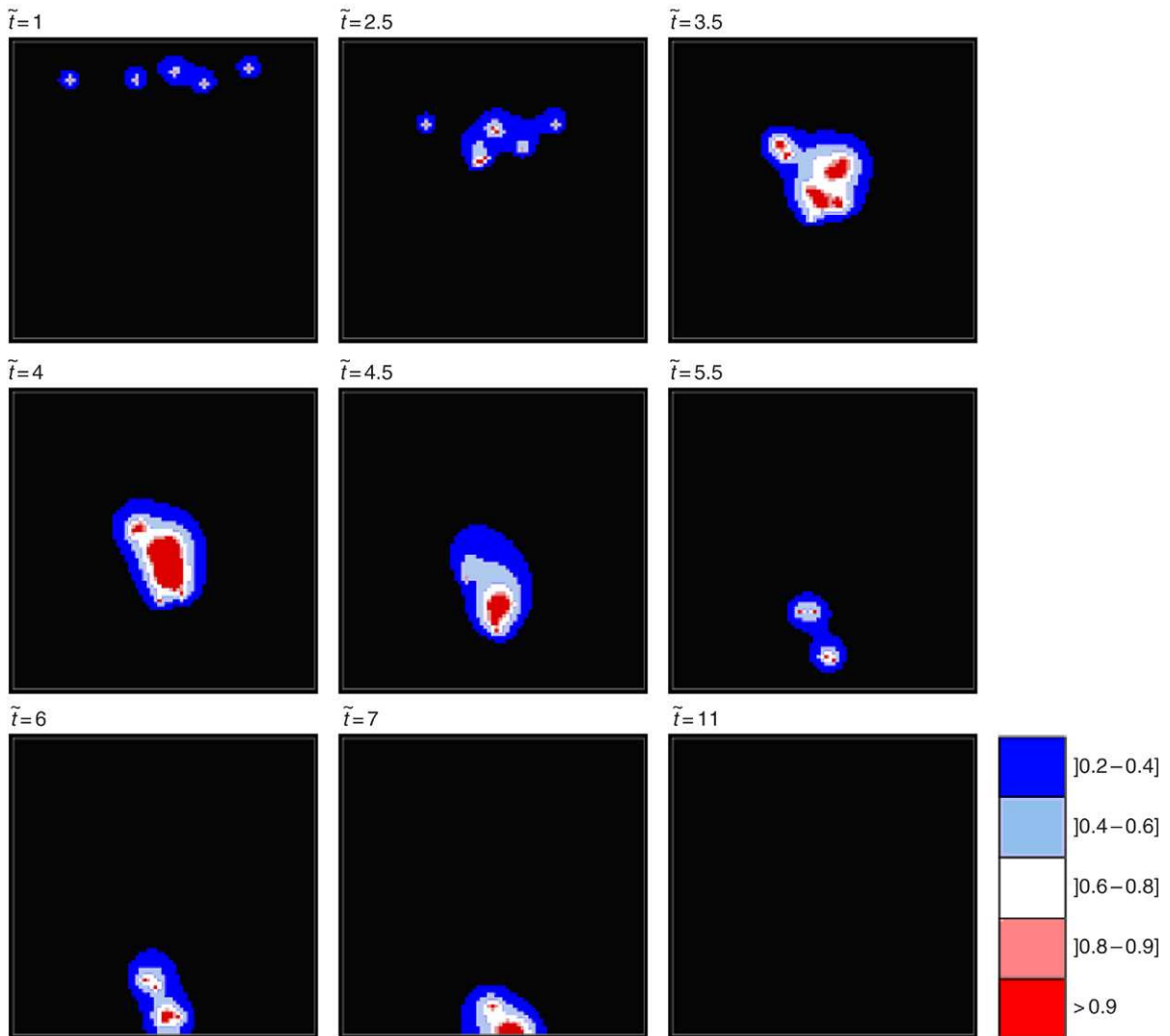


Fig. 10. MDE density produced by the endothelial cells of the growing sprouts. The density profile of MDE is associated with the capillary network growth in response to a circular gradient of TAF. The colour code represents the MDE density as a fraction of its maximum value.

blood and shear stresses are exerted on vessel walls. The effect is highlighted in Fig. 15, where new capillaries can be seen branching from mature vessels once more distal arcades have formed and begun to flow. At this stage the vascular architectures diverge from the previous cases (Figs. 7 and 9) with high capillary densities close to the tumour surface.

The new emerging sprouts produce MDE and can therefore be easily spotted on the MDE density snapshots displayed in Figs. 12 and 14. For the linear case (Fig. 12) at time $\tilde{t} = 8$ new patches appear behind the initial MDE front produced via sprout branching. At time $\tilde{t} = 14$ additional isolated patches appear but rapidly vanish as the newly formed vessels reconnect to the dense network.

Vessel branching is even more apparent for the circular tumour case (Fig. 14), as the main growing vessels rapidly converge. Although the primary front of MDE (due to sprout branching) has already reached the tumour at time $\tilde{t} = 6$, a secondary front of MDE (now due to vessel branching) appears. This progresses once again towards the tumour, reinforcing network density and connectivity. Isolated patches of MDE can still be seen at later times until the network becomes fully mature and stabilised.

The evolution of the vasculature is dynamic and continuous, with local increases in network connectivity leading to a *global* redistribution of wall shear stress. This redistribution can, in turn, reinforce vessel connectivity in other parts

of the network and modify flow accordingly. We can see in all cases how the part of the network close to the surface of the tumour becomes particularly dense — the branching probability in this region is high and this contributes to the reinforcement phenomenon. The model consequently reproduces the brush border effect observed experimentally particularly well.

4.3. Comparison of the network architectures

Although differences in bed architecture with and without the influence of WSS may, at first glance, appear somewhat modest, the global effects of these variations can be very profound indeed, particularly with regard to drug delivery to the tumour [5]. The aim of this section is to quantitatively compare vasculatures generated from a sprout-only branching model with those obtained from a model that includes vessel branching. To this end, three vasculatures have been generated corresponding to three different values of the haptotactic coefficient ($\rho = 0.12$, $\rho = 0.16$ and $\rho = 0.20$). We then compare the density and spatial distribution of connectivity under sprout-only branching and sprout-plus-vessel branching. The connectivity is defined here as the number of capillary elements meeting at a given node (junction) of the vascular network. Hence for 2D vasculatures, the branching coefficient at each node can either be 4, 3, 2 or 1 (identified as z_4 , z_3 , z_2 and z_1 in Figs. 16 and 17). The global architecture of a network is then characterised by scanning the capillary bed parallel from the parent vessel to the tumour. At each cross-sectional ordinate (x) we calculate the sums:

$$z_i^{\text{tot}} = \text{total number of nodes having } i \text{ capillary elements connected to it } (i = 1, 2, 3, 4).$$

This procedure was proposed in an earlier paper [5] and allows us to construct a “fingerprint” for each network. Comparisons between vasculatures generated with and without the influence of wall shear stress show some major differences in vessel density and connectivity. In the first case presented in Fig. 16 (no WSS effect), the capillary density increases monotonically as we approach the tumour surface and the connectivity is essentially dominated by z_2 (i.e. connections between two vessels). In the second case (with WSS), the increase in vessel connectivity is more dramatic as we approach the tumour surface, dominated this time by z_3 and z_4 (i.e. involving connections between 3 or 4 vessels). These latter vascular beds thus exhibit a more complex level of organisation with distinct, identifiable regions, *viz* a ramified region proximal to the parent vessel and a dense, highly connected region close to the tumour surface. The reinforcement mechanism described previously is responsible for the amplification in vascular connectivity (branching and anastomosis) near the tumour.

