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Inflammation et régénération du complexe dentino-pulpaire : de l'étude des mécanismes immunomodulateurs aux stratégies modulaires de libération de principes actifs

Alexis Gaudin

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HABILITATION A DIRIGER DES RECHERCHES

Alexis Gaudin

Maître de conférences des Universités -Praticien Hospitalier

UFR Odontologie Nantes

Inserm/UN/ONIRIS UMRS 1229-RMeS, Regenerative Medicine and Skeleton

**INFLAMMATION ET REGENERATION DU COMPLEXE DENTINO-
PULPAIRE : DE L'ETUDE DES MECANISMES IMMUNO-
MODULATEURS AUX STRATEGIES MODULAIRES DE LIBERATION DE
PRINCIPES ACTIFS**

TABLE DES MATIERES

1. INTRODUCTION	5
2. CURRICULUM VITAE	7
2.1 ÉTAT CIVIL	8
2.2 SITUATION PROFESSIONNELLE.....	8
2.4 RESUME CHRONOLOGIQUE DES ACTIVITES DE RECHERCHE ET HOSPITALO-UNIVERSITAIRES :	10
2.5 DIPLOMES	10
<i>Concours</i>	12
2.6 PRODUCTION SCIENTIFIQUE.....	13
2.7 FINANCEMENTS DE RECHERCHE.....	14
2.8 PRIX SCIENTIFIQUES ET DISTINCTIONS	15
2.9 ENCADREMENTS	16
<i>Encadrements doctoraux</i>	16
<i>Détails des co-encadrements doctoraux</i>	16
<i>Encadrements de master 2</i>	17
<i>Encadrements de master 1</i>	18
<i>Encadrement de thèses d'exercice</i>	18
2.10 ACTIVITE DE PILOTAGE ET COORDINATION	20
2.11 COLLABORATIONS INITIEES.....	21
2.12 ACTIVITES D'EXPERTISE ET PARTICIPATION A DES JURYS	24
<i>Expertises</i>	24
<i>Jurys de thèse</i>	24
<i>Jurys de concours</i>	24
<i>Activités reviewer</i>	24
<i>Activités évaluation</i>	25
2.13 ORGANISATION DE COLLOQUES, ANIMATION ET CONSEIL SCIENTIFIQUE	25
3. ACTIVITES PEDAGOGIQUES.....	26
3.1 ENSEIGNEMENTS MAGISTRAUX	27
<i>PACES à T1</i>	27
<i>3ème cycle : sous section 57-01</i>	30
<i>3ème cycle : sous section 58-01</i>	30
3.2 ENSEIGNEMENTS DIRIGES	30
3.3 ENSEIGNEMENTS PRATIQUES	31
<i>PACES à T1</i> :	31
3.4 TABLEAU RECAPITULATIF DES ENSEIGNEMENTS EN FORMATION INITIALE ET CONTINUE PAR ANNEE UNIVERSITAIRE.....	32

3.5 ENSEIGNEMENT POST-UNIVERSITAIRE ET FORMATION CONTINUE	34
<i>Conférences ou actions de formation continue internes</i>	35
<i>Conférences ou actions de formation continue externes</i>	35
3.6 RESPONSABILITES PEDAGOGIQUES	38
3.7 FORMATION A LA PEDAGOGIE	39
3.8 SCORE SIAPS.....	40
3.9 ACTIVITE DE RECHERCHE EN PEDAGOGIE	40
<i>Travaux en pédagogie ayant donné lieu à publications :</i>	40
<i>Autres activités de recherche en pédagogie</i>	42
4. ACTIVITE HOSPITALIERE.....	44
4.1 FONCTIONS DE FORMATION ET DE SOINS.....	45
4.2 AUTRE(S) ACTIVITE(S) HOSPITALIERES.....	45
4.3 ACTIVITES DE RECHERCHE HOSPITALIERE	45
<i>Activité de recherche clinique</i>	45
<i>Activité de recherche appliquée</i>	47
5. ACTIVITES DE RECHERCHE FONDAMENTALE	50
5.1 TRAVAUX DE RECHERCHE DOCTORAUX (2011-2015)	51
5.2 TRAVAUX DE RECHERCHE POSTDOCTORAUX ET MOBILITE (2016-2020)	60
<i>Travaux dans le domaine de la régénération osseuse et pulpo-dentinaire</i>	60
<i>Activité de recherches réalisées en mobilité (2016-2017)</i>	65
5.3 ACTIVITES DE RECHERCHE EN COURS ET PROJETS DE RECHERCHE	69
<i>Contexte de l'activité de recherche</i>	69
<i>Axe 1 : Design et évaluation d'un nouveau microgel biphasique à libération de LXA4 dans le cadre de l'ingénierie tissulaire. Projet BILIPOX</i>	70
<i>Axe 2 : Vésicules extracellulaires dérivées de cellules souches mésenchymateuses dentaires (pDMSC-EVs). Projet EVENDO</i>	74
<i>Axe 3 : Évaluation de l'activité biologiques des ciments à base de silicates de calcium. Projet CALIMED</i> ... 77	77
6. PUBLICATIONS ET COMMUNICATIONS.....	81
6.1 PUBLICATIONS.....	82
<i>Publications originales internationales soumises à comité de lecture</i>	82
<i>Publications de revues dans des journaux à comité de lecture</i>	84
<i>Autres publications non soumises à comité de lecture</i>	85
6.2 COMMUNICATIONS	87
<i>Communications internationales invitées personnelles</i>	87
<i>Communications orales avec résumé</i>	87
<i>Communications affichées :</i>	89

<i>Résumés publiés</i>	90
7. REFERENCES BIBLIOGRAPHIQUES	91
8. ARTICLES REPRESENTATIFS	96
9. FICHE DE SYNTHESE	143
9.1 RESUME DES TRAVAUX	144
9.2 SYNTHESE DES ENCADREMENTS.....	145
<i>Encadrements doctoraux</i>	145
<i>Encadrements de Master 2</i>	146

Liste des figures

Figure 1: Répartition relative des cellules immunocompétentes parmi la population CD45+. (Gaudin et al., J Endod 2015)	56
Figure 2 : Identification des cellules HLA-DRhigh HO-1+. (Gaudin et al., J Endod 2015).....	57
Figure 3 : Répartition relative des principales cellules immunocompétentes dans les incisives de rat non traitées (contrôles) et dans les incisives stimulées par du LPS ou par une solution saline tamponnée au phosphate (PBS) à 9 heures et 3 jours. (Renard et al., J Dent Res 2016)59	59
Figure 4 : Effet du greffon osseux particulier et de la pâte osseuse sur la polarisation des monocytes/macrophages. (Tournier et al., Sci Rep 2021)	63
Figure 5 : La lipoxine A4 (LXA4) a renforcé les propriétés immunomodulatrices des SCAP sur la stimulation et la prolifération des PBMC. (Gaudin et al., Sci Rep 2018).	66
Figure 6 : La procédure expérimentale du modèle 3D de racine in vitro. (Gaudin et al., J Endod 2020)	68
Figure 7 : Représentation schématique du projet EVENDO	77

1. INTRODUCTION

Ce manuscrit, présenté en vue de l'obtention de l'Habilitation à Diriger les Recherches, décrit le fruit de mon travail de recherche et de formation à et par la recherche. Ce manuscrit présente ainsi mes travaux de recherche effectués à Nantes de septembre 2011 (début de ma thèse) au sein de l'unité Inserm 1064 (centre pour la recherche en transplantation et en immunologie, Directeur de thèse : Pr Brigitte Alliot-Licht, PU-PH, Co-directeur de thèse : Pr Cristina Cuturi-Anegon DR) à aujourd'hui au sein de l'unité Inserm UMRS 1229-RMeS (Regenerative Medicine and skeleton, Directeur Jérôme Guicheux) sous l'égide de mon directeur de recherche le Pr Pierre Weiss.

Mes activités de recherche depuis mes débuts et jusqu'à maintenant se sont majoritairement intéressées à la régulation de l'inflammation pulpaire. Mon parcours professionnel d'enseignant chercheur m'a également permis de réaliser de la recherche en pédagogie au sein de l'UFR odontologie de Nantes (Doyen Pr Assem Soueidan) et de la recherche clinique au sein de l'unité d'investigation clinique UIC 11 (responsable Dr Gilles Amador Del Valle).

Après la présentation de ma candidature selon le format classique du *curriculum vitae*, ce manuscrit vous donnera un aperçu de mes activités de formation et d'enseignement. Enfin je vous présenterai mes activités de recherche, de leur initiation avec mes travaux doctoraux, postdoctoraux et mes différents encadrements effectués jusqu'à mes projets de recherche actuels et encadrements en cours.

2. CURRICULUM VITAE

2.1 ÉTAT CIVIL

Nom de naissance : Gaudin

Prénom : Alexis

Date et lieu de naissance : 21 septembre 1979 à Nantes

Nationalité : Française

Situation familiale : marié, 3 enfants

Domicile privé : 7 rue de la Pierre Nantaise 44100 Nantes

Tél. cellulaire : 06 49 67 70 83

Adresse électronique : alexis.gaudin@icloud.com

Domicile(s) professionnel(s) et personnels :

Faculté de chirurgie dentaire de Nantes,
U.F.R. Odontologie
1 place Alexis Ricordeau
44042 Nantes Cedex 01

Tél. fixe professionnel : 02 40 31 29 13

Adresse électronique professionnelle : alexis.gaudin@univ-nantes.fr

Situation militaire : exempté promotion 1979

Indicateurs scientifiques :

- Researcher ID : S-1188-2018
- ORCID : 0000-0003-4139-8927
- Loop profile : 995068

2.2 SITUATION PROFESSIONNELLE

N° d'inscription à l'ordre : 44022313

2017-... **Maître de Conférences des Universités – Praticien Hospitalier Temps Plein**
Faculté de chirurgie dentaire, Université de Nantes. Sous-section : 58-01
Odontologie Conservatrice-Endodontie (Responsable : Pr Pérez)
Service d'odontologie conservatrice et pédiatrique (Pr Fabienne Pérez)

2012-2017 **Maître de Conférences des Universités – Praticien Hospitalier Temps Partiel**
Faculté de chirurgie dentaire, Université de Nantes. Sous-section : 58-01
Odontologie Conservatrice-Endodontie (Responsable : Pr Pérez)
Service d'odontologie conservatrice et pédiatrique (Pr Fabienne Pérez)

2010-2012 **Attaché d'Enseignement**
Faculté de chirurgie dentaire, Université de Nantes. Sous-section : 58-01
Odontologie Conservatrice-Endodontie (Responsable : Dr Marion)
Service d'odontologie conservatrice et pédiatrique (Pr Fabienne Pérez)

2006-2010 **Assistant Hospitalo-Universitaire**
Faculté de chirurgie dentaire, Université de Nantes. Sous-section : 58-01
Odontologie Conservatrice-Endodontie (Responsable : Dr Marion)
Service d'odontologie conservatrice et pédiatrique (Dr Gilles Amador Del Valle)

2002-2005 **Interne en Odontologie**
Faculté de chirurgie dentaire, Université Paul Sabatier, Toulouse III
Service d'Odontologie CHU Toulouse Rangueil (Pr Calas)

Situation dans le cadre extra-universitaire :

2006 à 2016 Chirurgien-dentiste libéral à Sautron, Loire-Atlantique (44)

2006 Chirurgien-dentiste collaborateur à Sautron, Loire-Atlantique (44)

2.4 RESUME CHRONOLOGIQUE DES ACTIVITES DE RECHERCHE ET HOSPITALO-UNIVERSITAIRES :

Année	Statut	Formations diplômantes		Année	Activités en équivalent temps plein)			
		Recherche	Clinique		Recherche/Labo	Clinique/service	Enseignement/UFR	Libérale/lieu
2002	Concours internat			2002				
	Internat 1 ^{ère} année			2003				
	Internat 2 ^{ème} année					CHU Toulouse (0.9 ETP)	UFR Toulouse (0.1 ETP)	
	Internat 3 ^{ème} année							
2006	AHU		DU parodontologie (1)					
			DU parodontologie (2)	2007		CHU Nantes (0.6 ETP)	UFR Nantes (0.4 ETP)	
		M1		2008	LIOAD 791			
2009		M2		2009	UPRES EA 3826 (0.9 ETP)		UFR Nantes (0.4 ETP)	
	AHU		DU pédagogie médicale	2010	CRTI 1064 (0.2 ETP)	CHU Nantes (0.4 ETP)	UFR Nantes (0.4 ETP)	Exercice libéral Sautron (44°)
2010				2010	CRTI 1064 (0.8 ETP)		UFR Nantes (0.2 ETP)	
2011				2011				
2012	CNU 58-01	Thèse		2013	CRTI 1064 (0.4 ETP)	CHU Nantes (0.3 ETP)	UFR Nantes (0.3 ETP)	
	MCU-PH temps partiel			2016	CRTI 1064 (0.1 ETP)	CHU Nantes (0.4 ETP)	UFR Nantes (0.5 ETP)	
2016				2017	Mobilité recherche (1 ETP)			
	MCU-PH temps plein			2018	RMeS 1229 (0.2 ETP)	CHU Nantes (0.4 ETP)	UFR Nantes (0.4 ETP)	
2017					RMeS 1229 (0.5 ETP)	CHU Nantes (0.25 ETP)	UFR Nantes (0.25 ETP)	
2020	Pré-CNU 58-01							

2.5 DIPLOMES

Acquis :

2011-2015 Thèse de Doctorat. Université de Nantes, École Doctorale Biologie Santé Nantes-Angers

Intitulé : Étude des mécanismes immuno-modulateurs dans la pulpe dentaire en réponse aux pathogènes buccaux

Directeur de thèse : Pr Brigitte Alliot-Licht, PU-PH (57.03)

Co-directeur de thèse : Pr Cristina Cuturi-Anegon DR INSERM coordinateur équipe 1

Laboratoire d'accueil : INSERM-UMR 1064 : centre pour la recherche en transplantation et en immunologie

Directeur du laboratoire : Pr Ignacio Anegón DR INSERM U1064

2011 **Diplôme inter-universitaire et professionnel de pédagogie et de communication médicale**

Universités d'Angers, Bretagne Occidentale (Brest), Nantes et Rennes 1, U.F.R. Médecine, Nantes

2010 **Diplôme universitaire Parodontologie**

Université Paul Sabatier, U.F.R. Odontologie, Toulouse

2009 **Attestation de formation à la radioprotection des patients exposés aux rayonnements ionisants**

Université de Nantes, U.F.R. Médecine, Nantes

2009 **Master 2 : Sciences, Santé et Applications, Mention Biologie Cellulaire, Physiologie, Pathologie, Spécialité Biologie Ostéo-articulaire et Cranio-faciale. Biomatériaux et Biomorphologie**

Mention : Assez Bien

Laboratoire d'accueil : UPRES EA 3826 – LIOAD INSERM U791

Responsables : Dr Jocelyne Caillon, Dr Gilles Amador Del Valle

2008 **Master 1 : Maîtrise Sciences-Santé-Technologies, mention Sciences Biologiques et Médicales**

Université de Nantes, U.F.R. Médecine, Nantes

Unité d'Enseignement : Biotechnologies de la santé

Unité d'Enseignement : Biostatistiques

Stage d'initiation à la recherche

2006 **Diplôme d'État en Chirurgie Dentaire**

Université Paul Sabatier, U.F.R. Odontologie, Toulouse

Mention très honorable

2005 **Attestation d'Études Approfondies (AEA)**
Université Paul Sabatier, U.F.R. Odontologie, Toulouse
Service d'Odontologie du Pr Calas, CHU Toulouse Rangueil

1996 **Baccalauréat série S**
Mention Bien
Académie de Nantes

En préparation :

Diplôme d'Habilitation à diriger des recherches

Concours

Acquis :

2012 **Concours National de Maître de Conférence des Universités-Praticien Hospitalier**
Sous-section Odontologie Conservatrice-Endodontie (58-01)
Service d'Odontologie Conservatrice et Pédiatrique
Université de Nantes, U.F.R. Odontologie, Nantes

2006 **Concours d'Assistant Hospitalo-Universitaire du CSERD de Nantes**
Sous-section Odontologie Conservatrice-Endodontie (58-01)
Service d'Odontologie Conservatrice et Pédiatrique
Université de Nantes, U.F.R. Odontologie, Nantes
1^{er} renouvellement : février 2008
2nd renouvellement : octobre 2009

2002 **Concours National de l'Internat en Odontologie**
Rang : 6

1998 **Concours de PCEM 1**

Université de Nantes, U.F.R. Médecine, Nantes

En préparation :

Concours de recrutement de Professeur des Universités – Praticien Hospitalier dans la sous-section 58.01.

2.6 PRODUCTION SCIENTIFIQUE

- 22 articles internationaux et 5 chapitres de livre (1^{er} juin 2021)
- Indicateurs
 - H-index 8 – nombre de citations totales 422
 - SIGAPS : 294

Tableau synoptique des publications internationales (une liste complète se trouve dans la partie 6 de ce manuscrit).

	<i>Nombre total</i>	<i>1^{er} auteur</i>	<i>Dernier auteur</i>	<i>Autres</i>
Articles originaux indexés	14	8	3	3
Articles de revues indexés	3	-	2	1
Articles de revues non indexés	5	-	5	-
Chapitres livre	5	2	2	1

2.7 FINANCEMENTS DE RECHERCHE

<i>Année</i>	<i>Financier</i>	<i>Titre du projet</i>	<i>Rôle</i>	<i>Montant</i>
2020	ESE	EVENDO: Extracellular Vesicles derived from primed Dental Mesenchymal Stem Cells (pDMSC-EVs): Toward cell-free therapeutic applications to control pulp inflammation and facilitate dental pulp regeneration	Coordinateur	20 000€
2019	ANR	BILIPOX: A new biphasic microgel as lipoxin delivery system for tissue engineering	Coordinateur	285 000€
2019	Université de Nantes	ORGANITE Design of an organoid culture model for tissue engineering	Co-requérant	3 900€
2018	IFRO	LIPOXH: Design and in vitro assessment of a new biphasic microgels of silylated HPMC as lipoxin delivery system for periodontitis and pulpitis treatment	Coordinateur	20 000€
2018	Université de Nantes	Comparison of changes in cardiovascular parameters and efficacy of osteocentral (Quicksleeper) and locoregional anesthesia in mandibular pulpitis. Randomized controlled study	Co-requérant	5 000€
2017	IFRO	Analysis of the antibacterial and immunogenic properties of an innovative fibrin-chitosan hydrogel for human dental pulp regeneration	Co-requérant	20 000€
2016	Research Committee, University of the Pacific	Involvement of lipoxin A4 (LXA4) of the immunosuppressive paracrine and activity of Dental Pulp Stem Cells (DPSCs) and Stem Cells of the Apical Papilla (SCAPs)	Coordinateur	10 000 dollars

2.8 PRIX SCIENTIFIQUES ET DISTINCTIONS

- 2021** 1^{er} prix SOP – COMIDENT Bourse Michel Degrange
Gaudin A, Tolar M, Peters OA
Sujet : Cytokine production and cytotoxicity of calcium silicate-based sealers in two- and three-dimensional cell culture models. Journal of endodontics 2020, 46(6) :818-926.
- 2020** 1^{er} prix ESE-Annual Research Grant
Gaudin A, Galvani A, Aubeux D, Tessier S, Geoffroy V, Pérez F, Weiss
Sujet : Extracellular Vesicles derived from primed Dental Mesenchymal Stem Cells (pDMSC-EVs): Toward cell-free therapeutic applications to control pulp inflammation and facilitate dental pulp regeneration.
- 2016** 1^{er} prix IADR-Pulp Biology Regeneration Group
Gaudin A, Renard E, Bienvenu G, Amiaud J, Farges JC, Cuturi MC, Moreau A, Alliot-Licht B
Sujet : Immune cells and molecular networks in experimentally induced pulpitis.
- 2013** Lauréat Prix Paul Calas
Gaudin A, Renard E, Licht B, Farges JC, Hill M, Cuturi MC.
Sujet : Identification and characterization of CD45+ cells in human healthy dental pulp.
- 2011** 1^{er} prix du CNEOC
Gaudin A, Renard E, Licht B, Hill M, Cuturi MC.
Sujet : Characterization of dendritic cells expressing HO-1 in human dental pulp.

2005 1^{er} prix séance des internes Congrès national de parodontologie et implantologie SFPIO. **Gaudin A.** Sujet : Greffes osseuses autogènes : intérêt du prélèvement pariétal.

2.9 ENCADREMENTS

Encadrements doctoraux

<i>Doctorants co-encadrés</i>	<i>Directeurs de thèse</i>	<i>Année de soutenance</i>	<i>Nombre de publications co-signées</i>	<i>Dernier auteur</i>
D. Aubeux	Valérie Geoffroy/Fabienne Pérez	2	2
P. Tournier	Pierre Weiss/Jérôme Guicheux	2021	2	2

Détails des co-encadrements doctoraux

2020-2025 **Davy Aubeux.** Sujet : Évaluation in vitro et in vivo de microgels injectables de HPMC silylée en tant que système d'administration multimodal de médicaments pour l'ingénierie tissulaire.

Financement : projet BILIPOX (expérimentations) et auto-financement (thésard)

Co-encadrant (50%). Directrices de thèse : Valérie Geoffroy/Fabienne Pérez

Publications :

- Aubeux D, Peters OA, Hosseinpour S, Tessier S, Geoffroy V, Pérez F, **Gaudin A.** Specialized pro-resolving lipid mediators in endodontics: a narrative review. BMC Oral Health. 2021;21:276. doi:10.1186/s12903-021-01619-8.

- Aubeux D, Renard E, Pérez F, Tessier S, Geoffroy V, **Gaudin A**. Review of Animal Models to Study Pulp Inflammation. *Front Dent Med.* 2021;2 May:21. doi:10.3389/fdmed.2021.673552.

2016-2019 Pierre Tournier. Sujet : Caractérisation et amélioration des propriétés ostéogéniques d'un nouveau substitut osseux allogéniques

Financement : thèse CIFFRE société BIOBank SAS

Co-encadrant (50%). Directeurs de thèse : Pierre Weiss/Jérôme Guicheux

Devenir : post doctorant unité Inserm UMRS 1229-RMeS

Publications :

- Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, Bardonnnet R, Blanquart C, Corre P, Geoffroy V, Weiss P, **Gaudin A**. An Extrudable Partially Demineralized Allogeneic Bone Paste Exhibits a Similar Bone Healing Capacity as the “ Gold Standard ” Bone Graft. *Front. Bioeng. Biotechnol* 2021, 9, 1–10. doi:10.3389/fbioe.2021.658853.
- Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, Bardonnnet R, Blanquart C, Corre P, Geoffroy V, Weiss P, **Gaudin A**. A partially demineralized allogeneic bone graft: in vitro osteogenic potential and preclinical evaluation in two different intramembranous bone healing models. *Sci. Rep* 2021, 11, 1–13. doi:10.1038/s41598-021-84039-6.

Encadrements de master 2

2020-2021 Florian Segeron. (Étudiant pharmacie). Master 2 Sciences du Médicament et des Produits de Santé parcours BMTI - université de Nantes. Soutenance prévue en septembre 2021.

2019-2020 **Cassandra Lepetit.** Master 2 – Biologie Moléculaire et Cellulaire (BMC).
Université de Rennes 1. Devenir : technicien de recherche à Porsolt
(laboratoire de pharmacologie préclinique).

2018-2019 **Houman Noela Danielle Kacou.** Master 2 mention Biologie – Santé
Parcours Recherche clinique. UIC 11. Devenir : étudiante en médecine

Encadrements de master 1

2020 **Emmanuel Pellerin.** (Étudiant en odontologie). Devenir : (chirurgien-dentiste)

2020 **Camille Cabezon.** (Interne en Odontologie). Devenir : (chirurgien-dentiste)

2019 **Nuala Blanchflower.** (Étudiant en odontologie)

2018 **Quy-linh Do.** (Chirurgien-dentiste)

2016 **Maxime Carpentier** (Étudiant en odontologie). Devenir : (chirurgien-dentiste)

2015 **Alix Corbel.** (Étudiant en odontologie)

2014 **Lucie Ferrand.** (Étudiant en odontologie). Devenir : (chirurgien-dentiste)

Publications :

- Do QL, Gaudin A, Review L. The Efficiency of the Er : YAG Laser and Photon- Induced Photoacoustic Streaming (PIPS) as an activation method in endodontic irrigation: a literature review. J Lasers Med Sci. 2020;11(3):316–31
- Aubeux D, Blanchflower N, Badran Z, Bray E, Remaud, Clouet R, Prud'homme T, Gaudin A. Educational gaming for dental students: Design and assessment of a pilot endodontic-themed escape game. Eur J Dent Educ 2020, 24:449–57.

Encadrement de thèses d'exercice

Type	Date	Candidat	Sujet
Thèse	2021	Basile Sauvaget	Analyse des facteurs de l'hôte sur le pronostic endodontique
Thèse	2020	Roselyne Clouet	Élaboration et évaluation d'une application mobile (docop) pour l'accompagnement administratif des étudiants au centre de soins, d'enseignement et de recherche dentaires de Nantes

Thèse	2020	Clément Sabourin	Enquête sur l'enseignement initial de l'endodontie en France et à l'étranger
Thèse	2020	Marion Pépin	Les structures organoïdes appliquées à la biologie pulpaire
Thèse	2020	Bastien Lecaplain	Conséquences de la maladie parodontale sur la santé sexuelle masculine : revue systématique de la littérature
Thèse	2020	Camille Cabezon	Intérêts des guides chirurgicaux imprimés tridimensionnellement dans le cadre de la microchirurgie endodontique (étude sur modèle animal)
Thèse	2020	Nicolas Burot	Étude des biomatériaux situés dans le tiers cervical des dents permanentes immatures : comparaison de la résistance à la fracture dans un modèle in vitro
Thèse	2019	Thomas Denis Le Sève	Économie tissulaire et aménagement des voies d'accès en endodontie
Thèse	2019	Loïc Buffard	Évaluation ex vivo d'un guide chirurgical imprimé tridimensionnellement pour la chirurgie endodontique
Thèse	2018	Paul-Emile Allain	Utilisation de laser Erbium : YAG pour l'activation des solutions d'irrigation en endodontie : analyse de la littérature
Thèse	2018	François Martineau	Peut-on utiliser l'hypnose en urgence odontologique ?
Thèse	2018	Maxime Carpentier	Utilisation des loupes en odontologie : impact sur la posture du praticien ?
Thèse	2017	François Rocher	Rôle de la Chlorhexidine sur la stabilisation de la couche hybride
Thèse	2017	Lucie Ferrand	Le diagnostic pulpaire, limites et perspectives
Thèse	2017	Jérôme Garnier	Intérêts et indication du cone beam computerized tomography en endodontie
Thèse	2017	Karl Mallet	Défauts superficiels de l'émail dans le secteur antérieur, approches thérapeutiques
Thèse	2016	Carole Maestriperi	Les contentions en traumatologie
Thèse	2016	Charlène Laillé	Les lésions endo-parodontales
Thèse	2016	Grégoire Potaux	Intérêt de la documentation photographique dans la réhabilitation globale esthétique
Thèse	2015	Marie Gambert	Intérêts du cone beam dans la prise en charge des résorptions radiculaires
Thèse	2015	Valérie Lecomte	Les minéralisations pulpaires. Etiologies, manifestations et traitements
Thèse	2015	William Sinet	Ankylose et résorption de remplacement. Analyse critique de la littérature

Thèse	2014	Marion Senotier	Élaboration d'un protocole de recherche clinique comparant l'anesthésie ostéocentrale à l'anesthésie loco-régionale dans le cadre de pulpite irréversible des molaires mandibulaires
Thèse	2009	Valérie Guyader	Accidents per-opératoires iatrogènes en endodontie orthograde
Thèse	2006	Frédérique Piedvache	Gestion de la papille en implantologie

Publications :

- Lecaplain B, Badran Z, Soueidan A, Prud'homme T, **Gaudin A**. Periodontitis, erectile dysfunction, reproductive hormones, and semen quality: A systematic review. *Andrology* 2020 Dec 15. doi: 10.1111/andr.12961.
- Carpentier M, Aubeux D, Armengol V, Pérez F, Prud'homme T, **Gaudin A**. The Effect of Magnification Loupes on Spontaneous Posture Change of Dental Students During Preclinical Restorative Training. *J Dent Educ* 2019;83:407–15

2.10 ACTIVITE DE PILOTAGE ET COORDINATION

- 2021** 1^{er} vice-Doyen UFR Odontologie Nantes
- 2021** Responsables du groupe de travail sur les innovations pédagogiques UFR Odontologie Nantes
- 2020** Responsable groupe **INFLAMED** (Inflammatory mediators and microenvironment in bone and teeth) au sein de l'unité Inserm UMRS 1229-RMeS
- 2020** Référent Université Numérique en Santé et Sport (**UNESS**) pour UFR Odontologie
- 2018** Responsable de l'**Unité Fonctionnelle d'Odontologie Conservatrice et Endodontie**
- 2018** Responsable **UE Gradient thérapeutique (DFSAO 1)**
- 2017** Chargé de mission internationale (Endodontie) par le **CNEOC**
- 2017** Responsable **UE Master 1 : Sciences de la Santé Oro-Faciale**
- 2017** Responsable des **Travaux Pratiques d'Odontologie Conservatrice (DFGSO 2-DFSAO 1)**

2.11 COLLABORATIONS INITIEES

Dans le cadre des projets de recherche fondamentaux, j'ai pu créer une collaboration étroite avec le Pr Philippe Legrand et Corine Tourné-Peteilh (UMR 5253 CNRS).

UMR 5253 CNRS-UM-ENSCM :

Les recherches menées à l'ICGM s'intéressent à l'élaboration, la caractérisation et la mise en forme de matériaux fonctionnels à différentes échelles, de la molécule au dispositif en passant par les nanomatériaux. L'unité contribue au développement de solutions innovantes pour la résolution des enjeux sociétaux actuels que sont l'énergie, la santé et l'environnement, et développe de très nombreuses collaborations et projets qui sont pour la plupart de dimension internationale, avec des partenariats établis avec des laboratoires et universités du monde entier. L'ICGM est labélisé Institut Carnot Chimie Balard Cirimat et accompagne à ce titre, les industriels de plusieurs secteurs dans leurs projets R&D en science chimique et ingénierie, de la molécule au matériau.

Tutelles : Université de Montpellier, CNRS, ENSCM

Label : UMR 5253 (CNRS)

Département scientifique : Chimie

Écoles doctorales : CBS2, Sciences Chimiques Balard

Ces projets nous ont également permis de d'intégrer la société Cayman à notre projet. L'intérêt de ce projet est de bénéficier de leur expertise dans le domaine de la synthèse lipidique et de pouvoir discuter avec leur département R&D.

Cayman chemicals® :

Cayman chemicals® est un acteur industriel ancien dans la synthèse de médiateurs lipidiques présent depuis les années 1980. Cayman Chemicals est un expert de la synthèse, de la purification et de la caractérisation de médiateurs spécialisés dans la résolution de l'inflammation. Cayman est totalement équipé pour effectuer de la lipoxine ou d'autres médiateurs pro-résolveurs dans les bonnes pratiques de fabrication (BPF), un point crucial lorsque l'on considère la traduction humaine de notre projet.

Dans le cadre de ma mobilité, j'ai pu initier une collaboration avec le département et le laboratoire d'Endodontie du Pr Ove A Peters. Nous avons rédigé à ce jour 3 articles ensemble, et un chapitre d'ouvrage sur la régénération du complexe pulpo-dentinaire ; un autre article est a été accepté (15/11/2021, International endodontic journal IF : 5.26).

Pr Ove A Peters (University of Queensland, Australie) :

Le Pr Peters a au cours de ses 25 années de carrière dans le milieu universitaire été impliqué dans une variété de contextes scientifiques : de la neurobiologie à l'endodontie clinique. Le Dr Ove A. Peters a rejoint l'Université du Queensland, à Brisbane, en Australie, en 2020 après avoir occupé des postes de professeur à Heidelberg, en Allemagne et à Zurich, en Suisse, ainsi qu'à l'Université de Californie à San Francisco. Plus récemment, il a été le directeur fondateur du programme d'endodontie de troisième cycle à la Arthur A. Dugoni School of Dentistry de San Francisco. Le Dr Peters possède une vaste expertise clinique et de recherche et a publié plus de 160 manuscrits liés à la technologie et à la biologie endodontiques. Il est l'auteur de deux livres et a contribué à plusieurs ouvrages de premier plan en dentisterie.

Autres collaborations en cours :

Pr Julian Leprince et le Pr Anne Desrieux (Université de Louvain, Belgique) :

Cette collaboration a pour objectif de rassembler les complémentarités des laboratoires LDRI (Belgique) et RMeS (France) dans le domaine des dispositifs de libération de médiateurs lipidiques spécialisés dans la résolution de l'inflammation.

Pr Prasanna Neelakantan (University of Hong-Kong, Hong-Kong) :

Le Pr Prasanna Neelakantan est professeur d'Endodontie à la Faculté de médecine dentaire de l'Université de Hong Kong. Il est également vice-doyen à la recherche et à l'innovation de la Faculté. Son groupe de recherche se concentre sur la biologie des biofilms, la nano-thérapeutique, la réticulation du collagène et le débridement canalaire minimalement-invasif. Le projet de collaboration que nous menons est intitulé : "A novel multifunctional DNA-peptide towards optimization of tissue engineering to revitalize infected dead teeth (DNATE)". Dans ce projet nous souhaitons développer une approche complémentaire entre l'ingénierie tissulaire

(laboratoire RMeS) et l'expertise sur la désinfection et les nanothérapeutiques du laboratoire dirigé par le Pr Neelakantan.

Pr Elisabetta Cotti (University of Cagliari, Italie) :

Le Pr Elisabetta Cotti dirige le département de dentisterie conservatrice et d'endodontie à l'École de médecine dentaire de l'Université de Cagliari, en Italie. Elle est l'auteur de plusieurs articles dans le domaine de l'endodontie et s'intéresse particulièrement à la recherche clinique sur la pathologie périapicale, les techniques d'imagerie et les médiateurs lipidiques impliqués dans la résolution de l'inflammation. La collaboration avec le Pr Cotti se fait dans le cadre d'un projet de recherche clinique basé dont le but est de mesurer l'impact des régimes alimentaires basés sur les acides gras polyinsaturés et la thérapeutique endodontique.

Pr Jean-Christophe Farges (Université de Lyon, France)

Dans le cadre d'un appel offre IFRO et suite à ma thèse d'Université, nous avons entrepris une collaboration avec le Pr Jean-Christophe Farges et le Dr Maxime Ducret de Lyon. Le Pr Jean-Christophe Farges était membre rapporteur lors de ma thèse d'Université. Nous avons pu associer nos complémentarités respectives afin de mener le projet ; Analyse des propriétés antibactériennes et immunogènes d'un hydrogel innovant fibrine-chitosane pour la régénération de la pulpe dentaire humaine.

2.12 ACTIVITES D'EXPERTISE ET PARTICIPATION A DES JURYS

Expertises

Société savantes/groupe de recherche :

- Société Française d'Endodontie (SFE)
- Société Européenne d'Endodontie (ESE)
- Collège National des enseignants en Odontologie Conservatrice (CNEOC)

Jurys de thèse

Fleur Béres : 27 novembre 2017. Développement d'un matériau biomimétique à base de silicate tricalcique pour la régénération des tissus biominéralisés. (Directeur de thèse Stéphane Simon). Université Paris Descartes. Examineur

Jurys de concours

2020	Jury concours d'assistant hospitalo-universitaire interne à l'établissement : Dr Cécile Hemming
2020	Jury concours d'assistant hospitalo-universitaire interne à l'établissement : Dr Matthieu Remaud
2020	Jury concours d'assistant hospitalo-universitaire interne à l'établissement : Dr Lise Tisserand
2017-...	Jury DFGSAO1/TCEO1 interne à l'établissement

Activités reviewer

<i>Revues</i>	<i>Nombre de revues réalisées</i>
International Endodontic Journal	41
Clinical Oral Investigation	3
European Endodontic Journal	3
Scientific Reports	1

European Journal of Dental Education	1
Journal of Dental Education	1
Journal of Laser in Medical Sciences	1
Journal of Endodontics	2
Prostaglandins and Other Lipid Mediators	1

Activités évaluation

- 2021** Rapporteur Master 2 sujet : Étude des communications entre macrophages et fibroblastes dans la polyarthrite rhumatoïde
- 2019** Évaluation dossier thèse Université CIFRE
- 2017** Évaluation Master 2 Sujet : Étude in vivo de l'effet biomimétique et du potentiel régénératif endodontique d'un matériau à base de brushite
Mémoire réalisé sous la direction du Pr Stéphane Simon
- 2014-2019** Responsable de la titularisation SFE
- 2014-2019** Responsable du prix Paul Calas SFE

2.13 ORGANISATION DE COLLOQUES, ANIMATION ET CONSEIL SCIENTIFIQUE

- 2019** Responsable de l'Endodontie au sein du conseil scientifique congrès de l'Association Dentaire Française 2019
- 2018** Membre élu du conseil scientifique local Odontologie – Université de Nantes
- 2015-...** Co-organisateur du congrès international de la Société Française d'endodontie
- 2012-...** Co-organisateur des Entretiens de Ricordeau et trésorier de l'association des Entretiens de Ricordeau depuis 2018

3. ACTIVITES PEDAGOGIQUES

3.1 ENSEIGNEMENTS MAGISTRAUX

PACES à T1

- DFGSO 2 :
 - L'examen clinique en Odontologie-Conservatrice-Endodontie (1h) (années 2008-2009-2010)
 - Problématique de la zone inter proximale (2h) (depuis 2017)
 - Protection pulpaire (2h) (depuis 2017)

- DFGSO 3 :
 - Champ opératoire en Endodontie (3h) (années 2007/2008 à 2012/2013)
 - Cavité d'accès en Endodontie (2h) (années 2007/2008 à 2012/2013)
 - L'instrumentation en Endodontie (2h) (années 2007/2008)
 - La mise en forme canalaire (2h) (années 2007/2008)
 - Les urgences pulpaires inflammatoires (1h) (années 2008/2009 à 2012/2013)
 - L'hypersensibilité dentinaire (1h) (années 2006/2007 à 2012/2014)
 - Diagnostic clinique des pathologies pulpaires (3h) (années 2017/2018 et 2018/2019)
 - Thérapeutiques des pathologies pulpaires irréversibles (9h) (années 2017/2018 et 2018/2019)
 - Introduction Gradient thérapeutique (2h) (depuis 2019)
 - Restauration des dents cuspidées et techniques adhésives

directes

(4h) (depuis 2019)

– DFSAO 1 :

- Rapport entre Gastro-Entérologie et Odontologie
(1h) (années 2006/2007 à 2010/2011)
- Introduction Gradient thérapeutique
(2h) (depuis 2013)
- Erosion/infiltration
(1h) (années 2013-2016)
- Restauration des dents cuspidées et techniques adhésives
directes
(4h) (depuis 2013)
- Usures dentaires : diagnostic et prise en charge
(2h) (depuis 2017)
- Stratification antérieure des composites
(2h) (depuis 2013)

- DFSAO 2

- Urgences en Odontologie Conservatrice-Endodontie
(1h) (années 2007/2008 à 2012/2013)
- Plan de traitement global
(1h) (2014-2016)

– T1

- Chirurgie endodontique
(2h) (années 2006/2007 à 2012/2015)
- Aides optiques en Odontologie Conservatrice-Endodontie
(1h) (années 2007/2008 à 2011-2015)
- Perspectives d'avenir en Odontologie Conservatrice - Endodontie

(1h) (depuis 2007)

- Master 1
- Thérapeutiques de vitalité pulpaire, actualités et perspectives thérapeutiques en endodontie

(3h) (depuis 2017)

Préparation à l'Internat : état néant

- Cours préparation internat Odontologie Conservatrice-Endodontie
(2h) (années 2012 à 2016)

Mission(s) à l'étranger :

- Cours et TP sur la chirurgie endodontique
(1 semaine) (2014) Faculté de Monastir - Tunisie

Réalisations de documents pédagogiques

- Polycopiés : sujet : Rédaction d'un système d'évaluation de l'enseignement dans mon domaine d'activité d'enseignant.
Travail dirigé par le Pr Jacques-Henri Barrier (Unité de Recherche Pédagogique - évaluation et innovation)
- Films : état néant
- Cassettes vidéo-numériques : état néant
- Didacticiels : Livret des actes techniques en Odontologie Conservatrice et Endodontie (Dr Cécile Dupas)
Rédaction des items suivant :
 - Pose d'un pansement sédatif d'urgence (en collaboration avec le Dr François Coirier)
 - Pulpotomie d'urgence (en collaboration avec le Dr François Coirier)

- Parage d'une dent infectée d'urgence (en collaboration avec le Dr Pierre Nguyen)
- Traitement endodontique d'une mono ou bi-radiculée (en collaboration avec le Pr Olivier Laboux)
- Traitement endodontique d'une pluriradiculée (en collaboration avec le Pr Olivier Laboux)

3ème cycle : sous section 57-01

- C.E.S. parodontologie : Les relations endo-parodontales
(2h) (analyse d'article et cours magistral) (années 2012 à 2017)

3ème cycle : sous section 58-01

- C.E.S. Odontologie Conservatrice-Endodontie
(24h) (années 2015-2016 et 2017/2018)
- D.E.A. : état néant
- A.E.A. : Recommandations de l'AFFSAPS antibiothérapie et antibioprofylaxie
(1h) (années : 2004/2005)
- Séminaire : les urgences en Odontologie Conservatrice-Endodontie
(2h) (2010)
- Séminaire : stratification des composites antérieurs
(3h) (2014)
- Réalisations de documents pédagogiques :
- Cahier de l'internat 12 (juin 2013) question en Odontologie Conservatrice Endodontie

3.2 ENSEIGNEMENTS DIRIGES

Formation initiale : sous-section 58-01

Préparation à l'Internat :

- Odontologie Conservatrice et Endodontie (3h) (2017-...-
- Séance d'entraînement au raisonnement clinique (2X3H)

3.3 ENSEIGNEMENTS PRATIQUES

Formation initiale : sous-section 58-01

PACES à T1 :

– DFGSO 2 :

2 séances hebdomadaire de 3 heures

26 séances/an

Travaux pratiques d'Odontologie Conservatrice

Depuis 2012

– DFGSO 3 :

(78h) (années 2006/2007 à février 2009 et d'octobre 2009 à septembre 2010)

1 séance hebdomadaire de 3 heures

26 séances/an

Travaux pratiques d'Odontologie Conservatrice

Travaux pratiques d'Endodontie

– DFSAO 1 :

Travaux pratiques : utilisation de la rotation continue (3h)

(Années 2007/2008 à 2009/2010)

– DFSAO 2 : (3h)

- Travaux pratiques : les contentions collées en technique directe (années 2008/2009 et 2009/2010)

- Stratification des composites
(9h) (depuis 2014)

- Éclaircissement des dent pulpées et déulpées
(9h) (depuis 2014)

- Inlay/onlay
(9h) (depuis 2014)
- T1 : (3h)
- La stratification des composites (cours et travaux pratiques)
(1 journée) (années 2013-2016)
- Les aides optiques en Odontologie Conservatrice-Endodontie
(1h) (années 2008/2009 et 2009/2010)
- La chirurgie endodontique
(6h) (depuis 2017)

Réalisation de documents pédagogiques

- Film : pose de la digue, utilisé lors de la séance sur le champ opératoire
- Didacticiel : réalisation de fichiers pour la réalisation de la cavité d'accès

3.4 TABLEAU RECAPITULATIF DES ENSEIGNEMENTS EN FORMATION INITIALE ET CONTINUE PAR ANNEE UNIVERSITAIRE

Nombre d'heures de cours magistraux (CM), travaux pratiques (TP) et Enseignements Dirigés (ED) assurés par le candidat en formation initiale et en formation continue par année universitaire.

