

Bio-based elastomeric composites for antibacterial and antifouling applications: methodology for the synthesis and grafting of functionalized oligomers issued from natural rubber

Thi Nguyet Tran

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Par **« Thi Nguyet TRAN »**

« Bio-based elastomeric composites for antibacterial and antifouling applications. Methodology for the synthesis and grafting of functionalized oligomers issued from natural rubber »

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Table of Contents

CHAPTER	I. Natural rubber and methodologies to obtain functional telechelic oligomers	. 10
I.1 Int	troduction	. 11
I.2 Na	atural rubber	. 11
I.2.1	Specifications, industrial applications and world context	. 11
I.2.2	Rubber Producing Plants	. 14
I.2.3	Biosynthesis pathways in Hevea brasiliensis	. 16
I.3 Sy	nthesis of acrylate telechelic oligomers via chemical modification of natural rubber	. 18
I.3.1	Bibliography	. 18
I.3.1. telecl	1 Chemical degradation of natural rubber: existing methods for the synthesis of helic liquid natural rubber	19
I.3.1.	2 Reactivity and reactions of telechelic liquid natural rubber	. 24
I.3.2 differen	Synthesis of acrylate telechelic natural rubber, optimization of reaction conditions for nt targeted molecular weights	25
I.3.2.	1 Controlled degradation of natural rubber using periodic acid	. 26
I.3.2.	2 Synthesis of hydroxyl telechelic natural rubber	28
I.3.2.	3 Synthesis of Acrylate Telechelic Natural Rubber	. 29
I.4 Co	onclusions	. 31

CHAPTER II. Natural Rubber based materials integrating a new guanidine monomer for II.1 II.2 II.2.1 II.2.1.1 II.2.1.2 II.2.1.3 II.2.2 Synthesis and Mechanism of action of Cationic Antimicrobial Polymers 43 II.2.2.1 II.2.2.2 Factors Affecting Behavior of Cationic Antimicrobial Polymers 44

II po	.2.2.3 Why guanidine-based polymers are an attractive new class of cationic antimicrobial olymers?	i 50
II.2.	3 Natural Rubber based Cationic Antimicrobial Polymers	52
II.3	Conclusions	55
II.4 guanid	Submitted paper: Antibacterial activity of natural rubber based coatings containing a new inium-monomer as active agent	57
II.5	Supporting Information : Submitted paper	€

CHAPT	ER III. 1	Natural Rubber based films integrating a new monomer derived fro	m Zosteric
	ew Appro	ach to Reduce Biofouling	
111.1	Introduc	ction	
111.2	Bibliogi 104	raphy: Zosteric acid, a new promising anti-biofilm compound coming fr	om seagrass
III.	2.1 An	tifouling properties and toxicity of Zosteric Acid	
III. its a	2.2 Me analogs	echanism of action and the structure-activity relationships (SARs) study	of ZA and 108
III.3	Natural	Rubber integrating a new monomer issued from Zosteric acid	
III.	3.1 Но	w to design and synthesize new monomers derivated from Zosteric acid	for
anti	ifouling p	roperties	
Ι	II.3.1.1	Synthesis of <i>p</i> -CAA Monomer (1)	
Ι	II.3.1.2	Synthesis of <i>p</i> -CAB Monomer (2)	
Ι	II.3.1.3	Conclusions	
III.	3.2 Pre	eparation of thick films: formulations and kinetic study of the photopoly.	merization
III.	3.3 Lea	aching test results	
III.	3.4 Co	atings thermal properties and surface contact angle.	
III.	3.5 Fil	ms activity against pathogenic bacteria	
III.	3.6 Ex	perimental part	
Ι	II.3.6.1	Synthesis of <i>p</i> -CAA Monomer (1)	
Ι	II.3.6.2	Synthesis of <i>p</i> -CAB Monomer (2)	
Ι	II.3.6.3	Films preparation procedure	
Ι	II.3.6.4	Leaching tests protocole	
Ι	II.3.6.5	Biological assays	
III.4	Conclus	ions	