4.4. Capillary network adaptation to rheological constraints

The second feedback mechanism we consider in the context of dynamic angiogenesis involves vascular remodelling, where the radii of vessels within a pre-existing homogeneous network evolve dynamically under the influence of different haematocrit distributions. The vasculatures generated in the previous sections were homogeneous in radius, in the sense that the radii of all the vessels were assumed to remain invariant throughout a simulation. In reality, the distribution of capillary radii is highly heterogeneous, as the radius of a capillary strongly depends upon its associated flow, blood haematocrit, blood viscosity and upon a range of stimuli, both haemodynamic (shear stress, intravascular pressure) and metabolic. Obviously, adaptation of the vessel radii begins as soon as blood begins flowing through capillary arcades. This means that the vasculature is constantly adapting and remodelling itself during the growth process and this is the underlying ethos of adaptive tumour-induced angiogenesis. As a precursor to our ultimate aim of producing a fully-adaptive model, we begin by considering how perfusion within an initially homogeneous capillary bed influences radii adaptation, i.e. vascular remodelling. We note that a similar approach, coupled to a cellular automaton model of tumour growth, has recently been used in [13] to investigate heterogeneous distributions of haematocrit *within* tumours.

In the simulations presented below, the initial condition is that of a homogeneous bed, corresponding to that generated in Fig. 7. The initial radius of each capillary segment is 6 mm except the radius of the parent vessel, which is 14 mm and is assumed to remain constant. Remodelling of the vessels is permitted within a range, from a minimum radius of 2 mm to a maximum radius of 12 mm. These values correspond to vessel radii at the capillary level, where the size of the vessels are very close to the size of the red blood cells [39]. The parameters of the adaptation model given in Eq. (19) are:

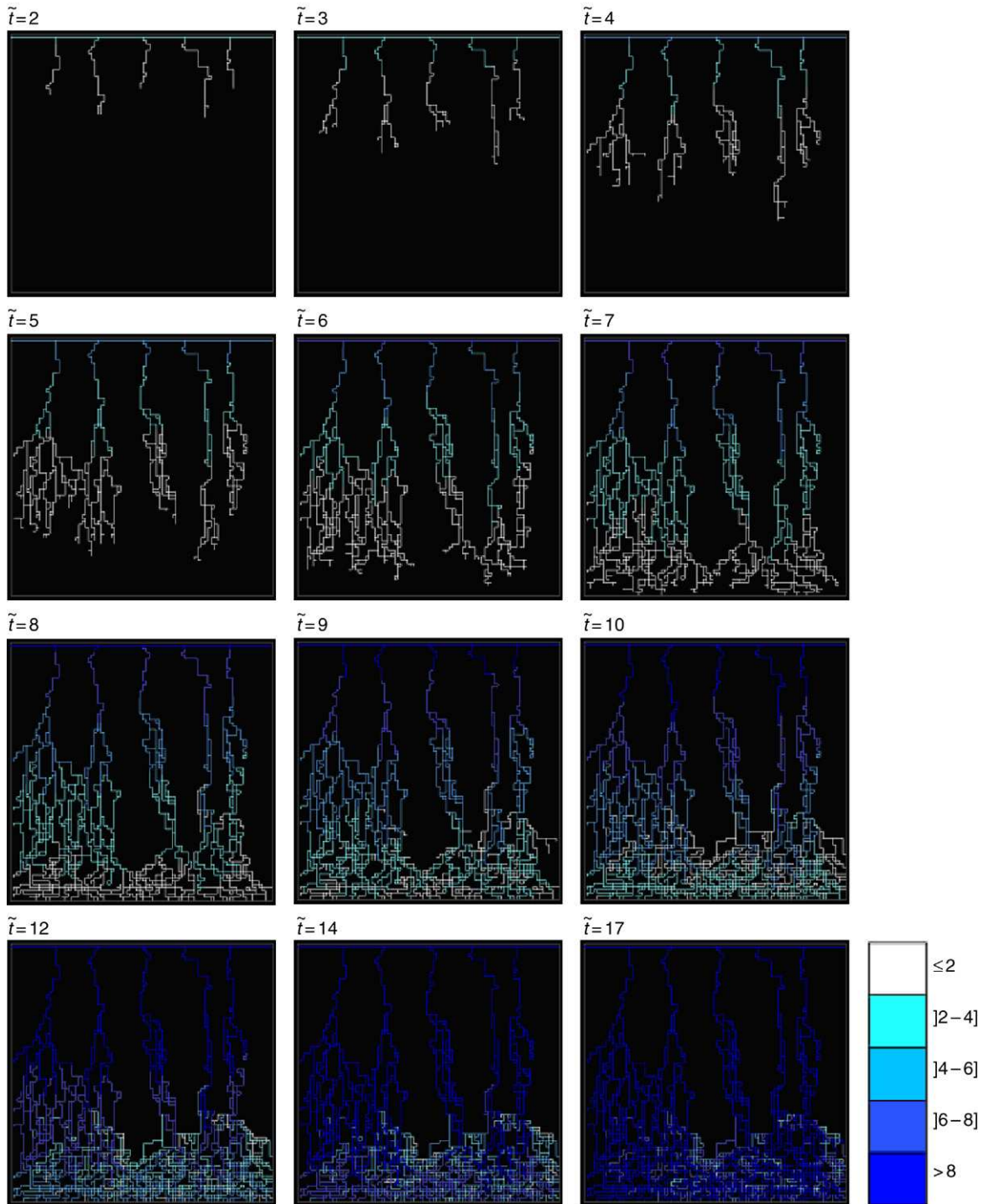


Fig. 11. Capillary network growth in response to an initial linear gradient of TAF. Vessel branching influenced by the local wall shear stress and TAF concentration is considered in addition to sprout branching. The colour code used allows us to identify the age of each vessel from $\tilde{t} \leq 2$ (i.e. $t \leq 3$ days) to $\tilde{t} > 8$ (i.e. $\tilde{t} = 12$ days).

$$\begin{aligned}
 k_s &= 0.35 & k_p &= 0.5 & k_m &= 0.12 \\
 \tau_{\text{ref}} &= 0.103 & Q_{\text{ref}} &= 1.909e^{-11}
 \end{aligned}$$