Année Universitaire	Cibles	Matière/CNU	Formation initiale			Formation continue		
			CM*	ED	TP	CM*	ED	TP
2012-2013	DFGSO 2	OCE			156			
	DFGSO 3	OCE	7 ^P					
	DFSAO 2	OCE	1 ^P					
	T1	OCE	7 ^P					
	CES	Parodontologie					2	
2013-2014	DFGSO 2	OCE			156			
	DFGSO 3	OCE	2 ^P					
	DFSAO 1	OCE	9 ^P					
	DFSAO 2	OCE	1 ^P	3	27			
	T1	OCE	4 ^P		9			
	CES	Parodontologie					2	

2014-2015	DFGSO 2	OCE			156			
	DFGSO 3	OCE	2 ^p					
	DFSAO 1	OCE	9 ^p					
	DFSAO 2	OCE	1 ^p	3	27			
	T1	OCE	4 ^p		9			
	CES	Parodontologie					2	
	Mission Tunisie	OCE				30 ^p		6
	DU	Esthétique				6 ^p		
	DUEE	OCE				3 ^d		
2015-2016	DFGSO 2	OCE			156			
	DFGSO 3	OCE	2 ^p					
	DFSAO 1	OCE	9 ^p					
	DFSAO 2	OCE	1 ^p	3	27			
	T1	OCE	4 ^p		9			
	CES	Parodontologie					2	
	CES	OCE					31	
	DU	Esthétique				6 ^p		
	DU	OCE				9 ^d		
2016-2017		OCE	16 ^p			3 ^d		
2017-2018	DFGSO 2	OCE	4 ^p		156			
	DFGSO 3	OCE	12 ^p					
	DFSAO 1	OCE	11 ^p		27			
	DFSAO 2	OCE						
	T1	OCE	1 ^p		6			
	CES	OCE					24	
	Master 1	OCE	3 ^p					
	DU	OCE				6 ^p +3 ^d		
2018-2019	DFGSO 2	OCE	4 ^p		156			
	DFGSO 3	OCE	12 ^p					
	DFSAO 1	OCE	11 ^p		27			
	DFSAO 2	OCE						
	T1	OCE	1 ^p		6			
	Certificat Endodontie	OCE				4 ^p		
	Master 1	OCE	3 ^p	2				
	DU	OCE				3 ^d		

2019-2020	DFGSO 2	OCE	4 ^P		104			
	DFGSO 3	OCE	6 ^P		27			
	DFSAO 1	OCE	11 ^P		27			
	DFSAO 2	OCE						
	T1	OCE	1 ^P					
	Certificat Endodontie	OCE				4 ^P		
	Master 1	OCE	3 ^P	2				
	DUPIC	Parodontologie				1 ^P	3	
	DU	OCE				6 ^P		
2020-2021	DFGSO 2	OCE	4 ^P		104			
	DFGSO 3	OCE	6 ^P					
	DFSAO 1	OCE	11 ^D					
	DFSAO 2	OCE						
	T1	OCE	1 ^D					
	Certificat Endodontie	OCE				4 ^P		
	Master 1	OCE	3 ^D	2				
	DU	OCE				6 ^P		

* : préciser ; ^P : présentiel en Amphi ; ^D : à distance sur ENT ou autre

3.5 ENSEIGNEMENT POST-UNIVERSITAIRE ET FORMATION CONTINUE

2020-... Diplôme Universitaire Endodontie Paris

Cours : le contrôle de l'inflammation pulpaire (4h ED)

Cours : Relations entre l'Endodontie et les pathologies systémiques (4h ED)

Cours : la chirurgie guidée (2h ED)

2019 Diplôme Universitaire Parodontologie Implantologie Clinique Nantes

Cours : La photographie dentaire (1h + 3h ED)

2018-... Certificat d'Endodontie Bruxelles

Cours : le contrôle de l'inflammation pulpaire (4h)

2016-2017 Diplôme Universitaire Formation Continue Odontologie Nantes

Cours : Reconstitution directe/indirecte du secteur postérieur en composite (6h)

2015-... Diplôme Universitaire Endodontie Marseille (DESUEM)

- Cours : Les ultrasons en Endodontie (3h) -
- 2014-2016** **Diplôme Universitaire Esthétique Nantes**
Cours photographie dentaire (3h)
Cours Stratification des composites (3h)
- 2011** **Diplôme Universitaire d'Endodontologie université Paris Diderot Paris 7**
Endodontie et pathologies en collaboration avec le Pr Olivier Laboux (2h)

Conférences ou actions de formation continue internes

- 2019** Troubles du comportement alimentaires et dents
- 2016** Les thérapeutiques de vitalité pulpaire
- 2014** Séance nouveauté et présentation en endodontie sur le TF adaptive
- 2012** Démarche thérapeutique pour la prise en charge d'un cas difficile en prothèse
- 2011** Applications cliniques du Cone Beam en endodontie
- 2011** Les clés du succès en chirurgie endodontique (SFE Bordeaux)
- 2010** Soirée Endodontie-Endocardite
- 2010** Les aides optiques : mieux voir pour mieux faire ?
- 2010** Travaux pratiques d'Endodontie : « Venez bousculer vos habitudes »
- 2010** Cone beam et Endodontie
- 2008** Implantologie chez le sujet âgé versus chez le sujet jeune
- 2008** Techniques d'obturation en endodontie
- 2008** Travaux pratiques d'endodontie : l'endodontie moderne
- 2006** Travaux pratiques d'Endodontie
- 2006** La chirurgie endodontique

Conférences ou actions de formation continue externes

- 2021** La désobturation en Endodontie (journée SOP paris)
- 2021** Utilisation des biocéramiques en endodontie (BAET Bruxelles)
- 2021** Les thérapeutiques de vitalité pulpaire (SFE Toulouse)

2021	Les patients à risque (SFE Nancy)
2020	Le diagnostic en Endodontie et conduite à tenir en urgences (SFE Paris)
2019	Approche biologique de l'endodontie (SFE Rennes)
2019	Les thérapeutiques de vitalité pulpaire (Clermont Ferrand)
2018-...	Les lésions endo-parodontales (SPIO Paris)
2018	Les thérapeutiques de vitalité pulpaire (SFE Montpellier)
2017	L'Obturation en Endodontie (ONFOC Niort)
2017	Le retraitement endodontique, quelle(s) stratégie(s) adopter ? (RDV formation 85)
2016	Rationaliser la mise en forme en Endodontie (ONFOC 76)
2016	La douleur en Odontologie (journée SOP paris)
2016	Regeneration ou revascularization", espoirs cliniques et réalités cliniques de la régénération en endodontie Auteurs (ADF Paris)
2015	La microchirurgie endodontique (ADF Paris)
2015	Les fêlures (Société Française d'Endodontie) (SFE Nancy)
2014	Les fêlures (Société Française d'Endodontie) (SFE Paris))
2013	Le diagnostic en Endodontie (ADF Paris)
2013	La chirurgie endodontique (SFE Lyon)
2013	Exposé magistral Qualité, efficacité, Ergonomie en Endodontie (SFE Poitiers)
2013	Les relations endo-parodontales, La gestion des complexités du traitement initial - Analyse et diagnostic (SFE Paris)
2012	Qualité, efficacité et ergonomie en Endodontie (SFE Nantes)
2011	La chirurgie endodontique (SFE Toulouse)
2011	Les clés du succès en chirurgie endodontique (SFE Bordeaux)
2010-2011	TP la chirurgie endodontique (ADF Paris)
2010-2011	La préparation canalaire en endodontie (ADF Paris)
2011	La microdentisterie (ADF Paris)
2011	Impact de L'Endodontie sur l'état général (ADF Paris)
2010	Les troubles du comportement alimentaire, prise en charge odontologique (Nantes)
2010	L'Endodontie au cœur de la santé (ADF Paris)

- 2009 La digue et ses griefs : la preuve par 9 (ADF Paris)
- 2009 Traitement des dents immatures nécrosées (ADF Paris)
- 2007 Gestion des instruments fracturés (AAAAFON Nantes)
- 2007 Indications et contre-indications du retraitement endodontique (ADF Paris)
- 2007 La stratification des composites secteur antérieur (ADF Paris)
- 2006-2007 Travaux pratiques rotation continue (ADF Paris)
- 2006 Prévention des échecs en implantologie (ADF Paris)
- 2005 Trois sources de complications en chirurgie endodontique : le sinus maxillaire, le nerf alvéolaire inférieur, l'artère grande palatine (ADF Paris)

- **Action de formation continue : article à visée pédagogique** (Article publié dans une revue éditée par la faculté d'Odontologie de Toulouse)

2005 Conditions du succès en chirurgie endodontique. Alexis Gaudin, Paul Calas. Synthèse clinique 2005 (1):11-20

2005 Migrations secondaires des incisives maxillaires en parodonte réduit, nécessité d'une contention définitive en fin de traitement. Alexis Gaudin, Nathalie Garnier. Synthèse clinique 2005 (1):33-40

- **Action de formation continue : article à visée pédagogique**
(Article paru en ligne réalisé pendant l'internat sur odontonline)

2013 Rationalisation de la mise en forme canalaire en endodontie. – intérêts du système One Shape® MICRO-MEGA®. Alexis gaudin, Fabienne Pérez. Dentoscope novembre 2013.

2008 Indications et pratiques actuelles en chirurgie endodontique. Alexis Gaudin odontonline 2008.

- **Action de formation continue :**

- Participation à l'établissement des recommandations COVID-19 – Stade 3 de l'épidémie -Guide praticien. Direction du groupe de travail pour le CNEOC et la SFE

3.6 RESPONSABILITES PEDAGOGIQUES

- **Co-coordonateur et co-créditeur d'un master 2 en médecine 4R**

Equipe coordinatrice (Pr Johann Clouet, Claire Vinatier, Dr Alexis Gaudin)

Lauréat appel offre NeXT 2019

<p>Etablissement(s) porteur(s) : Université de Nantes – CHU de Nantes</p> <p>Composante(s) concernée(s) au sein de la structure : Pôle Santé (UFR Pharmacie – UFR Odontologie – UFR Médecine)</p>
<p>Etablissements participants partenaires sur le site (composante(s)) :</p> <p>ONIRIS, CEISAM, IMN, ECN, IFREMER, INRA, SFR, Université Angers, Le Mans</p>
<p>Autres partenaires du projet (établissements nationaux ou internationaux / partenaires industriels) :</p> <p>Ensemble des partenaires du cluster BIOREGATE / Universités internationales : NUI Galway (Irlande) ; Université Catholique de Louvain (Belgique), Université de Laval (Canada) / Centre de recherche : CURAM, OIRM, CUSTOM, REMEDI, ThéCell. Laboratoires académiques ligériens : UMRS1229-RMeS, U1064-CRTI, U1089-TGT, U1238-Phy.Os, U1235-TENS. Partenaires non-académiques ligériens : Atlantic Bone Screen, Atlantic Bio GMP, Biomatlante, clean cells, ...</p>

Ce M2 M4R propose le développement d'une offre de formation internationale de niveau master 2 autour d'une thématique en plein essor de la santé du futur : la médecine 4R (M2 M4R). Cette formation unique, transdisciplinaire, évolutive, tournée vers l'innovation pédagogique et l'employabilité des étudiants répondra aux besoins en nouveaux métiers en médecine du futur.

Basée sur l'employabilité et sur la création de parcours individuels, cette formation permettra d'apporter l'ensemble des compétences aux futurs diplômés pour des débouchés vers le doctorat mais également vers le milieu professionnel après le master 2. D'un point de vue pratique, l'étudiant suivra des interventions relatives à : (i) un socle commun d'acculturation ;

(ii) des interventions de spécialités ; (iii) des interventions d'intérêt général. La mobilité à l'international des étudiants sera favorisée par la présence des partenaires internationaux et l'attribution de bourses. Les étudiants réaliseront un stage d'une durée de 6 mois.

Cette formation s'intégrera dans la politique volontariste d'internationalisation de la Nouvelle Université de Nantes. Elle sera organisée en anglais autour de la mobilisation de différents partenariats internationaux (Irlande, Belgique, Etats-Unis, Canada), tant au niveau universitaire que dans des centres d'excellence de recherche. Ce consortium de partenaires répond à l'organisation du RFI BIOREGATE qui comprend également l'ensemble des acteurs de la médecine 4R au niveau ligérien. Ainsi, à terme, le M2 M4R pourrait préfigurer le développement d'un nouveau graduate program s'intégrant dans la graduate school « Santé du Futur » de NEXT.

- Traduction de vidéos de vulgarisation endodontique (réalisée par L'ESE)
- Cours en ligne, podcasts : rédaction de 3 posts sur le patient à risque dans IDweblog
- Participation aux journées de formation service sanitaire 3 1/2 journées par an pour des étudiants de santé.

3.7 FORMATION A LA PEDAGOGIE

Formation personnelle à la pédagogie :

- **Diplôme inter-universitaire** et professionnel de pédagogie et de communication médicale
Universités d'Angers, Bretagne Occidentale (Brest), Nantes et Rennes 1, U.F.R. Médecine, Nantes
Année : 2011
- Participation à des **ateliers pédagogiques CNEOC 2019**
- **Participation Séminaires sur les innovations pédagogiques** organisé par le CNEOC, Mannheim, mars 2019
- **Mobilité à objectifs pédagogiques** (participation aux journées pédagogiques (4 journées complètes (pédagogie innovante, flipped classroom, serious game, team based learning) University of the Pacific, San Francisco, 2016-2017)

- **Formation à divers outils utilisés en pédagogie (atelier SPIN santé)**
Depuis 2019

3.8 SCORE SIAPS

Le score SIAPS Odontologie est calculé à 548,8

3.9 ACTIVITE DE RECHERCHE EN PEDAGOGIE

Travaux en pédagogie ayant donné lieu à publications :

- **Travaux sur les avantages et les défis ergonomiques de l'introduction des aides optiques au début du programme de formation dentaire.**

Les preuves scientifiques validant l'effet bénéfique des loupes dans la prévention des troubles musculo-squelettiques sont très rares. L'objectif de cette étude était d'évaluer l'impact des loupes dentaires sur la posture des étudiants en odontologie pendant un cours préclinique de dentisterie restauratrice. Cette étude a été menée à l'UFR de Nantes en 2017 avec un design « crossover ». Quarante étudiants en deuxième année d'études dentaires ont été répartis au hasard en deux groupes de 20 personnes chacun : le groupe A a utilisé des loupes, tandis que le groupe B n'en a pas utilisé. La semaine suivante, les étudiants ont inversé les configurations. Les étudiants ont été enregistrés sur vidéo pendant la préparation de la cavité. Les positions du tronc, de la tête et du cou, et du bras ont été analysées à l'aide de scores continus basés sur le Posture Assessment Instrument modifié. En outre, les cavités ont été évaluées et les étudiants ont rempli un questionnaire sur leurs perceptions des loupes. Sur une échelle où des scores plus faibles indiquent une meilleure posture, les résultats ont montré des scores ergonomiques de posture par minute significativement plus élevés pour les étudiants sans loupes ($146,3 \pm 6,64$ points/min) qu'avec loupes ($123,2 \pm 6,77$ points/min ; $p < 0,05$). La majorité des étudiants (32/39, 82%) ont montré des améliorations des postures ergonomiques avec l'utilisation de loupes. Le tronc, la tête et le cou ont été positivement impactés par l'utilisation des loupes, mais pas les bras. Les préparations de la cavité n'ont pas été améliorées par l'utilisation de loupes. Le questionnaire a révélé des aspects négatifs (douleur et difficulté d'adaptation) mais a souligné l'impact positif perçu sur la posture. Cette étude a documenté les avantages et les défis

ergonomiques de l'introduction du grossissement au début du programme de formation dentaire.

Ce travail a donné lieu à l'encadrement d'une thèse d'exercice et une publication :

- Carpentier M, Aubeux D, Armengol V, Pérez F, Prud'homme T, **Gaudin A**. The Effect of Magnification Loupes on Spontaneous Posture Change of Dental Students During Preclinical Restorative Training. J Dent Educ 2019;83:407–15.

- **Travaux sur la motivation des étudiants**

La société a évolué, les réseaux sociaux et l'avènement des générations X, Y et Z nous remettent en question sur nos manières de transmettre des informations, sur la manière dont ces informations sont perçues, retenues et appliquées. Nous avons pu participer et encadrer des travaux de thèse d'exercice décrivant très bien ces phénomènes. De plus, les échanges fructueux lors des CNEOC et des journées dédiées aux innovations pédagogiques nous ont fait prendre conscience de la nécessité de faire évoluer nos enseignements. Dans le cadre de mon activité pédagogique auprès d'étudiants et/ou de confrères, j'ai eu l'occasion de travailler sur différents projets (approche par compétence, matrice QX, portfolios, classes inversées, serious game, utilisation de smartphone pour le m-learning). Chaque initiative, prise individuellement est séduisante et semble prometteuse. Le risque néanmoins est de se perdre dans la « technique pédagogique » et gaspiller du temps et de l'énergie. On oublie souvent d'analyser la situation lorsque l'on est attiré vers la nouveauté.

A une échelle pilote, j'ai pu participer à la réalisation d'un projet d'escape game pédagogique. L'intérêt de cette expérience est d'avoir créé une dynamique au sein du corps enseignant et d'avoir pu valoriser ce travail *in fine* par la publication d'un article dans le domaine de la pédagogie médicale.

Ce travail a donné lieu à :

- Communication CNEOC 2019 – présentée par le Dr Davy Aubeux
1^{er} Prix: Educational Escape Room as an Alternative Teaching Strategy in Dentistry.
Aubeux D, Badran Z, Bray E, Remaud, Clouet R, Prud'homme T, **Gaudin A**

- Une publication :
Aubeux D, Blanchflower N, Badran Z, Bray E, Remaud, Clouet R, Prud'homme T,
Gaudin A. Educational gaming for dental students: Design and assessment of a
pilot endodontic-themed escape game. Eur J Dent Educ 2020, 24:449–57.
- Co-direction d'un **groupe de travail** sur les applications des concepts de "serious game" médicaux à l'Endodontie. Exemple de l'escape game.
- Co-encadrement **thèse d'Université** : « Entre jeux et simulation, l'escape game peut-il être un outil pertinent d'apprentissage dans le domaine de la Santé » ED Polytechnique Hauts-de-France
Étudiant : Roselyne Clouet
Début : 1^{er} décembre 2021 –...

Autres activités de recherche en pédagogie

Dans le cadre du Diplôme en communication et pédagogie médicale :

- Mémoire de pédagogie « Pourquoi la digue est-elle peu utilisée en endodontie ? Réflexions sur l'apprentissage, à propos de jeunes chirurgiens-dentistes de Loire Atlantique. »
- Communication orale : Pourquoi la digue est-elle peu utilisée en endodontie ? Réflexions sur l'apprentissage, à propos de jeunes chirurgiens-dentistes de Loire Atlantique.
Gaudin A, Hyon I, Barrier JH.
Congrès CNEOC; Strasbourg, 2012

En tant que coordinateur de la mission internationale du Collège des enseignants en Odontologie Conservatrice-Endodontie

Communication orale présentée lors du congrès l'ESE, Bruxelles, 2017
Irrigation trends among dental schools in France: a web-based survey
Guivarc'h M, ***Gaudin A**, Seux D, Bukiet F

4. ACTIVITE HOSPITALIERE

4.1 FONCTIONS DE FORMATION ET DE SOINS

Maître de conférences hospitalo-universitaire à temps plein

Centre de soins dentaires du CHU de Nantes

PHU 4 - ostéo-articulaire - tête et cou - odontologie - neurochirurgie - neuro-traumatologie ;
directeur : Dr Vabres Bertrand

Service d'Odontologie conservatrice et pédiatrique : chef de service : Pr Fabienne Pérez

- Nombre de consultations personnelles : **220/an**
- 47 semaines/an
- **12 unités de soins/vacation**
- 20 étudiants/vacation
- Années stagiaires encadrés : DFGSAO 1, DFGSAO 2, T1, Internes

4.2 AUTRE(S) ACTIVITE(S) HOSPITALIERES

- Responsable de l'**Unité Fonctionnelle d'Odontologie Conservatrice et Endodontie (depuis 2018)**
- Création et participation aux **staffs pluridisciplinaires** des étudiants de 5ème année et internes
- Création et participation aux **staffs de traumatologie** (pluridisciplinaires, à destinée des externes, internes et praticiens)
- **Encadrement étudiants** pour participer aux concours de restauration (Société GC, Dentsply...) Encadrement des 1^{er} et 2^{ème} prix obtenus par Alexandre Crozet en 2020 (Global clinical case contest)

4.3 ACTIVITES DE RECHERCHE HOSPITALIERE

Activité de recherche clinique

En tant qu'enseignant chercheur ayant une activité de soins spécialisées et odontologie conservatrice-endodontie, il m'a toujours semblé naturel de réaliser de la **recherche clinique**. En effet, ces études réalisées sur la personne humaine, en vue du développement des connaissances biologiques ou médicales me semblent indispensables pour mieux comprendre et/ou mieux traiter les pathologies endodontiques. J'ai eu l'opportunité de travailler au sein de

l'Unité d'investigation Clinique (UIC 11) dirigée par le Dr Gilles Amador Del Valle en tant qu'un **investigateur coordinateur ou investigateur.**

Le protocole de recherche clinique COQ sur l'anesthésie ostéocentrale que je porte actuellement (44 patients inclus sur les 72 escomptés, (étude débutée en janvier 2019) me permet de participer à la vie de cette UIC.

Intitulé Projet : Comparaison des variations des paramètres Cardio-vasculaires et de l'efficacité de l'anesthésie ostéocentrale (Quicksleeper) versus l'anesthésie locorégionale dans le cas de pulpite mandibulaire irréversible

Rôle dans le projet : responsable du projet - investigateur principal

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

En cours

Intitulé Projet : Comparaison des variations des paramètres Cardio-vasculaires et de l'efficacité de l'anesthésie ostéocentrale (Quicksleeper) versus l'anesthésie locorégionale dans le cas de pulpite mandibulaire irréversible

Rôle dans le projet : responsable du projet - investigateur principal

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

En cours

Intitulé Projet : Observational clinical trial, prospective and multicenter, measuring the long-term performance and safety of Biodentine™ in patients treated for endodontic indications.

Rôle dans le projet : investigateur associé

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

En cours

Intitulé Projet : Observational clinical trial, prospective and multicenter, measuring the long-term performance and safety of Biodentine™ in patients treated for endodontic indications.

Rôle dans le projet : investigateur associé

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

En cours

Intitulé Projet : REone

REone est issue de la synergie de 4 entités : le groupe RECOL, la société EndoData, les services d'odontologie hospitalo-universitaires (DU d'endodontie) et les cabinets d'endodontie libéraux. L'objectif de REone est de constituer une base de données dans le cadre d'une étude observationnelle prospective en soins courants. Dans le respect de la réglementation, cette base pourra servir de support à d'autres études à visées étiologiques et pronostiques.

Rôle dans le projet : investigateur associé

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

En cours

Intitulé projet : Traitement des dents immatures nécrosées post-traumatiques : essai randomisé multicentrique comparant la revascularisation pulpaire versus la barrière apicale

Responsable du projet : Pr Reza Arbab-Chirani (investigateur coordinateur)

Rôle dans le projet : investigateur associé

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

Stade des réévaluations

Intitulé Projet : Comparative randomized clinical comparison of pulp chamber pulpotomy and root canal treatment (pulpectomy) as a permanent endodontic treatment of mature permanent teeth: Analysis of relationship between inflammation biomarkers pulpal expression and treatment outcome

Responsable du projet : Pr Stéphane Simon (investigateur coordinateur)

Rôle dans le projet : investigateur associé

Lieu : service d'Odontologie Conservatrice et Pédiatrique, Pôle Odontologie, C.H.U. Nantes

Stade des réévaluations

Activité de recherche appliquée

- Impact du COVID-19 sur les pratiques professionnelles et sur l'enseignement de l'endodontie

La crise et la pandémie liées au COVID-19 ont paralysé et perturbé une partie de nos activités de pédagogiques, de soins et de recherche en 2020. Il a fallu s'adapter, modifier les enseignements et enfin envisager de nouvelles recommandations pour les chirurgiens-dentistes. J'ai eu l'opportunité de participer aux groupes de travail de recommandations de l'ordre et des différentes sociétés savantes ainsi qu'à la rédaction du guide des recommandations de la HAS (Haut autorité de santé). Ce groupe de travail a rédigé les recommandations et les différentes mises à jour en vigueur actuellement. De manière concomitantes à ces publications, j'ai également participé à un groupe de travail international sur l'impact du COVID-19 sur les enseignements en endodontie.

Publications

- Galicia JC., Mungia R, Taverna MV, Mendoza Marissa J, Estrela C, **Gaudin A**, Zhang C, Vaughn BA, Khan AA. Response by Endodontists to the SARS-CoV-2 (COVID-19) Pandemic: An International Survey. *Frontiers in Dental Medicine* 2021; 1(January).
- **Gaudin A**, Badran Z, Chevalier V, Aubeux D, Prud'homme T. COVID-19 and Oral Fluids. *Frontiers in Dental Medicine* 2020;1(September):1-9.
- **Gaudin A**, Arbab-chirani R, Pérez F. Effect of COVID-19 on Dental Education and Practice in France. *Frontiers in Dental Medicine* 2020;1(August):5-8.
- Groupe de travail coordonnée par le Conseil national de l'ordre des chirurgiens-dentistes. Recommandations d'experts pour la prise en charge des patients nécessitant des soins buccodentaires en période transitoire dans le cadre de l'épidémie de COVID-19. Mise à jour Février 2021. <https://www.ordre-chirurgiens-dentistes.fr/actualites/communiqu%C3%A9-mise-a-jour-des-reponses-rapides-dans-le-cadre-du-covid-19-mesures-et-precautions-essentielles-lors-des-soins-bucco-dentaires-en-cabinet-de-ville/>
- Réponses rapides dans le cadre du COVID-19 - Mesures et précautions essentielles lors des soins bucco-dentaires en cabinet de ville. Mise à jour Janvier 2021.

https://www.has-sante.fr/upload/docs/application/pdf/2020-05/rr_391_soins_bucco-dentaires_apres_le_deconfinement_14_05_20_mel.pdf

- **Mesure et évaluation des pratiques professionnelles**

L'exercice professionnel qu'il soit en milieu hospitalier ou libéral évolue. Tout professionnel de santé se doit d'offrir aux patients des soins correspondant aux données acquises, avérées de la science. Dans ce cadre j'ai eu l'opportunité de coordonner et de rédiger des articles de revues, soit sur des techniques innovantes soit sur les relations entre état de santé général et les maladies bucco-dentaires.

Publications

- Lecaplain B, Badran Z, Soueidan A, Prud'homme T, **Gaudin A**. Periodontitis, erectile dysfunction, reproductive hormones, and semen quality: A systematic review. *Andrology*. 2021;9 June 2020:1–12.
- Do QL, **Gaudin A**, Review L. The Efficiency of the Er : YAG Laser and Photon- Induced Photoacoustic Streaming (PIPS) as an activation method in endodontic irrigation: a literature review. *J Lasers Med Sci*. 2020;11(3):316–31.

5. ACTIVITES DE RECHERCHE FONDAMENTALE

5.1 TRAVAUX DE RECHERCHE DOCTORAUX (2011-2015)

Le cahier des charges pour notre projet de recherche était le suivant : nous avons souhaité élaborer un projet au caractère novateur, avec une finalité clinique, des débouchés en termes de publications, de retombées pour le département d'Odontologie Conservatrice-Endodontie, et enfin avec un encadrement de qualité permettant des avancées rapides compte-tenu d'un emploi du temps dense.

Le sujet retenu était l'étude des mécanismes immuno-modulateurs au niveau de la pulpe dentaire en réponse aux pathogènes buccaux.

Un point clé pour envisager une finalité clinique de ce projet était l'élaboration d'un modèle animal chez lequel différentes stratégies thérapeutiques pourraient être envisagées. L'expérience de la mise au point d'un protocole en Master 1 puis l'élaboration d'un nouveau modèle expérimental en master 2, qui a donné lieu à plusieurs communications internationales et une publication internationale nous ont donc servis pour l'élaboration de notre modèle de pulpite réversible chez le rat. Les collaborations avec les différents laboratoires (LIAOD et UPRES EA 3826) nous ont permis d'acquérir des méthodes (histologie, HPLC, dénombrement bactérien par ensemencement spiral, biostatistiques) utiles dans notre projet actuel de contrôle de la réponse immunitaire face aux pathogènes buccaux.

Nous savons notamment grâce aux travaux réalisés par l'équipe du Pr Jean-Christophe Farges de Lyon (avec qui nous collaborons) qu'en présence de pathogènes dans la pulpe dentaire, les odontoblastes ont la capacité d'attirer les cellules dendritiques via les TLR [1–3]. Les progrès récents de la recherche en immunologie ont permis de découvrir que la réponse immunitaire adaptative pouvait être contrôlée. Ces avancées scientifiques ont permis de développer les thérapies cellulaires et moléculaires visant à amplifier ces phénomènes naturels et à améliorer considérablement le pronostic des greffes. Existe-t-il un potentiel naturel du contrôle de la réponse immunitaire au sein de la pulpe dentaire ? Peut-on amplifier cette capacité de la pulpe dentaire ? C'est pour répondre à ces questions que nous avons travaillé avec le Pr Cristina Cuturi qui dirige une équipe de recherche au sein du laboratoire UMR 1064. Cette équipe est spécialisée dans l'étude des mécanismes de la tolérance allogénique et étudie les cellules dendritiques et leurs mécanismes. Lors de notre thèse d'exercice nous avons mené deux projets relativement distincts : améliorer les connaissances des acteurs clés du contrôle de la

réponse immunitaire pulpaire et l'établissement et la caractérisation d'un modèle animal d'inflammation pulpaire.

Ces choix nous ont amené à publier deux articles dans le *Journal of Endodontics* et le *Journal of Dental Research*. Nous avons également pu échanger nos points de vue lors de congrès nationaux (CNEOC) et internationaux (IADR, ESE) avec des spécialistes du sujet (Jacques Nör, Ken Hargreaves, Anibal Diogenes, Stéphane Simon, Kerstin Galler). Ces échanges nous ont fait prendre conscience des perspectives qui pouvaient découler de nos premiers travaux mais également du chemin à parcourir si l'on voulait progresser dans ce domaine.

L'endodontie est une discipline de l'odontologie permettant de prévenir ou de guérir les pathologies pulpaires et péri-apicales. Cette définition est associée traditionnellement aux traitements endodontiques initiaux orthogrades, aux retraitements endodontiques orthogrades et à la chirurgie endodontique [4]. Les progrès de la biologie, associés à une meilleure compréhension des phénomènes inflammatoires et cicatriciels, ont remis au goût du jour des thérapeutiques anciennes dites de « maintien de la vitalité pulpaire » [5–7]. Contrairement à la dent permanente immature, où la valeur d'une pulpe vitale est indéniable, le maintien de la dent vitale fait l'objet de débats dans les dents matures avec lésion carieuse profonde ou exposition pulpaire. Les thérapeutiques de vitalité pulpaire (VPT) ont une place tout à fait légitime dans l'arsenal thérapeutique endodontique [8, 9]. Leur objectif est de maintenir les propriétés biologiques naturelles de la pulpe (signal d'alarme, capacité cicatricielle, édification radiculaire etc...). L'un des facteurs clés du succès des thérapeutiques de vitalité pulpaire est la création de conditions permettant de contrôler la réponse immunitaire afin d'éviter les effets néfastes conduisant à la nécrose pulpaire. Cependant, il est important d'avoir à l'esprit que « bloquer » ou « empêcher » la réponse immunitaire pourrait ne pas être une bonne approche. En effet, la réponse immunitaire contribue de manière significative à la cicatrisation.

L'objectif est plutôt d'avoir des stratégies ciblées capables de contrôler la réponse immunitaire. Cela pourrait limiter les dommages aux tissus après une blessure, favoriser un environnement dit résolutif au niveau de l'inflammation pulpaire. De nombreuses stratégies ont été proposées et testées *in vitro*, peu ont été comparées et évaluées *in vivo*. Même si ces stratégies convergent pour contrôler l'inflammation de la pulpe, elles sont très diverses et parfois complexes.

Pourquoi vouloir contrôler l'inflammation pulpaire ?

Les bactéries sont la principale cause d'inflammation et d'infections de la pulpe. Dans les lésions carieuses, les traumatismes, l'usure ou les fissures, les bactéries et leurs sous-produits ont la possibilité d'atteindre la pulpe en traversant les tubules dentinaires [10]. L'inflammation pulpaire correspond à une réaction de défense de l'hôte contre des agents pathogènes étrangers. Il existe deux systèmes immunitaires complémentaires chez les vertébrés qui reconnaissent et éliminent les agents pathogènes : le système immunitaire inné et le système immunitaire adaptatif. La pulpe dentaire est capable de déclencher d'abord une réponse immunitaire innée, puis une réponse adaptative spécifique d'antigène [11–13].

L'immunité innée joue un rôle important dans les caries superficielles et provoque la réponse inflammatoire aiguë. La réponse inflammatoire s'accompagne de vasodilatation systémique et de migration des leucocytes. Dans une courte période d'activation du système immunitaire inné, les cellules immunitaires sécrètent diverses cytokines et chimiokines afin de recruter d'autres cellules immunitaires sur le site de l'infection. Les neutrophiles sont les premières cellules à adhérer aux cellules endothéliales et commencent à migrer à travers la paroi vasculaire au niveau du site de l'infection pour éliminer les agents pathogènes envahissants et sécréter des médiateurs vasoactifs et pro-inflammatoires. Dans les caries superficielles, le tissu pulpaire n'est pas en contact direct avec les agresseurs. Par conséquent, l'activité de la phagocytose n'est pas possible. Les réponses pulpaires sont faibles et chroniques. Si le système immunitaire inné dépasse sa capacité ou si sa fonction défensive devient limitée, il engage le système immunitaire adaptatif, activant ainsi des lymphocytes T et B pour éliminer les agents pathogènes. Le passage de la réponse innée à la réponse adaptative se produit probablement dans le cas de pulpes à inflammation irréversible dans lesquelles le front de la lésion carieuse est à moins de 2 mm de la pulpe [14, 15]. Dans les lésions actives à croissance rapide avec exposition pulpaire, les réactions inflammatoires deviennent aiguës et incontrôlées à mesure que la pénétration bactérienne se produit dans le tissu pulpaire. L'environnement pulpaire, la diversité des agressions, la microflore des sites des lésions carieuses et les possibilités d'apport en nutriments conduisent à une efficacité relative de la réponse immunitaire au sein du tissu pulpaire [16–18].

Comme dans d'autres tissus, de plus en plus de preuves suggèrent que la réponse inflammatoire pulpaire est non seulement impliquée dans la réponse immunitaire mais oriente également le processus de guérison. En ce qui concerne les dommages cardiaques, la littérature récente a renforcé le rôle central des monocytes / macrophages dans la stabilisation du rafraîchissement des cardiomyocytes en cas d'infarctus du myocarde [19]. Pendant de nombreuses années, l'importance de l'inflammation dans la guérison de la pulpe a été sous-estimée, considérée uniquement comme un effet indésirable. Il est maintenant prouvé que l'inflammation est une condition préalable à la cicatrisation des tissus et à la régénération de la pulpe. La réponse inflammatoire initiée peut évoluer vers la mort cellulaire et la destruction des tissus ou vers la cicatrisation et / ou la régénération des tissus. Cet équilibre qui peut évoluer dans un sens ou dans l'autre est lié à la présence et à la concentration de médiateurs de l'inflammation.

À ce jour, bien qu'il reste encore beaucoup à faire en ce qui concerne la connaissance de la régulation de la réponse immunitaire au sein de la pulpe dentaire, quelques molécules ou propositions thérapeutiques ont été identifiées afin de contrôler la réponse inflammatoire de la pulpe dentaire. Le contrôle de la réponse inflammatoire ouvrirait la voie à une optimisation des thérapeutiques de vitalité pulpaire. Les objectifs sont d'obtenir des résultats plus prévisibles, d'éviter ou de réduire la douleur, d'obtenir davantage de tissu fonctionnel et, éventuellement, de régénérer le tissu pulpaire afin d'éviter les pulpectomies.

Dans ce contexte, mes travaux de thèse réalisés au sein de l'équipe 1 unité INSERM UMR 1064 et sous la direction de Pr Brigitte Alliot-Licht PU-PH sous-section 57-03, responsable du département de Sciences Biologiques, U.F.R Odontologie Nantes, Equipe 1 unité INSERM UMR 1064 et en co-direction avec le Pr Cristina Cuturi DR INSERM, coordinateur de l'équipe 1 (Rôle des cellules dendritiques et des cellules régulatrices dans la tolérance immune) unité INSERM UMR 1064 eurent pour objectifs de :

- Caractériser des cellules et des médiateurs impliqués dans la régulation de la réponse immunitaire au niveau de la pulpe dentaire humaine
- Caractériser des cellules et de médiateurs impliqués dans la régulation de la réponse immunitaire au niveau de la pulpe dentaire saine et inflammatoires de rat.

- Mettre au point un modèle animal permettant d'étudier différentes stratégies thérapeutiques en rapport avec la modulation de la réponse immunitaire pulpaire.

Projet 1 : Caractérisation des cellules et des médiateurs impliqués dans la régulation de la réponse immunitaire au niveau de la pulpe dentaire humaine saine puis enflammée

A partir de pulpes dentaires issues de troisièmes molaires saines (extraites pour des raisons orthodontiques), nous avons pu par différentes techniques (cytométrie de flux, immunohistologie, RT-PCR) caractériser un ensemble de cellules impliquées dans la réponse immunitaire (lymphocytes B, cellules NK, lymphocytes T CD4+, lymphocytes T CD8+, cellules dendritiques) (Figure 1). Parmi ces cellules, nous nous sommes intéressés en particulier aux lymphocytes T régulateurs (Treg) caractérisés par les marqueurs suivants : CD3+CD4+CD25+CD127-Foxp3+. Nous avons pu quantifier ces cellules. Elles représentent environ 1% des lymphocytes T. Les lymphocytes T régulateurs sont essentiels pour maintenir la tolérance périphérique et limiter les réponses immunes excessives.