CHAPTER IV. Functionalization and covalent grafting of Polyisoprene Oligomers from
Natural Rubber to Surfaces
IV.1. Introduction
IV.2. Polymers at interfaces for coating applications
IV.1.1 State of the art
IV.1.1.1 Physisorption versus Chemisorption147
IV.1.1.2 Conformation of Oligomers and Polymers with a chain-end grafted at a surface148
IV.1.2 Bifunctional silane coupling agents for grafting polymer chains to surfaces
IV.1.2.1 Silanization and grafting of silane coupling agents to silicon-based material surfaces.149
IV.1.2.2 Molecular structure of silane coupling agents to obtain reactive surface coatings.151
IV.3. Thin coating characterization methods: X-ray reflectivity
IV.1.3 Basic concepts for XRR analysis
IV.1.4 How to "read" X-ray reflectivity data
IV.4 Submitted paper: Natural Rubber covalent coatings. New bio-based isoprene-oligomers for grafting polyisoprene molecular layers and thick films
IV.5 Supporting Information : Submitted paper

Abbreviations

ACLC: acryloyl chloride AcOH: Acetic acid ACTNR: Acrylate Telechelic Oligomer **AFM**: Atomic Force microscopy **APDMES**: 3-aminopropyl-(ethoxy) dimethylsilane **APMDES:** 3-aminopropyl-(diethoxy) methylsilane **APS**: 3-aminopropylalkoxysilanes **APTES:** 3-aminopropyltriethoxylsilane **ATR**: attenuated total reflection ATR-FTIR: Attenuated Total Reflection Fourier-transform Infrared CAPs: cationic antimicrobial polymers **CD₃OD**: methanol- d_4 **CDCl₃**: chloroform-*d* CTNR: Carbonyl Telechelic Oligomer **D**: distance **DBTL**: dibutyltin dilaurate DCC: N,N'-Dicyclohexylcarbodiimide **DCM**: dichloromethane **DCM**: Dichloromethane **DIAD**: Diisopropyl azodicarboxylate **DMAP**: Dimethylallyl pyrophosphate DMF: N, N-diméthylformamide DMSO: dimethylsulfoxide **DOSY:** Diffusion Ordered Spectroscopy **DSNR**: Dibutylphosphate supported Natural Rubber

E.Coli : Escherichia coli **ENR**: Epoxidized Natural Rubber EtOAc: Ethyl acetate **EtOH**: Ethanol **GPC**: Gel Permeation Chromatography h: Thickness HDDA: Hexamethylenediacryate HPLC: High Performance Liquid Chromatography HTNR: Hydroxyl Telechelic Oligomer **IPP:** Isopentenyl pyrophosphate **IR**: Infrared spectroscopy LNR: Liquid Natural Rubber MALDI-TOF MS: Matrix Assisted Laser Desorption Time of Fight Mass Spectroscopy **MAPTES**: Methacryloxylpropyl triethoxysilane **MEMS**: Microelectromechanical systems MIC: Minimum inhibitory concentration **MMS**: α-methacryloxymethyltrimethoxy silane MNR: Maleated Natural Rubber \overline{M}_n : Number Average Molecular weight MsCl: Mesyl chloride (1) NA: Avogadro's number NMR: Nuclear magnetic resonance NPAs: Natural products antifoulant **Oligo-DiSiHTNR**: Silano Diethoxyl Hydroxyl

Téléchelique Natural Rubber Oligomer

Oligo-MonoSiHTNR: Silano Monoethoxyl Hydroxyl Natural Rubber Oligomer **Oligo-SiHTNR**: Silano Hydroxy Natural Rubber Oligomer Oligo-TriSiHTNR: Silano Triethoxyl Hydroxyl Natural Rubber Oligomer PA: Pseudomonas aeruginosas, **p-CA**: *Para*-coumaric acid *p*-CAA: *Para*-coumaric A monomer *p*-CAB: *Para*-coumaric B monomer **PETA**: Pentaerythritol acrylate **PHMB**: Polyhexamethylene biguanidine hydrochloride **PHMG**: Polyhexamethylene guanidine hydrochloride **PI**: Polyisoprene **PNR**: Purified Natural Rubber **PS**: Polystyrene **PVC**: Polyvinyl chloride **QAC**: Quaternary ammonium compounds **QAS**: Quaternary ammonium salts **QPS**: Quaternary phosphonium salts **R**_F: Reflected intensity R_{g} : Radius of gyration of the polymer chains **Rpm**: Round per Minute **RT**: Room temperature **SA**: *Staphylococcus aureus* **SAM**: Self-assembled monolayer **SARs**: Structure-activity relationships