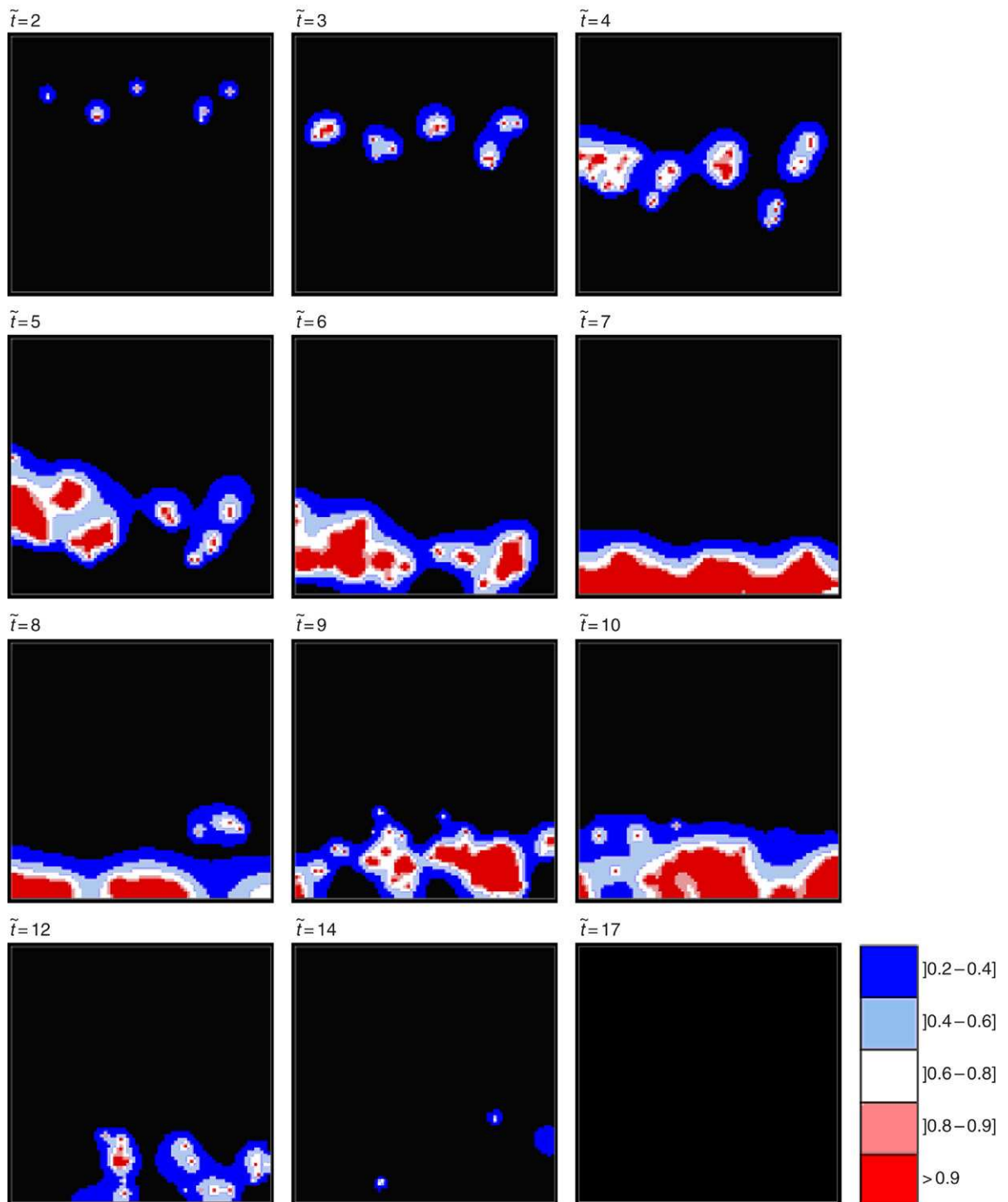


Fig. 12. MDE density produced by the endothelial cells of the growing sprouts. The density profile of MDE is associated with the capillary network growth in response to a linear gradient of TAF where both sprout and vessel branching are considered. The colour code represents the MDE density as a fraction of its maximum value.

Q_{ref} to the flow in the parent vessel, calculated from Eq. (11) with $R = 14 \mu\text{m}$, $L = 2 \text{ mm}$ and $\Delta P = 8000 \text{ Pa}$ (60 mmHg) (pressure drop across the parent vessel). The apparent viscosity μ_{app} is measured

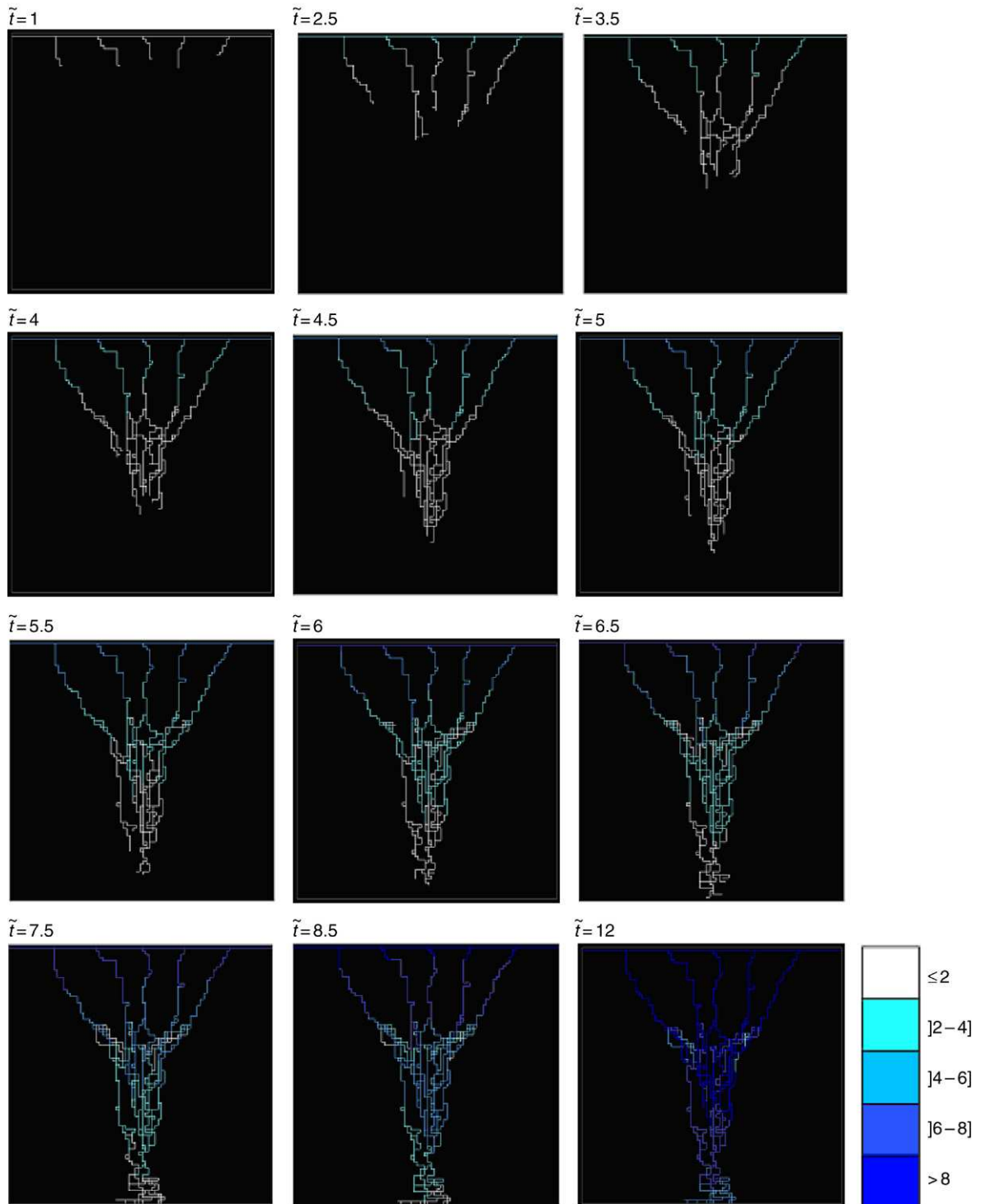


Fig. 13. Capillary network growth in response to an initial circular gradient of TAF. Vessel branching influenced by the local wall shear stress and TAF concentration is considered in addition to sprout branching. The colour code used allows us to identify the age of each vessel from $\tilde{t} \leq 2$ (i.e. $t \leq 3$ days) to $\tilde{t} > 8$ (i.e. $\tilde{t} = 12$ days).

from Fig. 3 for a discharge haematocrit $H_D = 0.45$ which is assumed to remain constant in the parent vessel [2].