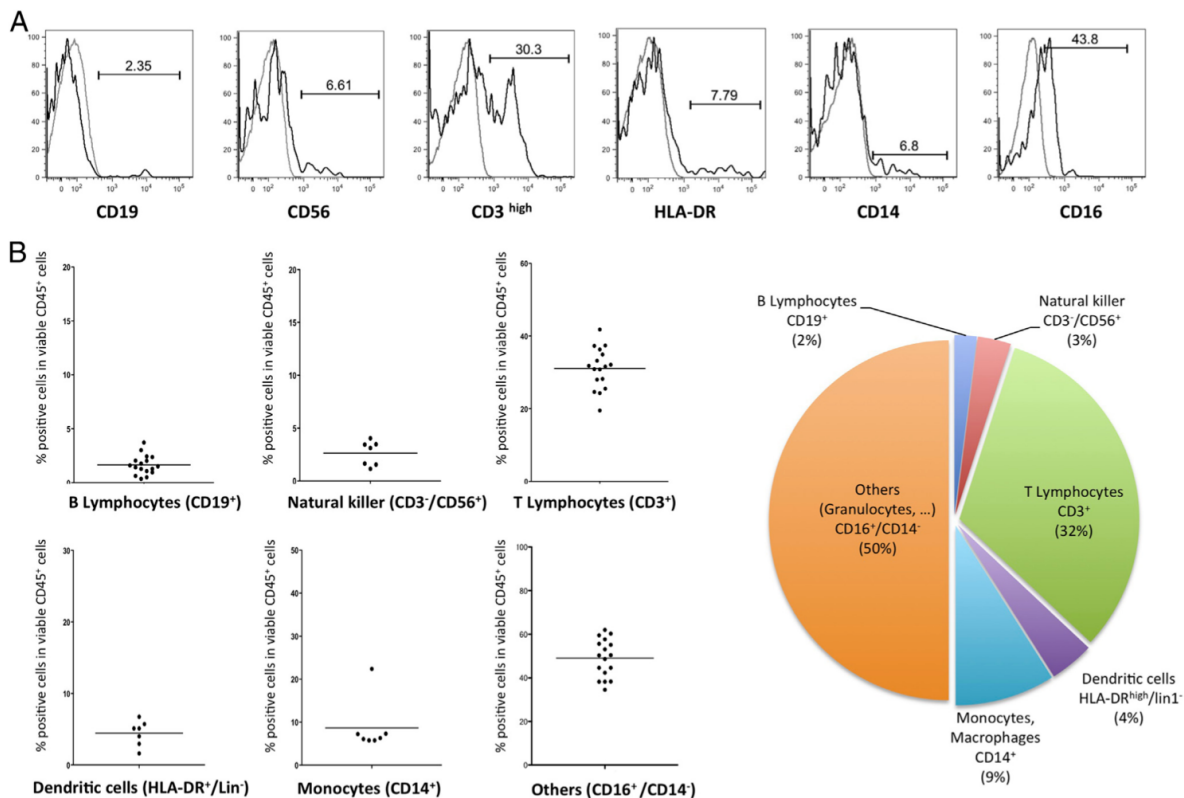


Figure 1: Répartition relative des cellules immunocompétentes parmi la population CD45+. (Gaudin et al., J Endod 2015)

- (A) Histogrammes de cytométrie en flux des marqueurs spécifiques (histogramme noir), CD19, CD3, CD56, HLA-DR, CD14 et CD16, exprimés parmi les cellules CD45+ par rapport aux isotypes contrôles (histogramme gris) d'un patient représentatif. Les chiffres représentent le pourcentage de cellules positives.
- (B) (B) Le niveau d'expression des marqueurs phénotypiques suivants : CD19+ pour les lymphocytes B, CD3+ pour les lymphocytes T, CD3CD56+ pour les NK, HLA-DR^{high}lin1- pour les cellules dendritiques, CD14+ pour les monocytes et les macrophages, et CD16+CD14 pour les autres (granulocytes et neutrophiles) parmi les cellules CD45+. Le graphique circulaire résume la répartition relative des principales cellules immunocompétentes parmi la population CD45+.

Nous avons également pu mettre en évidence par cytométrie de flux, immuno-histologie, RT-PCR, une sous-population de cellules dendritiques d'intérêt (HLADR+, CD14+, CD16+, CD83+, CD163+, CD45+) qui possède le même phénotype que les cellules dendritiques produisant de l'IL-10 (DC-10). Cette sous-population est présente *in vivo* et peut être induite en présence d'IL-10. Peu décrites, ces cellules sont particulièrement intéressantes car elles auraient la capacité d'induire la différenciation de lymphocytes T régulateurs via la production d'IL-10 et donc de représenter une sous-population de cellules dendritiques tolérogènes (Tol DC).

Au niveau de la pulpe dentaire humaine, nous avons retrouvé par immunohistologie la présence de cellules doublement marquées IL-10 et HLA-DR. Les DC-10 que nous avons pu caractériser au niveau de la pulpe dentaire humaine ont la capacité d'exprimer HO-1. HO-1 est une enzyme ayant entre autres, des propriétés immunosuppressives pouvant jouer un rôle dans les phénomènes de tolérance (Figure 2).

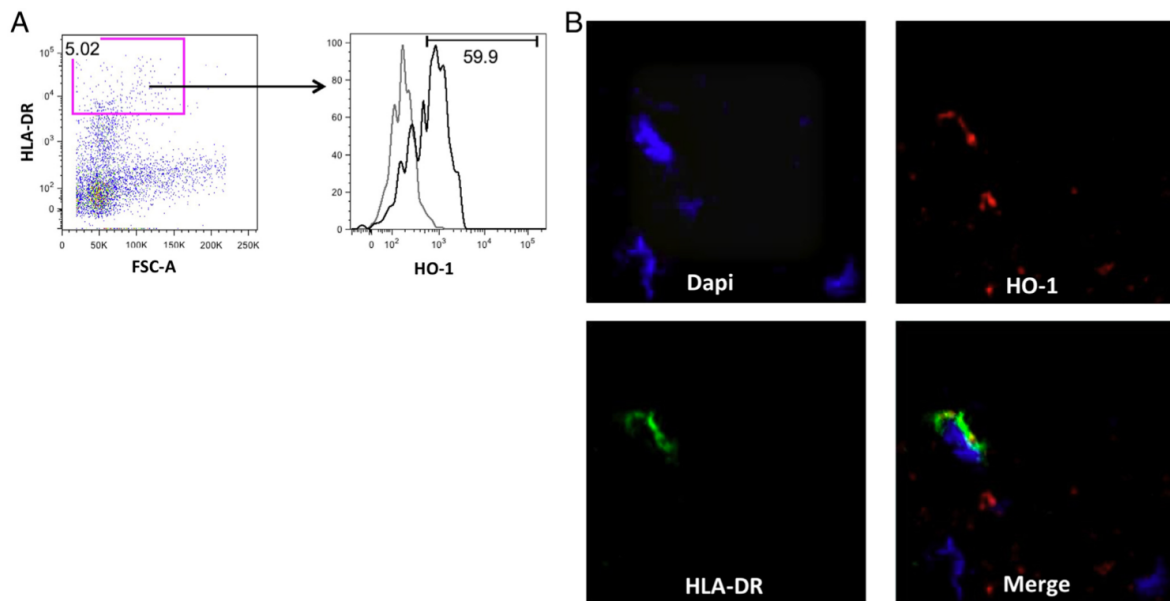


Figure 2 : Identification des cellules HLA-DRhigh HO-1+. (Gaudin et al., J Endod 2015)

(A) Analyse par cytométrie en flux montrant les cellules HLA-DR+ et HO-1+ dans la pulpe dentaire humaine. Un profil représentatif d'un donneur est montré (n = 4).

(B) Micrographies confocales de sections cryostatées de pulpe dentaire humaine montrant la coloration de l'acide nucléique par le DAPI (fluorescence bleue), les cellules HLA-DR+ (fluorescence verte), les cellules HO-1+ (fluorescence rouge), et images fusionnées avec double marquage (grossissement original 63). Les résultats sont représentatifs de 4 pulpes dentaires différentes analysées de la même manière.

- **Projet 2 : Caractérisation de cellules et de médiateurs impliqués dans la régulation de la réponse immunitaire au niveau de la pulpe dentaire saine de rat :**

Dans un premier temps, nous avons étudié et caractérisé un ensemble de cellules immunitaires chez le rat. Nous avons retrouvé comme chez l'homme, des cellules dendritiques exprimant HO-1 (cytométrie de flux et immunohistologie) sur des pulpes saines d'incisives centrales.

Ces études sur le rat nous ont également permis de retrouver des cellules ayant un phénotype compatible avec des MDSC (Myeloid Derived Suppressive Cells), cellules exprimant fortement HO-1 et possédant d'importantes propriétés immunorégulatrices.

- **Projet 3 : Création d'un modèle animal permettant d'étudier différentes stratégies thérapeutiques en rapport avec la modulation de la réponse immunitaire.**

Dans le but de comprendre le rôle des mécanismes de tolérance dans la pulpe dentaire, nous avons mis au point un modèle d'inflammation bactérienne de pulpite réversible chez le rat. Il existe peu, à l'heure actuelle, de modèle permettant d'étudier le contrôle de la réponse immunitaire chez l'animal. Nous avons donc développé un modèle d'inflammation induit par du lipopolysaccharide (LPS) (Figure 3).

La compréhension des mécanismes de régulation de la réponse immunitaire sur un modèle animal (ici le rat) nous a permis de définir une ou plusieurs stratégies thérapeutiques. Après avoir éliminé les éléments pathogènes bactériens lors du traitement de la carie, ces agents thérapeutiques appliqués via les tubuli dentinaires ou directement sur la pulpe dentaire devraient avoir un effet sur la réponse immunitaire et favoriser la régénération.

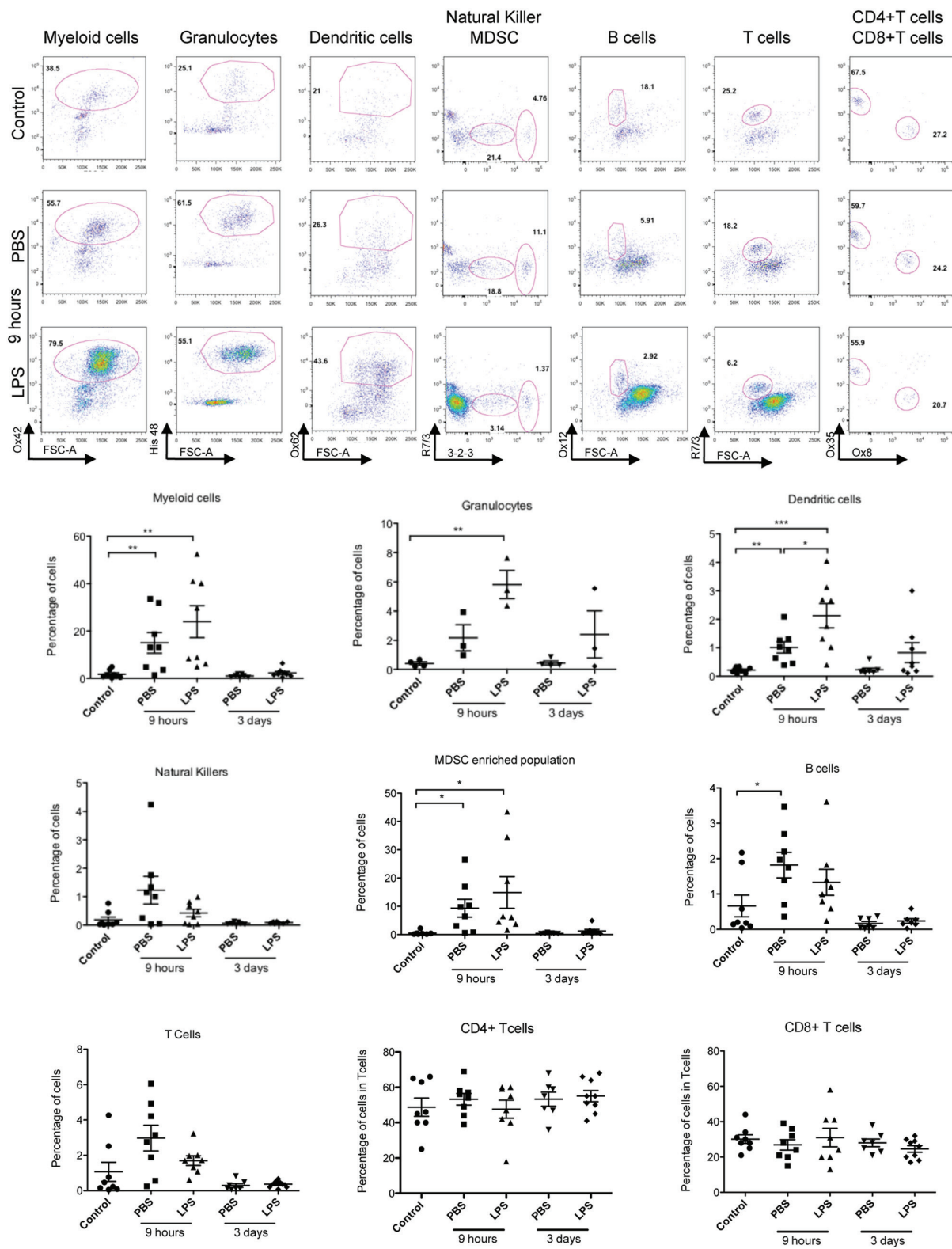


Figure 3 : Répartition relative des principales cellules immunocompétentes dans les incisives de rat non traitées (contrôles) et dans les incisives stimulées par du LPS ou par une solution saline tamponnée au phosphate (PBS) à 9 heures et 3 jours. (Renard et al., J Dent Res 2016)

*Une analyse par cytométrie en flux a été effectuée pour la caractérisation et la quantification des cellules myéloïdes (Ox42+), des granulocytes (His48+), des cellules dendritiques (Ox62+), des cellules NK (R7/3- 3.2.3high), des Myeloid Derived Suppressive Cells (R7/3- 3.2.3low), les lymphocytes B (Ox12+), les lymphocytes T (R7/3+), les lymphocytes T CD8+ (R7/3+ Ox8+) et les lymphocytes T CD4+ (R7/3+ Ox35+) dans la population de leucocytes (Ox1-Ox30+). Pour chaque population cellulaire et chaque condition, des diagrammes à points représentatifs du tri cellulaire activé par fluorescence de l'échantillon animal, 9 h après le traitement. Les graphiques représentent la moyenne \pm SEM ; n = 8. Pour chaque population, le pourcentage de cellules a été défini par le pourcentage de cellules exprimant les marqueurs spécifiques rapporté au pourcentage de leucocytes vivants. Pour les lymphocytes T CD4+ et les lymphocytes T CD8+, les pourcentages ont été obtenus à partir du pourcentage de cellules positives sélectionnées sur les lymphocytes T (R7/3+ ; n = 8). *P < 0.05. **P < 0.001. ***P < 0.0001.*

Publications issues du doctorat :

- **Gaudin A**, Renard E, Hill. M, Bouchet-Delbos L, Bienvenu-Louvet G, Farges JC, Cuturi MC, Alliot-Licht B. Phenotypic analysis of immunocompetent cells in healthy human dental pulp. Journal of endodontics 2015, 41(5) :621-7
- Farges JC, Alliot-Licht B, Renard E, Ducret M, **Gaudin A**, Smith AJ, Cooper PR. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm. 2015:230251
- Renard E, **Gaudin A** (co-premier), Bienvenu G, Amiaud J, Farges JC, Cuturi MC, Moreau A, Alliot-Licht B. Immune Cells and Molecular Networks in Experimentally Induced Pulpitis. J Dent Res. 2016 Feb;95(2):196-205.

5.2 TRAVAUX DE RECHERCHE POSTDOCTORAUX ET MOBILITE (2016-2020)

Travaux dans le domaine de la régénération osseuse et pulpo-dentinaire

- **Co-encadrement du doctorat de Pierre Tournier (2016-2019)**

Au travers de l'encadrement de la thèse de Pierre Tournier, nous nous sommes intéressés à la caractérisation et à l'amélioration des propriétés ostéogéniques d'un nouveau substitut osseux allogénique. Ce travail d'encadrement faisait appel à des problématiques découvertes et

approfondies lors de notre M2 et thèse d'Université (régénération osseuse) et réponse immunitaires (notamment polarisation des macrophages classiquement activés/phénotype régénératifs). Ce co-encadrement m'a également permis de découvrir l'unité Inserm UMRS 1229-RMeS, d'échanger avec les personnes qui y travaillent et le directeur Jérôme Guicheux. Le nouveau substitut osseux allogénique développé par la banque d'os BIOBank présente une alternative intéressante aux greffes osseuses autologues. En effet, le prélèvement d'os autologue présente des inconvénients majeurs comme le volume limité de greffon récoltable ou encore l'allongement du temps chirurgical, avec de nombreuses complications per- et post-opératoires décrites : douleurs, saignements, infections, cicatrices inesthétiques.... Parmi les alternatives à l'autogreffe, l'utilisation d'os allogénique est fiable et largement utilisé depuis des décennies. Ces greffons en blocs ou particuliers restent difficiles à utiliser dans des défauts osseux irréguliers ou difficiles d'accès, particulièrement en comblement osseux maxillo-faciaux ou en chirurgie dentaire post-extractionnelle. Cette proposition thérapeutique permet grâce à une déminéralisation partielle de poudre d'os cortico- spongieuse de pallier ces inconvénients. Cette pâte d'os est constituée de grains avec un centre minéralisé entouré de matrice osseuse déminéralisée, le tout baignant dans une gélatine de collagène de type I. Les objectifs du travail de thèse de Pierre Tournier étaient de caractériser et d'améliorer les propriétés ostéogéniques de cette pâte d'os allogénique. Ces propriétés ostéogéniques ont d'abord été démontrées *in vitro* au contact de cellules stromales mésenchymateuses et de monocytes humains, puis dans deux modèles *in vivo* de régénération osseuse intramembranaire : en régénération osseuse guidée et dans des défauts critiques de calvaria de rat.

De manières intéressantes ces travaux ont montré en immunohistochimie que la pâte osseuse a soit accéléré le recrutement de monocytes/macrophages sur place et/ou leur passage d'un phénotype de type M1 à un phénotype de type M2 par rapport à la greffe osseuse particulière. En effet, le petit nombre de cellules iNOS⁺ et l'abondance de cellules CD163⁺ et CD68⁺/CD163⁺ dans les défauts remplis avec la pâte osseuse suggèrent un environnement inflammatoire approprié pour la régénération osseuse, avec la polarisation des macrophages vers un phénotype pro-régénératif (Figure 4). Enfin, l'association de nanocristaux d'hydroxyapatite à des doses croissantes à la pâte d'os dans deux modèles de défauts critiques de calvaria chez le rat n'a pas permis d'augmenter ses propriétés régénératrices où la repousse osseuse s'est montrée équivalente à celle induite par une autogreffe osseuse.

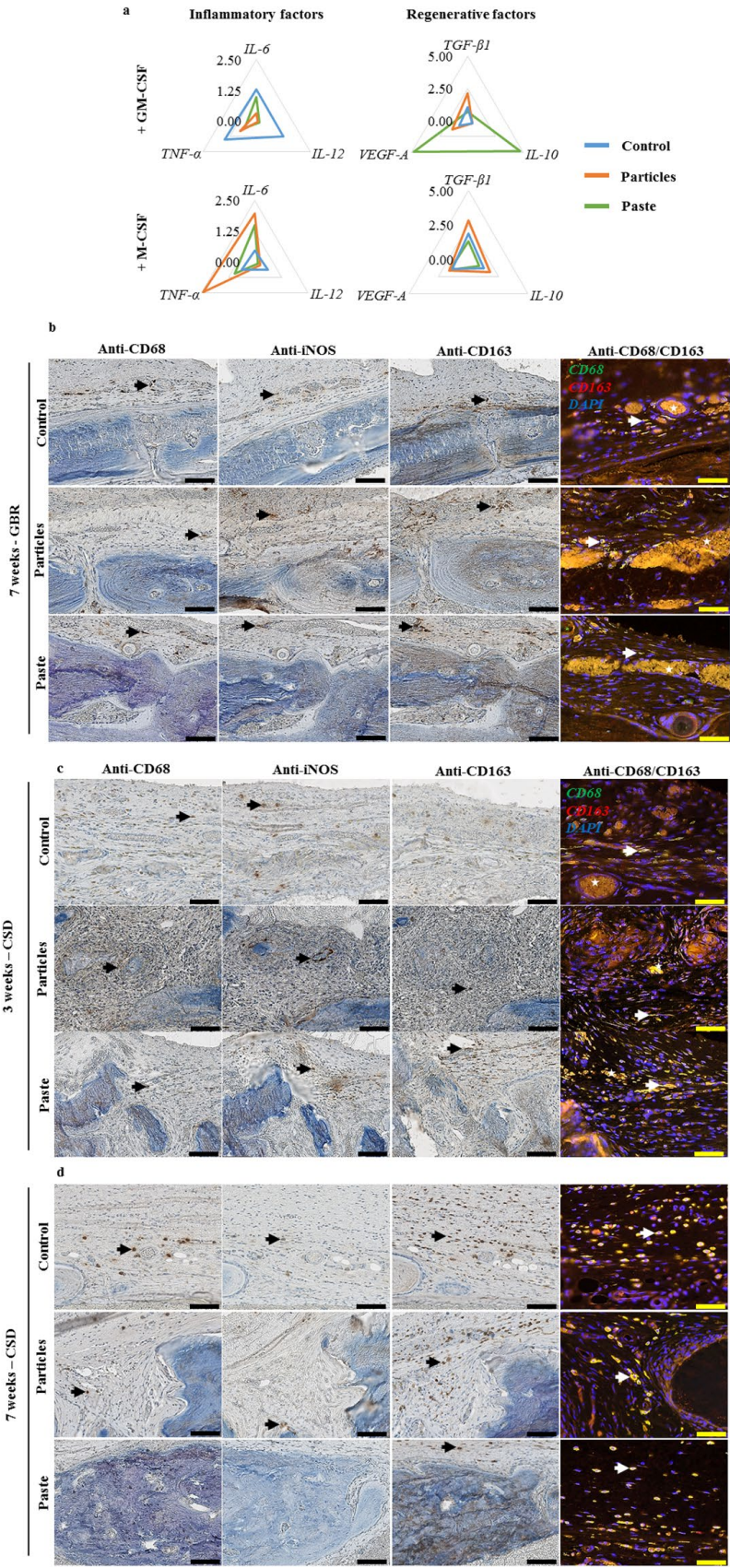


Figure 4 : Effet du greffon osseux particulier et de la pâte osseuse sur la polarisation des monocytes/macrophages. (Tournier et al., Sci Rep 2021)

(a) Expression de l'ARNm dans les monocytes humains cultivés en présence de GM-CSF ou de M-CSF pendant 3 jours en contact avec le greffon osseux sous forme de poudre ou la pâte osseuse. L'axe Y représente le niveau d'expression relatif moyen de l'ARNm des gènes spécifiés (N = 3). Colorations immunohistochimiques et immunofluorescentes représentatives sur des sections successives non décalcifiées de 7- μ m d'épaisseur, (b) dans le modèle de régénération osseuse guidée après 7 semaines, et (c) dans le modèle de défaut critique dans la calvaria de rat après 3 et 7 semaines. Des colorations immunohistochimiques ont été réalisées pour CD68, iNOS, CD163, et une immunofluorescence avec double marquage de CD68 (vert)/CD163 (rouge) et DAPI (bleu) pour les noyaux. Pour le marquage simple, les cellules positives étaient colorées par un précipité brun (flèche noire), pour le double marquage, les cellules doublement positives apparaissaient en jaune clair (flèche blanche). Des globules rouges auto-fluorescents typiques étaient visibles (étoiles blanches). Barres d'échelle : 100 μ m pour l'immunohistochimie, 50 μ m pour les images d'immunofluorescence.

Publications issues de la thèse :

- Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, Bardonnnet R, Blanquart C, Corre P, Geoffroy V, Weiss P, **Gaudin A**. An Extrudable Partially Demineralized Allogeneic Bone Paste Exhibits a Similar Bone Healing Capacity as the “ Gold Standard ” Bone Graft. *Front. Bioeng. Biotechnol* 2021, 9, 1–10. doi:10.3389/fbioe.2021.658853.
- Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, Bardonnnet R, Blanquart C, Corre P, Geoffroy V, Weiss P, **Gaudin A**. A partially demineralized allogeneic bone graft: in vitro osteogenic potential and preclinical evaluation in two different intramembranous bone healing models. *Sci. Rep* 2021, 11, 1–13. doi:10.1038/s41598-021-84039-6.
- **Analyse des propriétés antibactériennes et immunogènes d'un hydrogel innovant fibrine-chitosane pour la régénération de la pulpe dentaire humaine.** (Projet réalisé en collaboration avec l'équipe du Pr Jean-Christophe Farges)

Ce projet collaboratif avait pour but d'étudier la pertinence d'une stratégie thérapeutique innovante de revitalisation complète des dents humaines ayant subi une pulpotomie, c'est-à-

dire dont la pulpe dentaire a été partiellement éliminée par le chirurgien-dentiste pour des raisons thérapeutiques. En effet, les caries profondes conduisent très souvent à une inflammation irréversible de la pulpe dentaire dont le traitement comprend aujourd'hui l'élimination totale de la pulpe (pulpectomie). Or ce traitement ne prend pas en compte le fait que la partie la plus apicale de la pulpe dentaire n'est, le plus souvent, pas affectée et pourrait être conservée en réalisant une simple pulpotomie. La pulpe dentaire résiduelle pourrait cicatriser et régénérer le tissu pulpaire qui a été éliminé. De plus, suite à la pulpectomie, la cavité pulpaire (endodontique) est aujourd'hui obturée avec un biomatériau inerte à base de gutta-percha qui n'est pas toujours totalement étanche et peut être colonisé par des bactéries orales qui vont provoquer une infection dentaire. Ainsi, les thérapeutiques endodontiques actuelles ne peuvent pas être considérées comme totalement satisfaisantes.

Plusieurs études récentes réalisées chez l'animal ont montré que la régénération d'un tissu pulpaire est possible grâce à l'utilisation d'un biomatériau de type hydrogel. Nous avons récemment conçu un hydrogel innovant associant la fibrine, le constituant matriciel naturel des caillots sanguins, particulièrement bien toléré, et le chitosan, un polysaccharide naturel issu de la carapace des crustacés, connu pour ses propriétés antibactériennes. Nous avons démontré que cet hydrogel est cytotocompatible, et donc apte à être colonisé par les cellules souches de la pulpe résiduelle qui vont progressivement le remplacer par un nouveau tissu pulpaire.

Nous avons également évalué *in vivo*, dans un modèle de pulpotomie d'incisive de rat, la réponse immunitaire déclenchée par l'hydrogel placé au contact de la pulpe résiduelle.

Cette étude nous a permis de nous assurer que des bactéries et/ou une réponse immunitaire inappropriée ne viendront pas empêcher la dégradation correcte de l'hydrogel, sa colonisation par les vaisseaux sanguins et les cellules souches pulpaires de l'hôte, et la régénération d'une pulpe dentaire en lieu et place de l'hydrogel. Elle représente une étape majeure et indispensable dans le développement de ce biomatériau innovant destiné à régénérer la pulpe dentaire humaine dans le contexte clinique de la pulpotomie.

Publication :

- Renard E, Amiaud J, Delbos L, Charrier C, Montembault A, Ducret M, Farges JC, David L, Alliot-Licht B, **Gaudin A**. Dental pulp inflammatory/immune response to a chitosan-enriched fibrin hydrogel in the pulpotomized rat incisor. *Eur Cell Mater* 2020, 20(40) :74-87.

Activité de recherches réalisées en mobilité (2016-2017)

Pendant la période allant du 12 juillet 2016 au 12 juillet 2017, j'ai pu effectuer une année de mobilité en tant que « visiting researcher » dans le département d'Endodontie dirigé par le Pr Ove Peters University of the Pacific, San Francisco, USA.

J'ai choisi de rejoindre cette équipe constituée de chercheurs, cliniciens, résidents en endodontie et assistants de recherche afin de travailler sur les réponses des cellules souches de la papille apicale (SCAP) face à différents stimuli inflammatoires. Ce projet est en continuité avec mon sujet de recherche en France (immunologie de la pulpe dentaire). Par conséquent, j'ai pu exploiter mon expertise de certains outils (cytométrie en flux, RT-PCR, immunohistologie) mais également acquérir d'autres techniques (culture cellulaire, western blot, ELISA et immuno-analyse multiple système Luminex).

Lors de cette année de mobilité j'ai pu travailler principalement sur 2 sujets. Afin de mener ces 2 projets à terme, j'ai dû tout d'abord me former aux différents outils du laboratoire (durée : environ 15 jours), réaliser les demandes d'autorisation sur le plan éthique du fait de l'utilisation de matériel humain (dents de sagesse) et écrire 2 demandes de financements qui ont été acceptées.

1. Implication des lipoxines A4 (LXA4) sur l'activité immunosuppressive paracrine des SCAPS

Les lipoxines sont des médiateurs endogènes impliqués dans la résolution de l'inflammation et sont actuellement source de nombreuses recherches car elles permettent d'activer la résolution de l'inflammation sans déséquilibrer les voies classiques de cicatrisation contrairement à d'autres molécules anti-inflammatoires. Ces propriétés sont particulièrement intéressantes dans un contexte d'inflammation pulpaire ou de régénération endodontique.

Lors de cette année de mobilité, nous avons pu démontrer et confirmer, par différentes techniques, que les SCAPs possédaient de manière constitutive le récepteur pour les LXA4. De plus, ce récepteur était surexprimé lors de stimuli inflammatoires (différentes doses de Lipopolysaccharides (LPS), ou TNF- α). Nous avons également étudié l'action de LXA4 sur les SCAP stimulées par du LPS. Les SCAPS ont montré une diminution de sécrétion de différentes

cytokines pro-inflammatoires, facteurs de croissance et chémokines sous l'influence de LXA4 et ceci de manière dose-dépendante. Lorsque ces SCAP ont été mises en co-culture avec des cellules périphériques mononucléaires du sang (PBMC), les LXA4 ont augmenté la capacité régulatrice des SCAP (diminution de la prolifération des PBMC) (Figure 5).

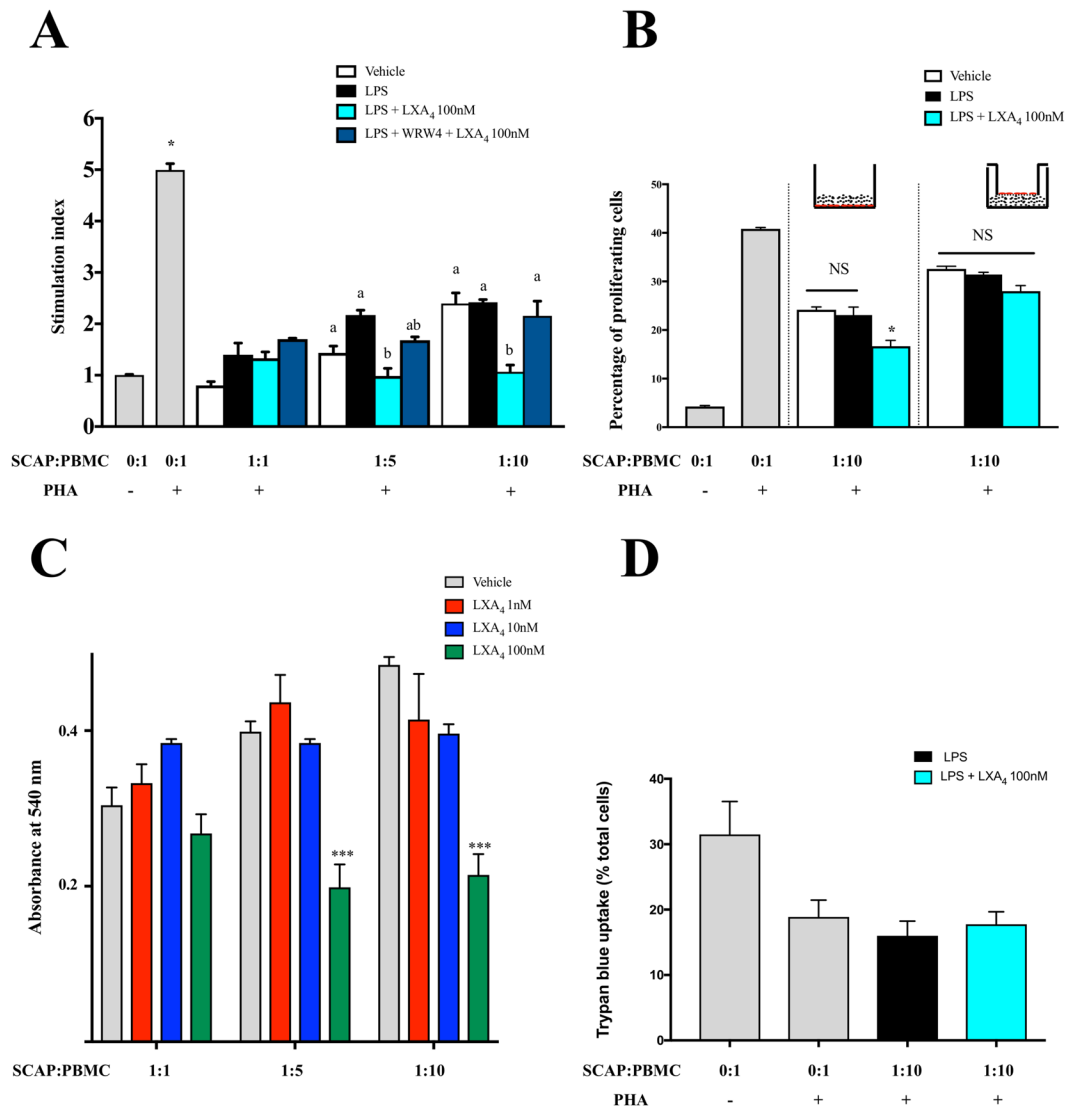


Figure 5 : La lipoxine A4 (LXA4) a renforcé les propriétés immunomodulatrices des SCAP sur la stimulation et la prolifération des PBMC. (Gaudin et al., Sci Rep 2018).

(A) Les SCAP ont d'abord été stimulées avec LXA4 100 nM ou LXA4 100 nM + LPS (1 µg/ml) ou WRW4 + LXA4 100 nM + LPS (1 µg/ml) pendant 24 h. Les PBMC ont été stimulées avec PHA et cocultivées avec les SCAP à différents ratios (1:1, 1:5, 1:10). Après 5 jours, l'indice de stimulation a été calculé à l'aide du test MTT. * $p < 0.05$. Les colonnes contenant la même lettre ou les mêmes lettres ne sont pas significativement différentes ($p > 0.05$). (B) Les SCAP (points rouges) ont d'abord été stimulées avec LXA4 uniquement ou LXA4 + LPS (1 µg/ml) pendant 24 h. Les PBMC marquées au CFSE (cellules rondes pleines) ont été stimulées avec PHA dans la chambre inférieure d'un Transwell. Les SCAP ont été mises en co-culture avec les PBMC stimulées (ratio SCAP/PBMC

1:10), soit en contact direct, soit séparées par une membrane Transwell. Après 5 jours de co-culture, les PBMC en prolifération ont été évaluées par coloration CFSE et analyse cytométrique en flux. * $p < 0.05$. (C) Les SCAP ont d'abord été stimulés avec LXA4 (0, 1, 10 ou 100 nM) pendant 24 heures et ont été co-cultivés avec des PBMC stimulés par le (PHA) selon différents ratios (1:1, 1:5, 1:10). Après 5 jours, l'effet des différentes doses de LXA4 sur la prolifération des PBMCa été évalué par MTT. *** $p < 0,001$ par rapport aux cellules témoins (véhicule). (D) Les SCAP ont d'abord été stimulées avec LXA4 100 nM ou LXA4 100 nM + LPS (1 $\mu\text{g/ml}$) pendant 24 h. Les PBMC ont été stimulées avec PHA et co-cultivées avec les SCAP à différents ratios (1:1, 1:5, 1:10). Après 5 jours, l'évaluation des cellules mortes a été calculée par absorption de bleu trypan. PHA phytohémmagglutinine A, CFSE carboxyfluorescéine succinimidyl ester.

2. Évaluation in-vitro des propriétés anti-inflammatoires des ciments endodontiques à base de silicate de calcium

Les nouveaux ciments endodontiques à base de silicate de calcium sont d'apparition récentes et peuvent être considérés comme des dérivés du Mineral Triaggregate (MTA). Le MTA a démontré depuis plus de 30 ans des succès cliniques et histologiques. Cependant, le changement de formulation afin de pouvoir créer un ciment endodontique est susceptible d'entraîner des variations des propriétés biologiques. Pendant mon année de mobilité, j'ai pu comparer les effets de 3 ciments à base de silicate de calcium sur des cellules souches parodontales (PDLSC) soit en culture classique en 2D, soit dans un modèle de culture 3D, soit dans un modèle *ex-vivo* (Figure 6). Nous avons pu démontrer des différences en termes de viabilité, migration et prolifération cellulaire. Nous avons également mis en évidence des variations en termes de sécrétion de cytokines et chemokines pro-inflammatoires.

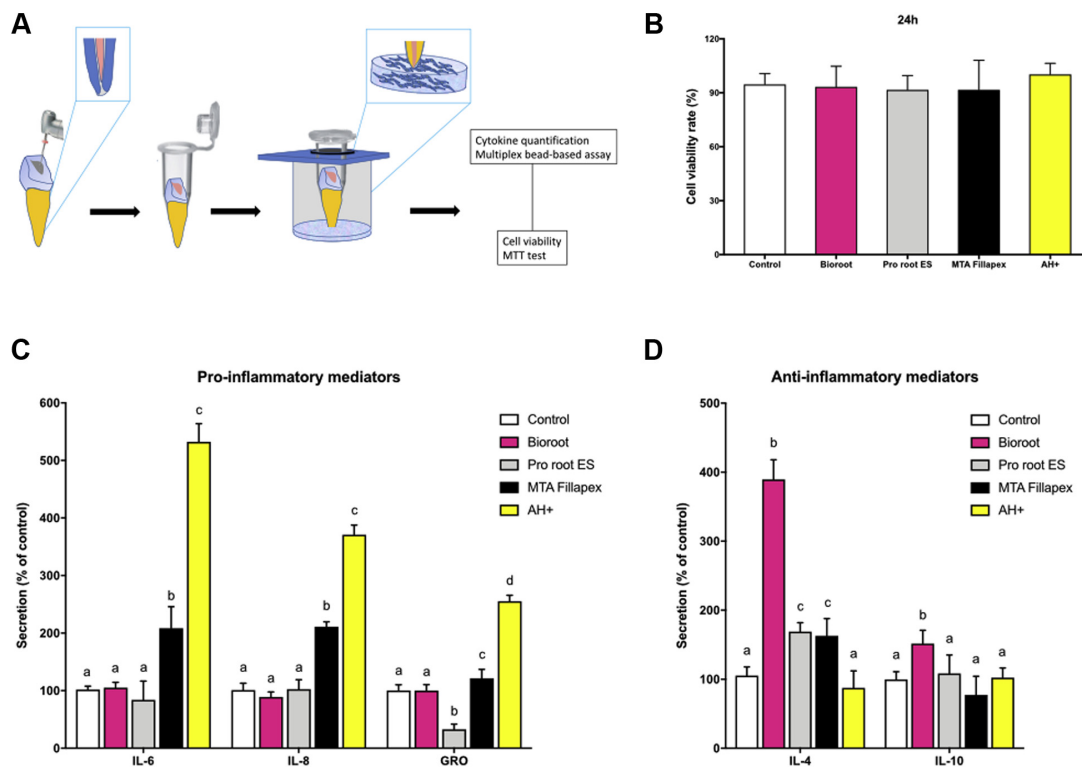


Figure 6 : La procédure expérimentale du modèle 3D de racine in vitro. (Gaudin et al., J Endod 2020)

(A) Procédure expérimentale du modèle de culture 3D en relation avec l'apex radiculaire. Le fond des tubes Eppendorf a été coupé pour permettre la protrusion de l'apex de la dent à travers cette ouverture et ajusté dans une plaque de culture cellulaire à 6 puits à l'aide d'un joint torique en caoutchouc avec 3 mm d'un apex de racine plongé dans le milieu de culture cellulaire 3D. Le milieu qui est resté en contact avec l'apex pendant 24 heures a été utilisé pour la quantification des cytokines, tandis que la viabilité des cellules encapsulées en 3D dans PuraMatrix a été évaluée à l'aide du test MTT. (B) Le taux de viabilité cellulaire (%) selon l'essai MTT dans les cellules souches du ligament parodontal (PDLSC) encapsulées en 3D après 24 heures d'exposition à différents scellements (BioRoot RCS, ProRoot ES, MTA Fillapex et AH Plus) ; les cellules encapsulées dans le milieu de culture seul ont servi de contrôle négatif. (C) Résultat du test multiplex montrant les médiateurs pro-inflammatoires (IL-6, IL-8 et GRO) sécrétés par les PDLSC dans le milieu de culture. (D) Résultat du test multiplex les montrant les médiateurs anti-inflammatoires (IL-4 et IL-10) sécrétés par les PDLSC dans le milieu de culture. Les résultats sont exprimés sous forme de moyennes et d'écart types. Des lettres différentes représentent des différences significatives entre les groupes dans chaque extrait de dilution. Analyse de la variance suivie du test post hoc de Bonferroni ($P < .05$).