SE: Staphylococcus epidermidis **SEC** Size exclusion chromatography **SLD**: Scattering length **TBAF**: Tetra-*n*-butylammonium fluoride **TBS**: *Tert*-butyldimethysilyl ether TBSCI: Tert-butyldimethylsilyl chloride TFA: 2,2,2-trifluoroacetic acid T_g: Glass transition temperature **TGA**: Thermogravimetric Analyzer **THF**: Tetrahydrofuran **TLC**: Thin-layer chromatography TLNR: Telechelic Liquid Natural Rubber T_{max}: Maximum temperature **XPS**: X-Ray photoelectron spectroscopy **XRR**: X-Ray reflectivity ZA: Zosteric acid σ : Surface grafting density **ρ**: Density

Introduction

1. Background

Biofouling or biocontamination is the colonization of a substrate by a range of micro- and macroorganisms and by their by-products [1-5]. It is recognized as a major problem for numerous applications such as biomedical implants and devices [6-8], biosensors, textiles [9], food packaging, food storage, water purification systems[10, 11], industrial and marine equipment [12].

The topic of antimicrobial properties in medical materials.

In the hospitals, pathogenic bacteria are a large problem since they form a well-defined bacterial network on the surfaces (named biofilm), which allows them to develop and acquire resistance to antibiotics, causing numerous infections and being one of the main primary causes of death worldwide [13, 14]. The contamination is due to several sources such as the surgical acts; the patient's own skin or mucous membrane; a contaminated medical device or equipment; contact with family members, other patients or even air suspensions after surgery [15].

The topic of antifouling properties in marine environment.

In marine or fresh water environment, biofilms start with the adhesion of molecules (proteins, inorganic compounds, ...) on the immerged objects followed by bacterial cells, which provokes the modification of the surface physicochemical properties[16], allowing microorganisms such as microalgae, and macro organisms, such as mussels and barnacles, to adhere. The attachment of this biofilm on boat hulls can dramatically increase drag and therefore fuel consumption, causing a major issue in the marine industry [1, 17]. On water treatment devices, the accumulation of organisms damages their function [10], while the efficiency of immerged captors is reduced.

As a result, in the recent past, great efforts have been invested to make functional polymeric devices or coatings, possessing antimicrobial and/or antifouling properties that prevent biofilm formation and subsequent corrosion [18, 19]. These coatings are generally classified as either antimicrobial or antifouling, the first being able to kill microbes when they contact the surface while the other being able to limit the initial attachment by their repellent properties[15]. A general method frequently used to prepare antimicrobial/antifouling polymers is to introduce a number of biocidal agents into ordinary polymers such as quaternary ammonium salts [13, 20-22], phosphonium salts, sulfonium salts [23, 24], guanidinium or bi-guanidinium group [25-27], chlorophenyl derivatives [28], natural antifouling compounds [29, 30]. However, in general, the potency of such antimicrobial/antifouling polymers is directly proportional to their toxicity towards humans and can induce environmental hazards. Therefore, the development of potent but non-toxic and no-releasing antimicrobial/antifouling polymers is indispensable and pursued by academy and industry.

Why natural rubber is attractive?

Most organic polymers are produced from petrochemicals and this is disadvantageous for many reasons, first of all because petroleum is a non-renewable resource. The price, the availability of petroleum and, in particular society concerns over global climate change continue to create increasing market pressure on the industry and domestic use of petrochemicals. In addition, nowadays, bio-based polymers featuring ecological advantages toward sustainable development are of great commercial interest due to the growing environmental awareness. Indeed, natural rubber is an attractive bio-based elastomer to be preferred in engineering applications due to its excellent properties including high elasticity, mechanical strength, fatigue resistance, wide range of operating temperatures and tear strength together with environmental friendliness [31]. For these reasons, the use of Natural Rubber (NR), a sustainable source, as a starting material becomes a great alternative.

2. Objectives

The objective of this PhD research work was to synthesize new environmentally friendly, non-releasing antifouling and/or antimicrobial bio-based materials from natural rubber. Two different strategies were followed to obtain freestanding films (Blue line) and functionalized surfaces (Yellow line) (Figure 1).