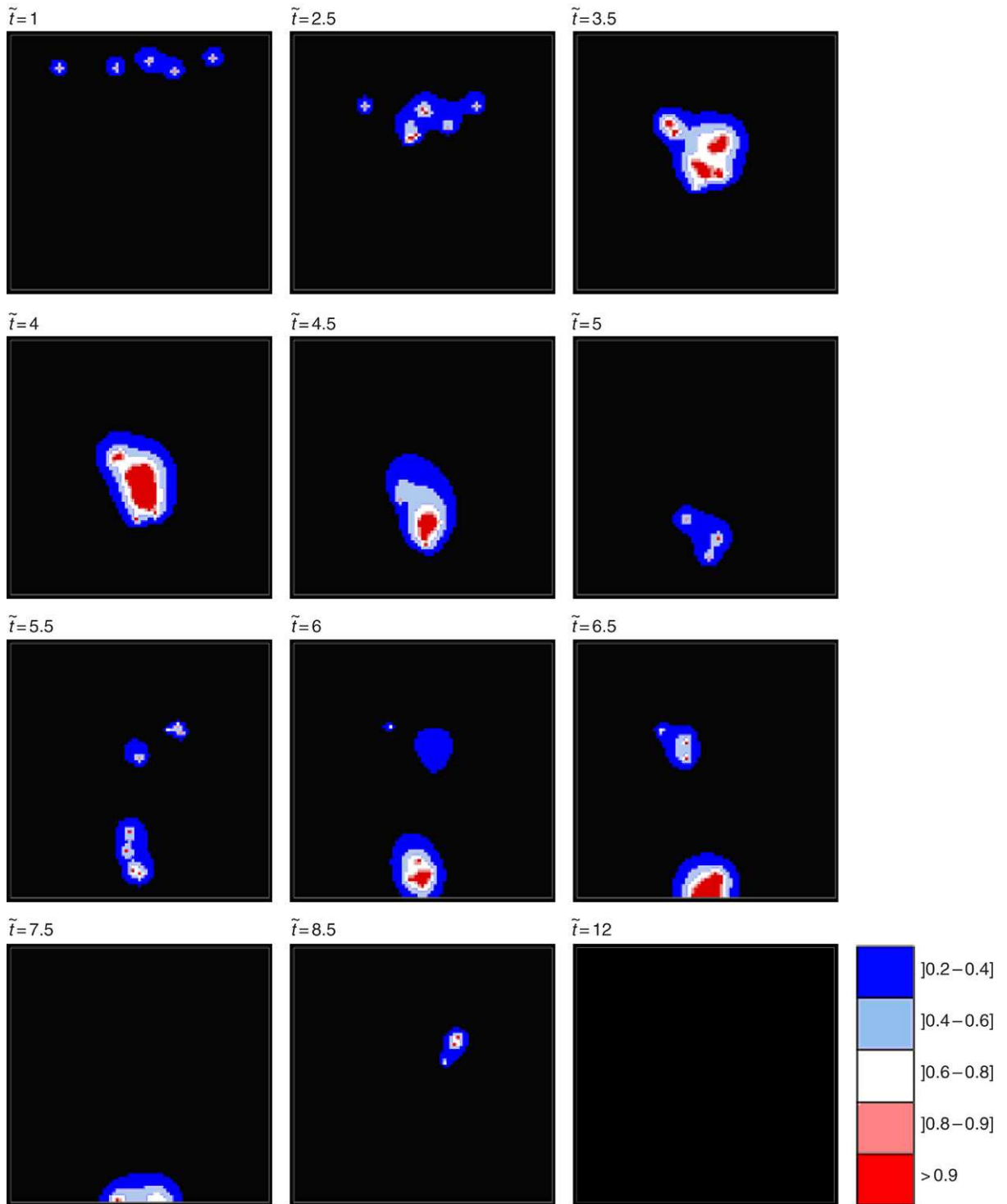


Fig. 14. MDE density produced by the endothelial cells of the growing sprouts. The density profile of MDE is associated with the capillary network growth in response to a circular gradient of TAF where both sprout and vessel branching are considered. The colour code represents the MDE density as a fraction of its maximum value.

Fig. 18 shows the results of vascular remodelling. Initially, the vasculature is filled only with plasma i.e. the blood haematocrit $H_D = 0$ (note that there is no contribution from the metabolic stimulus, i.e. $k_m = 0$ until H_D is non-zero).

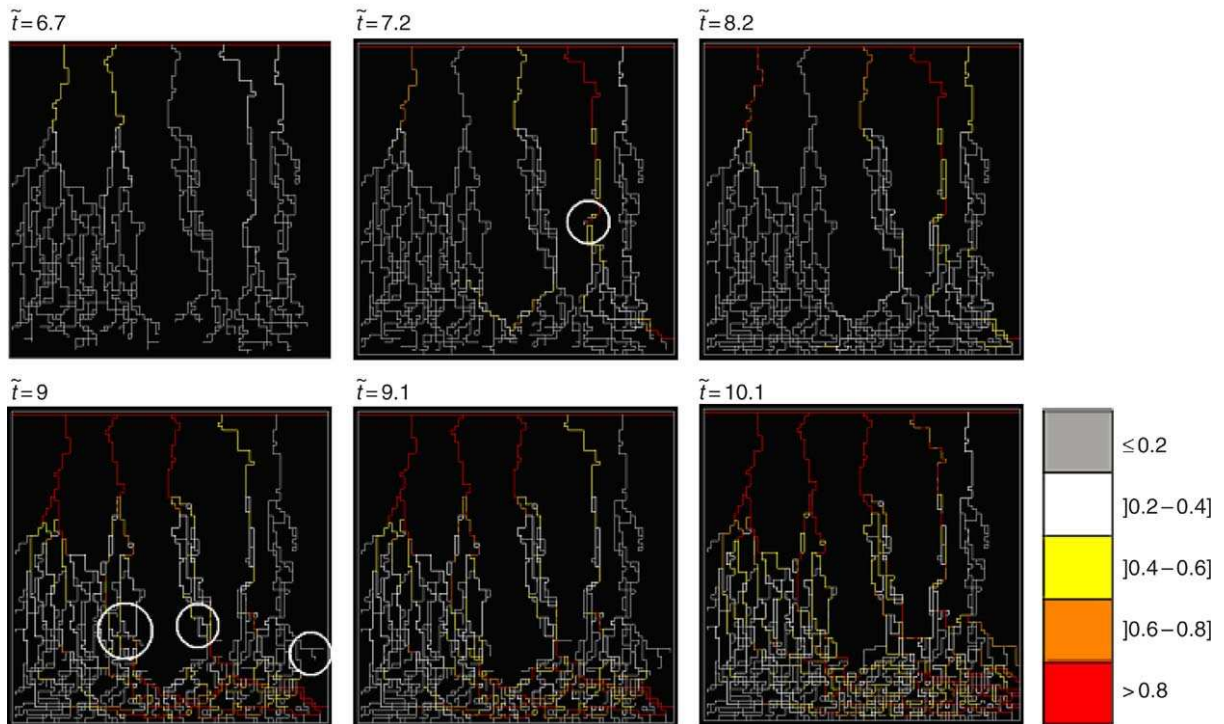


Fig. 15. Successive snapshots of the capillary bed taken at different stages of the growth process showing the associated magnitude of the wall shear stress in the vessels. Each vascular tree grows independently until it connects with another. Each connection leads to a sharp increase in the local wall shear stress, as the blood is able to flow in the recently-connected structure (for $\tilde{t} = 7.2$ a connection occurs in the middle of the bed and for $\tilde{t} = 9$ other connections are made). This leads to the emergence of new sprouts (highlighted by the white circles) in upper parts of the network where the wall shear stress is sufficiently high. The colour code represents fractions of the maximum WSS.