Publications :

- Gaudin A, Tolar M, Peters OA. Cytokine production and cytotoxicity of calcium silicate-based sealers in two- and three-dimensional cell culture models. Journal of endodontics 2020, 46(6) :818-926.

- **Gaudin A**, Tolar M, Peters OA. Lipoxin A4 Attenuates the Inflammatory Response in Stem Cells of the Apical Papilla via ALX/FPR2. *Sci. Rep.* 8, 1–12 (2018).

5.3 ACTIVITES DE RECHERCHE EN COURS ET PROJETS DE RECHERCHE

Contexte de l'activité de recherche

Importance et du rôle des médiateurs lipidiques spécialisés dans la résolution de l'inflammation

La résolution de l'inflammation est coordonnée et régulée par un large panel de médiateurs, notamment des médiateurs lipidiques spécialisés (SPM) [20, 21]. Les SPM permettent le retour à l'homéostasie, diminuent le recrutement des leucocytes, favorisent la clairance des cellules apoptotiques (notamment par les macrophages) et la reprogrammation d'un macrophage de type pro-inflammatoire à un phénotype pro-résolutif [22–24]. Parmi ces SPM, les lipoxines A4 (LXA4) ont été proposées et étudiées. Les LXA4 ont été testés dans différentes situations cliniques et ont montré des résultats prometteurs dans un large éventail de maladies inflammatoires, fibrose rénale, ischémie cérébrale et cancer [25–29].

Le professeur Ove Peters est à l'origine de notre travail sur les médiateurs lipidiques (SPM) impliqués dans la résolution de l'inflammation. Nous avons pu démontrer *in vitro* par exemple le rôle immunomodulateur de ces molécules sur des cellules souches de la papille apicale dentaire (SCAP) [30]. Les LXA4 ont freiné la sécrétion de médiateurs pro-inflammatoires par les SCAPs lorsque celle-ci étaient stimulées par du lipopolysaccharide. Les LXA4 ont également favorisé l'inhibition de la prolifération de lymphocytes lors de réaction de Mixed leucocyte Reaction (MLR). Enfin nous avons pu démontrer que ces actions étaient possibles par la fixation des LXA4 sur un récepteur ALX/FPR2 [30].

Fort de cette expérience, nous avons souhaité à notre retour, poursuivre dans cette voie et pouvoir étudier le rôle des SPM au niveau pulpaire. Afin de pouvoir envisager au mieux ce projet, Je me suis tourné vers le Pr Pierre Weiss (unité Inserm UMRS 1229-RMeS) dans lequel j'avais effectué une partie de mon stage de M2 et la première année de thèse d'Université. Après discussion et afin de pouvoir bénéficier des plateformes et de l'expertise de l'unité Inserm UMRS 1229-RMeS dans l'élaboration de dispositifs à libération de principes actifs j'ai

donc décidé de changer de laboratoire d'accueil. **Le Pr Weiss est actuellement mon directeur de recherche.**

Grâce à l'environnement facilitant de l'unité Inserm UMRS 1229-RMeS, j'ai pu développer mes axes de recherche, monter mes propres projets de recherche et développer ainsi une autonomie sur la thématique de recherche suivante : **les systèmes à libération de principes actifs pour favoriser la résolution de l'inflammation.** J'ai eu la chance d'obtenir un premier financement suite à l'appel offre de l'IFRO en 2018 qui a permis de générer des données et de pouvoir prétendre l'année suivante à une demande dans le cadre d'un projet ANR Jeune chercheur. Ce projet a été financé à hauteur de 285 KE sur 3 ans, ainsi, j'ai pu recruter un ingénieur, un thésard (Davy Aubeux, ancien AHU dans la sous-section 5801) et développer notre réseau de collaboration avec les P^{rs} Philippe Legrand (Montpellier), Julian Leprince (Belgique), Laurent David (Lyon), Ove Peters (USA), Christophe Blanquart (Nantes), Prasanna Neelakantan (Hong-Kong), Elisabetta Cotti Italie).

Axe 1 : Design et évaluation d'un nouveau microgel biphasique à libération de LXA4 dans le cadre de l'ingénierie tissulaire. Projet BILIPOX

Financements

- **ANR JCJ**, projet BILIPOX, A new biphasic microgel as lipoxin delivery system for tissue engineering. Coordinateur principal
- Université Nantes (**AOI odontologie**), projet ORGANITE, Design of an organoid culture model for tissue engineering Co-coordonateur.
- **IFRO**, projet LIPOXH, LIPOXH: Design and in vitro assessment of a new biphasic microgels of silylated HPMC as lipoxin delivery system for periodontitis and pulpitis treatment. Coordinateur principal.

Encadrement

- Doctorant : Davy Aubeux (2020-2025)
- Assistante-ingénieure : Solène Tessier (2020-2022)

- Master 2 :
 - Florian Segeron (2021)
 - Cassandra Lepetit (2019)

Rationnel

Le principal défi posé par l'utilisation de lipoxines ou d'analogues est leur instabilité chimique, une caractéristique qui nuit à leur utilisation pour traiter des maladies inflammatoires ou des lésions tissulaires.

Ainsi, pour préserver leurs activités biologiques, l'intégration dans des microparticules s'est révélée être une approche efficace pour traiter les lésions cutanées du rat dorsal et réduire l'inflammation de l'articulation temporo-mandibulaire. Il est intéressant de noter que l'incorporation de SPM dans des microparticules ou des nanoparticules a récemment été testée dans des modèles précliniques de parodontite et est apparue comme une approche efficace pour réduire l'inflammation parodontale et réduire la perte osseuse.

Cependant, les microparticules ne sont pas exemptes de limitations telles que l'utilisation de solvant organique, la capacité de charge limitée et la libération rapide du principe actif. Pour remédier à ces inconvénients, notre approche pharmacologique consiste à créer un dispositif permettant la libération *in situ* de la lipoxine. L'objectif est d'obtenir un relargage contrôlé directement au niveau des sites lésés. Pour cela, nous avons imaginé utiliser des hydrogels. Ce matériau constitué en grande partie d'eau existe sous de nombreuses formes et constitue un environnement favorable pour la régénération des tissus détruits. Ces propriétés, allié à une très bonne tolérance en font des candidats idéaux en tant que dispositifs à libération de principes actifs. En revanche, du fait de leur composition, il est très difficile d'incorporer des molécules hydrophobes tels que les médiateurs lipidiques. Cependant, grâce à un procédé novateur permettant la création d'un hydrogel à deux phases un cœur hydrophobe avec une partie extérieure hydrophile, nous avons pu envisager la possibilité d'incorporer des molécules hydrophobes. **L'objectif spécifique de ce projet est d'évaluer les avantages potentiels d'un nouveau système de distribution de microgel biphasique comme lipoxine A4 (LXA4) pour l'ingénierie tissulaire. Ces microgels biphasiques fonctionnalisés visent à la fois à moduler l'inflammation et également à favoriser la réparation ou la régénération des tissus**

Déroulé du projet

Pour traiter ces questions, le projet BILIPOX est organisé en 3 parties :

Partie 1 : Préparer et analyser les microgels biphasiques (BPMG) de Si-HPMC comme système de libération de LXA4 *in vitro*

Le Si-HPMC est obtenu par la réaction unique de condensation des fonctions alcoxysilane dans des conditions physiologiques de température et de pH. Un processus de modélisation d'émulsion sera appliqué en utilisant uniquement les propriétés interfaciales de l'HPMC sans utiliser de surfactant. Le choix du noyau huileux des BPMGs est réalisé en fonction de la solubilité et de la stabilité de la lipoxine dans les huiles végétales injectables. La formulation et les conditions de fabrication des BPMGs sont optimisées en terme de ratio huile/eau et en terme de pH pour respectivement optimiser la charge en lipoxine et prévenir le risque de son oxydation notamment en pH acide. La stabilité de LXA4 pourrait être améliorée par l'ajout d'antioxydants dans la phase huileuse lipophile (α tocopherol).

La caractérisation physico-chimique et les propriétés mécaniques sont évaluées. (La viscosité dynamique de la phase continue sera mesurée avec un rhéomètre à cylindre coaxial. L'étude des propriétés nanomécaniques et le balayage topographique de la surface seront effectués par un microscope à force atomique (AFM). Le profil de libération et l'absence de dégradation de LXA4 sont également mesurées). Le dosage de la lipoxine est réalisé par chromatographie liquide en tandem avec spectrométrie de masse ou par technique ELISA.

Partie 2 : Étudier l'effet des LXA4-BPMG sur le phénotype des macrophages et dans des conditions inflammatoires *in vitro*

Dans cette partie, il est prévu d'analyser la réponse de la polarisation des macrophages aux LXA4-BPMGs de Si-HPMC *in vitro*.

Les monocytes humains sont incubés avec les LXA4-BPMGs de Si-HPMC. La caractérisation des macrophages sera évaluée par cytométrie de flux en utilisant CD14, CD163, HLA-DR et CD16. IL-6, IL-10, IL-12, IL-1 β , TNF α , IL-23, IP-10, IL-1RA et TARC seront évalués par RT-PCR et ELISA multiplex grâce à notre collaboration avec le CRCINA, INSERM UMR 1232. La sécrétion de TGF β est étudiée en utilisant une lignée cellulaire rapporteuse commerciale. Des expériences de

réaction leucocytaire mixte (MLR) sont réalisées pour confirmer le phénotype du macrophage. Pour compléter l'étude, des macrophages M1 et M2 différenciés sont incubés avec des LXA4/BMP2-BPMGs de Si-HPMC pendant 3 jours.

Dans un second temps, il est prévu de mesurer l'impact des LXA4-BPMGs dans des conditions inflammatoires *in vitro* simulées. Deux types de cellules seront cultivés, des macrophages humains et des cellules souches orales (OSC). Un système transwell (pores de 0,4 μm) permet l'échange paracrine de facteurs solubles et est utilisé pour évaluer la communication entre ces deux types de cellules. Les macrophages sontensemencés dans l'insert transwell. Les LXA4-BPMGs de Si-HPMC sont ajoutés au milieu de culture dans l'insert transwell. Différents environnements inflammatoires simulés sont générés :

- Phase d'inflammation aiguë : les OSC sont confrontées à des M1.
- Inflammation chronique : Les OSC sont confrontés à des applications répétées de M1.
- Phase de résolution : les OSC sont confrontées à des M2.
- Phase de régénération/différenciation : le milieu de culture est remplacé par un milieu ostéogénique et les macrophages pré-stimulés sont remplacés par des macrophages frais ou par aucun macrophage.

Le profil d'expression des gènes inflammatoires (IL-6, IL-8, IL-10, IL-4, CXCL9, CXCL10, RANTES, TGF β , VEGF) et les marqueurs ostéogéniques clés que sont la phosphatase alcaline (ALP), l'ostéopontine et la BMP2 sont déterminés pour les OSC par RT-qPCR et dans le surnageant à l'aide de tests multiplex en luminex.

Partie 3 : évaluer l'effet des LXA4-BPMGs dans des modèles précliniques d'inflammation

La tâche 3 évaluera dans un premier temps, l'effet de LXA4 dans un modèle préclinique d'inflammation de la pulpe dentaire *in vivo*. En utilisant notre modèle original et établi de pulpite [31], nous allons (i) caractériser les événements inflammatoires et immunitaires, (ii) étudier l'interface entre l'hydrogel biphasique et la pulpe dentaire et aussi (iii) caractériser le tissu nouvellement formé. Les animaux seront répartis au hasard en cinq groupes différents. G1 : contrôle : dent intacte, G2 : contrôle positif : Ciment à base de silicate de calcium (gold standard), G3 : Si-HPMC BPMGs seul, G4 : Si-HPMC BPMGs encapsulé LXA4, G5 : Si-HPMC BPMGs seul + LXA4 libre. Les cytokines (IL6, IL1- β , TNF α , IL10, TGF β , IL23, IL12), chimiokines

(CCL2, CXCL1, CXCL2), et métalloprotéinases (MMP3, MMP8, MMP9) ainsi que iNOS et hème oxygénase-1 (HO-1), les principales cellules immunocompétentes (macrophages (M1 et M2), granulocytes, cellules dendritiques, cellules NK, lymphocytes B et T) seront étudiées respectivement par ELISA, qPCR et cytométrie de flux. La guérison de la pulpe dentaire sera évaluée par une analyse immunohistologique des marqueurs M1 (TNF α , iNOS, CCR7), M2 (CD206, Arg1 et CD163) ainsi que de la sialoprotéine dentinaire (DSP) et de la sialoprotéine osseuse (BSP).

Dans un second temps, l'effet de LXA4 sera évalué dans un modèle préclinique de parodontite *in vivo*. Pour le modèle de parodontite, des ligatures seront placées et des parodontopathogènes humains seront administrés dans un modèle de grand animal non cicatrisant (cochons miniatures) [32, 33]. Le parodonte des porcs miniatures présente de nombreuses similitudes avec celui des humains. La profondeur du sillon gingival est de 2 à 3 mm avec un long épithélium de jonction. L'évolution de la maladie suit les mêmes étapes que chez l'homme. Afin de quantifier l'arrêt de la perte osseuse et la régénération osseuse, une analyse tridimensionnelle par μ CT et une quantification du tissu osseux seront effectuées. Une histologie qualitative et quantitative sera réalisée pour différencier la croissance et la régénération osseuse de l'organe parodontal.

Axe 2 : Vésicules extracellulaires dérivées de cellules souches mésenchymateuses dentaires (pDMSC-EVs). Projet EVENDO

Vers des applications thérapeutiques acellulaires pour contrôler l'inflammation de la pulpe dentaire et faciliter la régénération du complexe pulpo-dentinaire

Financements

- ESE European Society for Endodontology (2020), Extracellular Vesicles derived from primed Dental Mesenchymal Stem Cells (pDMSC-EVs): Toward cell-free therapeutic applications to control pulp inflammation and facilitate dental pulp regeneration. Projet EVENDO. Coordinateur principal

Encadrement

- Doctorant : Davy Aubeux (2020-2025)
- Assistante-ingénieure : Solène Tessier (2020-2022)

Rationnel

Le contexte de ce projet est la résolution de l'inflammation dans des situations cliniques telle que la pulpite nécessitant la modulation de la réponse inflammatoire excessive des tissus afin d'améliorer la réparation ou la régénération de la pulpe. Les cellules souches mésenchymateuses dentaires (DMSC) sont actuellement considérées comme un outil intéressant pour la régénération des tissus [34–37]. En outre, les DMSC sont également connues pour leurs fortes propriétés immunomodulatrices [38, 39]. L'interaction réciproque entre les DMSC et les cellules immunitaires telles que les macrophages représente un mécanisme qui pourrait contribuer à l'homéostasie des tissus et à la guérison des maladies inflammatoires. Cependant, l'application clinique des DMSC s'est avérée difficile et a conduit à rechercher des alternatives acellulaires. Il a été démontré que les vésicules extracellulaires dérivées des MSC (MSC-EV) sont à l'origine d'une partie importante de l'effet thérapeutique observé, y compris l'immunosuppression [40]. Les MSC sont connues pour répondre à des stimulations par des environnements inflammatoires en passant à un phénotype immunosuppresseur "activé". Des études récentes ont démontré que le stimulus inflammatoire ou hypoxique pourrait augmenter la capacité immunomodulatrice des MSC-EV [41, 42]. L'objectif spécifique du projet "EVENDO" est de réaliser une caractérisation détaillée des vésicules extracellulaires dérivées de cellules souches mésenchymateuses dentaires stimulées (pDMSC-EVs) afin d'étudier leur implication en tant que modulateurs des effets anti-inflammatoires des DMSC en induisant une polarisation des macrophages et d'explorer si ces pDMSC-EVs peuvent devenir un outil pertinent pour promouvoir la régénération de la pulpe dentaire.

Déroulé du projet

Pour mener à bien le projet EVENDO (Figure 7), nous prévoyons une durée de 2 ans divisée en 3 phases (WP) avec des objectifs spécifiques décrits ci-dessous :

- WP1 : Isolation et caractérisation des vésicules extracellulaires dérivées des cellules souches mésenchymateuses dentaires dans différentes conditions de culture cellulaire (déprivation en sérum, hypoxie ou inflammation simulée). L'objectif de ce WP est de (1) déterminer l'influence des conditions de culture cellulaire sur le rendement de la production d'EVs, (2) évaluer la caractérisation des EVs à partir de différentes sources de cellules souches (hBMSC, SCAPs et DPSCs) (3) évaluer la concentration en particules et la distribution de taille des EVs dérivées de cellules souches mésenchymateuses dentaires stimulées (pDMSC-EVs) (4) optimiser les méthodes d'isolement pour obtenir des populations d'EVs purs.

- WP2 : Mesure des effets *in vitro* des vésicules extracellulaires dérivées de cellules souches mésenchymateuses dentaires stimulées (pDMSC-EVs) sur la polarisation des macrophages. L'objectif du WP2 est d'analyser les effets *in vitro* des vésicules extracellulaires dérivées de cellules souches mésenchymateuses dentaires stimulés (pDMSC-EVs) sur le phénotype des macrophages humains.

- WP3 : Optimisation des propriétés bioactives des vésicules extracellulaires dérivées de cellules souches mésenchymateuses dentaires (DMSCs) pour la régénération de la pulpe de la dentine. Le WP3 sera dédié à l'évaluation de l'impact des pDMSC-EVs sur la différenciation odontoblastique des SCAPs.

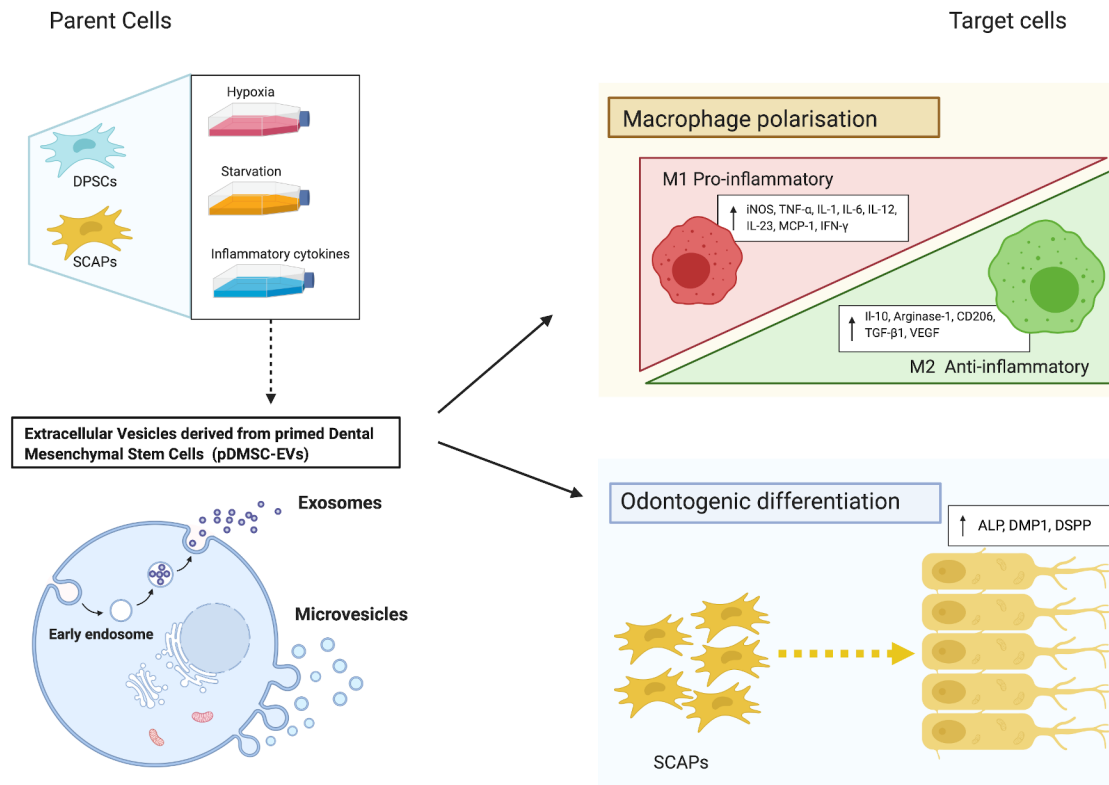


Figure 7 : Représentation schématique du projet EVENDO

Axe 3 : Évaluation de l'activité biologiques des ciments à base de silicates de calcium. Projet CALIMED

Financements

- Bourse Michel Degrange (2021), Évaluation de l'activité biologiques des ciments à base de silicates de calcium. Projet CALIMED. Coordinateur principal

Encadrement

Thèse d'exercice : Olivier Lapérine

Rationnel

La première génération de « biocéramique » ou de « ciment hydraulique » apparue dans les années 90 est le mineral trioxide aggregate (MTA). Le MTA a de nombreuses applications en endodontie allant des thérapeutiques de vitalité pulpaire, de l'obturation d'une perforation, jusqu'à l'obturation *a retro* lors de chirurgie endodontique. Bien que cette famille de matériau offre un bon pronostic à long terme, elle présente également certains inconvénients. Afin de pallier ces inconvénients, les ciments hydrauliques de nouvelle génération utilisent la nanotechnologie permettant de réduire la taille des particules à un mélange de nano et microparticules, et ainsi d'envisager une application pour l'obturation des canaux radiculaires. En plus de la bioactivité potentielle, ces ciments biocéramiques présentent une facilité d'emploi et de mise en place et constituent par conséquent un excellent choix clinique.

L'une des principales exigences pour les matériaux qui s'interfacent avec les tissus vivants de l'hôte est d'être non toxiques. Les macrophages sont considérés comme les principales cellules effectrices dans la régulation de la réparation des tissus et participent activement à l'évolution de la réponse inflammatoire en passant d'un phénotype inflammatoire (M1) à un phénotype régénératoire (M2) au fur et à mesure que la guérison progresse [43–45]. Des travaux récents ont révélé que les macrophages dans le tissu pulpaire en régénération subissaient une transition distincte, passant d'un phénotype M1 à un phénotype M2, ce qui suggère que la transition M1 à M2 des macrophages joue un rôle important dans la création d'un microenvironnement favorable nécessaire à la régénération du tissu pulpaire [46, 47]. En outre, le milieu conditionné des macrophages M2 a amélioré la différenciation odontogène/ostéogène des cellules souches de la pulpe dentaire humaine [48, 49]. Ces résultats suggèrent fortement que les événements inflammatoires délétères pourraient être inversés en créant un environnement local qui induit la polarisation du phénotype M2. À cet égard, le projet CALIMED propose de comparer les effets de différents ciments de silicate de calcium sur la reprogrammation des macrophages d'une phase M1 à une phase M2.

Déroulé du projet

L'objectif spécifique du projet est d'évaluer et de comparer l'effet de MTA, Biodentine (BD) et Bioroot (BR) sur la reprogrammation des macrophages d'une phase M1 à une phase M2,

mesurée par cytométrie de flux, qRT-PCR et ELISA. Afin de réaliser cet objectif, il est prévu de cultiver des monocytes humaines immortalisées (THP1) : Les cellules THP-1 seront maintenues dans un milieu RPMI 1640 (Gibco) complété par 10% de sérum fœtal bovin (FBS ; Gibco) et 50 U/mL de pénicilline-streptomycine (Gibco) dans des flacons de tissu de 75 cm². Les cellules seront incubées à 37°C dans une atmosphère humidifiée à 5% de CO₂ et 95% d'air.

Induction de la différenciation : Les monocytes THP-1 seront différenciés au stade M0 non polarisé par une incubation de 48h avec 100 nM de phorbol 12-myristate 13-acétate (PMA ; Sigma-Aldrich) dans des plaques 24 puits à 100 000 cellules/puits. La polarisation M1 et M2 des macrophages sera obtenue en incubant les macrophages M0 avec LPS (40 ng/mL ; Invitrogen)/IFN- (40 ng/mL ; R&D) et IL-4 (40 ng/mL ; R&D), respectivement, pendant 48h. Les états de polarisation des macrophages seront confirmés par cytométrie de flux en utilisant CD86 et CD206 comme marqueurs des sous-ensembles M1 et M2, respectivement.

Traitement avec le milieu conditionné (CM) : Les disques de MTA (Dentsply), BD (Septodont) et BR (Septodont) seront préparés conformément aux directives de l'Organisation internationale de normalisation 10993-5. Le MTA, la BD et le BR seront mélangés selon les instructions du fabricant et placés dans un moule cylindrique stérilisé en polyéthylène (5mm de diamètre et 3mm de hauteur) pendant 24h à 37°C et 5% de CO₂ dans un incubateur de culture cellulaire. Les éluats des différents matériaux seront ensuite extraits dans des conditions stériles en incubant les disques avec du Dulbecco's Modified Eagle's Medium (DMEM ; Invitrogen) complété par 10% de FBS et 50 U/mL de pénicilline/streptomycine pendant 24h à 37°C dans un incubateur à 5% de CO₂. Les milieux seront ensuite collectés et stérilisés par filtration sur une membrane de 0,22 µm de diamètre de pore, ce qui donnera les "milieux conditionnés" (MC). Des concentrations de CM non diluées, ½ et ¼ seront préparées. Les macrophages polarisés M1 seront divisés en quatre sous-ensembles : contrôle, MTA, BD, et BR, et cultivés pendant 24h, 48h, et 72h en utilisant le CM approprié.

Test de cytotoxicité de la lactate déshydrogénase (LDH) : La cytotoxicité de MTA, BD et ES sur les macrophages polarisés M1 sera mesurée en utilisant le test de cytotoxicité LDH (ThermoFisher) après 24h et 48h de culture.

Cytométrie en flux : Les culots cellulaires seront acquis après centrifugation et lavés deux fois avec du PBS contenant 0,5% de BSA. Les macrophages seront colorés avec l'anticorps anti-CD86

conjugué au PE et l'anticorps anti-CD206 pendant 15 minutes sur la glace et analysés avec un cytomètre en flux.

qRT-PCR : L'ARN total sera extrait à l'aide du mini kit RNeasy et du protocole DNase (Qiagen) et transcrit de manière inverse en ADNc à l'aide du kit de synthèse d'ADNc premier brin Transcriptor (Roche). La qRT-PCR sera réalisée à l'aide d'un kit PowerUp SYBR Green PCR (Applied Biosystems) pour analyser l'expression des gènes liés à des états de polarisation spécifiques des macrophages : TNF- α , IL-1 β , IL-6, et IL-12p40 pour M1 ; IL-10, et TARC pour M2. La GAPDH servira de gène de référence pour la normalisation de l'expression de l'ARN.

ELISA : Parmi toutes les cibles analysées par qRT-PCR, quatre de celles les plus modulées seront choisies pour être évaluées au niveau protéique par dosage immuno-enzymatique. Les cellules traitées par CM pendant les temps indiqués seront lavées trois fois dans du PBS et incubées dans un milieu sans sérum pendant 24 heures. Le surnageant sera ensuite recueilli et analysé à l'aide de kits de dosage immunologique ELISA (R&D) selon les instructions du fabricant.

6. PUBLICATIONS ET COMMUNICATIONS

6.1 PUBLICATIONS

Publications originales internationales soumises à comité de lecture

2021

1. Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, bradonnet R, Blanquart C, Corre P, Geofforu V, Weiss P, **Gaudin A**. A partially demineralized allogeneic bone graft: in vitro osteogenic potential and preclinical evaluation in two different intramembranous bone healing models. *Sci Rep.* 2021;11:1–13. doi:10.1038/s41598-021-84039-6. **(IF : 4.37)**
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2020

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2019

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10. **Gaudin A**, Tolar M, Peters OA. Lipoxin A4 Attenuates the Inflammatory Response in Stem Cells of the Apical Papilla via ALX/FPR2. *Sci. Rep.* 8, 1–12 (2018). **(IF : 4.37)**

2016

11. Renard E, **Gaudin A (co-premier)**, Bienvenu G, Amiaud J, Farges JC, Cuturi MC, Moreau A, Alliot-Licht B. Immune Cells and Molecular Networks in Experimentally Induced Pulpitis. *J Dent Res.* 2016 Feb;95(2):196-205. **(IF : 6.16)**

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20. **Gaudin A**, Arbab-chirani R, Pérez F. Effect of COVID-19 on Dental Education and Practice in France. *Frontiers in Dental Medicine* 2020;1(August):5–8.
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Autres publications non soumises à comité de lecture**Chapitres de livres, ouvrages**

1. Peters OA, Paranjpe A, **Gaudin A**. Dentine–Pulp Complex Regeneration. *Regenerative Approaches in Dentistry* 2021 Springer (Ed)
2. Boukpepsi T, **Gaudin A**. Biologie du complexe pulpo-dentinaire. *Endodontie JPIO* 2020 CDP

3. **Gaudin A**, Pérez F, Galicia J. Digital Technology in Endodontics. (2019). In: Tamimi F., Hirayama H. (eds) Digital Restorative Dentistry. Springer
4. Bronnec-Grall M, Eyzop E, Renard E, **Gaudin A**. Troubles liés aux toxicomanies et cavité buccale. La Bouche de L'enfant et de L'adolescent. 2019
5. Le Taillandier de Gabory L, **Gaudin A**, Catros S, Mortuaire G, Bastier PL. Pathologies infectieuses. Dents et sinus. RAPPORT SFORL 2017
6. **Gaudin A**, Laboux O. Endodontie et état général. Endodontie JPIO 2012 CDP (Ed)
7. Amador Del Valle G, Gautier H, **Gaudin A**, Le Mabeque V, Miegerville AF, Bouler JM, Caillon J, Weiss P, Potel G and Jacqueline C. Complete healing of severe experimental osseous infections using a calcium-deficient apatite as a drug-delivery system. Biomaterials Applications for Nanomedicine 2011 Rosario Pignatello (Ed.), ISBN: 978-953-307-661-4

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19. **Gaudin A**, Adou Akpe J, Marion D. Apport de la Régénération Tissulaire guidée à la chirurgie endodontique. Clinic 2006 ; 27(11) :543-0

6.2 COMMUNICATIONS

Communications internationales invitées personnelles

1. **Gaudin A**. Utilisation des biocéramiques en endodontie. Bruxelles 2021
2. **Gaudin A**. Modulation of the inflammatory response for dental pulp regeneration. Bruxelles 2020
3. **Gaudin A**. Les clés du succès en chirurgie endodontique. Colloques de Stomatologie, de Médecine Dentaire et de Chirurgie Maxillo-Faciale ; Lausanne 2012

Communications orales avec résumé

1. Renard E, **Gaudin A**, Bienvenu-Louvet G, Hill, M, Cuturi MC, Alliot-Licht. B. Quantification and Characterization of CD45+ Cells in Human Healthy Dental Pulp. International Congress of the French Endodontic Society, Nice 2014 ; 1er prix poster
2. **Gaudin A**, Renard E, Bienvenu-Louvet G, Hill, M, Cuturi MC, Alliot-Licht. B. Analyse par cytométrie de flux des cellules immunocompétentes de la pulpe humaine dentaire saine. Congrès CNEOC; Lille 2014.
3. **Gaudin A**, Renard E, Alliot-Licht B, Hill. M, Cuturi M.C. Identification of myeloid derived suppressive cells expressing HO-1 in healthy rat dental pulp. 16th European Society of Endodontology Biennial Congress, Lisboa, 2013

4. **Gaudin A**, Renard E, Alliot-Licht B, Hill. M, Cuturi M.C. Characterization of dendritic cells and myeloid derived suppressive cells expressing HO-1 in healthy rat dental pulp. CONSEURO : Paris, 2013
5. **Gaudin A**, Hyon I, Barrier JH. Pourquoi la digue est-elle peu utilisée en endodontie ? Réflexions sur l'apprentissage, à propos de jeunes chirurgiens-dentistes de Loire Atlantique. Congrès CNEOC; Strasbourg, 2012.
6. **Gaudin A**, Renard E, Licht B, Hill M, Cuturi C. Characterization of dendritic cells expressing HO-1 in human dental pulp. Congrès CNEOC; Nantes, 2011; 1er prix
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9. **Gaudin A**, Amador Del Valle G, Gautier H, Le Mabecque V, Miegerville AF, Potel G, Bouler JM, Weiss P, Caillon J, Jacqueline C. Evaluation d'une apatite déficiente en calcium à libération prolongée de linézolide dans un modèle d'ostéomyélite aiguë à staphylocoque doré méticiline résistant chez le lapin. Congrès CNEOC; Lyon, 2010
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16. **Gaudin A**. Greffes osseuses autogènes : intérêt du prélèvement pariétal (premier prix). Congrès national de parodontologie et implantologie SFPIO 2005 ; Bordeaux, 2005
17. Calas P, **Gaudin A**. Traitement endodontique chirurgical : évaluation du pronostic. Conférence magistrale congrès CNEOC ; Bordeaux, 2005
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Communications affichées :

20. Guivarc'h M, **Gaudin A**, Seux D, Bukiet F Irrigation trends among dental schools in France: a web-based survey. 17th European Society of Endodontology Biennial Congress, Bruxelles, 2017
21. **Gaudin A**, Renard E, Bienvenu G, Amiaud J, Farges J.C., Cuturi MC, Moreau A, and Alliot-Licht B. Immune cells and molecular networks in experimentally induced pulpitis. IADR – PBRG : Nagoya, 2016
22. **Gaudin A**, Renard E, Alliot-Licht B, Hill. M, Cuturi M.C. Identification and characterization of CD45+ cells in human healthy dental pulp. IADR Satellite Symposium of the Pulp Biology and Regeneration Group PBGR: San Francisco, 2013
23. **Gaudin A**, Renard E, Licht B, Farges JC, Hill M, Cuturi MC. Identification and characterization of CD45+ cells in human healthy dental pulp. International Congress of the French Endodontic Society, Aix-en Provence 2013

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25. **Gaudin A**, Renard E, Alliot-Licht B, Hill. M, Cuturi M.C. Identification of myeloid derived suppressive cells expressing HO-1 in healthy rat dental pulp. International Endodontic Journal, 47, 50–113, 2014

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REVIEW

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Specialized pro-resolving lipid mediators in endodontics: a narrative review



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Abstract

Endodontics is the branch of dentistry concerned with the morphology, physiology, and pathology of the human dental pulp and periradicular tissues. Human dental pulp is a highly dynamic tissue equipped with a network of resident immunocompetent cells that play major roles in the defense against pathogens and during tissue injury. However, the efficiency of these mechanisms during dental pulp inflammation (pulpitis) varies due to anatomical and physiological restrictions. Uncontrolled, excessive, or unresolved inflammation can lead to pulp tissue necrosis and subsequent bone infections called apical periodontitis. In most cases, pulpitis treatment consists of total pulp removal. Although this strategy has a good success rate, this treatment has some drawbacks (lack of defense mechanisms, loss of healing capacities, incomplete formation of the root in young patients). In a sizeable number of clinical situations, the decision to perform pulp extirpation and endodontic treatment is justifiable by the lack of therapeutic tools that could otherwise limit the immune/inflammatory process. In the past few decades, many studies have demonstrated that the resolution of acute inflammation is necessary to avoid the development of chronic inflammation and to promote repair or regeneration. This active process is orchestrated by Specialized Pro-resolving lipid Mediators (SPMs), including lipoxins, resolvins, protectins and maresins. Interestingly, SPMs do not have direct anti-inflammatory effects by inhibiting or directly blocking this process but can actively reduce neutrophil infiltration into inflamed tissues, enhance efferocytosis and bacterial phagocytosis by monocytes and macrophages and simultaneously inhibit inflammatory cytokine production. Experimental clinical application of SPMs has shown promising result in a wide range of inflammatory diseases, such as renal fibrosis, cerebral ischemia, marginal periodontitis, and cancer; the potential of SPMs in endodontic therapy has recently been explored. In this review, our objective was to analyze the involvement and potential use of SPMs in endodontic therapies with an emphasis on SPM delivery systems to effectively administer SPMs into the dental pulp space.

Keywords: Specialized pro-resolving mediators, Pulpitis, Apical periodontitis, Endodontics, Resolution of inflammation, Therapeutic potential

Background

The word endodontics comes from the old Greek “endo” meaning “inside” and “odont” for “tooth.” Endodontics is the branch of dentistry concerned with the morphology,

physiology and pathology of the human dental pulp and periradicular tissues. Dental pulp is a connective tissue that can trigger an immune response when challenged by different stimuli. In vital teeth, injuries such as dental caries, trauma, operative procedures, and periodontal diseases can lead to pulp inflammation, which is termed pulpitis. Pulpitis represents one of the main emergencies in dental practice, is the most prevalent form of orofacial pain, and has been associated with the prescription

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and potential overuse of opioid analgesics [1]. Similar to that of many chronic inflammatory diseases, uncontrolled or excessive inflammation during pulpitis can lead to chronic inflammation, scarring, and fibrosis [2]. Teeth are composed of soft connective tissue (dental pulp) enclosed within a mineralized hard tissue envelope (dentin and enamel). This inextensible envelope is responsible for the low compliance of the tooth: tissue swelling associated with pulp inflammation is limited, thereby influencing postinjury events that may ultimately lead to immune events that destroy pulp tissue [3]. Moreover, excessive dental pulp inflammation is a major culprit in the dysregulation of pulp healing and regeneration of injured tissues since the inherent mechanisms are significantly hampered [4].

How to best treat deep carious teeth with inflamed pulp is still controversial [5]. For many years, root canal treatment (RCT), which involves cleaning out all of the pulp from the root canal and filling the root canal system, has been the most appropriate treatment. RCT provides a reliable outcome; however, treated teeth are more brittle than nontreated teeth, especially the molars (hazard ratio, 7:1) [6]. The risk of bacterial dissemination throughout the body (bacteremia) always remains after root canal treatment [7–9]. The available literature on the incidence and diversity of odontogenic infection and the correlation between odontogenic bacteremia and systemic disease justifies the contraindication of such treatments in immunocompromised patients or those at risk for infective endocarditis [10–13].

An alternative approach is to remove only the inflamed portion to maintain and preserve the healing potential of the pulp. The ultimate goal of this concept, so-called vital pulp therapy (VPT) is the preservation of pulp vitality and function. The benefits of VPT include the maintenance of defense mechanisms, pain perception as a warning system, healing and tissue regeneration, completion of root formation in young patients to strengthen thin dentin walls and prevention of long-term complications. Despite these advantages, VPT has not become standard practice among dentists or gained general acceptance in the dental community. VPT outcomes have been difficult to predict due to the variable inflammatory status of the remaining pulp tissue. Indeed, pulp inflammation often remains high, leading to pulp necrosis [5, 14–16]. In most clinical situations, the decision to perform endodontic treatment is justified only by the absence of other available therapeutic tools to limit the immune/inflammatory process that impairs the healing and regenerative potential of the remaining and surrounding healthy tissues.