The first one (Blue line) would concern the synthesis of antimicrobial/antifouling thick freestanding films by photopolymerization between tailor-made acrylate monomers bearing the antibacterial/antifouling group and telechelic acrylate oligomers (ACTNR), prepared from natural rubber. The freestanding films obtained have been characterized by thermal analysis and contact angle to determine, respectively, their thermal properties and the surface wettability behavior. At the same time, biological tests have been performed to evaluate the antibacterial and/or antifouling activity. As the main goal of this work was to develop a new non-releasing antimicrobial/antifouling material, some

leaching tests have been carried out for each component of our films after immersion in water to ensure that active monomer is covalently linked to the NR network. The chemical structures used in this approach are shown in Figure 2.



Figure 2. Schematic representation of approach 1, concerning the synthesis of antimicrobial/antifouling thick freestanding films.

The second approach (Yellow line) to obtain composite surfaces (organic graft to inorganic materials) was to anchor covalently a thin or ultra-thin polymer layer of natural rubber on different supports. To perform this, the second strategy of this thesis was to synthesize a target acrylate surface (Figure 3) by covalently grafting the appropriate derivates of NR and modifying their free chain ends. This surface has been used as precursor for the future applications. For instance, it could be used as a protective layer for microelectromechanical devices thanks to barrier properties toward water of natural rubber or used as an antibacterial and/or antifouling layer by polymerizing from the acrylate free extremity with other bioactive monomers. This target acrylate surface was prepared following two alternative routes (Figure 3).



Figure 3. Schematic presentation of the two routes for approach 2, concerning the covalent grafting of NR oligomers on different surfaces.

The first one consisted in firstly functionalizing an activated surface, containing free hydroxyl groups, with aminated alkoxysilane commercial molecules (APTES, APMDES or APDMES, Figure 3) to obtain some amino groups at the surface. These amino groups have been reacted with oligoisoprenes bearing carbonyl groups at the chain ends via reductive amination reaction. Conversely, the second route consisted in the covalent grafting of a new oligomer Oligo-SiHTNR synthesized from NR, directly on the surface (Figure 3). These new oligomers have been synthesized from CTNR, by selective reaction of only aldehyde function with commercial silane coupling agents (ComSiA), generating a silane functional group at the chain-end and a hydroxyl group at the other end (after reduction of the ketone function).

As natural rubber is constituted by polyisoprene chains with *cis*-1,4-carbon-carbon double bonds with a stereoregular configuration on the polymeric backbone, it is possible to chemically modify it [32] to give new polymeric materials. Our group has achieved the *one pot* synthesis of carbonyl telechelic natural rubber oligomers via controlled degradation of NR, using periodic acid [33-37]. In this work, these CTNR oligomers are used as synthons to generate ACTNR oligomers (precursor for the first strategy) or new Oligo-SiHTNR oligomers (precursor for the second strategy) (Scheme1).



Scheme 1. General chemical pathways developed in this manuscript.

3. Presentation of the manuscript

This manuscript is divided into four chapters. It starts with a short introduction, dealing with the background, the interest and the objectives of the research work.

The first chapter will provide the methodology to obtain functional telechelic oligomers from NR, especially, to obtain ACNTR that is used as precursor for this work. In this short chapter, we briefly review the interest to use and the previous work on natural rubber, particularly, the chemical modification of natural rubber.

The synthesis of bio-based coatings containing a new Guanidine monomer will be presented in Chapter II, starting with a literature review about antimicrobial polymers, including the impact of microbial adhesion and how to prevent and eradicate bacterial adhesion. The interest to use a specific Guanidinium group as antimicrobial agent will be also discussed in this Section. After this general introduction, our work will be presented in a publication format, explaining the preparation of the active monomer, the effect of various parameters on the different films formulations, and the photopolymerization reaction to prepare thick films. The biological activity of the thick freestanding films against three strains of pathogenic bacteria has been studied, together with the leaching tests to understand how the thick materials age with time.

The chapter III will focus on the use of bio-inspired molecules, in particularly Zosteric acid (ZA), as an alternative biocide-free agent to control biofilm formation. A brief recent literature review about the antibiofouling properties of Zosteric acid and its analogs, including the mode of action against bacteria and structure-activity relationship, will be recalled. In this chapter, we will explain the choices of targeted Zosteric acid derivates monomers, how to synthesize and incorporate them in NR based networks. The kinetic study of the photopolymerization, the biological tests against five strains of pathogenic bacteria and a leaching study will be presented as well.