Red blood cells then begin flowing into the network from a parent vessel of constant haematocrit $H_D = 0.45$. The left column of Fig. 18 (a, c, e and g) shows the evolution of vessel radii at times $\tilde{t} = 1, 2, 3.5$ and 4 respectively. The right column of Fig. 18 (b, d, f and h) shows the corresponding haematocrit distribution in the vasculature. Fig. 19 shows the results of vascular remodelling for the same architecture containing an initial patch of red blood cells (haematocrit $H_D = 0.45$) located in the middle of the network (Fig. 19(b)). The aim here was to assess the importance of the initial haematocrit distribution in the network in determining its final morphology. Fig. 19 (a, c, e and g) shows the vessel radii at times $\tilde{t} = 1, 2, 3.5$ and 4 respectively, and Fig. 19 (b, d, f and h) the corresponding haematocrit profiles. Comparison of the two resulting adapted vasculatures shows that there are only minor differences in the final distributions of vessel radii.

4.5. Nutrient and drug transport through the adapted network

In the previous section, the implementation of vascular adaptation mechanisms allowed us to generate a network that appears morphologically realistic. The aim of this section is to quantify the efficiency of such a network vis à vis its ability to carry blood-borne material (nutrients, drugs) to the tumour. In order to achieve this, we infuse a tracer into the parent vessel and observe its evolution within the capillary bed. Two different drug delivery regimes have been considered, either a continuous infusion or a bolus injection.

Figs. 20 and 21 correspond to the continuous infusion case. Fig. 20 shows how the total mass of tracer in both the adapted and homogeneous vasculatures vary with time. We observe that the amount of tracer is significantly higher in the adapted vasculature and steady-state is reached far sooner. This is due to (i) radial adaptations resulting in a doubling of the total volume of the vasculature itself, and (ii) vascular remodelling at the bed scale, which mitigates the bypassing effects characteristic of highly connected homogeneous systems. This has important consequences for modelling cancer treatment using cytotoxic drugs — Fig. 21 presents a comparison of drug uptake between the adapted

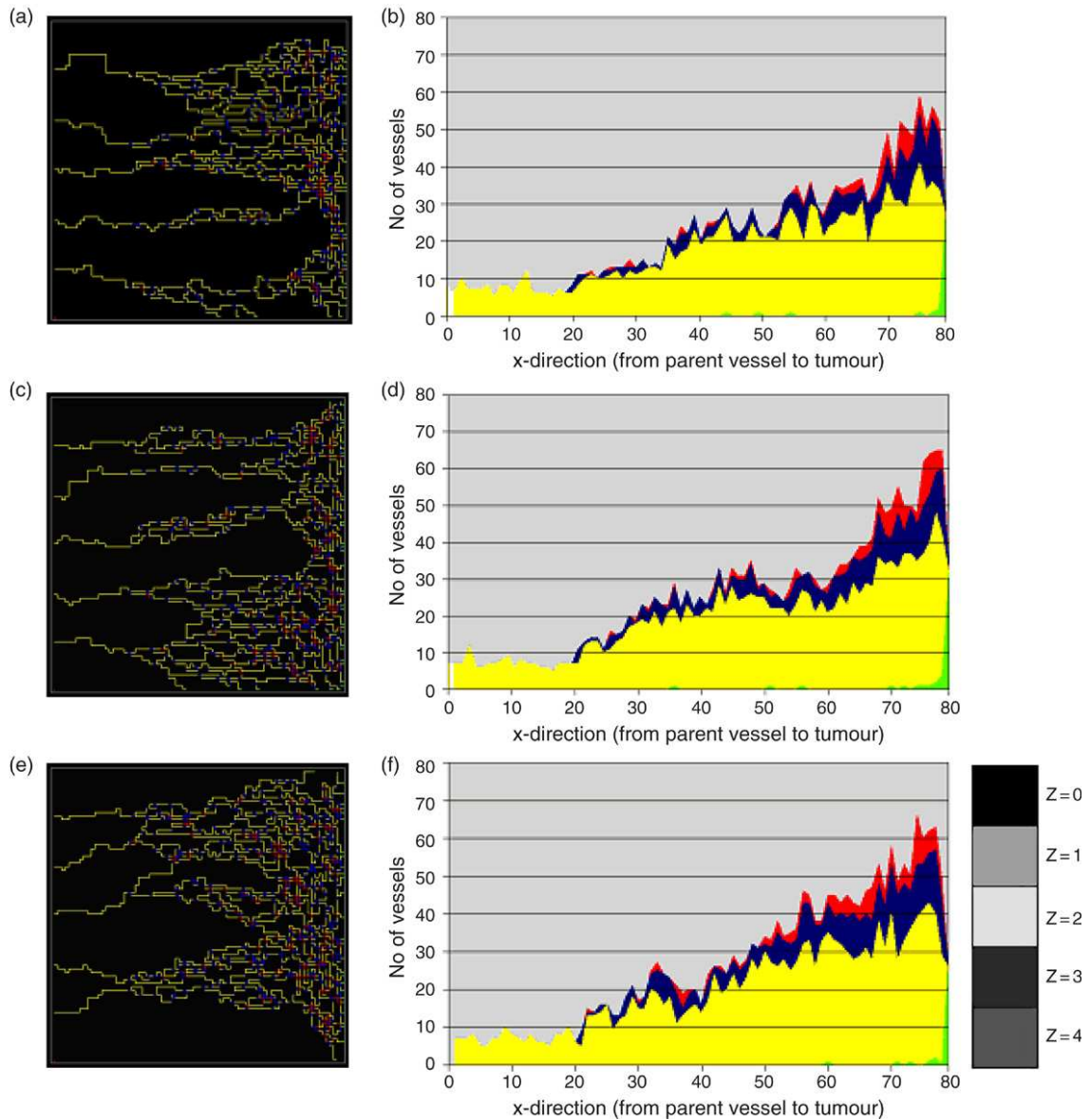


Fig. 16. (a, c, e) Vasculatures generated with sprout branching only (i.e. no influence of the wall shear stress) for various values of the haptotactic coefficient ($\rho = 0.12$, $\rho = 0.16$ and $\rho = 0.20$ respectively). Each vessel is coloured according to its connectivity (number of connecting vessels); (b, d, f) corresponding spatial density and connectivity maps. The lower region of the capillary bed (near the tumour surface, here on the right hand side) is dominated by a connectivity level z_2 .

and homogeneous vasculatures (assuming the tracer to be a cytotoxic agent). We find that delivery to the tumour under conditions of continuous infusion is faster and more effective in the adapted network.