Thus, it is crucial to identify molecular and cellular agents that can attenuate immune/inflammatory events

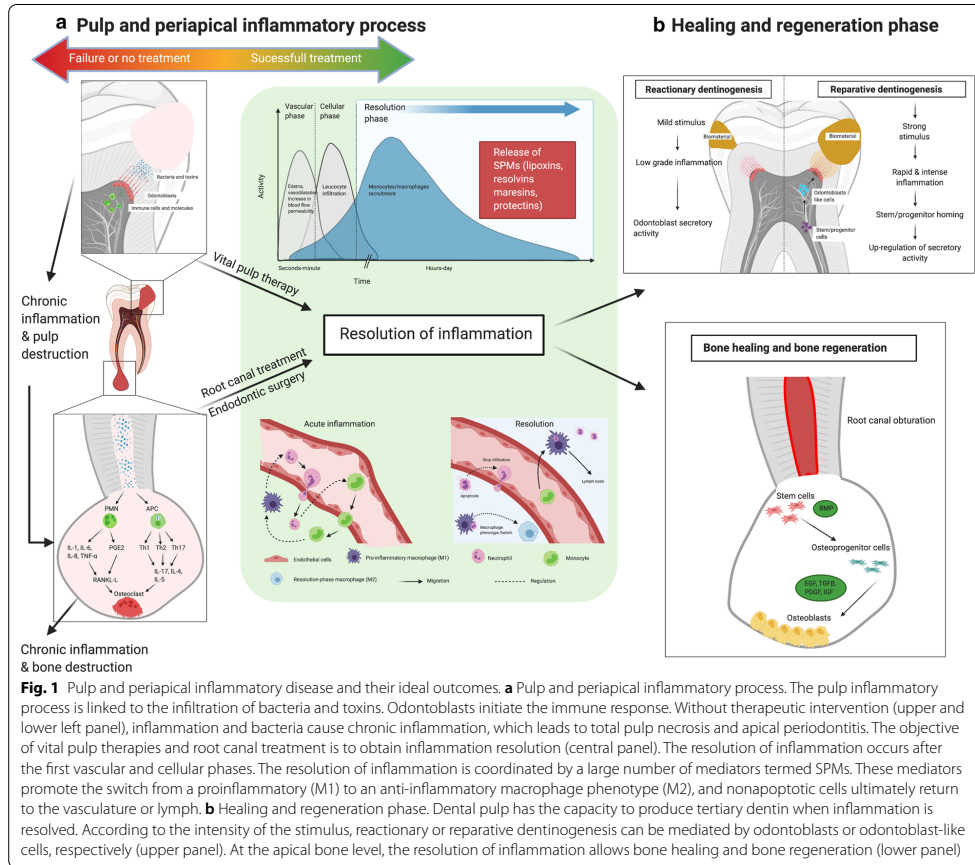
and promote the rapid return of tissue homeostasis. Blocking the immune response entirely with drugs such as anti-inflammatory or immunosuppressive agents is either ineffective, as the immune response contributes significantly to regeneration or has significant side effects [17]. Extensive work over the past few decades has revealed that the resolution of acute inflammation is critical in avoiding persistent chronic inflammation and supporting repair and regeneration [18]. The resolution of inflammation is coordinated and regulated by a large number of mediators, including SPMs. This review aims to provide fundamental information on the involvement of SPMs in endodontics.

Main text

Positive and negative aspects of pulp inflammation

Bacteria are the main and initial cause of inflammation and pulp infection. Carious lesions, trauma, wear and cracks provide bacteria and their byproducts access to the pulp through the dentinal tubules. Odontoblasts are the first cells to contact bacteria and their toxins due to their peripheral location in dental pulp tissue. As other cells (skin and mucosal epithelial cells) play multiple roles, these immunocompetent cells initiate and orchestrate the oral immune response [19]. Odontoblasts are first involved in fighting bacterial invasion and activating innate defense by producing beta-defensins and nitric oxide [20, 21]. In parallel, numerous *in vitro* studies have also shown that odontoblasts may secrete proinflammatory and immunomodulatory mediators, including chemokines and cytokines: interleukin (IL)-6, IL-10, chemokine C-X-C motif ligand (CXCL)1, CXCL2, CXCL8 (IL-8), CXCL10, and chemokine C-C motif ligand (CCL)2 [22–24].

The low compliance of the pulp environment, the absence of collateral replacement due to terminal vascularization, and the diversity of the microflora at the sites of the carious lesions are responsible for a relative inefficiency of the immune response leading more easily than other tissues to necrosis [25]. Innate immunity plays an important role in superficial caries [26–28] and is responsible for the acute inflammatory response, which is accompanied by systemic vasodilation, vascular leakage, and leukocyte migration. Within a short period after innate immune activation, immune cells (polymorphonuclear neutrophils, odontoblasts and macrophages) secrete various proinflammatory cytokines and chemokines to recruit other immune cells to the site of infection. Neutrophils are the first cells to adhere to endothelial cells and migrate across the vascular wall at the site of infection to engulf invading pathogens and secrete vasoactive and proinflammatory mediators (Fig. 1). In superficial caries, the pulp tissue is not in direct contact with the



pathogens; therefore, phagocytosis is not possible. The pulpal responses are subdued and tend to be subclinical. If the innate immune system exceeds its capacity or its defensive effect becomes limited, the adaptive immune system becomes involved, and once activated, specific T and B cells facilitate pathogen clearance. The transition from innate to adaptive immune responses likely occurs in cases of irreversibly inflamed pulp in which the carious lesion front is less than 2 mm from the pulp. In rapidly growing active lesions with pulpal exposure, inflammatory reactions become uncontrolled as the bacteria penetrate the pulp tissue [19, 29].

Until recently, the importance of inflammation in pulpal healing has been underestimated and merely considered an undesirable side effect. New evidence suggests

that inflammation is a prerequisite for tissue healing and pulp regeneration. Indeed, the initiated inflammatory response can lead to cell death and tissue destruction or toward wound healing and tissue regeneration. This dynamic balance is linked to the presence and concentration of pro- and anti-inflammatory mediators. A slowly progressing carious lesion can result in pulp healing that is no longer possible if inflammation progresses [29, 30]. Moreover, inflammation can also lead to a cascade of events resulting in excessive immune activity and pulp necrosis. To avoid irreversible damage to pulp tissue, the immune response must be limited to eliminating pathogens without destroying the host tissue (i.e., pulp). The therapeutic aim is to limit tissue damage following injury and control the immune response to promote a return to

homeostasis in the dental pulp environment leading to pulp healing.

Apical periodontitis relies upon a dynamic balance between inflammation and bone resorption

Apical periodontitis (AP) is an inflammatory lesion of the apical and peri-radicular areas mainly caused by bacterial elements from an infected tooth. During pulpitis, bacteria aggregate into a biofilm that adheres to the canal wall. Biofilm development in the root canals is initiated just after the first invasion of the pulp chamber by oral bacteria following inflammatory breakdown of pulp tissue [31]. This biofilm formation starts with surface attachment of bacteria, followed by microcolony development, secretion of extracellular polymeric substances, and then different stages of biofilm maturation and dissociation [32]. Concurrently, the inflammatory lesion front moves toward the apical portion of the root, providing a fluid vehicle for the invading biofilm to progress. The inflammatory response is usually unable to eliminate this biofilm [33]. Consequently, these infections progress to cause total pulp necrosis [34], which stimulates a secondary immune response in the periapical region [35]. As a defense mechanism, AP functions to confine bacteria to the infected tooth and prevent bacterial spread to both adjacent and distant sites. AP is the result of a dual inflammatory reaction that is aggressive and defensive, leading to bone destruction through osteoclastic activation and eventually to root resorption in severe cases [36]. Of note, there is a growing body of evidence showing that several systemic diseases (such as diabetes mellitus or hypertension) may affect the outcome of endodontic treatment and may be considered modulating factors that affect oral infection progression [11, 37–39].

Bacteria exert their pathogenicity through direct and indirect mechanisms. Bacterial factors such as enzymes, exotoxins, peptidoglycan, lipoteichoic acid, and lipopolysaccharides are released into the periradicular tissue, leading to phagocytic influx and the production of proinflammatory mediators. Neutrophils are the first cells involved in the defense and progression of AP, and these cells cause the chemotaxis of monocytes and lymphocytes [36, 40]. Bone macrophages, either resident or recruited from the peripheral blood, secrete inflammatory mediators such as IL-1 α , tumor necrosis factor- α (TNF- α), IL-6, IL-8, and transforming growth factor- β (TGF- β) [35] that modulate the inflammatory process. This secretion is implicated in the activation, proliferation, and differentiation of osteoclasts and fibroblasts [41]. Moreover, osteoclasts are also activated by prostaglandins and leukotrienes and, in turn, attract additional polymorphonuclear cells (PMNs) and macrophages to the inflammation site, thus generating an amplification

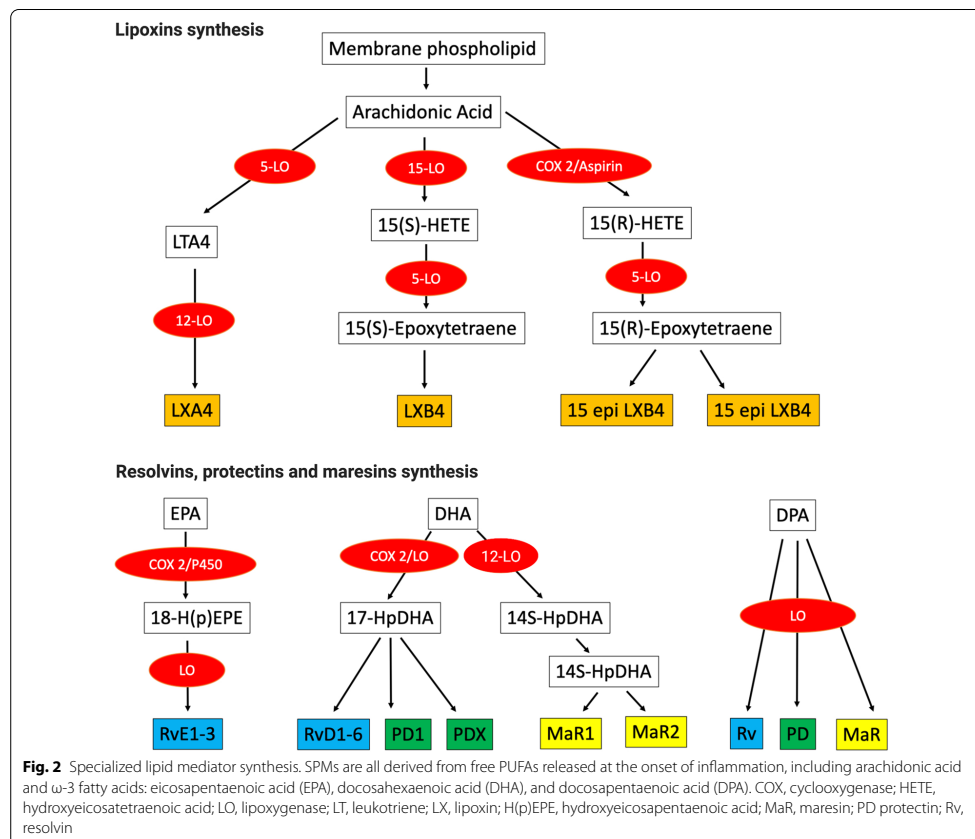
loop [34]. IL-8 and other chemoattractive peptides stimulate leukocyte and monocyte infiltration into periapical lesions. Antigen-presenting cells (APCs), especially dendritic cells (DCs) and macrophages, are crucial in the polarization of T helper (Th) cells toward Th1, Th2, Th17, or T regulatory cells (T regs) [42]. Th17 cells produce IL-17, a proinflammatory cytokine that coordinates with IL-8 to activate the inflammatory process by attracting neutrophils, inducing the production of RANK-L (also known as osteoprotegerin ligand (OPGL)) and activating osteoclasts [40]. RANKL binding to the RANK receptor on the surface of preosteoclasts induces the maturation and activation of these cells, whereas OPG, a naturally occurring inhibitor of RANKL, is a decoy receptor that prevents RANK-RANKL engagement [43, 44].

Advances have been made in understanding the pathogenesis of AP, especially the identification of cells and mediators capable of modulating the immune system. The dynamic balance between the protective effect of inflammation and its destructive capabilities is an active field of investigation that includes immunotherapy [45–47], antioxidants [48–59], selective estrogen receptor modulators [60, 61], stem cells [62–66], phototherapy [67–71], matrix metalloproteinase inhibitors [72, 73], anti-inflammatory agents [74–77], and SPMs [78–81].

The resolution of inflammation

Historically, the resolution of inflammation has been considered a passive process involving the dilution of chemokine gradients over time; thus, circulating leukocytes no longer sense gradients and are no longer recruited to the site of injury [82]. However, extensive work over the past few decades has revealed that the resolution of acute inflammation is crucial in avoiding the development of persistent chronic inflammation [9]. Resolving inflammation is an active, coordinated, anti-inflammatory, and pro-resolving program that facilitates a return to tissue homeostasis. Although inflammation and its resolution are natural, protective, and active programs inherent in an organism in response to external insult, uncontrolled, excessive, or unresolved inflammation can lead to different forms of tissue damage [83]. In teeth, this inflammation gives rise specifically to scarring and fibrosis in the context of pulpitis and prevents the return to homeostasis [84]. The healing, regeneration, and reconstruction of diseased tissues are significantly hampered [85].

The resolution of inflammation is coordinated and regulated by a large number of mediators, including SPMs. These mediators are derived from polyunsaturated fatty acids (PUFAs) and bind to G protein-coupled receptors (GPCRs) [86] (Fig. 2). Approximately 20 ligands (e.g., lipoxins, resolvins, maresins, and protectins) and



6 receptors (ALX/FPR2 [87, 88], GPR32, GPR18 [89], chemerin1, BLT1, and GPR37) have been identified to date, highlighting the complex and multifaceted nature of immune resolution [90–92].

Pro-resolving lipid mediators

Lipoxins

Arachidonic acid (AA) synthesis and its derivative products are key regulators involved in the host immune response. The oxygenation of AA initiates the synthesis of powerful bioactive compounds called eicosanoids. These metabolites include prostaglandins, leukotrienes, and lipoxins, which are lipoxygenase (LOX) interaction products. Lipoxin A4 (LXA4) and lipoxin B4 (LXB4) were the first mediators identified that possess specific proresolving actions [93, 94]. These mediators are bioactive autacoid metabolites of

omega-6 (ω -6) AA derived by LOX-mediated conversion, followed by the oxidation of 15-hydroxyperoxyeicosatetraenoic acid (15-HpETE).

Lipoxin has three synthetic routes that depend on the cellular environment and result in the formation of lipoxygenase eicosanoid products [95] (Fig. 2). The first route is associated with mucosal and vascular cell–cell interactions. 15-LOX is present in macrophages, monocytes, erythrocytes, and reticulocytes and is induced and activated by IL-4 and IL-13 [96]. Under the action of 15-LOX, an oxygen molecule is inserted on carbon 15 of AA and leads to the formation of 15-HpETE. 15-HpETE serves as a substrate for 5-LOX in neutrophils, which generates an epoxytetraene intermediate that is converted to LXA4 and LXB4. This 5-LOX is regulated by cytokines, including granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-3 [97].

The second route is linked to platelet-leukocyte interactions. This synthetic route takes place in the vascular system and involves 12-LOX, which is activated during peripheral blood platelet – leukocyte interactions [98].

Interestingly, the third biosynthesis route is dependent on aspirin and leads to the generation of 15 epi-lipoxin A4, which is also known as aspirin-triggered lipoxin (ATL), and 15 epi-lipoxin B4. Aspirin induces the acetylation of cyclooxygenase-2 (COX-2), transforming AA into 15R-HETE. 15R-HETE is then transformed by leukocytic 5-LOX into 15-epi-LXA4 and 15-epi-LXB4 [99]. The considerable proresolving effects of aspirin compared with those of other nonsteroidal anti-inflammatory drugs (NSAIDs) can be attributed to these processes [100]. Similarly, the clinical benefits and anti-inflammatory effects of statins are associated with the conversion of AA into 15-epi-lipoxin [101].

During inflammatory reactions in vivo, the synthesis of lipid mediators occurs in two stages. In the initial acute phase of inflammation, the leukotriene B4 concentration increases with the infiltration of neutrophils. The accumulation of neutrophils also coincides with increasing prostaglandin E2 (PGE2) concentrations. This prostaglandin induces LOX activity, and the resultant lipoxins contribute to the resolution of inflammation [102, 103].

Lipoxins play multiple roles in the inflammatory response, even at picogram-level concentrations [104]. Lipoxins regulate innate immune system function (e.g., attenuate monocyte recruitment [95]), induce a proresolving M2 phenotype [86, 105], inhibit the functions of DCs [106], and modulate the adaptive immune system by decreasing memory B-cell responses [107]. Lipoxins also inhibit the activation of eosinophils and lymphocytes [108, 109]. In parallel, these factors promote the non-phlogistic infiltration (without the release of proinflammatory mediators) of monocytes and macrophages [100]. Lipoxins stimulate the return of vascular permeability homeostasis and are now recognized as anti-edemogenic mediators [110]. Furthermore, lipoxins improve stem cell (periodontal and apical stem cell) proliferation, migration, and wound healing capacity. The binding of LXA4 to its receptor ALX/FPR2 regulates inflammatory mediators (inhibits cytokine, chemokine, and growth factor secretion) and enhances immunomodulatory properties (inhibits the proliferation of T lymphocytes in mixed leukocyte reactions) [88, 111].

E- and D-series resolvins

Resolvin (Rv) is a term for resolution-phase interaction products. These factors are natural exudate products defined by their potent bioactivities. These endogenous mediators are synthesized during the resolution phase of inflammation from ω -3 fatty acids,

including eicosapentaenoic acid (EPA) to produce E-series resolvins (RvE1, RvE2, and RvE3) and docosahexaenoic acid (DHA) to produce D-series resolvins (RvD1–RvD6), following COX-2 acetylation or cytochrome p450 activity [112] (Fig. 2). Even at pico-nanogram doses, resolvins counteract the proinflammatory state and actively promote resolution via monocyte/macrophage uptake of debris, apoptotic PMNs, and the killing/clearance of microbes [100, 113]. RvE1 reduces dendritic cell IL12 production [114] and potently stimulates IL10 production [115, 116].

RvD1–RvD6 protect against PMN-mediated reperfusion-induced organ injury [117]. RvD2 prevents leukocyte infiltration via the production of nitric oxide and inhibits the production of cytokines, thereby improving the clearance of microbes [118]. RvD5 decreases the amount of bacteria present in circulating blood and exudates, improves phagocytosis, and counteracts proinflammatory markers [100].

Maresins and protectins

The biosynthesis of protectins and maresins involves the formation of epoxide intermediates of DHA (Fig. 2) [90]. When produced in neural systems, protectins are termed neuroprotectin D and exert potent protective effects on the retina, brain, and pain [100, 119]. Maresin 1 can reduce proinflammatory cytokines by inhibiting the NF- κ B pathway and activating M2 macrophages and is involved in pain mechanisms and tissue regeneration [120].

Specialized pro-resolving lipid mediators and pain control

There is increasing evidence that SPMs play an important role in the reduction of pain [93, 121]. Some studies, particularly those in rheumatology and gastroenterology, have shown anti-inflammatory effects and resolution leading to the potential control of pain [122, 123]. For example, in an induced rat model of arthritis, it has been shown that resolvin can reduce pain associated with acute or chronic inflammation more effectively than steroid or analgesic treatments [124]. In addition, compared with COX-2 inhibitors, resolvin has been shown to attenuate inflammatory pain via central and peripheral actions in mice [125]. Recent studies have also shown that SPMs can reduce inflammatory pain, postoperative pain and neuropathic pain in animal models via immune, glial and neuronal modulation [126]. SPM receptors (ALX/FPR2, ChemR23) are present on neuronal bodies, nerve terminals and synaptic terminals [127]. Additional studies need to be performed to assess the effect of SPMs on postoperative endodontic pain and to determine whether the results observed in previous studies are translatable to pulpitis or apical periodontitis pain.

Specialized pro-resolving lipid mediators in endodontics

SPMs have been studied extensively and their potent effects on reducing inflammation have been documented, along with the development of a wide range of preclinical disease models using validated commercially available SPMs [18]. Most of these studies focused on the role of SPMs in a variety of medical pathologies, such as asthma, Alzheimer's disease, and rheumatoid arthritis. Furthermore, independent evidence supports the putative role of impaired inflammation resolution mechanisms in periodontal disease [128]. A recent systematic review investigated the biological effects of SPMs on periodontal tissues in animals with experimental periodontitis [129]. Six studies from the same research team using an experimental periodontitis model applied RvE1 and lipoxins to treat experimental periodontitis. RvE1 and lipoxins were topically applied to treat experimental periodontitis. The application of SPMs significantly prevented alveolar bone loss and promoted bone regeneration compared with those in the control group. The doses of SPMs and the periods of disease induction varied based on the preclinical model used. Two studies further demonstrated the positive shift in microbial composition (a trend for returning the microbiota to a state associated with health), which was consistent with a positive shift in inflammatory status (a decrease in inflammatory cell infiltration and a reduction in osteoclastic activities), and this effect was regulated by SPMs [129]. Finally, a recent study highlighted the role of maresin 1 and RvE1 in promoting the regenerative properties of periodontal ligament stem cells under inflammatory conditions [130]. However, very few studies (7 PubMed citations) have examined the role of SPMs in pulpal and periapical disease (Fig. 1). One systematic review investigated the potential of SPMs as an adjunct in the treatment of endodontic infection [131].

Role of SPMs in pulp inflammation (pulpitis)

A protective effect of RvE1 was shown in an animal model of pathogen-mediated inflammation (rat dental molar pulp exposed to the oral environment). Histological analysis was performed at 24 and 72 h posttreatment and revealed that RvE1 reduced inflammation and induced milder pulp alterations (a mild cellular inflammatory response and less degenerative alterations of the pulp tissue) than the other treatments. These results also demonstrated the lack of benefit of directly applying corticosteroids to the pulp tissue [80]. Moreover, in another pulpitis model (rat dental incisors exposed to the oral environment), RvE1 was able to attenuate the inflammatory response in the exposed pulp of rat incisors [132]. Interestingly, the expression of ChemR23, a transmembrane receptor to which RvE1 can bind, was upregulated

during inflammatory processes and downregulated in inflamed teeth that were treated with RvE1, demonstrating an inhibitory effect against leukocyte infiltration.

SPMs in apical periodontitis

Numerous studies support systemic oral administration of omega-3 PUFAs for their beneficial effects on inflammatory diseases, including rheumatoid arthritis, bowel diseases, and chronic periodontitis [133, 134]. A study by El Khouli et al. [135] demonstrated that dietary supplementation with omega-3 significantly reduced inflammatory symptoms such as aphthous stomatitis by altering the cellular functions of leukocytes, blocking proinflammatory cytokine production, and inhibiting the production of AA metabolites, thus dampening the proinflammatory response.

This omega-3 regimen could also be used to treat AP because its pathological mechanisms of action involve comparable inflammatory and cellular mediators. Systemic oral administration of proresolving mediators (omega-3 PUFAs) decreased the expression of the proinflammatory cytokines TNF- α , IL-1 β , IL-6, and IL-17 and increased production of the anti-inflammatory cytokine IL-10 in a rat model of AP [78]. These results are consistent with a previous report from the same team [81]. In this earlier study, systemic oral PUFA administration suppressed bone resorption and induced bone regeneration by decreasing osteoclastogenesis and increasing osteoblastogenesis in AP.

In a rat model of apical periodontitis in immature rat teeth, RvE1 promoted root formation and reduced inflammation [136]. Radiographic and histologic analysis at 3 and 6 weeks demonstrated that RvE1 reduced the periapical lesion size compared with that in control teeth (without RvE1 treatment). The periapical inflammatory reaction was reduced in the resolvin group. Finally, radiological root lengths and the thickness of the dentinal walls were also greater in the resolvin group than in the control group. This increase may lead to improved resistance to root fracture. Although these results are promising, the authors emphasize the need for a delivery system that can potentiate the stability of RvE1.

In an animal model of tooth revascularization, RvD2 promoted the resolution of inflammation [79]. RvD2 administration within the root canals of necrotic teeth with apical lesions allowed continued calcification around the root apex and prevented and reversed periapical periodontitis compared with those of the control group without RvD2 treatment. RvD2 reduced overall inflammation by decreasing myeloperoxidase activity in phagocytes and reducing cell infiltration. Moreover, RvD2 treatment increased the expression of the receptor GPR18 (mainly expressed on leukocytes, monocytes,

and macrophages) inside and outside root canals [79], underscoring the role of RvD2 in apical closure and tissue regeneration around the apex. In summary, these studies demonstrate that in cases of pulpitis or AP, topical and systemic applications of SPMs stimulate regeneration and resolve inflammation.

Limits, potential, and therapeutic perspectives

SPMs are highly potent, efficacious endogenous ligands associated with positive outcomes in preclinical animal models. Additional studies are required to assess the safety and efficacy of SPMs as therapeutic agents in humans. The development of stable analogs and drug delivery systems is still in the research stage. SPMs are delicate in their physicochemical nature, require complex chemical synthesis and are prone to metabolic inactivation [90]. Clinical trials with SPM analogs that resist metabolic inactivation are underway but are still in early phases [133]. Topical applications (e.g., nanomicellar

solutions) have been advocated to treat ocular inflammation and pain in cataract surgery [137]. Furthermore, an LXA4 agonist exhibited efficacy and safety in a pilot study for children with asthma [138]. Interestingly, the incorporation of SPMs into microparticles or nanoparticles has recently been explored in a few preclinical models of periodontitis and has proven to be an effective approach for decreasing periodontal inflammation and bone loss [139]. However, these particles have some drawbacks, including nonspecific biodistribution, poor water solubility, and limited bioavailability [140].

To overcome these limitations, different pharmacological approaches are currently under investigation to effectively administer SPMs in the dental pulp space. Access to the dental pulp is generally easier than access to the periapical region. However, the root canal may be narrow with a small volume. Therefore, it is challenging to implant a preformed scaffold into the root canal that would seamlessly cover the entire space of the canal. This

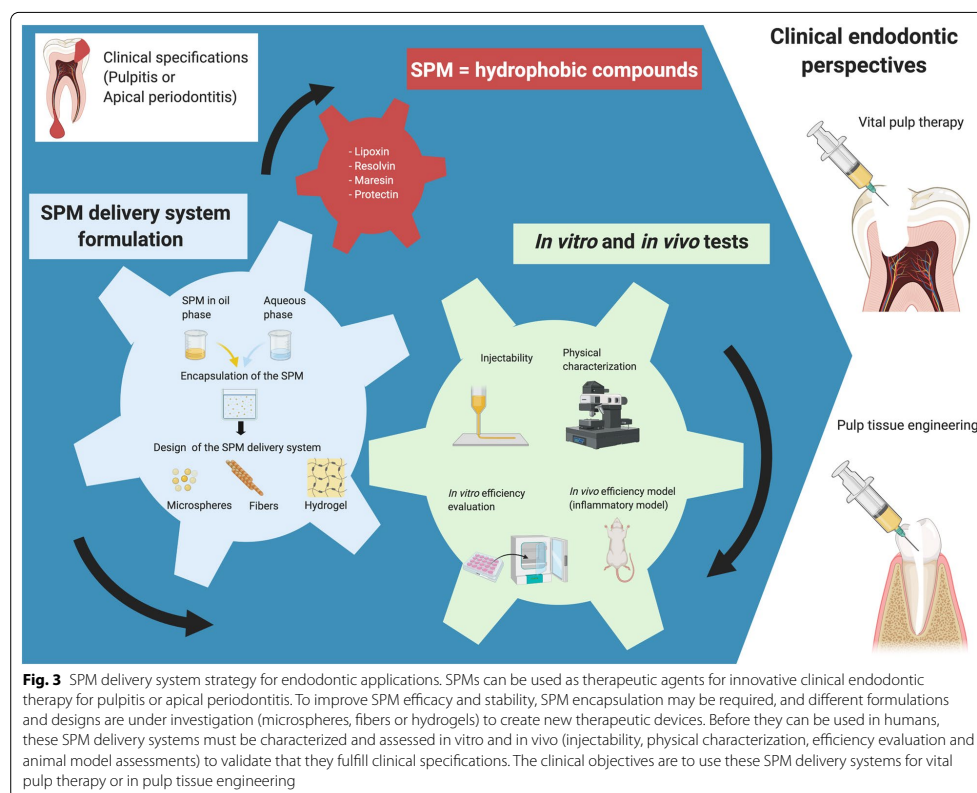


Fig. 3 SPM delivery system strategy for endodontic applications. SPMs can be used as therapeutic agents for innovative clinical endodontic therapy for pulpitis or apical periodontitis. To improve SPM efficacy and stability, SPM encapsulation may be required, and different formulations and designs are under investigation (microspheres, fibers or hydrogels) to create new therapeutic devices. Before they can be used in humans, these SPM delivery systems must be characterized and assessed *in vitro* and *in vivo* (injectability, physical characterization, efficiency evaluation and animal model assessments) to validate that they fulfill clinical specifications. The clinical objectives are to use these SPM delivery systems for vital pulp therapy or in pulp tissue engineering

concern is one of the main issues that must be addressed when considering drug delivery systems to the dental pulp or the periapical region [141, 142]. The ideal biomaterial should be injectable, biodegradable, biocompatible, and nonimmunogenic, be characterized by high porosity, have adequate mechanical properties, and be easily biofunctionalized or combined with biomolecules such as SPMs. The most common carriers for sustained drug release include microspheres, fibers, and hydrogels [143–145]. Hydrogels are three-dimensional materials that can incorporate a large quantity of water while maintaining their structural and functional integrity. These unique biomedical-grade materials represent a promising delivery system for drugs, proteins, cells, and more because of their biocompatibility, solute permeability and tunable release characteristics (Fig. 3) [146, 147]. Naturally, hydrogels have traditionally been limited to carrying hydrophilic drugs rather than hydrophobic drugs [148]. To improve the drug loading capacity of a hydrophobic compound (e.g., LXA4), different hydrogel formulations are being tested for periodontal applications. Poloxamer 407 (P407) and thermoresponsive polyisocyanopeptide (PIC) gels have been compared in vitro; LXA4 remained bioactive after its release from PIC gels, and no cytotoxicity was observed for the 1% PIC gel [149] in contrast with other concentrations and P407. In another study, the same team proposed loading LXA4 into acid-terminated, ester-capped poly(lactic-co-glycolic acid) (PLGA) microspheres using an electrospray procedure combined with PIC gels [150]. In this proof-of-concept study, the authors indicated that the PIC-PLGA vehicle exhibited suitable injectability, long-term structural stability and no obvious in vivo inflammatory response. Moreover, the ability of SPMs to be incorporated into a scaffold to resolve the inflammatory responses associated with pulpitis and apical periodontitis has yet to be established in the literature.

Conclusion

Unlike currently available anti-inflammatory drugs, such as anti-TNF α antibodies, SPMs can dampen excessive inflammation without compromising host defense. Furthermore, SPMs do not impair endogenous healing pathways but rather act locally to halt leucocyte recruitment and promote the resolution of the inflammatory response. The few studies that have explored the role of SPMs in endodontics have generated promising results regarding controlling pulp inflammation, promoting the resolution of apical inflammation, and reversing the bone tissue destruction caused by excessive neutrophil activity. Scientific and technical barriers remain due to the instability, complex and delicate physicochemical nature, and potential metabolic inactivation of SPMs.

Hydrogel-based drug delivery systems represent a promising approach to overcoming the aforementioned obstacles, and these systems are currently being investigated for periodontal applications in particular. Although the tissues and treatments vary, pulpal and periapical diseases share many features; therefore, advances in SPM delivery systems for periodontal applications will greatly benefit both conditions. This would be an important milestone for modular hydrogel strategies and open a new way to design drug delivery systems for specific applications, such as the treatment of pulpitis and apical periodontitis.

Abbreviations

SPMs: Specialized pro-resolving lipid mediators; RCT: Root canal treatment; VPT: Vital pulp therapy; AP: Apical periodontitis; IL-: Interleukin; CXCL: Chemokine C-X-C motif ligand; CCL: Chemokine C-C motif ligand; TNF- α : Tumor necrosis factor- α ; TGF- β : Transforming growth factor- β ; PMNs: Polymorphonuclear cells; APCs: Antigen-presenting cells; DCs: Dendritic cells; Th: T helper; T regs: T regulatory cells; RANK-L: Receptor activator of nuclear factor κ B-ligand; OPGL: Osteoprotegerin ligand; PUFAs: Polyunsaturated fatty acids; GPCRs: G protein-coupled receptors; PtdSer: Phosphatidylserine; CRT: Calreticulin; ICAM: Intercellular adhesion molecule; CD: Cluster of differentiation; AA: Arachidonic acid; LOX: Lipoxygenase; LX: Lipoxin; HpETE: Hydroxyperoxyeicosatetraenoic acid; GM-CSF: Granulocyte-macrophage colony-stimulating factor; ATL: Aspirin-triggered lipoxin; COX-2: Cyclooxygenase-2; NSAIDs: Nonsteroidal anti-inflammatory drugs; PGE2: Prostaglandin E2; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; PIC: Polyisocyanopeptide; PLGA: Ester-capped poly(lactic-co-glycolic acid); DPA: Docosapentaenoic acid; HETE: Hydroxyeicosatetraenoic acid; LO: Lipoxygenase; LT: Leukotriene.

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Authors' contributions

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A partially demineralized allogeneic bone graft: in vitro osteogenic potential and preclinical evaluation in two different intramembranous bone healing models

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In skeletal surgical procedures, bone regeneration in irregular and hard-to-reach areas may present clinical challenges. In order to overcome the limitations of traditional autologous bone grafts and bone substitutes, an extrudable and easy-to-handle innovative partially demineralized allogeneic bone graft in the form of a paste has been developed. In this study, the regenerative potential of this paste was assessed and compared to its clinically used precursor form allogeneic bone particles. Compared to the particular bone graft, the bone paste allowed better attachment of human mesenchymal stromal cells and their commitment towards the osteoblastic lineage, and it induced a pro-regenerative phenotype of human monocytes/macrophages. The bone paste also supported bone healing in vivo in a guide bone regeneration model and, more interestingly, exhibited a substantial bone-forming ability when implanted in a critical-size defect model in rat calvaria. Thus, these findings indicate that this novel partially demineralized allogeneic bone paste that combines substantial bone healing properties and rapid and ease-of-use may be a promising alternative to allogeneic bone grafts for bone regeneration in several clinical contexts of oral and maxillofacial bone grafting.

In oral and maxillofacial bone grafting procedures, autologous bone grafting is still considered to be the reference technique. This technique has a high success rate as a result of the combination of osteoconductivity (the ability to provide a structural support for bone growth), osteogenicity (the promotion of osteoblastic differentiation of progenitor cells), and osteoinductivity (the induction of bone growth in large bone defects or heterotopic sites, mainly due to growth factors in the bone and marrow environment)^{1–4}. Despite these advantages, autologous bone grafts (ABGs) suffer from numerous serious peri- and post-operative drawbacks. Among these, the potential donor site morbidities (e.g., pain, hematoma, blood loss, nerve injury, the risk of bone fracture), the additional operative time, the limited available bone (e.g., iliac crest, calvarial bone), and the occasional need for a second surgical team are the main limitations of this procedure and consequently hindering the clinical benefits. To

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overcome these limitations, several alternatives such as alloplastic and xenogenic bone substitutes or allogeneic bone grafts are used in oral and maxillofacial surgeries^{5–7}.

Allogeneic bone grafts are reliable and have been used extensively for decades as an alternative to ABGs^{8,9}. The amount of allogeneic bone is virtually unlimited, and it can be obtained in various sizes and shapes (e.g., blocks, blades, paste, putty, powder, chips, injectable forms). The duration of the surgery is shortened compared to ABG-based reconstructions because there is no need for a bone harvesting procedure. Moreover, allogeneic bone grafts exhibit the same bone healing capacities as ABGs in multiple indications such as bone augmentation before dental implant placement (e.g., sinus floor elevation, ridge augmentation)^{10,11}.

Although allogeneic bone grafts are mainly used in powder or block forms, they exhibit a lack of versatility and/or handling, especially in specific indications related to pre-implant surgery, such as sinus floor elevation, guided bone regeneration in horizontal/vertical augmentation, and alveolar socket preservation. To address these unmet clinical needs, a novel ready-to-use, easy-to-handle, extrudable, and moldable human bone graft for bone regenerative medicine has been developed¹². This allogeneic bone paste is made of partially demineralized bone particles, consisting of a mineralized core (Sup. Fig. 1, upper panel, white arrows) surrounded by a demineralized bone matrix (Sup. Fig. 1, upper panel, black arrows). This bone paste does not require any mixing, rehydration, or reconstitution prior to its use, which is a key feature for clinical use. Such a bone paste could replace the bone substitutes that are in powder form, particularly for the irregular or hard-to-reach areas frequently encountered in oral and maxillofacial bone surgery. However, aside from its handling and physical characteristics, proof of concept of its bone regenerative capacity should be established prior to its clinical transfer. In this context, the present work aimed to provide a thorough assessment of the bone healing capacity of this allogeneic bone paste compared with an allogeneic particular bone graft before its partial demineralization.

The purpose of this study was hence to assess the effects of the bone paste *in vitro* (1) on the attachment and osteoblastic commitment of primary human mesenchymal stromal cells from bone marrow (hBM-MSCs), (2) on the polarization of primary human monocytes isolated from circulating blood, and (3) to test and validate the *in vivo* capacity of this innovative bone paste to support and promote bone healing in two preclinical models: a guided bone regeneration (GBR) and a critical size defect (CSD) model in rat calvaria to mimic intramembranous bone healing of oral and maxillofacial defects.

Results

Attachment and osteoblastic commitment of hBM-MSCs. The attachment of hBM-MSCs was first investigated after short periods (1, 3, and 6 h) of contact with the particular bone graft or the bone paste. After 1 h of contact, the cells on the surface of the particular bone graft exhibited a round shape, while they had an elongated and spread-out shape on the surface of bone paste. Interestingly, at 3 h and 6 h, all of the cells exhibited an elongated and spread-out shape (Fig. 1a) irrespective of the graft. These data strongly suggest that the bone paste allows faster cell adhesion compared to the particular bone graft. Quantification of the DNA content of the adherent cells revealed no difference after 1 h of contact. However, after 3 and 6 h of contact, the DNA content was significantly higher (60% and 70%, respectively) for the cells cultured in contact with the bone paste compared to the cells in contact with the particular bone graft (Fig. 1b).

To determine whether the particular bone graft or the bone paste may affect the osteoblastic differentiation of hBM-MSCs, the cells were cultured in contact with the bone grafts in culture medium with or without osteogenic factors for 14 and 21 days. As seen by the SEM analyses, the osteogenic factors did not influence the colonization of the particular bone graft or the bone paste by the hBM-MSCs. Under all of the culture conditions, the cells had elongated cellular bodies and cytoplasmic expansions (Fig. 1c, white arrows). Of interest, the cohesion between the bone paste allowed the cells to create a network from particle to particle, with or without osteogenic factors, at all of the time points (Fig. 1c). It is also worth noting that this phenomenon was not observed with the cells cultured in contact with the particular bone graft. Finally, to evaluate the osteogenic commitment of the hBM-MSCs cultured in contact with the particular bone graft or the bone paste, the ALP activity was measured in cell lysates, and it was found to be significantly higher in the cells cultured in contact with the bone paste compared to the cells in contact with the particular bone graft: 3.3 and 2.7 times higher, respectively, in the absence of osteogenic factors and 7.3 and 4.6 times higher, respectively, in the presence of osteogenic factors after 14 and 21 days of culture (Fig. 1d).

Intramembranous bone healing in a GBR model. To mimic clinical indications of guided bone regeneration (e.g., alveolar socket preservation), the ability of the bone paste to support bone healing was first assessed in a GBR model in rat calvaria. The μ -CT analyses of the control defects at 7 weeks post-surgery revealed a significant degree of centripetal bone ingrowth from the edges of the defects (Fig. 2a), as expected in this GBR model. In the grafted defects, homogenous bone ingrowth (Fig. 2a, yellow arrows) was observed, but the particular bone graft and the bone paste were still visible in the defects (Fig. 2a, white arrows). The three-dimensional quantification of the MV/TV, increasing over time, indicated that new bone formation occurred in the control and in the grafted defects (Fig. 2b). However, the increase in the MV/TV between 0 and 7 weeks was greater in the group grafted with the bone paste than in the group grafted with the particular bone graft (2.2 vs. 1.6-fold, respectively).

In all of the defects, the histological observations revealed the collagen-rich and layer-organized nature of the newly formed bone (yellow matrix), with numerous osteocytes, that was identical to the native bone outside the defects (Fig. 3). These observations confirmed the μ -CT analyses, with significant bone formation after 7 weeks in the control defects, yet without continuous newly formed bone tissue (i.e., full closure). In the defects implanted with the particular bone graft and the bone paste, the grafts were perfectly osteointegrated and still visible (Fig. 3, *gr*) and recognizable due to their shape and the absence of osteocytes inside the grafts.