The strategy elaborated to make thin and ultra-thin polyisoprene (PI) coatings and the methodology built for anchoring the antibacterial films presented in the previous chapters to surfaces will be discussed in chapter IV, in the format of a scientific publication. An appropriate literature review and specific techniques for functionalizing surfaces will be introduced. In this chapter, we will also review the general methods for the characterization of thin coatings and how to study the interface structure at the molecular scale. The choice of silane coupling agent will be explained too.

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CHAPTER I. Natural rubber and methodologies to obtain functional telechelic oligomers

I.1 Introduction

This chapter focuses firstly on a brief summary about Natural Rubber including what is NR, how NR is produced, and why it is interesting for industrial applications, followed by a brief bibliography on the chemical degradation of NR.

An overview of the existing methods for obtaining Telechelic Liquid Natural Rubber (TLNR) is reported. Then the various chemical modifications achievable on these oligomers will be detailed, introducing the last part of this bibliographic study devoted to the various materials obtained from TLNR and their applications. In the last section, the procedure followed in this research work to obtain diacrylate oligomers (ACTNR) is described.

I.2 Natural rubber

I.2.1 Specifications, industrial applications and world context

An elastomer is a polymeric material that displays elastics properties including failure resistance at high deformation, compared to thermoplastics or thermosets, metals or alloys. Among the classes of existing elastomers, described in Figure I-1, natural rubber is one of the most useful in manufacturing highly technical products such as tires, conveyor belts, joints, as well as many objects used in various fields, including household, engineering materials, medical, etc.[31, 38-41] (Figure I-3, Left).



Figure I-1: Elastomer rubber compounds types.

The demand for natural rubber has increased throughout the XXI century [42] [43] (Figure I-3, Right) because of its excellent properties such as high elasticity, mechanical strength, fatigue resistance and tear strength (Figure I-2). In addition, NR is also an interesting material made from a renewable source, considered as a "bio" and "green" raw material. It exhibits unique and special chemical and physical properties that have no equivalent in synthetic elastomers [44, 45]. Owing to its vegetal origin, NR is a

complex material, containing not only hydrocarbon polymeric chains, but also various non-rubber substances such as amino acids, proteins, carbohydrates, neutral and polar lipids and inorganic substances (Table II-1), which in part explain the superior mechanical properties, mainly cold crystallization [46, 47].



Figure I-2: General properties of Elastomer Classes and Rubber compounds, ref. [48, 49].



Figure I-3:Annual global production, consumption of Rubber & NR from 2010-2017 (Left) (Data Copyright from ref. [42] [43]); Distribution of the world Natural Rubber applications estimated from the International Rubber Study Group, ref. [50] (Right).

	Latez	K	Dry Rubber	
% w/w	Fresh latex	Dry matter of latex	Dry matter	
Rubber hydrocarbon	35.0	87.0	94.0	
Proteins	1.5	3.7	2.2	
Carbohydrates	1.5	3.7	0.4	
Lipids	1.3	3.2	3.4	
Organic solutes	0.5	1.1	0.1	
norganic substances	0.5	1.2	0.2	

Table I-1: Composition of natural rubber latex and raw dry rubber, ref. [51].

I.2.2 Rubber Producing Plants

More than 2500 plant species can produce natural rubber (Figure I-4), but for the totality of the present applications, it comes from the latex of the *Hevea brasiliensis* tree. However, *Hevea brasiliensis* crops have very little genetic variability, leaving rubber plantations at risk of serious pathogenic attacks. The residue of proteins in latex products derived from *Hevea brasiliensis* has led to serious and widespread allergic hypersensitivity [52-56]. In addition, complete protein removal cannot be easily or cheaply achieved and, when it is accomplished, it negatively affects latex product performance. Therefore, there is an active search for an alternative source of NR with comparable properties.



Parthenium argentatum (Guayule)

Taraxacum kok-saghyz

Figure I-4: Examples of Rubber producing plants.