Figs. 22 and 23 correspond to the bolus injection case, with the aim to investigate dilution effects. The injection is turned off at the normalized instant $\tilde{t} = 1.45$. Fig. 22 shows, as in the previous continuous case, that the resistance to the flow has been significantly reduced in the adapted network as the radii of the main vessels are bigger. This effect is not only reflected in the differences of amplitude between the curves but also by the shapes of the curves. Whereas in the adapted case the amount of drug decreases linearly as soon as the injection of drug is turned off, the decrease is non-linear in the homogeneous case and reflects the resistance effect. This means that no main path exists to direct the flow towards the tumor and blood has to find its way through the gap in connectedness existing between the few

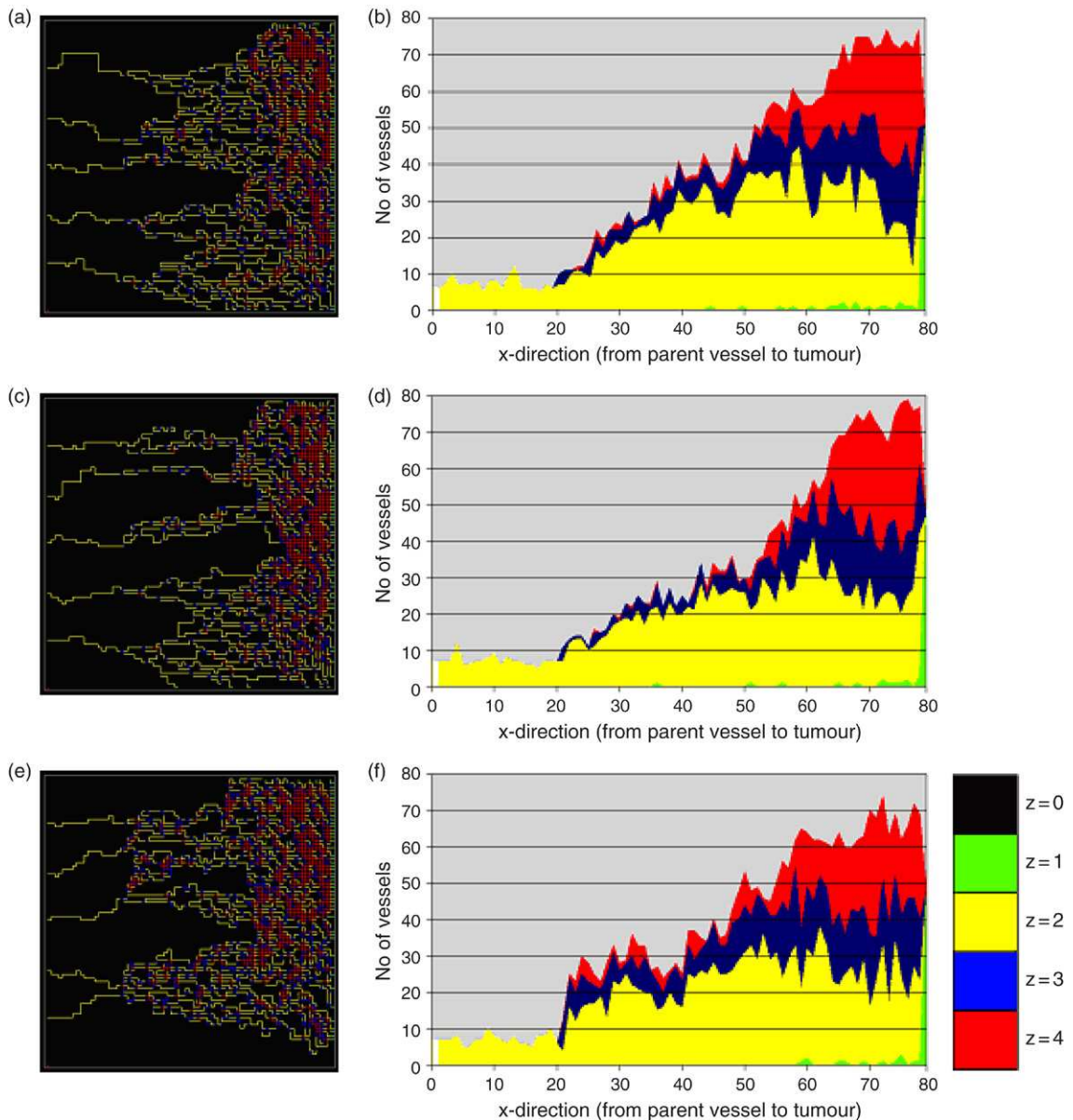


Fig. 17. (a, c, e) Vasculatures generated with sprout and vessel branching (i.e. taking into account the influence of the wall shear stress) for various values of the haptotactic coefficient ($\rho = 0.12$, $\rho = 0.16$ and $\rho = 0.20$ respectively). Each vessel is coloured according to its connectivity (number of connecting vessels); (b, d, f) corresponding spatial density and connectivity maps. The lower region of the capillary beds (near the tumour surface, here on the right hand side) is dominated by a connectivity level z_3 and z_4 .

main upper vessels and the bulk of the network (more connected). This obstacle is reduced in the adapted network where preferential paths for blood flow have been formed to connect the parent vessel to the tumour. Although the same amount of drug has been perfused in both the homogeneous and the adapted network, we observe from Fig. 22 that most of it does not even penetrate into the vasculature in the homogeneous case. Consequently, the amount of drug reaching the tumour is significantly smaller for this vasculature compared to the adapted one as exhibited in Fig. 23.

These simulations demonstrate that vascular adaptation and the resulting bed architecture are important issues that cannot be ignored when modelling angiogenic processes at the micro-scale.