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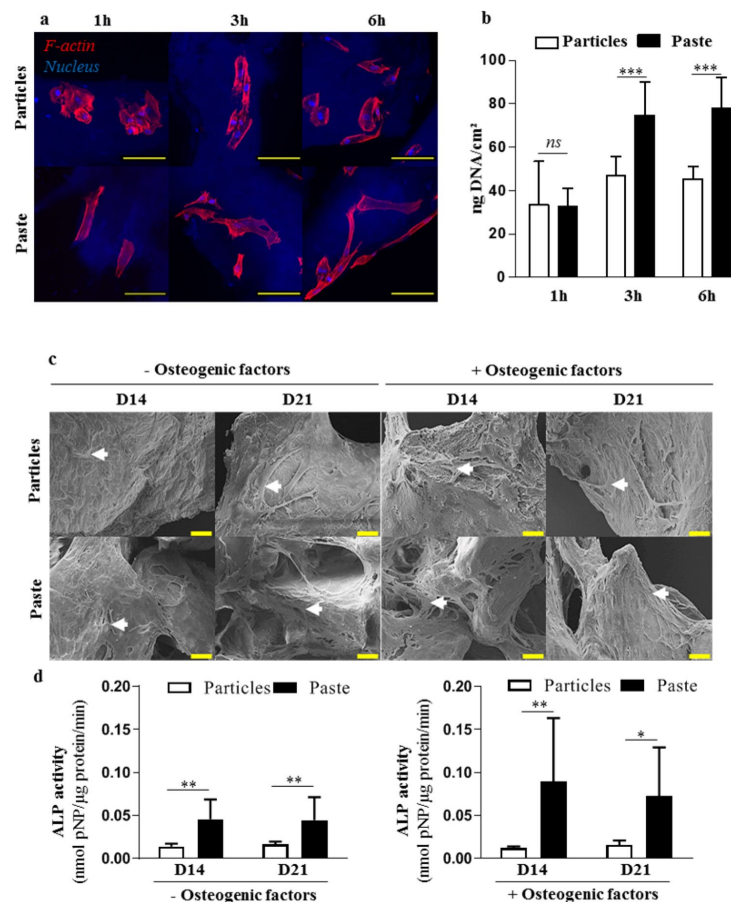


Figure 1. Attachment and osteoblastic commitment of hBM-MSCs cultured in contact with the particular bone graft or the bone paste. (a) Representative confocal images of stained hBM-MSCs. Red (phalloidin-AF568): F-actin, blue (Hoechst): nucleus. The particular bone graft and the bone paste were visible as a result of their blue auto fluorescence. Scale bars: 100 μm. (b) Quantification of the total DNA in hBM-MSCs in contact with the particular bone graft or the bone paste. (c,d) SEM images and ALP activity in lysates of hBM-MSCs grown in contact with the particular bone graft or bone paste with or without osteogenic factors for 14 and 21 days. Scale bars: 20 μm. The results are expressed as means ± the SD (N = 3, n = 3; **p* < 0.05, ***p* < 0.01 ****p* < 0.001).

Intramembranous bone healing in a CSD model. The intrinsic bone-forming ability of the bone paste was then tested using a challenging CSD model of intramembranous bone healing. Based on the reconstructed μ-CT images, it appeared that no newly formed bone was observable in the control defects or filled with the particular bone graft at 3 and 7 weeks post-surgery (Fig. 4a,b white arrows). This was confirmed by the MV/TV quantifications (Fig. 4c). By contrast, in the defects filled with the bone paste, newly formed bone was clearly visible after 3 and 7 weeks (Fig. 4a,b yellow arrows), thus indicating healing of the defects in the presence of the bone paste.

The histological observations confirmed the radiological analyses and showed that a barely detectable amount of new bone had formed at the edges but not at the center of the control defects at 3 weeks (Fig. 5a) and 7 weeks (Fig. 5b) post-surgery. The same observation was made in the defects filled by the particular bone graft at 3 weeks (Fig. 5a) and 7 weeks (Fig. 5b) post-surgery. In this group, three weeks after the implantation, the soft tissue appeared broadly vascularized and highly cellularized in close proximity to the particular bone graft (Fig. 5a). The surface of these particular bone graft was light blue, probably due to the fact that the surfaces had lost their collagen content (which appeared yellow by Movat's pentachrome staining). Seven weeks after the particular

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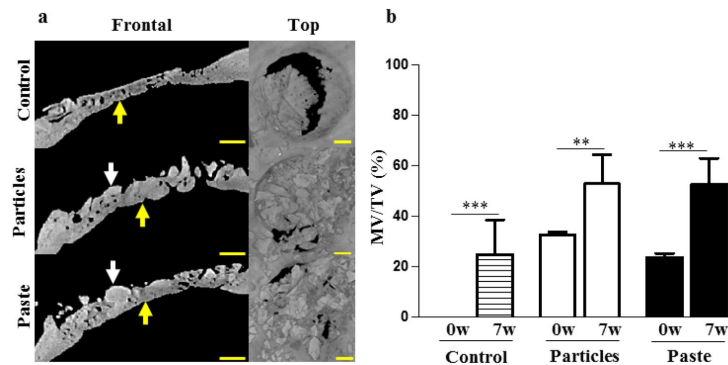


Figure 2. Rat calvaria healing 7 weeks after surgery in a guided bone regeneration model. (a) Representative images of the reconstructed μ -CT acquisitions (yellow arrows: newly formed bone, white arrows: grafts). The control represents the defects with the GBR membrane without bone grafts (unfilled defects). Scale bars: 1 mm. (b) Three-dimensional μ -CT quantitative analysis of the mineral volume (MV) within the tissue volume (TV) in the defects at the time of surgery (0w, baseline) and 7 weeks (7w) after surgery. The results are expressed as means \pm the SD (n = 6) * p < 0.05, ** p < 0.01, *** p < 0.001.

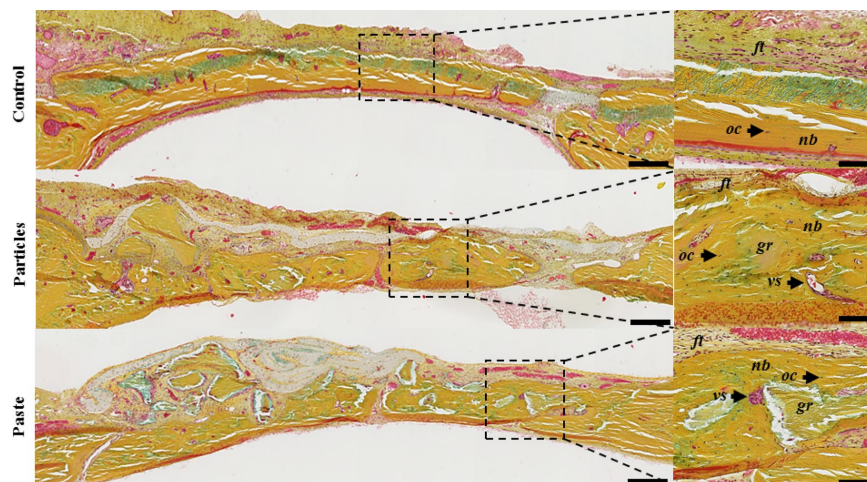


Figure 3. Rat calvaria healing 7 weeks after surgery in a guided bone regeneration model. Representative images of the defects with Movat's pentachrome staining of undecalcified 7- μ m thick frontal sections. The control represents the defects with the GBR membrane without bone grafts (unfilled defects). Scale bars: 250 μ m for the histological full defects and 100 μ m for the histological magnification (dashed square). Dark yellow: collagen in the bone tissue, light red: cytoplasm, dark red: osteoid borders, brown: nuclei, ft: fibrous tissue, oc: osteocytes, nb: newly formed bone, vs: blood vessels, gr: grafts.

bone graft implantation, the soft tissue appeared to be less vascularized and less cellularized compared to the defects observed 3 weeks after the implantation. On the other hand, the defects filled with the bone paste exhibited a substantial proportion of newly formed bone, which was already visible 3 weeks post-surgery (Fig. 5a, nb). Despite the fact that the newly formed bone was distributed heterogeneously in the defects 3 weeks after the surgery, lamellar bone structures with numerous osteocytes and a number of blood vessels could be observed (Fig. 5a, nb, vs). Of interest, the bone paste had become fully integrated in the regenerated bone 7 weeks after the surgery (Fig. 5b, gr), as discernible in the defects with their characteristic acellular mineralized inner core and demineralized outer part.

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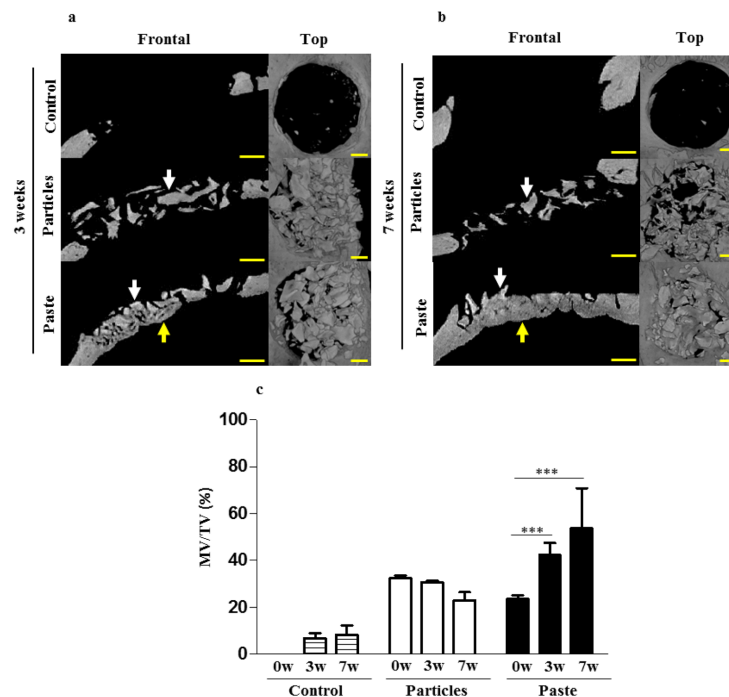


Figure 4. Rat calvaria healing at 3 and 7 weeks after surgery in a critical size defect model. (a,b) Representative images of the reconstructed μ -CT acquisitions (yellow arrows: newly formed bone, white arrows: grafts). The control represents the unfilled defects. Scale bars: 1 mm. (c) Three-dimensional μ -CT quantitative analysis of the mineral volume (MV) within the tissue volume (TV) in the defects after 0 weeks (baseline), 3, and 7 weeks of regeneration. The results are expressed as means \pm the SD ($n=6$) ** $p < 0.01$, *** $p < 0.001$).

At this time point, the bone regrowth in the defects filled with the bone paste was much more abundant and homogeneous compared to at 3 weeks (Fig. 5b), with a limited amount of soft tissue. Moreover, the regenerated bone (Fig. 5b, nb) exhibited a typical lamellar osseous structure, with numerous osteocytes (Fig. 5b, oc) and vasculature reminiscent of that observed in native bone tissue outside the grafted area.

Monocyte/macrophages phenotypic changes. Having demonstrated that the bone paste had greater regenerative properties than the particular bone graft in two complementary models of intramembranous bone healing, we sought to determine whether monocytes/macrophages may play a pivotal role in the discrepancy found in the regenerative efficiency of the two types of bone grafts.

When cultured under pro-inflammatory conditions, the macrophages in contact with the particular bone graft expressed lower levels of *TNF- α* , *IL-6*, and *IL-12* mRNA level (classical markers of inflammation) compared to the control without grafts (Fig. 6a). The macrophages cultured in contact with the bone paste also expressed lower levels of *TNF- α* , *IL-6*, and *IL-12* compared to the control. However, they exhibited higher *VEGF-A* and *IL-10* mRNA expression (classical pro-regenerative markers), thereby suggesting a substantial pro-regenerative phenotype (Fig. 6a). On the other hand, when cultured under pro-regenerative conditions, the macrophages exhibited no change in *VEGF-A*, *TGF- β* , or *IL-10* mRNA expression when in contact with the particular bone graft or the bone paste compared to the control, thus indicating that the pro-regenerative phenotype of the macrophages was maintained in these conditions (Fig. 6a). However, the macrophages in contact with the bone paste also expressed lower *IL-6* and *TNF- α* mRNA levels than those in contact with the particular bone graft, supporting the role of the bone paste to enhance the regenerative phenotype of the macrophages (Fig. 6a).

To further determine whether this in vitro observation may have a degree of in vivo relevance, the phenotype of the macrophages was analyzed in the calvarial defects by immunohistochemical labeling [CD68 (total macrophages), iNOS (pro-inflammatory macrophages), and CD163 (pro-regenerative macrophages)].

In the GBR model, CD68⁺, iNOS⁺, or CD163⁺ cells (black arrows), as well as CD68⁺/CD163⁺ cells (white arrows) were found in the control group as well as in the vicinity of the particular bone graft (Fig. 6b). However, in the group grafted with the bone paste, while CD68⁺, CD163⁺, and CD68⁺/CD163⁺ cells were found in

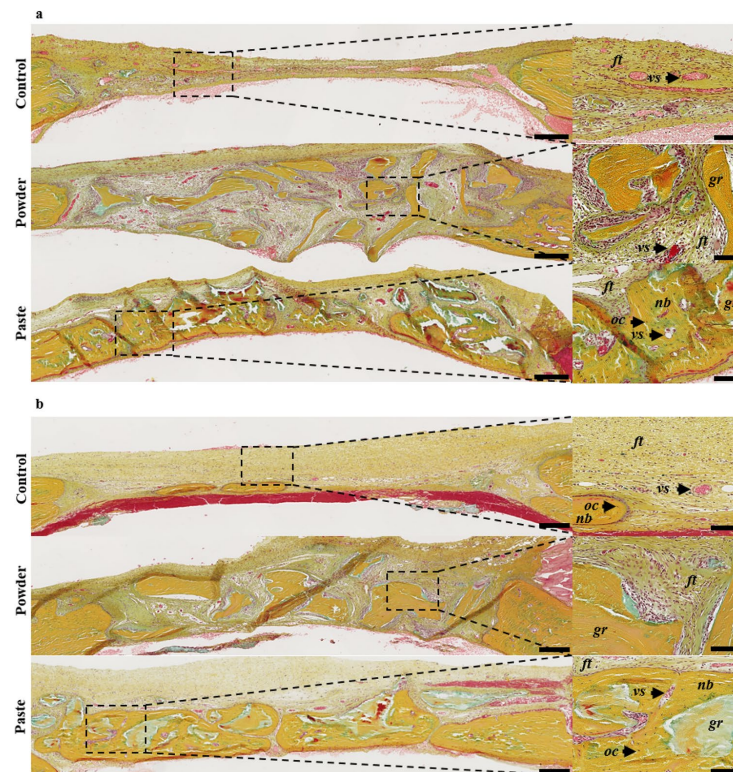


Figure 5. Rat calvaria healing at 3 and 7 weeks after surgery in a critical size defect model. Representative images of the defects with Movat's pentachrome staining of undecalcified 7- μ m thick frontal section, 3 (a) or 7 (b) weeks after the surgery. The control represents the unfilled defects. Scale bars: 250 μ m for the full defects, 100 μ m for the high-magnification image (dashed square). Dark yellow: collagen in the mature bone, light red: cytoplasm, dark red: osteoid borders, brown: nuclei, *ft*: fibrous tissue, *oc*: osteocytes, *nb*: newly formed bone, *vs*: blood vessels, *gr*: grafts.

the regenerated tissue, few iNOS⁺ cells were observed. This strongly suggests that the macrophages had largely assumed a pro-regenerative phenotype 7 weeks after the implantation.

In the CSD model, after 3 and 7 weeks, CD68⁺, iNOS⁺, CD163⁺, or CD68⁺/CD163⁺ cells were present in the control group (Fig. 6c). In the grafted groups, our data suggest that the macrophages had a more pronounced pro-regenerative engagement in the defects filled with the bone paste than with the particular bone graft. This difference was clearly observable after 3 weeks of regeneration. In the defects filled with the particular bone graft, CD68⁺ and iNOS⁺ cells could be seen, while CD163⁺ and CD68⁺/CD163⁺ cells were hard to discern. Conversely, in the defects filled with the bone paste, CD68⁺, CD163⁺, CD68⁺/CD163⁺ cells were present, and few iNOS⁺ cells could be seen. After 7 weeks of regeneration, the unfilled defects and the defects filled with the particular bone graft contained CD68⁺, iNOS⁺, CD163⁺, and CD68⁺/CD163⁺ cells. At this time point, the defects grafted with bone paste were filled with newly formed bone, and hence few monocytes/macrophages could be seen in the defects. Indeed, only CD163⁺ and CD68⁺/CD163⁺ cells were present in the soft tissue in the regenerated defects (Fig. 6d).

Discussion

Allogeneic bone grafts are a suitable and widely used alternative to ABGs in oral and maxillofacial bone graft procedures. However, allogeneic bone is mainly used in powder or block forms, and its lack of versatility and adaptability limits its use in irregular or hard-to-reach areas. In this work, the regenerative potential of an easy-to-handle and ready-to-use moldable allogeneic bone graft in a paste form made of partially demineralized bone allograft was assessed, with the aim of supplanting the use of allogeneic particular bone grafts in oral and maxillofacial bone grafting procedures.

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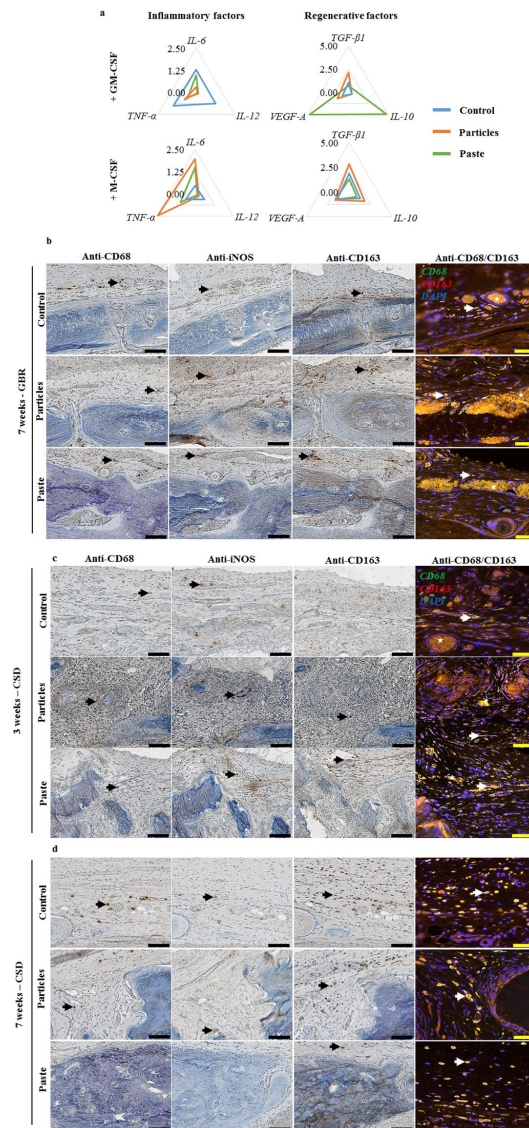


Figure 6. Effect of the particular bone graft and the bone paste on monocyte/macrophage polarization. (a) mRNA expression in human monocytes cultured in the presence of GM-CSF or M-CSF for 3 days in contact with the particular bone graft or the bone paste. The Y-axis represents the mean relative mRNA expression level of the specified genes (N = 3). Representative immunohistochemical and immunofluorescent stainings on successive undecalcified 7-µm thick sections, (b) in the GBR model after 7 weeks, and (c) in the CSD model after 3 and 7 weeks. Immunohistochemical stainings were performed for CD68, iNOS, CD163, and immunofluorescence with double labeling of CD68 (green)/CD163 (red) and DAPI (blue) for the nuclei. For single labeling, positive cells were stained with a brown precipitate (black arrow), for double labeling, double-positive cells appeared as light yellow (white arrow). Typical auto fluorescent red blood cells were visible (white stars). Scale bars: 100 µm for the immunohistochemistry, 50 µm for the immunofluorescence images.

Our data first of all demonstrated better attachment of hBM-MSCs to the surface of the bone paste compared to the particular bone graft. The greater attachment could be due to the partial demineralization and heating of the bone paste, which modifies the surface of the graft and hence also the cell-material interaction. Although further analyses (e.g., particle topography, kinetics of focal adhesion formation) are required to properly characterize the nature of the interactional changes, it has been reported that the denaturation of collagen can expose cryptic adhesion sites and RGD (Arg-Gly-Asp) integrin-binding sites that promote the adhesion of hBM-MSCs¹³⁻¹⁸. Additionally, the bone paste was found to remain cohesive for up to 21 days in an aqueous medium, and it promoted the osteogenic commitment of hBM-MSCs. This latter property is of great relevance for bone regeneration since the bone paste could act as a cohesive scaffold in the bone defects. This may lead to better osteointegration and osteoconduction than with independent and non-demineralized bone grafts. The higher ALP activity observed in hBM-MSCs cultured in contact with the bone paste compared to the particular bone graft may be due to the different interaction between the cells and the bone grafts, and/or to the presence of a cellular network at the surface of the bone paste, thereby enhancing the osteogenic commitment of hBM-MSCs. Moreover, type I collagen is thought to drive osteogenic fate, and the exposure of RGD binding sites is known to enhance the osteogenic phenotype of mesenchymal stromal cells¹⁸⁻²¹.

These positive *in vitro* effects of the bone paste on the mesenchymal progenitors were also confirmed through the regenerative potential of the bone paste investigated in a GBR and a CSD model in rat calvaria. In light of the similar organization and ossification between the cranial and maxillary bones, these calvarial models are widely used for preclinical studies focused on the development of bone substitutes for oral and maxillofacial surgeries²². Additionally, this operating site allows easy and rapid examination of the bone healing properties of the grafted materials²³⁻²⁵. Moreover, such regenerative properties of bone graft have to be investigated in GBR and CSD models, as they are representative of bone healing in techniques that are clinically used in several oral and jaw surgery indications (e.g., sinus lift, socket preservation). However, the atrophic environment of the rat calvaria (poor vasculature and bone marrow) is a challenge when it comes to achieving bone regeneration.

The covering membrane in the GBR model prevents non-osteoblastic cells from migrating from the surrounding soft tissues, thereby indirectly favoring invasion of the bone defects by osteoprogenitors, which improves the bone regeneration²⁶⁻²⁸. In the CSD model, as the bone regeneration is slower compared to the GBR model, an intermediate time point, 3 weeks in addition to 7 weeks of regeneration, was chosen to investigate the early events that are crucial for bone regeneration in these challenging conditions.

The bone regeneration observed in the defects filled with the bone paste in the GBR model validated the ability of this graft to support bone healing. Moreover, the more pronounced increase in the MV/TV between 0 and 7 weeks in the group grafted with the bone paste compared to the group grafted with the particular bone graft can be explained either by the lower MV/TV of the bone paste at the implantation, due to the demineralized part of the bone paste, or by a higher amount of newly formed bone. In the CSD model, the radiological and histological observations showed that the particular bone graft failed to support bone repair. The fibrous tissue observed 3 weeks post-implantation suggests that a fibrous encapsulation of the graft occurred, thereby preventing the bone formation. Longer time points would have been necessary to evaluate the healing properties of the particular bone graft in this CSD model.

Nonetheless, the absence of calvarial bone healing has been observed for similar time points, 7 weeks post implantation, with alternative bone filling materials such as synthetic biphasic calcium phosphate^{29,30} or xenogenic bone³¹ which are widely clinically used. Those particular bone grafts substitutes are known to be mainly osteoconductive, which is not sufficient to allow a bone regrowth in an atrophic site (i.e., low vasculature and the absence of bone marrow) such as calvaria. The particular bone graft used in this study (i.e., cleaned, grinded and sterilized bone tissue) displayed insufficient bone healing properties to promote bone regrowth in the CSD model. Since the particular bone graft had no effect on cell viability (Sup. Fig. 2), the lack of new bone formation in the particular bone graft-filled defects is likely to be related to the low self-healing capacity of the calvarial bone.

In contrast with the particular bone graft, the bone paste induced bone regeneration. This effect is probably not due to the release of growth factors^{32,33} from the demineralized bone matrix, since the heat treatment that the bone graft was subjected to has been reported to denature growth factors³⁴. Therefore, we hypothesize that bone regeneration could be induced by the cell-particle interaction and cellular network that is known to promote osteoblastic differentiation and to foster bone regeneration^{35,36}. Additionally, the cohesive nature of the bone paste may prevent it from leaking out of the defect, which is notably advantageous in irregular and hard-to-reach areas, which are common in oral and maxillofacial surgery.

Lastly, we investigated the phenotype of the macrophages in contact with the particular bone graft and the bone paste, as these cells are among the first to invade bone defects and interact with grafted materials. Indeed, pro-inflammatory "M1-like" macrophages in early immune responses³⁷⁻³⁹ and pro-regenerative "M2-like" macrophages in the later stages of bone healing⁴⁰⁻⁴² are both critically required for proper bone regeneration. The macrophages in contact with the bone paste expressed lower *IL-12* and higher *IL-10* mRNA levels than those in contact with the particular bone graft. This result supports the hypothesis that an "M1-like" to "M2-like" phenotypic switch known to promote inflammation resolution, wound repair, and bone healing⁴³⁻⁴⁶ was induced by the bone paste. In addition, the increase in *VEGF-A* mRNA expression in the macrophages in contact with the bone paste may also contribute to acceleration of the bone repair process, since angiogenesis plays a pivotal role in bone growth and healing⁴⁷⁻⁵¹.

Our overall immunohistochemical observations also strongly suggest that the bone paste either accelerated the on-site monocyte/macrophage recruitment and/or their "M1-like" to "M2-like" phenotypic switch compared to the particular bone graft. Indeed, the small number of iNOS⁺ cells and the abundance of CD163⁺ and CD68⁺/CD163⁺ cells in the defects filled with the bone paste suggest a suitable inflammatory environment for bone regeneration, with the polarization of the macrophages towards a pro-regenerative phenotype. As the initial inflammation phase may be shortened in these defects, the mineralization phase of the bone tissue could start

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earlier as well and thus explain the higher MV/TV after 7 weeks in the defects filled with the bone paste compared to the particular bone graft in both the GBR and the CSD model. This is of notably clinical relevance in oral and jaw surgeries, since shortening of the bone healing time may enhance the speed and stability of osteointegration of dental implants, thereby leading to a reduction of the associated treatments or temporary prostheses that often cause significant discomfort for patients.

Conclusions

To the best of our knowledge, this study is the first to demonstrate the bone regenerative capacity of a partially demineralized bone graft in a paste form *in vitro* and *in vivo*. In this study, we successfully demonstrated that a ready-to-use and easy-to-handle partially demineralized allogeneic bone paste has substantial bone regenerative properties. The ability of the allogeneic bone paste to induce bone formation by mesenchymal progenitor cells and macrophages, as well as its radio-opacity, are key features for clinicians. Moreover, as a ready-to-use bone substitute, this paste does not require any additional compounds prior to its use, which is an advantage when considering both regulatory approval and potential clinical indications. Our promising preclinical data have recently led us to initiate an interventional randomized clinical trial for pre-implant guided bone regeneration in oral surgery (NCT04141215).

Materials and methods

Process to obtain the bone paste. The allogeneic particular bone graft and bone paste were obtained from human donors who had provided their informed consent, and all of the protocols were approved by the French ethical committee "Comité de Protection des Personnes" (CPP), in accordance with the 1975 Helsinki declaration and its subsequent amendments. The bone allografts were harvested during total hip replacement surgeries and donated to the BIOBank bone bank (Presles-en-Brie, France), under the authorization of the "Agence Nationale de Sécurité du Médicament et des produits de santé" (ANSM, n°FR07703T-19-01) according to the relevant French regulations and ethical standards. Cleaning of the femoral heads was ensured by supercritical CO₂ lipid extraction followed by successive immersions in H₂O₂, NaOH, and EtOH, leaving the bone matrix defatted⁵³, virally inactivated^{53,54}, and unaltered⁵⁵⁻⁵⁷. The cleaned femoral heads were crushed to generate the particular bone graft (0.3 to 1 mm in diameter, Sup. Fig. 1, Sup Vid. 1). The particular bone graft was either sterilized by 25 kGy gamma irradiation and used as an internal comparator, or it was processed into a paste form. For this purpose, the particular bone graft was partially demineralized with HCl, then hydrated with water to make it an extrudable and moldable preparation (Sup. Fig. 1, Sup Vids. 2 and 3), and heated (121 °C) to ensure partial transformation of the outer demineralized collagen of the particles into gelatin and simultaneous sterilization of the entire bone graft¹⁸. All of the particular bone graft and the bone paste samples were made from pooled donor material in order to limit the intra-donor variability.

The bone and paste were imaged with transmitted light (Leica DM12 LED, Wetzlar, Germany) or reflected light (Leica M125). For all of the X-ray micro-computed tomography (μ-CT) acquisitions, the samples were scanned under the following conditions: resolution: 8 μm/px, single 360° scan, rotation: 0.710°, averaging frames: 4 (SkyScan-1072, Bruker, Billerica, USA). The three-dimensional datasets were reconstructed with the NRecon software (Micro Photonics Inc, Allentown, USA). For the scanning electron microscopy (SEM) imaging, the samples were sputtered with a gold plasma (Sputter Coater Desk Denton Vacuum, Moorestown, USA), and the images were taken using an electron microscope (Zeiss, Oberkochen, Germany) with a secondary electron detector set at 10 kV.

Analysis of hBM-MSCs attachment and osteoblastic commitment. Human BM-MSCs from two independent donors (one male and one female, PromoCell, Heidelberg, Germany) were amplified in PromoCell Growth Medium supplemented with 1% penicillin–streptomycin (P/S) (Invitrogen, Paisley, UK) in a humidified atmosphere at 37 °C, 5% CO₂ (Air Liquide, Paris, France). The medium was changed every other day. The cells were used between passage 2 and 5.

To measure the attachment of hBM-MSCs to the particular bone graft and the bone paste, the grafts (0.125 cc/well, 66 and 93 mg, respectively) were spread on the bottom of 24-well plates (Ultra-Low Attachment Surface, Corning). One milliliter of culture medium (DMEM/Ham's F12 (1:1), high glucose, GlutaMAX (Thermo Fisher Scientific) with 15% fetal calf serum (FCS, Dominique Dutscher, Brumath, France) and 1% P/S) was added to each well and the plates were incubated in a humid atmosphere at 37 °C, 5% CO₂ for 24 h. The medium was then removed and 1 × 10⁵ hBM-MSCs were seeded in each well. After 1 h, 3 h, and 6 h of contact between the hBM-MSCs and the particular bone graft or the bone paste, the cells were extensively rinsed with 1X phosphate buffered saline (PBS) at 37 °C to remove the non-adherent cells. The DNA content of remaining adherent cells in contact with the particular bone graft or the bone paste was measured with Quant-iT™ PicoGreen dsDNA Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions and then normalized to the surface of the bone grafts measured by μ-CT as mentioned in subsection 2.1.

For the confocal imaging, the cells were fixed in 4% paraformaldehyde (PFA) for 20 min and then permeabilized with Triton X-100 (1% in 1X PBS) for 10 min at room temperature. The cells were then labeled with 1:200 Alexa Fluor 568 phalloidin (Thermo Fisher Scientific) in 1X PBS at room temperature for 1 h, and the nuclei were stained with 1:50,000 Hoechst 33258 (Thermo Fisher Scientific) in 1X PBS for 15 min at room temperature. The cell samples were imaged using a confocal microscope (A1RS, Nikon, Champigny sur Marne, France).

To assess the ability of the particular bone graft and the bone paste to support osteogenic commitment, the level of alkaline phosphatase (ALP) activity was measured in cell lysates. The particular bone graft and the bone paste were placed in low-binding 24-well plates as described above, and 1 × 10⁵ hBM-MSCs were seeded in each well. After 48 h, the medium was replaced with culture medium with or without osteogenic factors (50 μM

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Gene	Common name	Primer names/sequence	Supplier
<i>IL-12</i>	Interleukin-12	QT0000364	Qiagen
<i>IL-6</i>	Interleukin-6	QT00083720	Qiagen
<i>TNF-α</i>	Tumor necrosis factor-alpha	QT01079561	Qiagen
<i>TGF-β</i>	Transforming growth factor-beta	QT00000728	Qiagen
<i>IL-10</i>	Interleukin-10	QT00041685	Qiagen
<i>VEGF-A</i>	Vascular endothelial growth factor-A	F: 5' GCTGTCTTGGGTGCATTGGA R: 5' ATGATTCTGCCCTCCTCCTC	Eurofins
<i>B2M</i>	Beta2-microglobulin	F: 5' CCTGGAGGCTATCCAGCGTA R: 5' GGATGACGTGAGTAAACCTGAATCT	Eurofins
<i>PPIA</i>	Peptidyl prolyl isomerase A	F: 5' GTCAACCCACCGTGTCTT R: 5' CTGCTGTCTTTGGGACCTGT	Eurofins

Table 1. Targets, names, and sequences of the primers used for the real-time PCR analysis of mRNA expression in human monocytes/macrophages. F: forward, R: reverse.

ascorbic acid, 10 mM β -glycerophosphate, and 100 nM dexamethasone (Thermo Fisher Scientific)). The medium was changed every other day. The ALP activity was measured after 14 or 21 days with an Alkaline Phosphatase Substrate kit (Bio-Rad, Hercules, USA) and normalized to the total protein content, measured using a BCA protein assay kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Monocyte/macrophage polarization. Monocytes and human serum (HS) were obtained from the clinical transfer platform of Nantes Hospital (PF DTC, CIC 0503). Monocytes were seeded in 12-well plates at a density of 1.25×10^6 cells in 2.5 mL of culture medium (RPMI 1640, Thermo Fisher Scientific) with 8% HS, 2 mM L-glutamine, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin. To induce pro-inflammatory "M1-like" macrophages, the medium was supplemented with GM-CSF (granulocyte-macrophage colony-stimulating factor) at 20 ng/mL (CellGenix GmbH, Freiburg im Breisgau, Germany) and to induce pro-regenerative "M2-like" macrophages, the medium was supplemented with M-CSF (macrophage colony-stimulating factor) at 50 ng/mL (Isokine, Kopavogur, Iceland), for 3 days, as previously described^{58,59}. The cells were cultured in contact with the particular bone graft, the bone paste (0.125 cc/well, 66 and 93 mg, respectively), or in the absence of the graft as a control. After 3 days, lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, USA) at 200 ng/mL was added to each well and the total RNA was extracted after 6 h.

To investigate the monocyte/macrophage polarization in contact with the particular bone graft and the bone paste, real-time PCR was used to measure the expression levels of genes encoding specific markers of the monocyte/macrophage phenotype. Total RNA was prepared with TRIzol Reagent (Thermo Fisher Scientific), followed by Nucleospin XS RNA columns (Macherey-Nagel, Hoerd, France) according to the manufacturer's instructions. A 0.5 μ g quantity of the total RNA was reverse transcribed and analyzed using a Bio-Rad CFX96 detection system using SYBR Select Master Mix (Thermo Fischer Scientific). The relative mRNA expression was normalized to the expression of the *B2M* and *PPIA* housekeeping genes and calculated using the $2^{-\Delta\Delta C_t}$ method with Bio-Rad CFX Manager software. The primer sequences and the names of the target and the housekeeping genes are indicated in Table 1.

In vivo analysis of the bone regeneration in a guided bone regeneration (GBR) and a critical size defect model (CSD). *Animals and ethical aspects.* The study was carried out in compliance with the ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations, the institutional guidelines of the European and French Ethics Committee and approved by the local ethics committee (Comité d'Ethique en Experimentation Animale, Pays de la Loire (CEEA.PdL) n°06, agreements n° 2016111513349765/8560 and n°201802021521/15410). Seven-week-old syngeneic male Lewis rats were purchased from an approved breeder (Charles River, Écully, France). A concerted effort was made to minimize physical and psychological suffering and to reduce the number of animals used.

Surgical procedures for the GBR and CSD models. All of the veterinary medicines were purchased from Centra-vet (Dinan, France). The animals were allowed to acclimate for a week at the animal facility before the surgery. The acclimated animals were anesthetized by inhalation of an air/isoflurane mixture (4% isoflurane) in a closed induction chamber. Throughout the surgical procedures, the animals were placed on a heating pad, and the anesthesia was maintained with an air/isoflurane mixture (2% isoflurane) through an inhalation mask. The operating site (the top of the skull) was shaved and the skin was cleaned with sterile water and iodized polyvidone (Betadine). The preoperative analgesia was performed by subcutaneous injection of lidocaine (Xylocaine) at 5 mg/kg on the operative area, and buprenorphine (BupreCare) at 0.02 mg/kg and meloxicam (Metacam) at 1 mg/kg were injected subcutaneously in the back of the rats. The skin and the periosteum were incised and pushed back on the sides, after which full-thickness bilateral paramedian parietal craniotomies were performed using a 5-mm outer diameter trephine (two defects per rat). During the surgery, the defects were abundantly irrigated with saline solution to avoid cauterization of the defect edges by the trephine. The defects were then either left

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unfilled as a negative control or filled with the particular bone graft (rehydrated with 0.9% NaCl, 0.6 mL/cc for 10 min) or the bone paste.

Two experimental procedures were independently carried out: a GBR and a CSD model. For the GBR model, the defects were covered with a polytetrafluoroethylene membrane. For the CSD model, the defects were left uncovered. For both models, the skin was sutured (5/0, non-absorbable suture (Ethicon, Bridgewater, USA)) and the animals were returned to their cages with water and food ad libitum. The postoperative analgesia was provided by subcutaneous injection of buprenorphine at 0.02 mg/kg twice daily for 3 days and meloxicam at 1 mg/kg mixed in their water supply for 5 days. After 7 weeks for the GBR model, and after 3 or 7 weeks for the CSD model, the animals were euthanized in a CO₂ chamber. The calvaria were collected using sharp scissors inserted through the *foramen magnum*, and the skulls were cut following the temporal crest to the frontal bone, above the coronal suture. The calvaria were fixed in 4% PFA for 72 h and then stored in 70% ethanol.

In vivo micro-computed tomography analyses. To quantify the mineral volume (MV) within the tissue volume (TV) (i.e., the bone in the defects), the calvaria were scanned using μ -CT, as mentioned in subsection 2.1. To calculate the MV/TV among the different groups, a hollow cylindrical volume of interest (VOI) of 4.5 mm in diameter and 1 mm in height were selected in the defects. The three-dimensional quantitative results were expressed as MV/TV (%) after 3 or 7 weeks (CTAn software, Bruker, Madison, WI).

Histological and immunological analyses. Fixed samples were dehydrated by means of a graded series of ethanol baths. Non-decalcified bone specimens were infiltrated and embedded in Technovit 9100 New (Heraeus Kulzer, Les Ulis, France). For each sample, a frontal section was performed through the defects of each explant using a circular diamond saw (Leica, Wetzlar, Germany) and serial 7- μ m thick sections were cut using a hard tissue microtome (Polycut, Leica). The sections were stained with Movat's pentachrome (Thermo Fisher Scientific).

Single immunostainings were performed with three different antibodies (anti-CD68: 1:500 ab125212, anti-CD163: 1:2,000 ab182422, or anti-iNOS (inducible nitric oxide synthase): 1:250 ab15323 (Abcam, Cambridge, UK)). The labeling was visualized with a horse anti-rabbit peroxidase-conjugated antibody (Vector, Burlingame, USA) and 3,3'-diaminobenzidine (DAB, Vector). The slides were counterstained with Mayer's hematoxylin (Microm Microtech, Brignais, France).

For double CD68/CD163 fluorescent labeling, the samples were processed as described above. The incubation with CD163 was followed by incubation with a secondary antibody (Alexa Fluor 568-conjugated goat anti-rabbit 1:200, ab17471). The CD68 was then added, followed by Alexa Fluor 488-conjugated goat anti-rabbit (1:200, ab150081). All of the slides were scanned with a slide reader (NanoZoomer, Hamamatsu).

Statistical analyses. The statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad, San Diego, USA). Statistical significance was determined using two-way ANOVA followed by Bonferroni's post-test for multiple group comparisons with different variables, or a *t*-test for two-group comparisons. Statistical significance was set at $p < 0.05$. Unless stated otherwise, the experiments were repeated at least three times. The results are presented as means \pm the SD.

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Author contributions

P.T. conceptualized the study, designed the experimental procedure, performed the experimentations, analyzed the data, wrote and edited the manuscript. A.B. produced the bone substitute samples, and helped to design the experimental procedures. C.B., A.P. and P.C. designed the experimental procedures, performed the experimentations, and analyzed the data. A.M., T.B., J.V., C.V., M.A., J.L. performed the experimentations and analyzed the data. R.B., V.G., J.G., P.W. and A.G. supervised the study, wrote and edited the manuscript. All authors reviewed and approved the manuscript.

Competing interests

The authors declare no competing interests.


Additional information

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BASIC RESEARCH – BIOLOGY

Cytokine Production and Cytotoxicity of Calcium Silicate-based Sealers in 2- and 3-dimensional Cell Culture Models



SIGNIFICANCE

Besides biocompatibility, BioRoot RCS and ProRoot ES did not induce proinflammatory cytokines and promoted anti-inflammatory cytokine secretion by human periodontal ligament stem cells that may have a positive influence and attenuate initial inflammatory response.