Hevea brasiliensis

Recently, Cornish *et al.* [56-58] proved that *Parthenium argentatum* (Guayule) has a considerable interest, producing a commercially acceptable quality rubber but non-allergenic in comparison to traditional NR. However, cultivation of Guayule is restricted to semi-arid regions. Moreover, it presents also some drawbacks concerning the processes of extraction from the plant because the rubber is produced in all compartments of the plant including leaves, roots, limb, etc. Consequently, the shrub has to be cut, milled and the latex is then extracted with organic solvents. Conversely, with *Hevea brasiliensis*, the latex is obtained by directly tapping the tree, making an incision in the trunk and thus collecting the sap freely oozing out from the ducts. This white liquid is collected and then coagulated to yield high molecular weight (Mn >1 million g/mol), as solid raw polymer (Figure I-5).



Figure I-5:Latex fractionation by centrifugation and NR ball obtained by chemical or acidic coagulation, ref. [59].

Depending on the fields of application, there are numerous methods for processing latex into commercial grades of dry natural rubber and stabilized latex, as shown in the diagram in Figure I-6. Our laboratory has been interested in the valorization of different types of rubber and in this PhD research work the NR-Standard CV60 was used as starting material: its production is highlighted in dark green color in Figure I-6.



Figure I-6: Methods of processing Hevea brasiliensis latex into commercial grades of dry natural rubber and into concentrated latex, ref.[60].

I.2.3 Biosynthesis pathways in *Hevea brasiliensis*

S.C. Nyburg *et al.* [61] were the first to show, by X-ray diffraction studies, that the backbone structure of NR was constituted by the carbon-carbon double bonds of the isoprene repeating units in 1,4-*cis*-configuration. Later, Tanaka *et al.* [62] demonstrated by ¹H-NMR and ¹³C-NMR spectroscopy analyses that the second and third units of the polyisoprene chains of *Hevea* rubber are in the *trans* configuration, followed by the *cis* enchainment; the terminal groups are pyrosphosphate (or a fatty acid ester) (Figure I-7, Left). Recently, Cornish *et al.* [63] pointed out that NR exists in latex as rubber particles (RPs), which primarily consists of a hydrophobic rubber core enclosed by a lipid monolayer (Figure I-7, Right).



Figure I-7: Microstructure of natural rubber from Hevea (Left), General representation of a rubber particle (Right), ref. [64, 65].

Despite extensive researches, the exact molecular mechanisms underlying NR biosynthesis has been not completely elucidated but the general view of NR biosynthesis, exposing the complexity of the biomachinery was pointed out by Cornish *et al.* [66], presented in Figure I-8.



Figure I-8: General view of the NR biosynthesis of the Natural Rubber, ref. [66].

It is accepted in the literature that the monomer to make NR is isopentenyl pyrophosphate (IPP), produced from sucrose *via* the cytosolic mevalonate pathway (in cytoplasm) or the methylerythritol pathway (in chloroplast or bacteria) [67, 68]. In the first step, IPP is isomerized into dimethyl allyl pyrophosphate (DMAPP) by the isomerase enzyme and subsequently forms geranyl-pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranyl geranyl pyrophosphate (GGPP) [69] (Figure I-9). These formed molecules will act as initiators in rubber polymerization (Figure I-10).



Figure I-9:Structure of isopentenyl pyrophosphate at pH=7 and initiator formation in NR biosynthesis, ref.[69].

In the presence of the metal cofactors (Mg^{2+} or Mn^{2+} *in vivo* and Mg^{2+} *in vitro*) and the rubber transferase enzyme, particularly, *cis*-prenyl transferase (EC 2.5.1.20), the first IPP monomers are added in the *trans* configuration and then the following ones in the *cis* configuration [57, 69-73]. Each step is repeated and consists on an addition of an IPP molecule and the successive abstraction of a proton to give the carboncarbon double bond, and it is accompanied by the liberation of pyrophosphoric acid (HOPP). The schematic steps of the rubber biosynthesis including the mechanism of interaction of metal cofactorenzyme-initiator are presented in Scheme I-1.

Concerning the last step of termination, so far, no particular evidence exists to explain the relative mechanism. Generally, chain termination occurs when a specific chain length is reached, and it is regulated by steric factors with the detachment of the enzyme from the rubber molecule. As mentioned previously, long-chain fatty acid ester groups linked to the polyisoprene backbone are clearly observed in ¹³C NMR, ¹H NMR spectra of NR, and confirmed by FTIR analysis, even after purification by deproteinization and acetone extraction. These fatty acids linked to rubber molecules can be removed only by transesterification or saponification with NaOH [71, 74, 75].