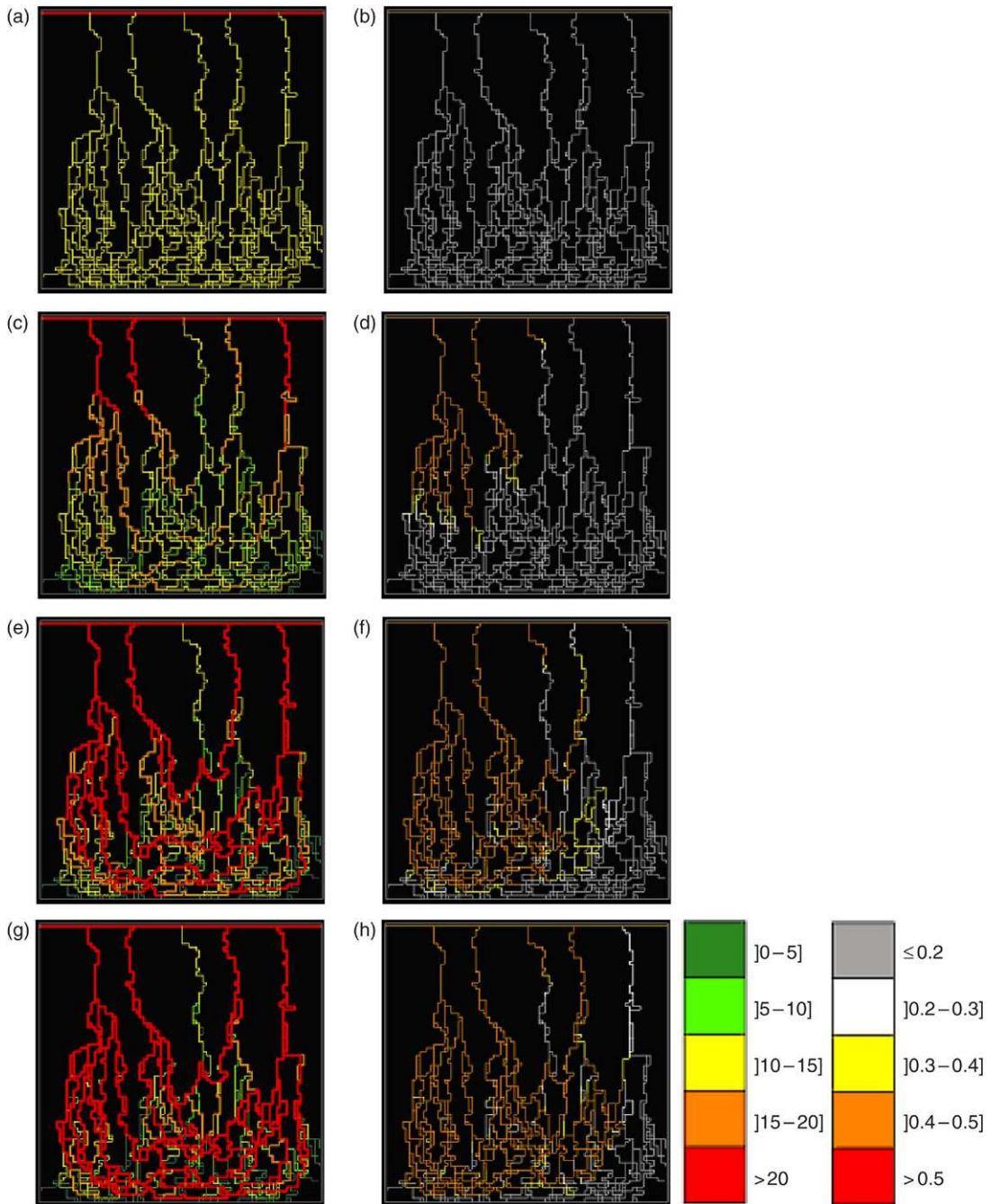


Fig. 18. Left column (a, c, e, g) evolution of the vascular adaptation process from an initial homogeneous network with vessel radius $R = 6 \mu\text{m}$ (the colour code on the left represents the vessel radius in μm). Snapshots correspond to times $\tilde{t} = 1, 2, 3.5$ and 4 respectively. Right column (b, d, f, h) the corresponding haematocrit evolution from an initial blood cell free network (the colour scale on the right represents the blood haematocrit).

5. Discussion and conclusion

The mathematical modelling of blood flow in a tumour-induced capillary network was considered in earlier papers [4] and [5]. The aim then was to investigate the process of drug delivery to a tumour via a static vascular network containing a Newtonian fluid of constant viscosity. These hypotheses were clearly very crude approximations of

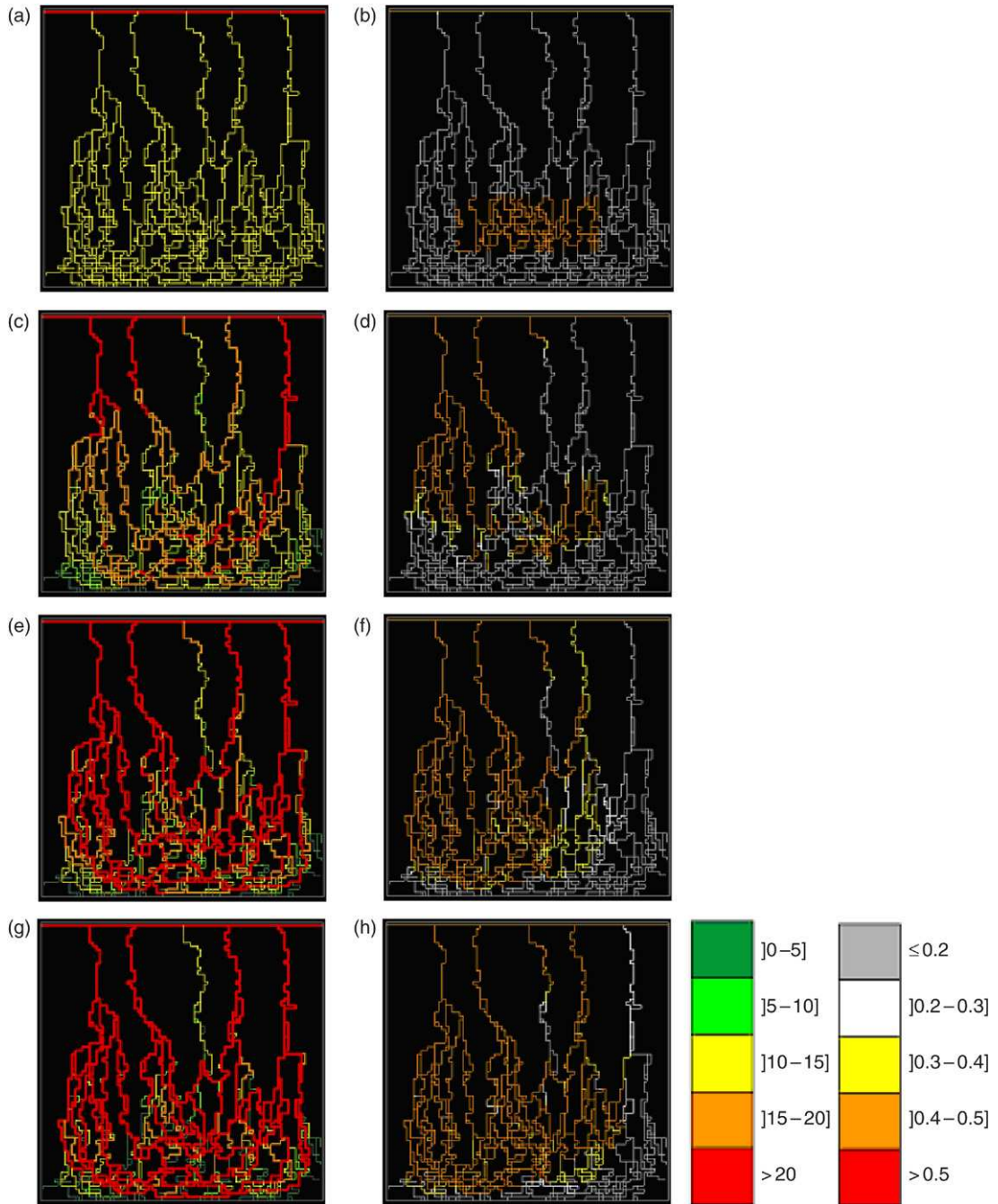


Fig. 19. Left column (a, c, e, g) evolution of the vascular adaptation process from an initial homogeneous network with vessel radius $R = 6 \mu\text{m}$ (the colour code on the left represents the vessel radius in μm). Snapshots correspond to times $\tilde{t} = 1, 2, 3.5$ and 4 respectively. Right column (b, d, f, h) corresponding haematocrit evolution from an initial localized patch or red blood cells in the centre of the vasculature (the colour scale on the right represents the blood haematocrit).

the actual physical situation and the present paper has sought to make significant improvements concerning both network growth and flow modelling. To this end, we have presented results corresponding to a number of different stages in the formulation of our adaptive tumour-induced angiogenesis (ATIA) model. Firstly, a new formulation for tumour-induced angiogenesis in the absence of flow has been proposed. This new model, utilized as a foundation

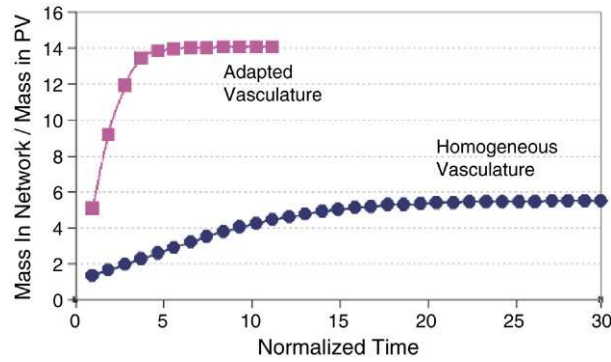


Fig. 20. Comparison of the total mass of drug in the adapted and homogeneous vasculatures (normalized by the total mass of drug in the parent vessel) for a continuous infusion regime of the drug into the parent vessel. (the normalized time unit \tilde{t} represents approximately $t = 0.2$ min).