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ABSTRACT

Introduction: The aim of the present study was to assess the effects of different silicate-based sealers (ie, BioRoot RCS [Septodont, Saint Maur des Fosses, France], ProRoot ES [Dentsply Sirona, York, PA], and MTA Fillapex [Angelus, Londrina, PR, Brazil]) on cytokine production and viability of human periodontal ligament stem cells (PDLSCs). AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) was used as a reference material. **Methods:** PDLSCs were cultured either in 2-dimensional or 3-dimensional conditions (in 0.15%–0.5% PuraMatrix [BD Biosciences, Bedford, MA]) for 24 hours with eluates from set endodontic sealers. Additionally, the toxicity of eluates from endodontic sealers was evaluated using an *in vitro* root model experimental procedure. PDLSC viability was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. PDLSC culture medium was used for cytokine quantification (interleukin [IL]-6, IL-8, growth-regulated oncogene, IL-4 and IL-10) using the HCYTMAG-60K-PX41 Milliplex kit (EMD Millipore, Burlington, MA). **Results:** In 2-dimensional culture conditions, BioRoot RCS revealed a good PDLSC viability rate. ProRoot ES had no effect on PDLSC viability regardless of the dilution. MTA Fillapex was strongly cytotoxic even at the lowest extract dilutions (1:1, 1:2, and 1:4). Encapsulation of PDLSCs in PuraMatrix tended to decrease the cytotoxic effect of the sealers. In the 3-dimensional *in vitro* root model experimental procedure, BioRoot RCS, ProRoot ES, and MTA Fillapex revealed a cytocompatibility pattern. Different calcium silicate-based sealers exhibited different proinflammatory cytokine production. BioRoot RCS greatly stimulated the release of IL-10 and, to a lesser degree, IL-4 by PDLSCs ($P < .05$). **Conclusions:** BioRoot RCS and ProRoot ES did not induce proinflammatory cytokines and promoted anti-inflammatory cytokine secretion by PDLSCs that may have a positive local impact by attenuating an initial inflammatory response. (*J Endod* 2020;46:818–826.)

KEY WORDS:

2D cell culture; 3D cell culture; calcium silicate-based endodontic sealers; cytokines; cytotoxicity; periodontal ligament stem cells; root canal filling material

The goal of endodontic obturation is to seal as much as possible root anatomic spaces in order to prevent reinfection and limit a growth of microorganisms that may be left behind after cleaning and shaping procedures. Recently, endodontic sealers based on various calcium silicate compositions emerged in the market. They were developed after the initial introduction of mineral trioxide aggregate (MTA). In light of studies performed on MTA, it may be expected that sealers could have interesting properties, such as supporting tissue regeneration, being bioinductive for bone and cementum, and not stimulating inflammatory processes or root resorption¹.

Indeed, studies showing biocompatibility and bioactivity of these sealers have emerged^{2–4}. For example, *in vitro* experiments showed cytotoxicity and up-regulated expression of proinflammatory cytokines interleukin (IL)-1 β and IL-6 by MTA Fillapex (Angelus, Londrina, PR, Brazil) compared with silicate-based sealers EndoSeal (Maruchi, Wonju, Korea) and EndoSequence BC Sealer (Brasseler,

TABLE 1 - Tested Materials

Materials	Manufacturer	Composition
BioRoot RCS	Septodont, Saint Maur des Fosses, France	Powder: tricalcium silicate, zirconium oxide, and excipients Aqueous solution: calcium chloride and excipients
ProRoot ES	Dentsply Sirona, York, PA	Powder: calcium sulphate, dicalcium silicate, tricalcium silicate, bismuth oxide, and a bit of tricalcium aluminate Liquid (viscous aqueous solution of a water-soluble polymer)
MTA Fillapex	Angelus, Londrina, PR, Brazil	Paste A: salicylate resin, bismuth trioxide, and fumed silica Paste B: fumed silica, titanium dioxide, mineral trioxide aggregate, and base resin
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany	Paste A: epoxy resins, calcium tungstate, zirconium oxide, silica, iron oxide pigments, and aerosil Paste B: adamantane amine, N,N-dibenzyl-5-oxanonane, TCD-diamine, calcium tungstate, zirconium oxide, and aerosil

Savannah, GA)⁵. It may be expected that formulations of silicate-based sealers differ from the original composition of MTA in order to have proper characteristics for use as endodontic sealers (working time, setting time, and difficulty of handling) and, therefore, may have modified biological and physical properties. The behavior of these endodontic sealers was generally evaluated using traditional 2-dimensional (2D) cell cultures. The main drawbacks of the 2D cultures are that they form monolayers in which extracellular matrix components and cell-to-cell and cell-to-matrix interactions are minimally expressed⁶.

Conversely, 3-dimensional (3D) cell culture models⁵ are closer to *in vivo* cellular conditions because the 3D scaffold supports cell growth and cell functions including morphogenesis, cell metabolism, and cell-to-cell interactions⁷. Therefore, the aim of this study, using a 3D cell culture model combined with an *in vitro* root model experimental procedure, was to assess the effects of 3 silicate-based sealers (ie, BioRoot RCS [Septodont, Saint Maur des Fosses, France], ProRoot ES [Dentsply Sirona, York, PA], and MTA Fillapex) on cytokine production and viability of human periodontal ligament stem cells (PDLSCs).

MATERIALS AND METHODS

This study was approved by the Institutional Review Board at the Arthur A. Dugoni School of Dentistry at the University of the Pacific, San Francisco, CA (institutional review board protocol #16-128). Human PDLSCs were obtained from impacted third molars from donors aged 16–25 years old with informed consent of the patients and their parents in accordance with the World Medical Association's Declaration of Helsinki. In addition, 30 intact, freshly extracted human maxillary incisor teeth were stored at 4°C and used for this study.

Preparation of Extracts

The tested sealers were BioRoot RCS, ProRoot ES, MTA Fillapex, and AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) (Table 1). AH Plus was used as a reference material. All materials were mixed according to the manufacturers' instructions. Discs of each sealer were prepared under aseptic conditions in sterile cylindrical polypropylene blocks 7 mm in diameter and 3 mm in height. The materials were placed in a humid chamber at 37°C for 48 hours⁸. The extraction of different materials' eluates was performed in general accordance with ISO 10993-12. The extraction was made in complete cell culture medium, and various dilutions (1/2, 1/4, and 1/8 v/v) were prepared according to a pretest cytotoxic sealer.

Isolation, Culture, and Characterization of PDLSCs

PDLSC culture was established as previously described⁹. PDLSCs at passage 4 were analyzed for cell surface antigen expression by flow cytometry using a Guava easyCyte 8HT flow cytometer (EMD Millipore, Burlington, MA). Fluorochrome-conjugated monoclonal mouse antihuman antibodies against CD45-APC/Cy7, CD90-PE-CY7, CD105-APC, CD146-PerCP/Cy5.5, and STRO-1-fluorochrome-conjugated mouse anti-human antibody or isotype controls (all from BioLegend, San Diego, CA) were used. Data were analyzed using InCyte 2.5 software (EMD

Millipore). Cells counts were performed at each passage, and the population doubling times were calculated.

Cell Encapsulation within PuraMatrix

PDLSCs were encapsulated in different concentrations (0.15%, 0.25%, and 0.5%) of self-assembling peptide scaffolds (PuraMatrix; BD Biosciences, Bedford, MA) at different cell densities (0.25–1 × 10⁶ cells/mL). Encapsulation was established as previously described¹⁰. Briefly, the necessary volume of the desired concentration of PuraMatrix was prepared by diluting the stock with sterile 10% sucrose (Table 2). Gelation of the PuraMatrix was initiated by gently running culture media down the side of the well on top of the hydrogel. The number of viable PDLSCs was determined by counting in the presence of 0.4% trypan blue stain. Proliferation data were expressed as the proliferation index (cell number after 72 hours/cell number after 24 hours).

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide Analysis

The cytotoxicity of the different endodontic sealers was evaluated using PDLSCs in 2 dimensions and in PuraMatrix cultures using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. PDLSCs incubated for 24 hours in Dulbecco modified

TABLE 2 - Preparation of the Different Concentrations of PuraMatrix

Final PuraMatrix concentration (%)	1% PuraMatrix (stock μ L)	20% sucrose (μ L)	Deionized water (μ L)	2 × x cell suspension in 10% sucrose (μ L)	Final volume (μ L)
0.5	500	—	—	500	1000
0.25	250	250	—	500	1000
0.15	150	250	100	500	1000

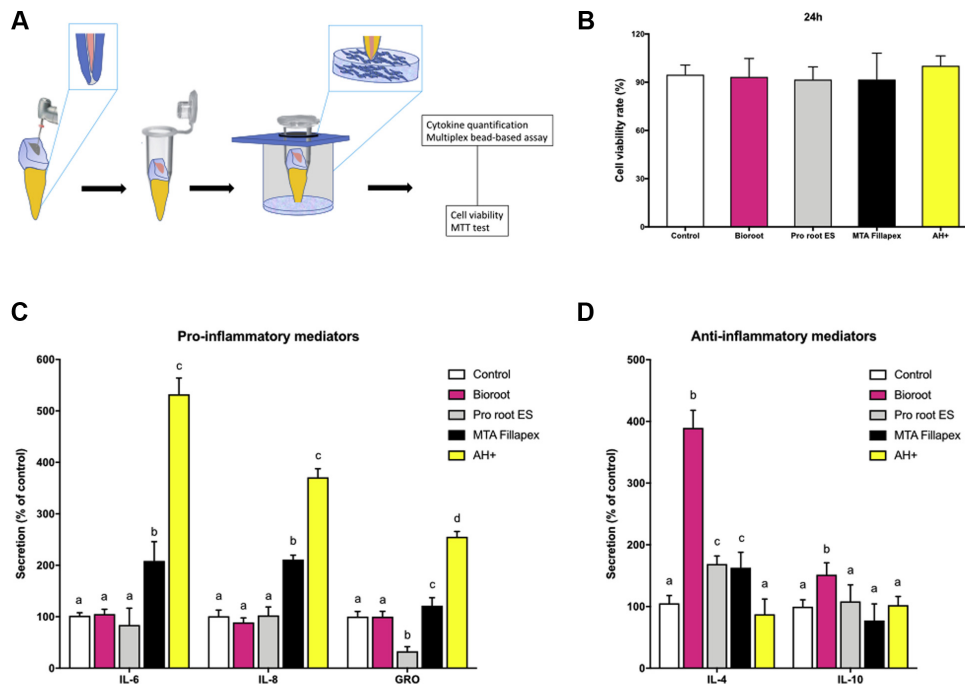


FIGURE 1 – The 3D *in vitro* root model experimental procedure. (A) *In situ* root-end filling model apparatus. The bottoms of Eppendorf tubes were cut to allow the protrusion of the tooth apex through this opening and adjusted into a 6-well cell culture plate using a rubber O-ring with 3 mm of a root apex dipped in the 3D cell culture medium. The medium that remained in contact with the apex for 24 hours was used for cytokine quantification, whereas the viability of 3D encapsulated cells in PuraMatrix was assessed using the MTT assay. (B) The cell viability rate (%) according to the MTT assay in 3D encapsulated PDLSCs after 24 hours of exposure to different sealers (i.e. BioRoot RCS, ProRoot ES, MTA Fillapex, and AH Plus); cells incubated in culture medium alone served as the negative control. (C) The result of the multiplex bead-based assay showing proinflammatory mediators (IL-6, IL-8, and GRO) secreted by PDLSCs in culture medium. (D) The result of the multiplex bead-based assay showing anti-inflammatory mediators (IL-4 and IL-10) secreted by PDLSCs in culture medium. The results are expressed as means and standard deviations. Different letters represent significant differences between groups in each dilution extract. Analysis of variance followed by Bonferroni's *post hoc* test ($P < .05$).

Eagle medium with 20% dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO) served as the positive cytotoxicity control, and PDLSCs incubated for 24 hours in full culture medium without sealer eluates served as the negative control. PDLSCs were incubated with 0.5 mg/mL of MTT (Thermo Fisher Scientific, Waltham, MA) in complete medium at 37°C in 5% CO₂. After 4 hours of incubation at 37°C, the medium was removed, and the formazan crystals were dissolved in dimethyl sulfoxide. Absorbance was measured at 540 nm using a VersaMax Microplate Reader (Molecular Devices, Sunnyvale, CA). The results were expressed as the percentage of surviving cells in test cultures when the number of viable cells in negative controls was taken as 100%.

Multiplex Analysis of Cytokines and Chemokines

Cytokine and chemokine elaboration was interrogated with the HCYTMAG-60K-PX41

Milliplex kit (EMD Millipore) following the procedure described in the manufacturer's manual. The Milliplex kits also provided a quality control material to confirm the accuracy of the assay. Plates were analyzed using a Magpix platform (Luminex, Austin, TX) equipped with xPONENT 3.1 software (Luminex), and the concentration of each cytokine was calculated from raw data. Analyte concentrations were determined in respect to a 5-parameter logistic standard curve.

In Vitro Root Model Experimental Procedure

The *in vitro* root model experimental procedure was described previously in detail¹¹. Briefly, 30 human maxillary incisor teeth were selected and sterilized by gamma ray irradiation. Root canals were instrumented to 1 mm short of the foramen using the WaveOne Gold reciprocating file tip size #45 (Dentsply Sirona) (Fig. 1A). Roots were then divided randomly

into 5 groups using a single-cone technique with one of the following sealers: BioRoot RCS, ProRoot ES, MTA Fillapex, or AH Plus ($n = 6$). Roots with unfilled canals served as negative controls. The bottoms of Eppendorf tubes were cut to allow the protrusion of the tooth apex and a 3-mm-long apical section of each root was placed in contact with the 3D cell culture (PuraMatrix 0.25%) for 24 hours. The medium that remained in contact with the apex for 24 hours was used for cytokine quantification, whereas the viability of 3D PuraMatrix-encapsulated cells was assessed using MTT assays as described previously.

Statistical Analysis

All results were expressed as means ± standard deviation; all experiments were performed with 5 replicates and performed at least 3 times, except for multiplex analysis of cytokines, which was performed twice.

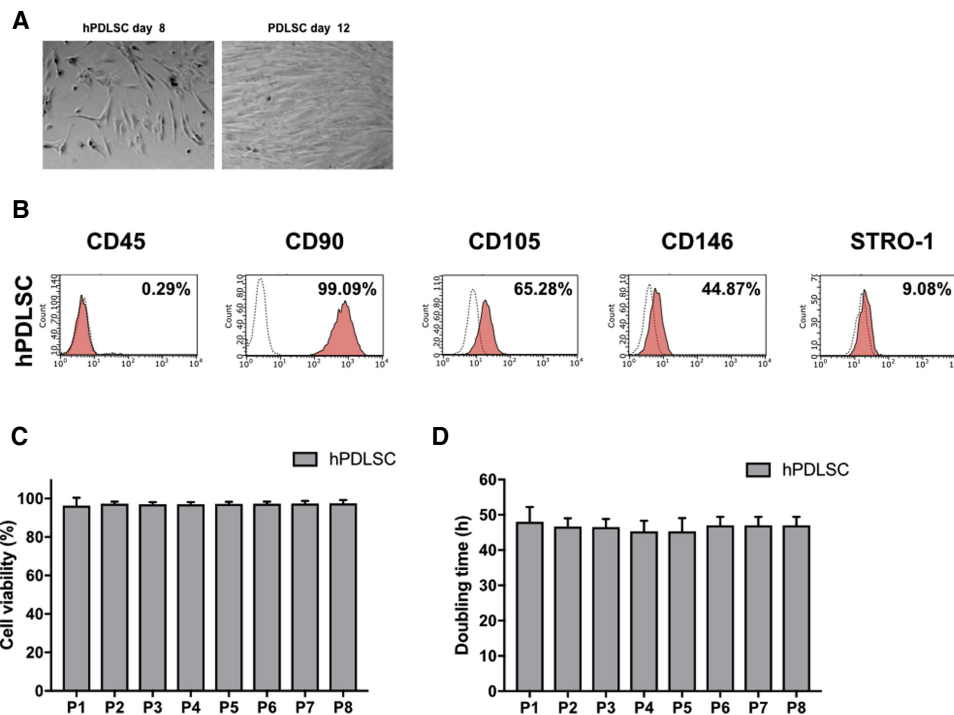


FIGURE 2 – Mesenchymal immunophenotype analysis and cell viability of PDLSCs. (A) Generation of fibroblast colonies from single cells after 8–12 days of culture. Representative phase-contrast microscopic photographs of the generation and expansion of PDLSCs. Cells have elongated shapes and grow attached to substrate. Scale bar = 25 μ m. (B) Flow cytometry analysis of representative histograms at passage (P) 3 showed that PDLSCs expressed cell surface human mesenchymal stem markers (CD90, CD105, CD146, and STRO-1) and lacked the expression for leukocyte common antigen (CD45) (in red) compared with their appropriate isotype controls (dashed line). The insert numbers represent the mean fluorescence intensity values from viable cells. The figure shows representative flow cytometry histograms obtained after 3 independent experiments. (C) The cell viability (trypan blue exclusion assay) of PDLSCs was stable from passage 1 to 8. (D) The cell doubling times were stable from passage 1 to 8.

Data were analyzed and visualized using GraphPad Prism 7.0 (GraphPad Software Inc, San Diego, CA). Statistical analyses were performed using 2-way analysis of variance followed by Bonferroni's *post hoc* tests with $P < .05$ regarded as statistically significant.

RESULTS

Identification of Mesenchymal Phenotype Expression of Isolated PDLSCs

Cell populations presented typical homogeneous fibroblastlike morphology and generated fibroblastlike colonies from single cells after 8–12 days of culture (Fig. 2A). PDLSCs expressed specific mesenchymal stem cell antigens (CD90, CD105, CD146, and STRO-1) and were negative for a hematopoietic marker (ie, CD45) (Fig. 2B). Cell viability of PDLSCs was comparable

and high from passages 1–8 (Fig. 2C). The cell doubling time was stable (around 48 hours) and similar from passage 1 to 8 (Fig. 2D).

Proliferation and Survival of PDLSCs in PuraMatrix

The trypan blue test showed that PDLSCs proliferated in PuraMatrix when seeded at a density of $0.25\text{--}0.5 \times 10^6$ cells/mL (Fig. 3A–C). In contrast, at the excessively high cell density (1×10^6 cells/mL), PDLSCs did not proliferate, and, instead, the cell population fell to around the same as the maximum 72-hour data for the 0.5×10^6 cells/mL (Fig. 3A–C). No difference was observed in PDLSC proliferation between the 0.15% and 0.25% concentrations of PuraMatrix that were tested ($P = .1715$) (Fig. 3D). Therefore, 0.25% gel was used for the majority of the applications because its increased rigidity

provided a more stable gel than the lower concentrations.

MTT Assay

In 2D cultures, PDLSCs grown with BioRoot RCS exhibited $124\% \pm 9.9\%$ (no dilution), $115\% \pm 8.2\%$ (dilution 1:2), and $118.41\% \pm 7.7\%$ (dilution 1:4) viability relative to the viability of the no-treatment control group. The increase in the viability of cells grown with BioRoot RCS was statistically significant ($P < .05$). ProRoot ES had no effect on PDLSC viability regardless of the dilution ($P > .05$). MTA Fillapex was strongly cytotoxic even at the lowest extract dilutions (1:1, 1:2, and 1:4), but the cytotoxic effect was reduced compared with the negative control at an extract dilution of 1:8. AH Plus showed mild toxicity at a high extract concentration (1:1), and there was no significant difference in cell viabilities at

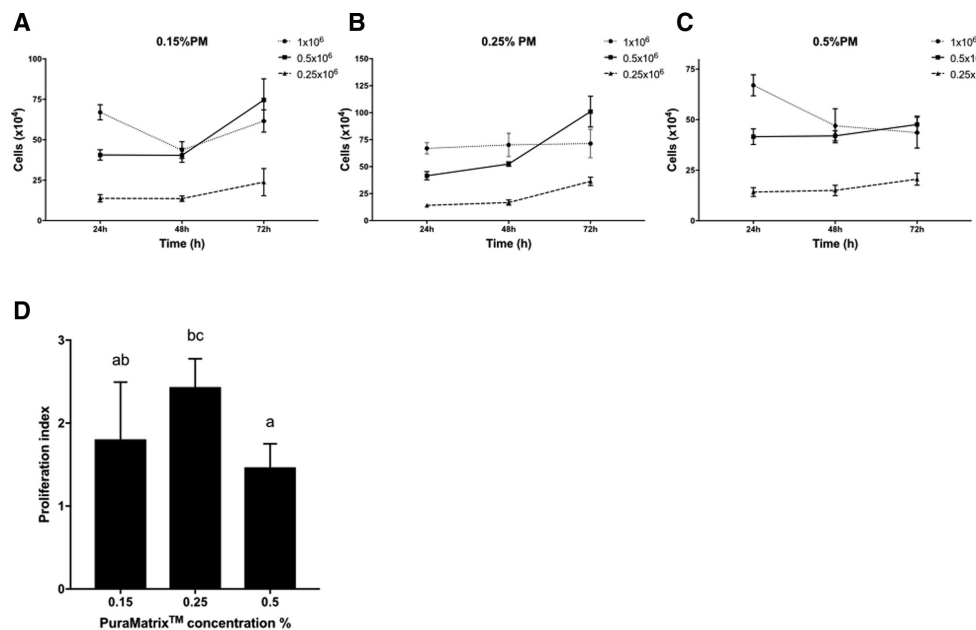


FIGURE 3—Proliferation of PDLSCs in PuraMatrix. PDLSCs were plated in (A) 0.15%, (B) 0.25%, and (C) 0.5% PuraMatrix at a cell density of $0.25\text{--}1 \times 10^6$ cells/mL and allowed to grow up to 72 hours. Trypan blue 0.4% was used to count cells at indicated time points. (D) Proliferation of PDLSCs according to the concentration of PuraMatrix. PDLSCs were plated in 0.15%–0.5% PuraMatrix at a cell density of 0.5×10^6 cells/mL. The results are expressed as means and standard deviations. Different letters represent significant differences between groups. Analysis of variance followed by Bonferroni's *post hoc* test ($P < .05$).

various extract dilutions that were reduced to negative control levels when the extract was diluted to 1:2 ($P > .05$) (Fig. 4A).

In 3D cell encapsulation with 0.25% PuraMatrix, BioRoot RCS and ProRoot ES showed an increase in the viability of PDLSCs at the lowest dilutions (1:1 and 1:2 and 1:1, respectively) ($P < .05$). ProRoot ES and BioRoot RCS remained noncytotoxic for the other dilutions ($P > .05$). MTA Fillapex remained cytotoxic at a high extract concentration (1:1). With extract dilutions of 1:2 and higher, MTA Fillapex was no longer cytotoxic. There were no significant differences in viabilities of cells exposed to extracts from AH Plus ($P > .05$) (Fig. 4B).

In an *in vitro* root model experimental procedure, BioRoot RCS, ProRoot ES, MTA Fillapex, and AH Plus had no toxic effects on cell viability, with MTT results very similar to the negative control group ($P > .05$) after 24 hours of exposure (Fig. 1B).

Evaluation of Cytokine Production

No significant differences among BioRoot RCS, ProRoot ES, and the negative control

group were observed for IL-6 and IL-8 ($P > .05$). However, in contrast with BioRoot RCS, ProRoot ES caused a significant decrease in growth-regulated oncogene (GRO) production. Both MTA Fillapex and AH Plus caused a significant up-regulation of IL-6, IL-8, and GRO in the culture medium compared with the negative control group ($P < .05$) (Fig. 1C). BioRoot RCS greatly stimulated the release of IL-10 and, to a lesser degree, IL-4 by PDLSCs when compared with the negative control group ($P < .05$). ProRoot ES and MTA Fillapex exhibited a mild increase in IL-10 production, whereas AH Plus had no effect on IL-10 production by PDLSCs. There was no statistical difference in the production of IL-4 by PDLSCs when exposed to ProRoot ES, MTA Fillapex, or AH Plus ($P > .05$) (Fig. 1D).

DISCUSSION

Calcium silicate–based sealers have received a lot of interest from endodontists because of their biocompatibility and bioactivity. Indeed, these sealers contain calcium phosphate, which enhances the setting properties and

results in a material with a chemical composition and crystalline structure similar to tooth and bone apatite materials, thereby improving sealer-to-root dentin bonding¹². The calcium silicate–based sealers were tested for cytocompatibility and bioactivity¹³. However, there are only a few studies regarding their effect on the immune response, a key process in respect to tissue healing and regeneration. Exploration of cytokine production and cell viability may help to better understand the behavior of the tested sealers.

Although recommendations of ISO 10993-5 are to use established cell lines in cytotoxicity assays, freshly isolated human PDLSCs were used in this study. PDLSCs were selected because they may get in contact with endodontic sealers *in vivo*. Although present in a lesser quantity than periodontal ligament fibroblasts and osteoblasts, PDLSCs are considered a prime cell source for periodontal regeneration¹⁴ and are supposed to be involved in the healing of apical periodontitis and tissue regeneration¹⁵.

Conventional 2D culture systems exhibit contact inhibition of cell growth in a cell monolayer and, hence, do not duplicate

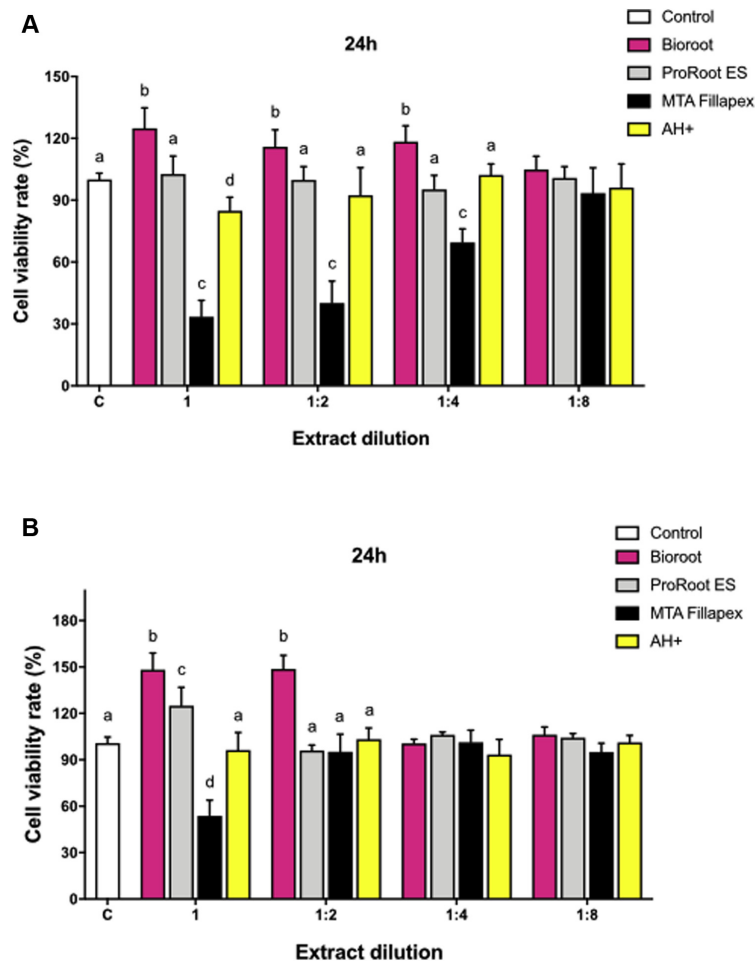


FIGURE 4 – Cell viability rate (%) according to MTT assay in PDLSCs after 24 hours of exposure to different dilutions of sealer extracts (1:1, 1:2, 1:4, and 1:8) of BioRoot RCS, ProRoot ES, MTA Fillapex, and AH PLUS; PDLSCs incubated in culture medium alone served as the negative control. (A) PDLSCs were plated in 2 dimensions at a cell density of 5×10^4 cells/mL in 24-well culture dishes. (B) PDLSCs were plated in 0.25% PuraMatrix at a cell density of 0.5×10^6 cells/mL. The results show mean \pm standard deviation of 3 parallel experiments performed in triplicate. Different letters represent significant differences between groups in each dilution extract. Analysis of variance followed by Bonferroni's *post hoc* test ($P < .05$).

original *in vivo* characteristics of cell morphology and functionality⁷. 3D cultures of PDLSCs in PuraMatrix may mimic the characteristics of connective tissue better and appear to be a more adequate model for cytotoxicity studies.

PuraMatrix is a synthetic peptide nanofiber scaffold that had also previously been shown to promote growth and proliferation of multiple cell types, including chondrocytes, hepatocytes, endothelial cells, and dental pulp stem cells^{16,17}. In the present

study, PuraMatrix enhanced proliferation of PDLSCs. However, the proliferation index varied with the PuraMatrix concentration and the PDLSC seeding density¹⁰. Depletion of nutrients and contact inhibition of PDLSCs may explain the reduction of proliferation.

To better mimic the *in vivo* situation, the *in vitro* root canal filling model was combined with the 3D PDLSC culture in the present study. The advantages of such an approach consist in using more realistic material amounts and the presence of cement

surrounding dentin that may increase clinical relevance of the results^{5,18}. This model also has some limitations (eg, any dentin in contact with cultured cells *in vitro* must be first sterilized to avoid microbial contamination). In the present study, this was done with gamma irradiation. Heat sterilization used in other studies was likely to denature some proteins contained in dentin, such as transforming growth factor beta^{5,11,18}.

In the present study, PDLSCs grown with BioRoot RCS exhibited increased cell

viability in comparison with PDLSCs grown in 2D culture or encapsulated in PuraMatrix. This favorable biocompatibility is in agreement with recent studies in which BioRoot RCS was also tested in a fresh or set state^{3,19-21}. It may be speculated that BioRoot RCS is not only noncytotoxic but also may release some components to the surrounding tissue that might have a beneficial effect on tissue healing and cell viability and proliferation. To a lesser extent, ProRoot ES also exhibited biocompatibility in 2D and 3D cultures in this study. In contrast, MTA Fillapex significantly decreased PDLSC viability. The cytotoxic effects of MTA Fillapex may be attributed to the presence of resinous components, mainly salicylate resin and silica^{22,23}. Moreover, MTA Fillapex probably has an unbalanced ratio among resin and MTA with higher values for salicylate resin that may explain the higher cytotoxicity and the properties of extended setting and working time, excessive flowability²⁴, and solubility, which were reported in previous studies^{25,26}.

As expected, encapsulation of PDLSCs by PuraMatrix decreased the impact of all of the sealers on the viability of PDLSCs. Indeed, cells behaved more natively when cultured in 3D environments allowing greater stability, longer life spans, and lesser toxin susceptibility than cells in 2D cultures^{7,27}.

This is the first study showing that BioRoot RCS and Pro Root ES did not increase proinflammatory mediators (IL-6, IL-8,

and GRO). IL-6 is a pleiotropic cytokine that acts as a major mediator in the host response after tissue injury, with a stimulatory effect on bone osteoclasts and bone resorption through an autocrine/paracrine manner²⁸. IL-8 and GRO are members of the CXC chemokine family that mediate various functions, such as attracting neutrophils to sites of inflammation and regulating angiogenesis. Therefore, it may be speculated that up-regulation of such proinflammatory cytokines and chemokines may be associated with the inflammatory response, periapical lesions, and an increase in osteoclastogenesis and bone resorption in case of extrusion of the root-filling materials. As expected, MTA Fillapex and AH Plus enhanced the production of proinflammatory mediators and confirmed previous results^{5,29,30}.

BioRoot RCS showed a significant increase in IL-10 and a lesser increase of IL-4 production by PDLSCs. IL-10 is a key anti-inflammatory cytokine that inhibits the production of proinflammatory cytokines including IL-1 β , IL-6, and tumor necrosis factor alpha. IL-4 is another anti-inflammatory cytokine that plays a role in the formation of Th2 lymphocytes and in shifting the macrophage phenotypes into IL-10-producing cells that would be compatible with alternatively activated macrophages/wound healing macrophages (M2)^{31,32}. IL-4 is also known to stimulate fibroblast proliferation and inhibit osteoclast activities³³. Interestingly, the combination of IL-4 and IL-10 induces

endocytic activity by monocytes. Monocytes derived from patients could be induced to become anti-inflammatory using these cytokines³⁴. In a recent study, MTA treatment increased CD206+ cells (M2 marker) in a time-dependent way. After MTA treatment, the expression of M2-related cytokines was up-regulated³⁵. Moreover, PDLSC transplantation is able to induce macrophage polarization toward the M2 phenotype, and the shift in the polarization toward M2 macrophages in the early stage of tissue repair contributed to the enhanced periodontal regeneration³⁶.

Within the limitations of this study, it may be concluded that BioRoot RCS and ProRoot ES showed better cytocompatibility compared with MTA Fillapex and AH Plus that may potentially trigger innate inflammatory response. Besides biocompatibility, BioRoot RCS and ProRoot ES did not induce proinflammatory cytokines and promoted anti-inflammatory cytokine secretion by PDLSCs, which may have a positive local impact by attenuating an initial inflammatory response.

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The authors deny any conflicts of interest related to this study.

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Research Reports: Biological**Immune Cells and Molecular Networks in Experimentally Induced Pulpitis**

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Abstract

Dental pulp is a dynamic tissue able to resist external irritation during tooth decay by using immunocompetent cells involved in innate and adaptive responses. To better understand the immune response of pulp toward gram-negative bacteria, we analyzed biological mediators and immunocompetent cells in rat incisor pulp experimentally inflamed by either lipopolysaccharide (LPS) or saline solution (phosphate-buffered saline [PBS]). Untreated teeth were used as control. Expression of pro- and anti-inflammatory cytokines, chemokine ligands, growth factors, and enzymes were evaluated at the transcript level, and the recruitment of the different leukocytes in pulp was measured by fluorescence-activated cell-sorting analysis after 3 h, 9 h, and 3 d post-PBS or post-LPS treatment. After 3 d, injured rat incisors showed pulp wound healing and production of reparative dentin in both LPS and PBS conditions, testifying to the reversible pulpitis status of this model. *IL6*, *IL1-β*, *TNF-α*, *CCL2*, *CXCL1*, *CXCL2*, *MMP9*, and *iNOS* gene expression were significantly upregulated after 3 h of LPS stimulation as compared with PBS. The immunoregulatory cytokine *IL10* was also upregulated after 3 h, suggesting that LPS stimulates not only inflammation but also immunoregulation. Fluorescence-activated cell-sorting analysis revealed a significant, rapid, and transient increase in leukocyte levels 9 h after PBS and LPS stimulation. The quantity of dendritic cells was significantly upregulated with LPS versus PBS. Interestingly, we identified a myeloid-derived suppressor cell-enriched cell population in noninjured rodent incisor dental pulp. The percentage of this population, known to regulate immune response, was higher 9 h after inflammation triggered with PBS and LPS as compared with the control. Taken together, these data offer a better understanding of the mechanisms involved in the regulation of dental pulp immunity that may be elicited by gram-negative bacteria.

Keywords: dental pulp, innate immune response, lipopolysaccharides, chemokines, cytokines, leukocytes

Introduction

Dental pulp is a highly dynamic tissue equipped with a network of resident immunocompetent cells that may play a major role in the defense against pathogens and during tissue injury (Izumi et al. 1995; Kawashima et al. 2006; Gaudin et al. 2015). Dental pulp is able to mount inflammatory/innate and later adaptive immune responses to fight bacteria and inactivate those of their components that gain access to the pulp during the caries process (Hahn and Liewehr 2007a, 2007b). The cariogenic microflora contains many bacterial species. Gram-positive bacteria greatly dominate the microflora in initial and moderate dentin caries lesions, with the proportion of gram-negative bacteria growing with the progression of tooth decay (Hahn et al. 1989; Love and Jenkinson, 2002; Martin et al. 2002; Chhour et al. 2005; Aas et al. 2008). Pathogen-associated molecular patterns (PAMPs) are released into the dental pulp through dentinal tubules. Odontoblasts, fibroblasts, leukocytes, and stem cells recognize PAMPs through pattern recognition receptors, in particular the Toll-like receptors (TLRs; Tokuda et al. 2001; Durand et al. 2006; Veerayutthwilai et al. 2007; Mutoh et al. 2009; Farges et al. 2011; Liu et al. 2014; He et al. 2015). Lipopolysaccharide (LPS) is an integral component of the membrane of gram-negative bacteria and is recognized by TLR4. The binding of LPS to TLR4 leads to the

development of innate immune responses, either by the MyD88-dependent pathway (He et al. 2014) or by the Trif-dependent pathway. This second pathway induces a type I IFN response (Elson et al. 2007). Type I IFN signaling is also required for TLR4-mediated induction of *IL10*, a potent anti-inflammatory molecule that regulates excessive production of inflammatory cytokines during infection (Iyer et al. 2010).

In the pulpitis process, a low-grade inflammation enables pulp tissue regeneration (tertiary dentinogenesis), whereas more intense and severe inflammation leads to necrosis (Izumi

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et al. 1995; Cooper et al. 2014). To characterize the immune response triggered by the gram-negative bacterial PAMPs in dental pulp, we investigated the expression of proinflammatory and anti-inflammatory mediators in rat incisor dental pulp tissue upon LPS stimulation. The expression of dentin sialophosphoprotein (DSPP), an odontoblastic marker, was also assessed as an indicator of dental pulp regeneration. In addition, the immune cells of the dental pulp were investigated by fluorescence-activated cell-sorting (FACS) analysis. This technique allows identification and quantification of small populations of cells with a set of different markers. We previously showed that total leukocytes represent <1% of the cell population obtained after enzymatic digestion in healthy human pulp, and we established the presence of specific populations of leukocytes involved in immunoregulation (Gaudin et al. 2015). In this study, we used a model of experimentally injured rat pulp to analyze the levels of the key cellular actors in immune response present in this tissue to throw light on the immune response process following gram-negative bacteria aggression, with the final aim of identifying new targets for vital pulp therapies.

Materials and Methods

Experimental Pulpitis Model

Female Sprague-Dawley rats (Charles River Laboratories, Saint-Germain-Nuelles, France) weighing 200 to 250 g were used throughout the study. The animal experiments were approved by the Animal Ethics Committee of the University of Nantes (CEEA No. 00286.01). Induction of pulpitis was performed under general anesthesia with an intramuscular mix of xylazine (8 mg/kg) and ketamine (805 mg/kg) and local anesthesia with periapical injection of articaine as previously described (Kawanishi et al. 2004). The portion of the upper incisor exposed to the oral cavity was removed horizontally with round tungsten steel burs to expose the pulp chamber at the pulp horn, and the entrance of the pulp horn was enlarged with K-files #40 to a length of 1 mm to create space to apply LPS or phosphate-buffered saline (PBS). Five microliters of LPS from *Escherichia coli* O111:B4 (Sigma Chemical Co., St. Louis, MO, USA) diluted in PBS at a final concentration of 10 mg/mL or PBS alone was dropped on the injured pulp, and the cavities were sealed with Cavit (ESPE, Seefeld, Germany). Animals were sacrificed 3 h, 9 h, and 3 d after injury, and the incisors were extracted.

Histologic Analysis

Teeth were fixed in paraformaldehyde (PFA) at 4 °C overnight, decalcified (4% ethylenediaminetetraacetic acid [EDTA], 0.2% PFA) at 50 °C in a KOS Microwave (Milestone, Kalamazoo, MI, USA) for up to 10 d, and then embedded in paraffin and cut into 3- μ m-thick sections for Masson trichrome staining ($n = 4$). Images were created with bright-field microscopy (Nikon Eclipse E600). For the immunohistochemistry ($n = 2$), 3- μ m paraffin sections were stained with a polyclonal goat

anti-Iba1 antibody (Abcam, Cambridge, UK) for macrophage detection (Rehg et al. 2012). A standard ABC method was used for detection with a biotinylated donkey anti-goat secondary antibody and peroxidase-conjugated streptavidin (Dako, Trappes, France) and a DAB substrate as a chromogen (Dako). Negative control without the primary antibody was performed. Counterstaining was carried out with Gill 2 hematoxylin. Stained slides were observed with a virtual microscope (NDP view; Hamamatsu, Japan).

Real-time Polymerase Chain Reaction

RNA extraction and reverse transcription were performed from pulp tissues of nontreated teeth and teeth treated with PBS or LPS ($n = 8$). Total RNAs were isolated with RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. For cDNA synthesis, 1 μ g of total RNA was used as a template for reverse transcription according to the manufacturer's instructions (Invitrogen, Waltham, MA, USA). Primer sequences are compiled in Table 1. Hypoxanthine phosphoribosyltransferase (HPRT) gene was used as endogenous control gene. The analysis was performed with a Viia7TM Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with SYBR Green qPCR Master Mix (Life Technologies, Foster City, CA, USA). The mRNA expression level was defined as the fold change in mRNA levels in a given sample relative to the level of a calibrator. The nontreated pulp was used as calibrator and defined the 1 \times expression of each gene. The mRNA expression level is calculated as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{Target} - Ct_{HPRT})_{sample} - (Ct_{Target} - Ct_{HPRT})_{calibrator}$.

Flow Cytometry

Pulp tissue was minced into small pieces and centrifuged. The tissue was digested in collagenase D (Roche, Paris, France) and incubated at 37 °C. After 45 min, digestion was stopped through 10mM EDTA. Cells were then washed 2 times with PBS supplemented with 2% fetal calf serum and 2mM EDTA. The cell suspension was filtered through a 100- μ m nylon filter. After resuspension in the same buffer, single-cell suspensions (20 million cells) were ready for antibody staining. For each staining, 1 million cells were incubated for 30 min on ice with isotype control or monoclonal conjugated antibodies (Table 2). After 2 washes, cells were fixed with PFA 2%. Cells were acquired with FACS Canto II (BD Biosciences, San Jose, CA, USA), and results were analyzed with Flowjo Software (TreeStar, Inc., Ashland, NC, USA). All results in this study were based on the same gating strategy; nonviable cells and doublets were excluded ($n = 8$). The spleen of healthy rat and isotypic monoclonal conjugated antibodies were used to determine the gate of positive cells in each experiment.