Scheme I-1:Biosynthesis of NR: (1) initiation and (2) propagation and mechanism of interaction of metal cofactor-rubber transferase-initiator, ref. [69].

I.3 Synthesis of acrylate telechelic oligomers via chemical modification of natural rubber

I.3.1 Bibliography

As described in the previous section (Figure I-2), NR has many singular properties but also some drawbacks, such as low flame resistance and gas permeability, limited resistance to chemicals and solvents, and poor ozone and weathering performance, mainly because of its unsaturated hydrocarbon chain structure. Moreover, its nonpolar character causes limitations in a variety of applications. As result, chemical modifications of NR have been widely developed for many years, as interesting methods to improve these limited properties, producing epoxidized natural rubber (ENR)[33, 76, 77], maleated natural rubber (MNR)[78-80], dibutylphosphate supported natural rubber (DSNR) [81], or graft copolymers of NR and PMMA (NR-*g*-PMMA)[82, 83], graft copolymers of NR and polystyrene(NR-*g*-PS)[82, 84, 85]. An alternative way to modify NR is by reducing its long chains into low molecular weight ones ($\overline{M}_n \leq 20000 \text{ g.mol}^{-1}$), which are known as Liquid Natural Rubber (LNR), because they behave as viscous liquid honey. LNR can flow at room temperature and hence the mixing process at

industrial scale is no longer a problem, and the process may be cheaper. LNR can be easily produced by oxidative degradation of NR by different processes: either from the coagulated rubber by mechanical (mastication) or radiation process, or from the latex phase by action of phenylhydrazine/oxygen system[33]. The main differences between NR and LNR are the molecular weight and the nature of the chain ends, which are dead for NR and reactive for LNR (which is also named as Telechelic Liquid Natural Rubber, TLNR). Bearing reactive terminal groups, which can be utilized in further chain extension reactions to synthesize new polymer structures, LNR presents a great industrial interest, especially in two areas: first as a reactive plasticizer, mainly in tire processing and high hardness compounds, and second in the production, as it is easier processing conventional rubber by blending it in the latex phase with LNR [33].

I.3.1.1 Chemical degradation of natural rubber: existing methods for the synthesis of telechelic liquid natural rubber

The development of methods for the preparation of TLNRs was started two decades ago. These methods involve controlled degradation of NR backbone via oxidative chains scissions, either by chemical or photochemical routes. The main chemical pathways described in the literature for the degradation of NR as well as the structure of the resulting TLNR are presented in Scheme I-2.





Advantages and inconvenients of each reaction are presented in Table I-2. Among all these methods, the oxidative degradation of NR using periodic acid is the most effective to obtain TLNR with controlled molecular weight and chemical structure (shown on Table I-2). Furthermore, it was firstly developed by our laboratory [35, 86]. In this manuscript, only this route is reported and has been used in the experimental part.

Category	Chain Scission method	End groups	M _n obtained (g/mol)	Dispersity (Đ)		Advantages/Inconvenient	Ref.
Redox	H ₂ O ₂ or organic peroxide/sulphanilic acid or Phenyl-hydrazine	Oxygenated groups (carbonyl, hydroxyl)		1.70-1.97	•	Can be carried out either in an organic solvent or directly in the latex phase	[87]
	Phenylhydrazine/FeCl ₂ /O ₂	Carbonyl, phenylhydrazone	3000-35000				
	Phenylhydrazine /O ₂	Carbonyl, phenylhydrazone			✓	Economically viable on an industrial scale	
					×	Poor control of functional group chain-end	
	UV/nitrobenzene	Carbonyl	~ 3000	-			
Photochemical	Sunlight/Fe and Co acetyl acetonate and nitrobenzene	Carbonyl	2000-8000	-	✓	Simple method, possible use on large amounts of NR	[88, 89]
	UV/H ₂ O ₂ in toluene/methanol or THF	Hydroxyl	8700 or 5000	1.93-1.97	✓	Economically viable	
			(Depending on irradiation time)		×	Number average functionality not estimated	
	Sunlight/H ₂ O ₂	Hydroxyl	7600 after 50h of irradiation	1.80-1.82	×	Presence about 10% side products consisting mostly of a	
	Sunlight/Benzophenone in toluene	Hydroxyl, hydroperoxide Ketone	10000-50000	-		cross-linked phase	
	Cis-but-2-ene-1,4-diol/Grubbs I catalyst	Hydroxyl	80000-380000	2.40-3.91	√	Giving access to TLNR with high molar masses	
Metathesis	Cis-but-2-ene-1,4-diol/Grubbs II catalyst	Hydroxyl, acetyl	5000-18000	5.28-23.30	×	Secondary reactions of intra- molecular cyclization	[90]
	Cis-but-2-ene-1,4-diacetate/Grubbs II	ate/Grubbs II Hydroxyl, 850-21000 2.18-3.64		Useh aget of Crubh agtal-set for	[34, 91,		
	cataryst β-pinene/ Ru-allylidene catalyst	Acetyi Monoterpene units	700-3000	1.60-2.50	~	industrial applications and no recovery of catalyst	72]