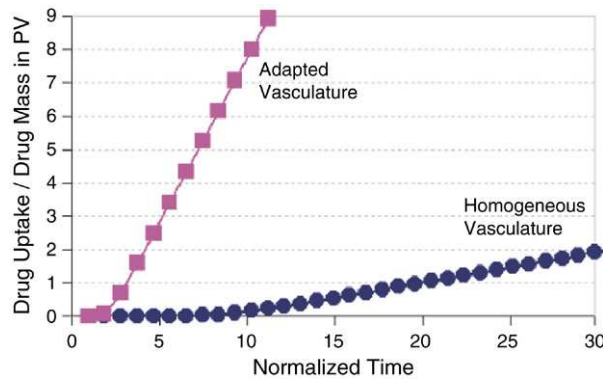


Fig. 21. Comparison of the total drug uptake by the tumour for the adapted and homogeneous vasculatures (normalized by the total mass of drug in the parent vessel) for a continuous infusion regime of the drug into the parent vessel (the normalized time unit \tilde{t} represents approximately $t = 0.2$ min).

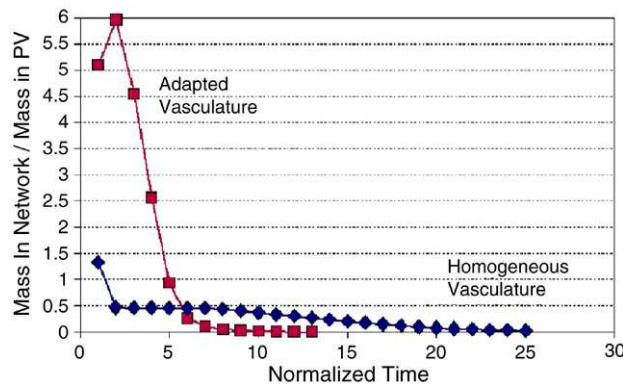


Fig. 22. Comparison of the total mass of drug in the adapted and homogeneous vasculatures (normalized by the total mass of drug in the parent vessel) for a bolus injection regime of the drug into the parent vessel (injection is turned off at normalized time $\tilde{t} = 1.45$, namely $t = 0.3$ min).

for subsequent development, is based on the earlier approaches in [6,15,16]. Here, we explicitly take account of the regulation of the growth process by a matrix-degrading enzyme (MDE), produced by the migrating endothelial cells. The motivation for taking this enzyme into account explicitly in the modelling formulation is due to its importance as a new target for therapy. Indeed, several studies [17–20] have shown that the formation of a functional vascular network is strongly dependent upon the proteolytic activity of this protein.

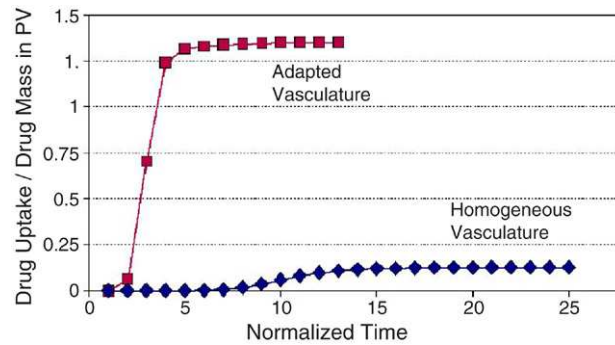


Fig. 23. Comparison of the total drug uptake by the tumour for the adapted and homogeneous vasculatures (normalized by the total mass of drug in the parent vessel) for a bolus injection regime of the drug into the parent vessel (injection is turned off at normalized time $\tilde{t} = 1.45$, namely $t = 0.3$ min).

Another aim of this study has been to increase the realism of vascular modelling algorithms, leading to improved simulations of therapy protocols and allowing treatment efficiency to be quantified more effectively. A more in-depth analysis of cancer therapy will be the focus of a forthcoming paper. Here, we have focused on network adaptation due to rheological constraints, where blood has been considered as a non-Newtonian fluid, in keeping with its biphasic nature.

We have first considered the influence of blood flow on the vascular growth process itself and our approach has been based upon experimental observations, which suggest that the wall shear stress associated with perfusion could be responsible for the emergence of new sprouts via endothelial cell stimulation. It has been assumed, not unreasonably, that vessel branching occurs preferentially in immature vessels that have not yet formed a basal lamina. The growth mechanism related to shear stress thus allows the network to adapt its architecture dynamically as the flow distribution within the capillary bed evolves. A reinforcement mechanism has emerged from our study, whereby the vessel density dramatically increases via vessel branching near the tumour surface. This is due to high levels of localised wall shear stress in parts of the vasculature far from the parent vessel. Increased anastomosis (i.e. the formation of capillary arcades) aids the progressive release of shear stress near the tumour, whilst ensuring vessel connection to the parent vessel. The resulting vasculatures are highly heterogeneous, consisting of a sparse network proximal to the parent vessel coupled to a dense distal ensemble of capillaries. Many of these structures contrast sharply with those generated without the influence of wall shear stress.

In the final part of the study, we have implemented a number of feedback mechanisms related to the adaptation of vessel radii to both haemodynamic and metabolic stimuli. We have adapted an existing model proposed in [1–3], based on experimental observations and measurements performed on the rat mesentery. It has emerged that both haemodynamic (shear stress, transmural pressure) and metabolic stimuli must be included in order to generate stable vascular structures. A remodelling algorithm has been used in the context of a tumour-induced vasculature, with the aim of assessing the efficiency of subsequent chemotherapy treatments (i.e. successful drug delivery to the tumour). Results presented here have focused upon a posteriori adaptation, i.e. adaptation of an existing fully-grown vasculature. In order to evaluate the importance of the initial haematocrit condition upon the final adapted network, we have considered two different initial conditions: first a network free of red blood cells ($H_D = 0$), and secondly, a network with a patch of red cells ($H_D = 0.45$) at its centre. The resulting networks are virtually identical, with only minor differences reflecting the variation in the initial conditions. Hence, if sufficient time is allowed for remodelling, the same optimised architecture appears to emerge regardless of the initial haematocrit distribution. Of course, the initial distribution is no longer an issue if adaptation is considered during growth. This work is ongoing and it will be interesting to see if the same optimised architecture is produced.

The natural and important extension to our model of allowing the simultaneous adaptation (vasodilation and vasoconstriction) of parts of the network as it grows is expected to have considerable implications for therapy. This formulation would allow us to investigate dynamically the effects of anti-angiogenic drugs on the growing vascular network. We then expect to be able to test and optimise, via numerical simulations, various treatment protocols that couple chemotherapy with anti-angiogenic therapies.

Acknowledgments

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