Statistical Analysis

Statistical analysis was performed by a Student's *t* test for FACS analysis data and nonparametric Mann-Whitney *U* test for

Table 1. Primer Sequences Used in the Current Study.

Primers	Forward 5' → 3'	Reverse 5' → 3'	Product Size, bp	Accession No.
HPRT	CCTTGGTCAAGCAGTACAGCC	TTCGCTGATGACACAAACATGA	188	NM_012583.2
IL6	GCAAGAGACTTCCAGCCAGTT	CATCATCGCTGTTACATAATCA	229	NM_012589.2
IL10	ACTGGCTGGAGTGAAGACCA	AAGGCTTGGCAACCAAGTAA	213	NM_012854.2
TNF- α	CTTCTCATTCTGCTCGTGG	GCTACGGGCTTGTCACTCG	174	NM_012675.3
HO-1	CCACAGCTCGACAGCATGTC	GTTTCGCTCTATCTCCTTTCCA	195	NM_012580.2
IL1- β	TCACTCGTGGGATGATGACG	TGTCGTTGCTTGTCTCTCT	201	NM_031512.2
MCPI	GCATCCACGTGCTGTCTCAG	TCTCTCTTTGAGCTTGGTGAC	168	NM_031530.1
INF- γ	AGTGTCTATCGAATCGCACCTG	TTCTGGTGACAGCTGGTGAAT	159	NM_138880.2
IL12p35	TGATGATGACCCTGTGCCTT	GCATGGAGCAGGATACAGAGC	250	NM_053390.1
IL12p40	TCATCAGGGACATCAAAACC	CGAGGAACGCACCTTTCTG	210	NM_022611.1
IL23p19	GGACTCGGACATCTTACAGG	GGAACGGAGAAGAACGCT	178	NM_130410.2
TGF- β	CTCAACACCTGCACAGCTCC	ACGATCATGTTGGACAACCTGCT	349	NM_021578.2
CXCL1	ACCCAAACCGAAGTCATAGCC	TGTCAGAAGCCAGCGTTCAC	173	NM_030845.1
CXCL2	GCCCAGACAGAAGTCATAGCC	TTCTGCCGTTGAGGTACAG	161	NM_053647.1
MMP9	GCTGGGAACTATCTGGAAA	GTTGTGGAAACTCACAGCC	176	NM_031055.1
MMP8	GCAGCTGTTTCTATAACGGG	GTGGTCCACTGAAGAAGAGGAA	187	NM_022221.1
MMP3	CCTCGTGTACCCACCAAT	GGATGGAAGAGACGGCCAAA	199	NM_133523.3
iNOS	GCTCCATGACTCTCAGCACA	TCTTGAACGTAGACCTTGGGT	186	NM_012611.3
DSPP	TCAAATGGGGTGCTTTA	TGCTCACTGCACAACATG	111	NM_012790.2

bp, base pairs.

Table 2. Monoclonal Conjugated Antibodies Used in the Current Study.

Antibody	Fluorochrome	Dilution	Specificity
Ox1-Ox30	Biotinylated+ streptavidin PE/Cy7	1/500 ^e	CD45
Ox12	FITC	1/1,000 ^e	Ig κ chain antibody
Ox42	FITC	1/1,000 ^e	CD11b/c
R7/3	Alexa 488	1/2,000 ^e	TCR $\alpha\beta$
Ox35	PE	1/200 ^e	CD4
Ox8	Alexa 647	1/500 ^e	CD8
3.2.3	Alexa 647	1/1,000 ^e	CD161 (NKRPI)
His48	FITC	1/500 ^e	Granulocyte
Ox62	Alexa 647	1/2500 ^e	CD103 (α E2integrin)
Ox6	PE	1/500 ^e	MHC class II
Iba1	—	1/4,000 ^e	Ionized calcium binding adaptor molecule I

quantitative polymerase chain reaction data through use of GraphPad Prism software (La Jolla, CA, USA). Results are presented as \pm SEM. Differences associated with probability values of $P < 0.05$ were considered statistically significant.

Results

Histology of Pulp Tissue and DSPP Gene Expression

To observe the effects of PAMPs on pulp tissue, histologic and immunohistochemistry observations were carried out on the incisor teeth at different times posttreatment with LPS or PBS. Noninjured teeth were used to perform a control (Fig. 1A, B). In teeth treated with PBS, infiltrated cells were observed close to the injured site as early as 9 h after cavity preparation (Fig. 1D, E), and osteodentin matrix was observed 3 d postinjury (Fig. 1J, K). In rats treated with LPS, the area of infiltration by inflammatory cells and the osteodentin deposition were larger

than in the PBS-treated teeth (Fig. 1G, H, M, N). However, the amount and the distribution of macrophages observed in the odontoblast and subodontoblastic zones were similar in all conditions (Fig. 1C, F, I, L, O). These data reveal that inflammation was limited to the horn of the teeth and disappeared after 3 d. Compared with controls, the expression of the *DSPP* gene increased 3 h after PBS and LPS application and remained significantly upregulated after 3 d. Furthermore, *DSPP* expression was significantly higher in the PBS-injured pulp as compared with LPS at day 3 (Fig. 1R).

RNA Expression of Inflammatory Mediator Genes in LPS-induced Pulpitis

To investigate the molecules involved in the inflammation, the expression of inflammatory mediators was analyzed at the transcript level in the injured pulp (Fig. 2). In contrast with the noninjured teeth and those with the PBS treatment, *IL6*, *IL1- β* , *TNF- α* , *IL10*, an anti-inflammatory cytokine, and *CCL2*,

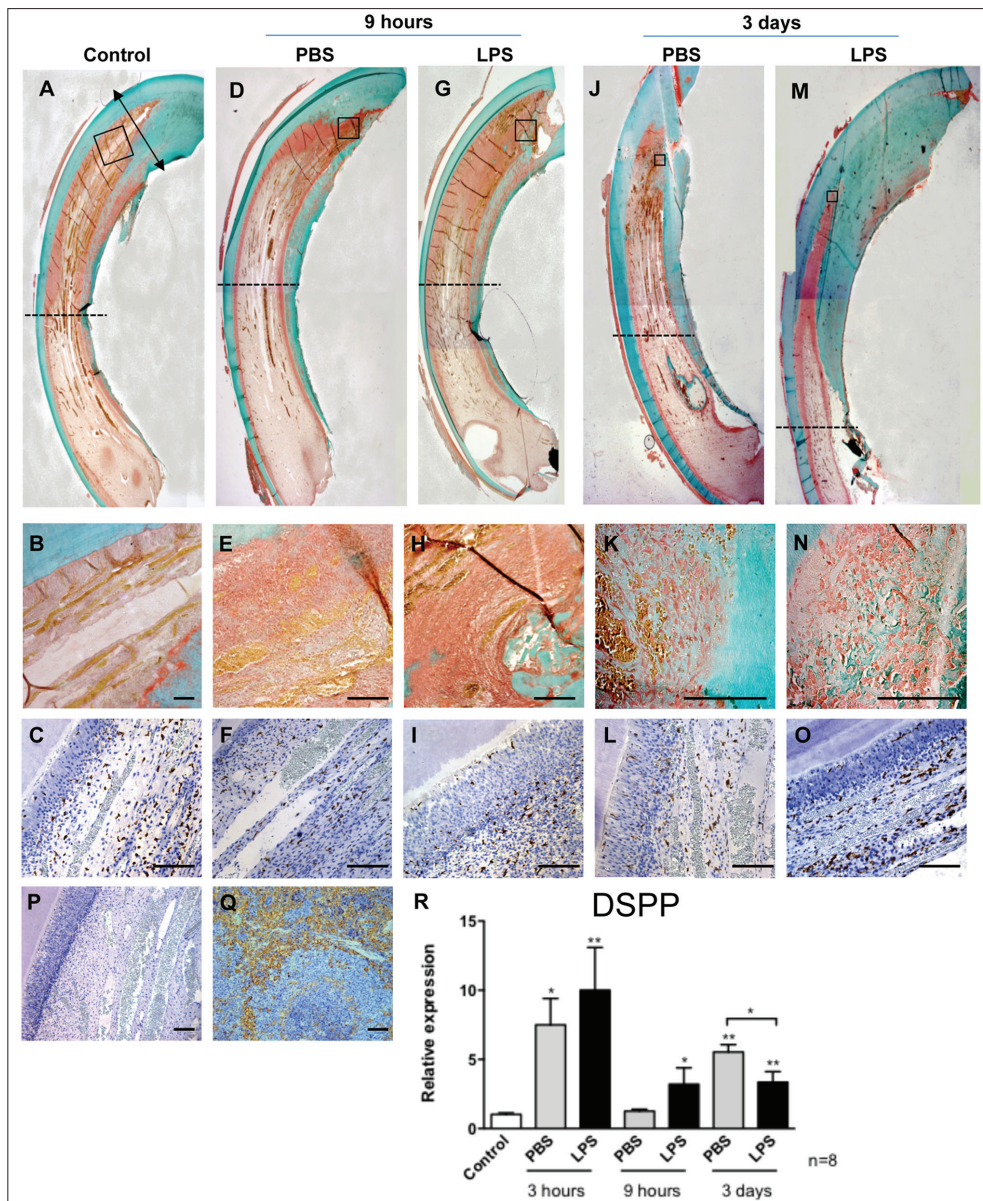


Figure 1. Masson trichrome staining and immunohistochemical analyses of nontreated pulp (control) and experimentally rat pulpitis induced by phosphate-buffered saline (PBS) or lipopolysaccharide (LPS) treatment observed after 9 h and 3 d. On control pulp, the amputated site is shown by an arrow (A). In this area, the box delineates the region shown at higher magnification revealing the odontoblastic palisade (B). Inflammatory infiltrate was observed in the injured portion of the pulp 9 h after treatment with PBS (D: low magnification; E: higher magnification from boxed area in D). Nine hours after treatment with LPS, intense inflammatory infiltrate was observed in a major part of the pulp tissue (G: low magnification; H: high magnification). Three days after PBS treatment, wound-healing tissue with collagenous matrix was observed (J: low magnification; K: high magnification).

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CXCL1, and *CXCL2* chemokine ligands were highly upregulated 3 h after LPS treatment, after which the levels of all these transcripts rapidly returned to the baseline (untreated teeth). The expression of *iNOS* was also dramatically increased 3 h after LPS treatment and decreased by 9 h, although it remained upregulated after 3 d as compared with the untreated teeth. As far as metalloproteinase is concerned, the *MMP3* transcript level was significantly upregulated 9 h and 3 d after LPS treatment. In addition, as compared with PBS, *MMP9* gene expression was significantly higher 3 and 9 h after LPS treatment. Furthermore, in LPS- and PBS-induced pulpitis, the levels of heme oxygenase-1 (*HO-1*) transcripts were significantly higher 3 and 9 h after treatment as compared with control. Interestingly, 3 d after PBS application, *HO-1* expression appeared to be significantly higher than that of LPS. Finally, expression of *IL12p35*, *IL12p40*, *IL23p19*, and *TGF- β* was always at the same level as control in PBS- and LPS-injured pulp during the experimentation (Fig. 2).

Flow Cytometric Analysis of Immunocompetent Cells in Rat Incisor Pulp

We then analyzed the percentage of leukocytes 9 h and 3 d after LPS application on the pulp horn as compared with PBS and control (untreated teeth). Dot plots of 1 representative experiment out of 8 were shown, and statistical analyses were performed either on the percentages of cells in total viable cells or on total viable cells and T cell population (Figs. 3, 4; Appendix Fig.). FACS analysis revealed a significant increase of leukocytes (Ox1-Ox30⁺ cells) 9 h after treatment with LPS and PBS when compared with control. The quantity of these cells returned to the baseline after 3 d (Fig. 3). Within the leukocyte population, a significant accumulation of myeloid cells (Ox42⁺) was in evidence 9 h after treatment with LPS and PBS versus control. More precisely, percentages of granulocytes (His48⁺) were significantly upregulated 9 h after treatment with LPS versus the untreated pulp. In addition, dendritic cell (Ox62⁺) population was significantly increased under LPS treatment as compared with PBS and control at the same time point. In contrast, the percentage of T cells (R7/3⁺) and natural killer cells (R7/3⁺ 3.2.3^{high}) remained unchanged, whereas in this model of pulpitis, the percentage of B cells (Ox12⁺) was increased only after 9 h of PBS exposure as compared with control. In addition, we identified, for the first time to our knowledge, a myeloid-derived suppressor cell (MDSC)-enriched cell population in dental pulp (R7/3⁺ 3.2.3^{low} subset). MDSCs are heterogeneous mixtures of myeloid cells at different stages of differentiation, including precursors of granulocytes, macrophages, dendritic cells, and early myeloid progenitors (Dugast et al. 2008). In our study, MDSC-enriched cell populations

were significantly higher 9 h after LPS and PBS treatments as compared with control, and they returned to the baseline after 3 d.

Discussion

This study gives details of an in-depth analysis of LPS-induced innate immune response in incisor rat dental pulp. Rodent incisors are continuously erupting teeth with high healing capability after pulp injury. They have been used to evaluate variations in biological factors in acute pulpal inflammation (Kawanishi et al. 2004; Kawashima et al. 2005; Zheng et al. 2009). We chose this model because it can provide the isolation of adequate cell amounts for the detection of subsets of immune cells by FACS analysis, which would not be possible with molars. The formation of tertiary dentine and the increase in DSPP transcripts confirm the reversible status of the induced pulpitis with PBS or LPS treatment. As the quantity of osteodentin deposit was greater in LPS compared with PBS, the model offers the opportunity to identify biological parameters induced by gram-negative bacteria.

LPS represents one of the most potent activators of the human innate immune system. It is well established that numerous cytokines are induced in response to LPS (TLR4 agonist). TLR4 activation leads to the induction of inflammatory genes, such as *TNF*, *IL6*, and *IL1- β* (through My-D88 dependent signaling pathway) and to the induction of the anti-inflammatory molecule *IL10* (through the Trif-dependent pathway; Iyer et al. 2010). In the present study, we demonstrate that the inflammatory/immune response of pulp injury with LPS is faster and stronger than without LPS (PBS), in concord with previous observations in human and rat pulp under bacterial infection (Hosoya and Matsushima 1997; Bletsas et al. 2006). In fact, in contrast to PBS treatment, proinflammatory cytokines (*IL6*, *IL1- β* , *TNF- α*) and chemokines (*CXCL1*, *CXCL2*, *CCL2*), as well as *iNOS*, the enzyme responsible for high nitric oxide production in infection settings, peaked after 3 h in response to LPS injury and decreased thereafter. The initiation of proinflammatory mediator production is likely to be due to direct activation by LPS and/or be related to the nitric oxide synthesis, as previously shown in the same model (Kawashima et al. 2005). Furthermore, *CCL2* and *IL1- β* were upregulated both under caries lesions and upon stimulation with pathogens (Durand et al. 2006; Silva et al. 2009). *IL10* is not expressed in healthy human pulp but is strongly upregulated in irreversible pulpitis (Farges et al. 2011). In our study, we show that *IL10* transcripts are also greatly increased under LPS treatment as compared with PBS. *IL10* has numerous immunomodulatory effects, including the inhibition of proinflammatory cytokine production and the inactivation of macrophages (Tokuda et al. 2002; Iyer et al. 2010).

magnification from boxed area in J). After 3 d with LPS, irregular osteodentin formation was observed in the pulp chamber (M: low magnification; N: high magnification). Micrographs are representative of 4 separate experiments. Immunohistochemical staining of the Iba1 marker observed in the area at equal distance between the coronal and apical limits of the incisors (dotted lines), where the odontoblastic layer is present, revealed a similar distribution of macrophages under all conditions (C, F, I, L, O; n = 2). Scale bars: 50 μ m. No staining was detected in negative control (P), and typical features of macrophages from rat spleen were observed in positive control (Q). *DSPP* transcript expression was studied by quantitative polymerase chain reaction 3 h, 9 h, and 3 d after treatment with PBS or LPS (R; n = 8 per condition). *DSPP* gene was significantly upregulated as early as 3 h until 3 d under PBS and LPS treatments as compared with the control (noninjured pulp). * $P < 0.05$. ** $P < 0.001$. DSPP, dentin sialophosphoprotein.

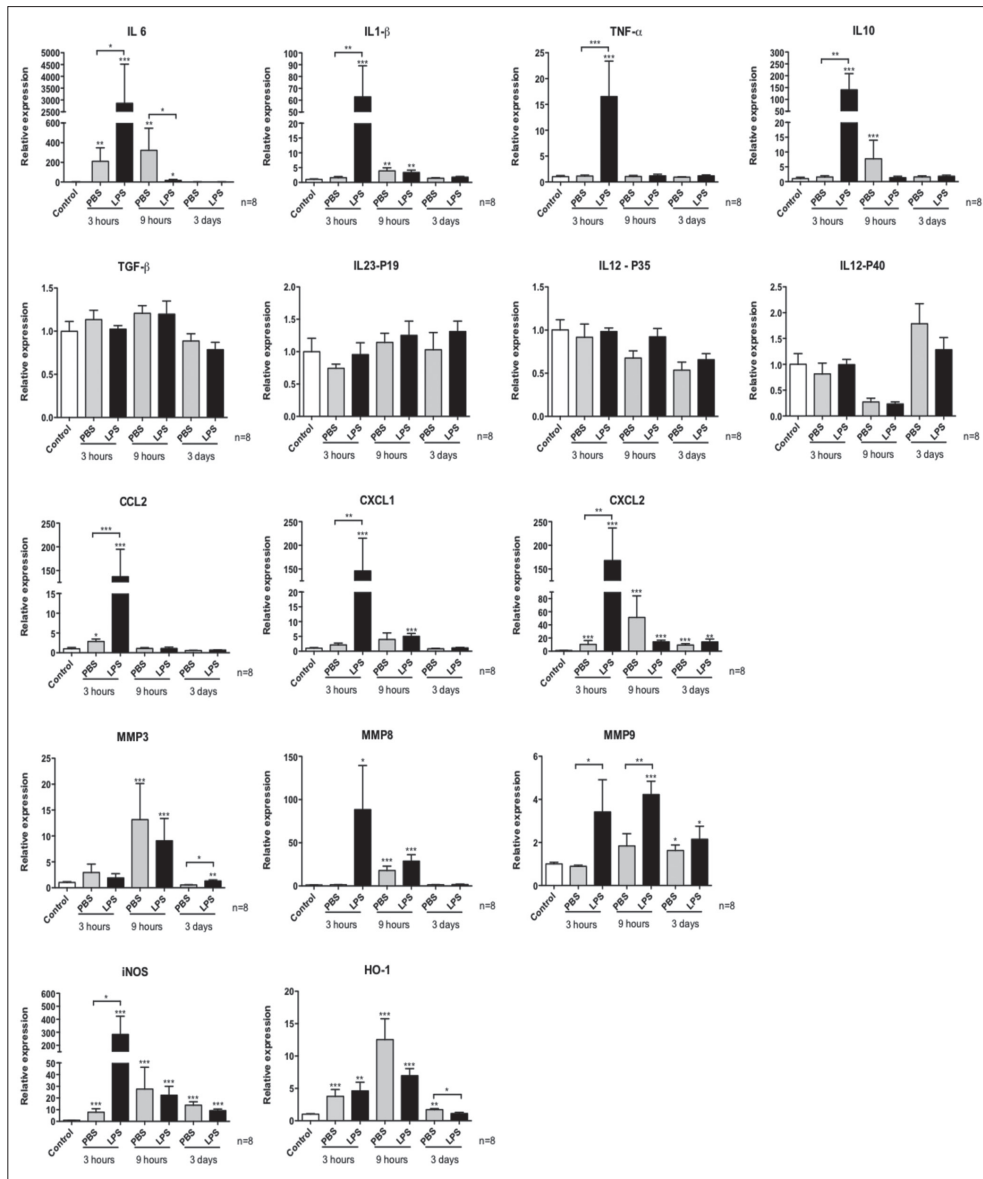


Figure 2. Analysis of transcripts of cytokines (IL6, IL1- β , TNF- α , IL10, TGF- β , IL23, IL12), chemokines (CCL2, CXCL1, CXCL2), and metalloproteinases (MMP3, MMP8, MMP9) as well as iNOS and heme oxygenase-1 (HO-1), by real-time reverse transcription polymerase chain reaction, 3 h, 9 h, and 3 d after phosphate-buffered saline (PBS) or lipopolysaccharide (LPS) treatment, compared with control (noninjured pulp). LPS significantly increased *IL6*, *IL1- β* , *TNF- α* , and *IL10* as well as *CCL2*, *CXCL1*, *CXCL2*, *MMP9*, and *iNOS* gene expression as compared with control and PBS as early as 3 h after treatment. *IL6* was the most upregulated. *iNOS* was upregulated versus control for each time and each condition. *TGF- β* , *IL23*, and *IL12* did not exhibit any modification of expression during experiment. *MMP3* transcripts increased after 3 d under LPS versus PBS, whereas *MMP9* was stimulated by LPS after 3 and 9 h versus PBS. Compared with control, *HO-1* gene expression was increased 3 h after PBS and LPS treatment and peaked after 9 h, and HO-1 was stimulated only by PBS treatment at day 3 ($n = 8$). * $P < 0.05$. ** $P < 0.001$. *** $P < 0.0001$.

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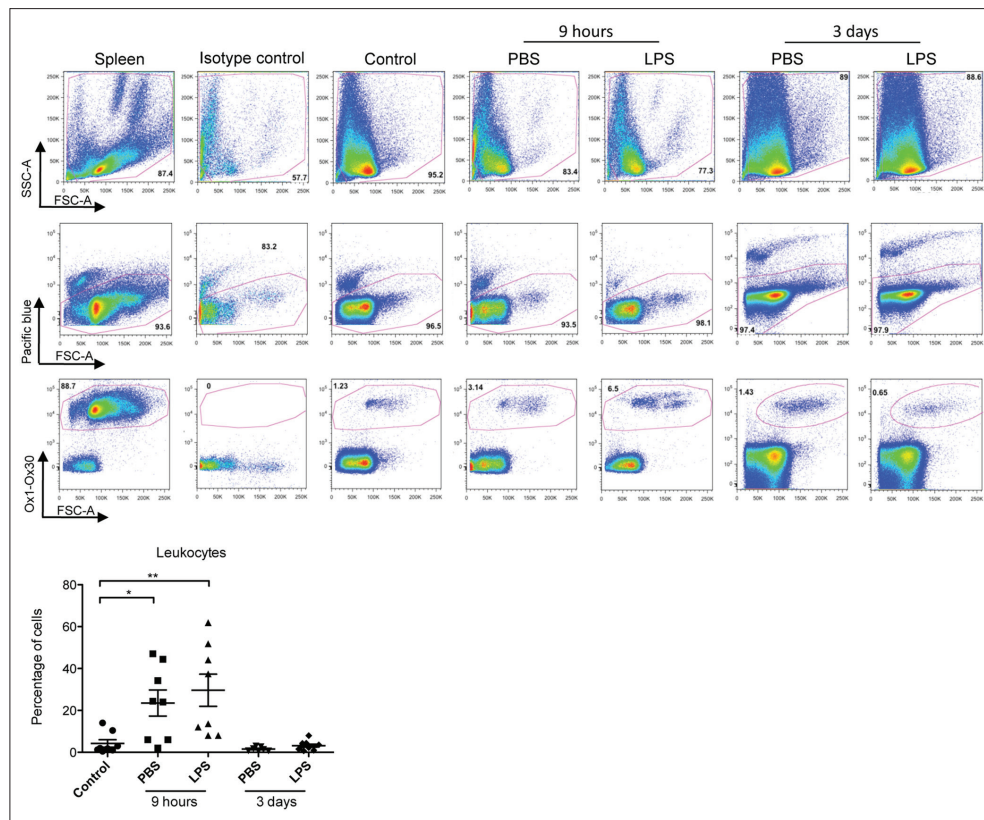


Figure 3. Percentages of leukocytes (Ox1-Ox30⁺) in rat incisors in nontreated animals (control) and in experimentally induced pulp inflammation analyzed by fluorescence-activated cell-sorting, 9 h and 3 d after phosphate-buffered saline (PBS) or lipopolysaccharide (LPS) treatment. Representative fluorescence-activated cell-sorting dot plots are shown for each condition. Gating strategy was used to eliminate dead cells (Pacific Blue–positive cells). Dot plots showing cells stained with Ox1-Ox30⁺ antibody and the appropriate fluorochrome-conjugated isotype control IgG are depicted. Cells obtained from the spleen of untreated rat were used as positive controls. Graph represents mean \pm SEM from 2 independent experiments (4 animals per experimental condition in each experiment; $n = 8$). * $P < 0.05$. ** $P < 0.001$.

IL12 and IL23 control the balance between immunity and immunopathology in patients with inflammatory and infectious diseases (Hoeve et al. 2006), and a previous study described an increase of TGF- β in human irreversible pulpitis (Piatelli et al. 2004). This growth factor controls the initiation and resolution of inflammation through the regulation of the activation, chemotaxis, and survival of leukocytes (Li et al. 2006). *IL12*, *IL23*, and *TGF- β* gene expression was not modified in our study. However, rodent incisors are continuously erupting teeth; as such, the physiology of their pulp is different from that in mature erupted teeth. As a result, the injury responses could be quite specific to this model (Kawanishi et al. 2004; Eba et al. 2012).

While MMPs have long been known to increase inflammation and the associated tissue damage, they can also dampen

inflammatory processes and promote wound healing and tissue remodeling (Parks et al. 2004). Zheng et al. (2009) analyzed MMP3 and MMP9 in rat incisor dental pulp in a reversible pulpitis model without LPS, and in accord with them, in our study, under PBS treatment, the MMP3 expression is upregulated at 9 h and then returns to the baseline, whereas MMP9 is upregulated (albeit to a lesser extent) 3 d after pulp injury. Results with PBS alone could be partially explained by the fact that exposed pulps were sealed with an interim restoration material with hygroscopic property. Interestingly, we show that LPS enhances *MMP9* gene expression at 3 and 9 h and *MMP3* gene expression 3 d after injury. Considering that topical application of MMP3 exhibits an anti-inflammatory effect in a canine mature pre-molar mild irreversible pulpitis model (Eba et al. 2012) and induces angiogenesis and tertiary dentinogenesis on injured rat teeth

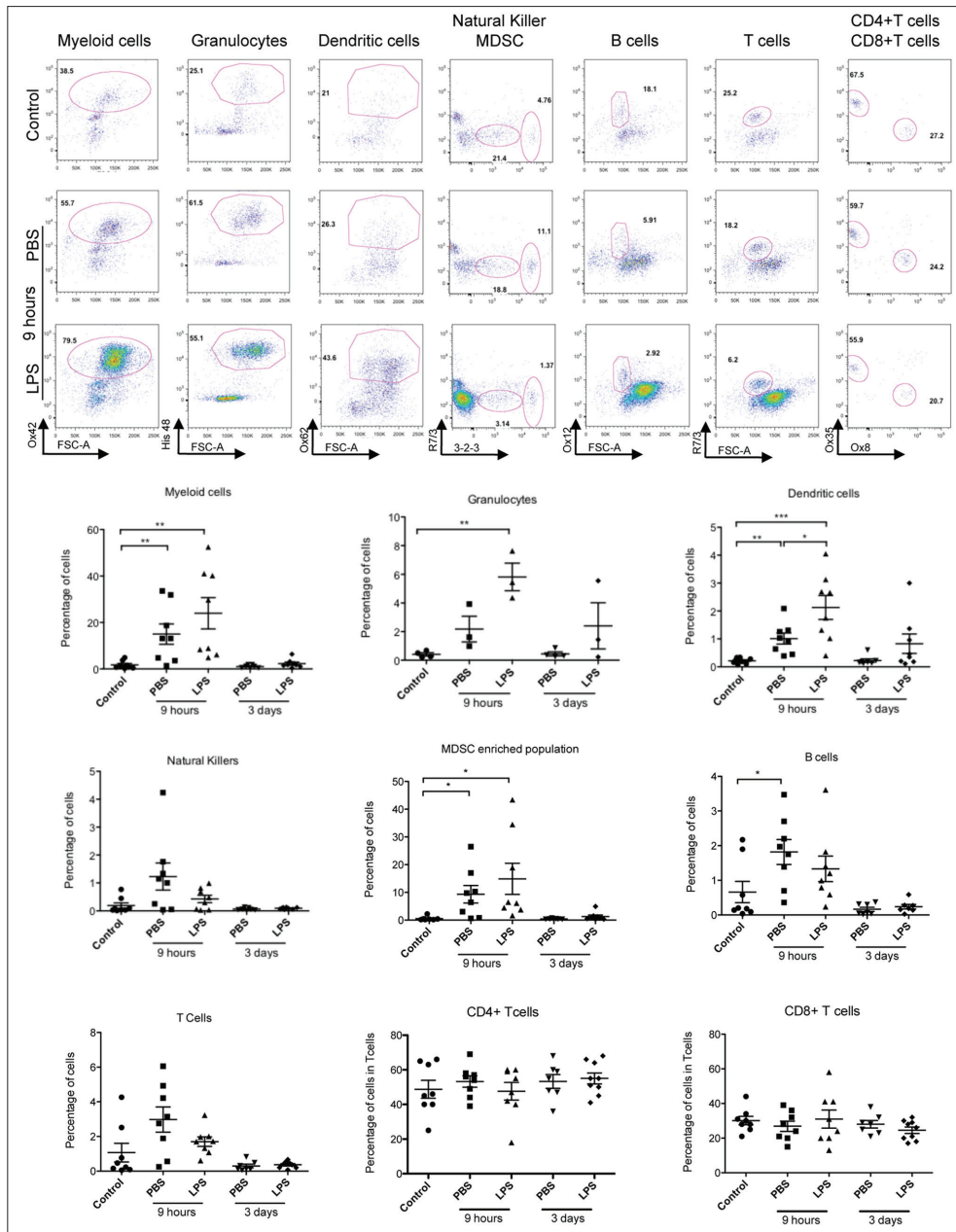


Figure 4. Relative repartition of key immunocompetent cells in nontreated rat incisors (control) and injured teeth 9 h and 3 d after treatment with phosphate-buffered saline (PBS) or lipopolysaccharide (LPS). Flow cytometry analysis was performed for the characterization and quantification of myeloid cells (Ox42⁺), granulocytes (His48⁺), dendritic cells (Ox62⁺), natural killer cells (R7/3⁺ 3.2.3^{high}), myeloid-derived suppressor cell (MDSC)–

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(Zheng et al. 2009), this augmentation of MMP3 by LPS could play a critical role in pulp wound healing. In addition, for the first time, we have shown that there is an increase in MMP8 during dental pulp-induced inflammation with PBS and LPS, without understanding at the moment its exact pro- or anti-inflammatory role.

HO-1 is an isoform of the enzyme that catabolizes the degradation of heme to biliverdin. HO-1 is induced by many stimuli, including oxidative stress, LPS, proinflammatory cytokines, and anti-inflammatory stimuli. A growing body of evidence points toward the capacity of HO-1 to inhibit immune reactions (Blancou et al. 2011). Studies on human dental pulp cells have demonstrated the cytoprotective effect of HO-1 in vitro with its contribution to odontoblast differentiation (Lee et al. 2013). In our study, HO-1 was first detected 3 h after treatment with LPS and PBS, then peaked at 9 h and decreased thereafter. HO-1 was significantly upregulated at 3 d under PBS as compared with LPS stimulation, suggesting that physical irritation (without PAMPs) is sufficient for the induction of HO-1.

In human, caries has been shown to induce an increased number of neutrophil granulocytes, macrophages, DCs, T cells, and B cells (Izumi et al. 1995; Hahn and Liewehr 2007a; Bruno et al. 2010). In our study, we evaluated the effect of LPS on these key immune cells. We observed that leukocytes, as early as 3 h, were significantly increased in pulp tissue injured with LPS and PBS, returning to the baseline after 3 d. Analysis of the different populations of innate effector immune cells indicated that the percentage of T cells and natural killer cells remained unchanged during the experiment. These results suggest that a single application of LPS does not increase the number of these cells in immune response. Furthermore, B-cell accumulation was observed only in response to surgical procedure without LPS. However, we observed an upregulation of myeloid cells after PBS and LPS application. More precisely, we saw that granulocytes levels were higher 9 h after treatment with LPS compared with control. Moreover, LPS treatment induced a higher accumulation of DCs in comparison with PBS, probably in response to CXC chemokines and cytokines. In our model, LPS acts as an activator of the inflammatory/innate immune response, yet repeated irritation could be essential to elicit an adaptive immune response.

MDSCs are populations of heterogeneous cells with a remarkable ability to regulate immune responses (Dugast et al. 2008; Drujont et al. 2014), and the MDSC population that is expanded after LPS exposure regulates alloreactive T cells in an HO-1- and IL10-dependent manner (De Wilde et al. 2009). To our knowledge, we describe for the first time the presence of an MDSC-enriched cell population in noninjured rodent incisor pulp. Additionally, LPS and PBS induced an accumulation of this cell population in inflamed pulp 9 h after treatment. Further studies are, however, needed to evaluate their immunomodulatory capacity in dental pulp.

Taken together, our data show that LPS enhances and regulates inflammation in a model of rodent reversible incisor pulpitis. We have characterized and quantified inflammatory/innate immune cells in response to the TLR4 agonist. As such, we have identified for the first time an MDSC-enriched cell population that could play a crucial role in the resolution of the inflammatory/innate response and enable repair processes. This study is a first step toward the identification of molecular or cellular targets for new therapeutic agents in mild reversible pulpitis in immature teeth.

Author Contributions

E. Renard, A. Gaudin, contributed to conception, design, data acquisition, analysis and interpretation, drafted the manuscript; G. Bienvenu, contributed to design, data acquisition, analysis and interpretation, critically revised the manuscript; J. Amiaud, contributed to data acquisition, drafted the manuscript; J.C. Farges, contributed to data interpretation, critically revised the manuscript; M.C. Cuturi, contributed to conception, data analysis, and interpretation, critically revised the manuscript; A. Moreau, B. Alliot-Licht, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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← enriched population (R7/3⁺ 3.2.3^{low}), B cells (Ox12⁺), T cells (R7/3⁺), CD8⁺ T cells (R7/3⁺ Ox8⁺), and CD4⁺ T cells (R7/3⁺ Ox35⁺) in the leukocyte (Ox1-Ox30⁺) population. For each cell population and condition, representative fluorescence-activated cell-sorting dot plots of the animal sample, 9 h after treatment, presented in Figure 3 are shown. Graphs represent mean ± SEM; n = 8. For each population, the percentage of cells was defined by the percentage of cells expressing the specific markers related to the percentage of living leukocytes (Ox1-Ox30⁺ cells gated). For CD4⁺ T cells and CD8⁺ T cells, the percentages were obtained from the percentage of positive cells gated on T cells (R7/3⁺; n = 8). *P < 0.05. **P < 0.001. ***P < 0.0001.

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9. FICHE DE SYNTHÈSE

9.1 RESUME DES TRAVAUX

Mes activités de recherche depuis mes débuts en 2009 et jusqu'à maintenant se sont majoritairement intéressées à la **régulation de l'inflammation pulpaire**. Tout d'abord par l'identification des cellules et molécules immunorégulatrices au sein de la pulpe dentaire lors de mon doctorat réalisé au sein de l'équipe 1 de l'unité INSERM 1064 entre 2011 et 2015 (Directeur de thèse : Pr Brigitte Alliot-Licht, PU-PH, Co-directeur de thèse : Pr Cristina Cuturi-Anegón DR).

Ensuite, lors de ma mobilité en 2016-2017, j'ai pu initier des travaux sur les **médiateurs lipidiques spécialisés** dans la résolution de l'inflammation. Afin d'envisager des dispositifs permettant d'utiliser ces molécules et après discussions avec mon directeur de recherche actuel le **Pr Pierre Weiss**, j'ai ensuite rejoint l'unité Inserm UMRS 1229-RMeS. Le co-encadrement de la thèse de Pierre Tournier m'a permis de me familiariser avec les équipes et les plateformes de recherche de mon laboratoire d'accueil. L'environnement propice pour développer ma thématique de recherche au sein de l'unité Inserm UMRS 1229-RMeS m'a permis de diriger plusieurs projets dont la finalité reste la régulation de l'inflammation pulpaire afin de promouvoir la guérison et la régénération du tissu pulpaire. Cette thématique appartient au **groupe INFLAMED** (Médiateurs inflammatoires et microenvironnement osseux et dentaire) nouvellement créé dans l'équipe REGOS de l'unité Inserm UMRS 1229-RMeS dont je suis le responsable.

L'ensemble de mes travaux de recherche (fondamental, clinique et pédagogique) m'a permis de générer **22 articles internationaux originaux et de revues, dont 8 en premier auteur, 10 en dernier auteur. J'ai également pu contribuer à l'encadrement d'1 doctorat d'université soutenu, 1 en cours et 3 masters 2**. Mes projets de recherches passés et actuels ont bénéficié de financements, notamment de l'ANR, de la société européenne d'endodontie, de la société française d'endodontie et de l'institut français pour la recherche en odontologie. Je coordonne actuellement une ANR jeune chercheur dédiée au design et à l'évaluation d'un nouveau microgel biphasique à libération de LXA4 dans le cadre de l'ingénierie tissulaire.

9.2 SYNTHESE DES ENCADREMENTS

Encadrements doctoraux

Tableaux synoptiques des co-encadrements doctoraux

<i>Doctorants co-encadrés</i>	<i>Directeurs de thèse</i>	<i>Année de soutenance</i>	<i>Nombre de publications co-signées</i>	<i>Dernier auteur</i>
D. Aubeux	Valérie Geoffroy/Fabienne Pérez	2	2
P. Tournier	Pierre Weiss/Jérôme Guicheux		2	2

Détails des co-encadrements doctoraux

2020-2025 : **Davy Aubeux**. Sujet : Évaluation *in vitro* et *in vivo* de microgels injectables de HPMC silylée en tant que système d'administration multimodal de médicaments pour l'ingénierie tissulaire.

Financement : projet BILIPOX (expérimentations) et auto-financement (thésard)

Co-encadrant (50%). Directrices de thèse : Valérie Geoffroy/Fabienne Pérez

Publications :

- Aubeux D, Peters OA, Hosseinpour S, Tessier S, Geoffroy V, Pérez F, **Gaudin A**. Specialized pro-resolving lipid mediators in endodontics: a narrative review. BMC Oral Health. 2021;21:276. doi:10.1186/s12903-021-01619-8.
- Aubeux D, Renard E, Pérez F, Tessier S, Geoffroy V, **Gaudin A**. Review of Animal Models to Study Pulp Inflammation. Front Dent Med. 2021;2 May:21. doi:10.3389/fdmed.2021.673552.

2016-2019 : **Pierre Tournier**. Sujet : Caractérisation et amélioration des propriétés ostéogéniques d'un nouveau substitut osseux allogéniques

Financement : thèse CIFFRE société BIOBank SAS

Co-encadrant (50%). Directeurs de thèse : Pierre Weiss/Jérôme Guicheux

Devenir : post doctorant unité Inserm UMRS 1229-RMeS

Publications :

- Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, Bardonnnet R, Blanquart C, Corre P, Geoffroy V, Weiss P, **Gaudin A**. An Extrudable Partially Demineralized Allogeneic Bone Paste Exhibits a Similar Bone Healing Capacity as the “ Gold Standard ” Bone Graft. *Front. Bioeng. Biotechnol* 2021, 9, 1–10. doi:10.3389/fbioe.2021.658853.
- Tournier P, Guicheux J, Paré A, Maltezeanu A, Blondy T, Veziers J, Vignes C, André M, Lesoeur J, Barbeito A, Bardonnnet R, Blanquart C, Corre P, Geoffroy V, Weiss P, **Gaudin A**. A partially demineralized allogeneic bone graft: in vitro osteogenic potential and preclinical evaluation in two different intramembranous bone healing models. *Sci. Rep* 2021, 11, 1–13. doi:10.1038/s41598-021-84039-6.

Encadrements de Master 2

2020-2021 **Florian Segeron**. (Étudiant pharmacie). Master 2 Sciences du Médicament et des Produits de Santé parcours BMTI - université de Nantes. Soutenance prévue en septembre 2021.

2019-2020 **Cassandra Lepetit**. Master 2 – Biologie Moléculaire et Cellulaire (BMC). Université de Rennes 1. Devenir : technicien de recherche à Porsolt (laboratoire de pharmacologie préclinique).

2018-2019 **Houman Noela Danielle Kacou**. Master 2 mention Biologie – Santé

Parcours Recherche clinique. UIC 11. Devenir : étudiante en médecine