Oxydation	$H_2O_2/200$ -300 psi and 150°C in toluene	Hydroxyl	2500-3000	1.4	~	Low functionality of hydroxyl chain-end	[93]
	O ₃ (Ozonolysis)	Carbonyl, carboxyl, hydroxyl	-	-	×	Utilization of O ₃ (toxic product in high concentration)	[20, 87]
	Periodic acid (H5IO6)/mCBPA system or only H5IO6 system	Carbonyl	1000-12000	1.5-3.0	~	Controlled molar weight and chemical structure with 2 different functional groups (ketone and aldehyde)	[20, 33, 35, 36]

Table I-2: Selected Methods for the production of telechelic liquid natural rubber, including advantages and inconvenients of each method.

I.3.1.1.1 Specific case of the controlled degradation of natural rubber using periodic acid

Our group is particularly specialized in the synthesis of carbonyl telechelic natural rubber oligomers (CTNR) *via* the controlled degradation of NR, using periodic acid [34, 86, 94-96]. Indeed, D. Reyx, I. Campistron *et al.* [86] developed firstly in 1997 the use of periodic acid (H_5IO_6) to prepare telechelic liquid natural rubber from NR in latex phase. The analysis of the obtained oligomers revealed the presence of aldehyde, ketone functional groups but also residual oxiranes, secondary furanic and cyclic structures.



Scheme I-3: Synthesis of carbonyl telechelic natural rubber, ref.[35].

Later, in 2003, Gilliet-Ritoit *et al.*[35] investigated the chain degradation of natural rubber (Scheme I-3, Blue arrow) with periodic acid in THF, and compared it to another pathway using as intermediate epoxidized *cis*-1,4-polyisoprene (ELNR)(Scheme I-3, Red arrow). This ELNR was previously prepared from NR, using *meta*-chloroperoxybenzoic acid (*m*-CPBA). They demonstrated for both cases that the oligomers with aldehyde and ketone functions at the chain ends were generated. However, the direct degradation of NR was slower than that of the epoxidized one and the molar masses of the oligomers obtained depended on the epoxide content. They proposed that the H₅IO₆ cleaved the ELNR through the trans-formation of epoxide function into *vic*-diol, but in the case of degradation directly of NR, they proposed a mechanism reaction *via* two steps. Firstly, H₅IO₆ reacts with a carbon-carbon double bond to form an epoxide or a α -glycol that is cleaved with the excess of H₅IO₆ to obtain the CTNR, bearing ketone and aldehyde groups at the chain ends (Scheme I-4).



Scheme I-4: Oxidative degradation mechanism using H_5IO_6 via one step for the synthesis of CTNR, ref. [34].

In 2005, P. Phinyocheep *et al.* [94] focused on the effect of periodic acid amount on the NR degradation process in latex phase. To perform that, they epoxidized firstly the purified NR with formic acid (HCO₂H) to generate epoxidized natural rubber (ELNR). This ELNR was subsequently treated with H_3IO_6 . They observed that the epoxide contents were approximately the same before and after periodic acid treatment. It seemed that the degradation process did not take place at the epoxide function, but occurred via the C=C double bond of the isoprene unit, thus generating *vic*-diols as appropriate intermediate. To confirm this hypothesis, they also investigated the treatment of NR and purified natural rubber (PNR) with periodic acid. The obtained results showed that H_3IO_6 promoted the breakage of NR/PNR chains, but not with as high efficiency as that of ELNR. Furthemore, H_5IO_6 might be able to introduce an epoxide functional group onto the rubber molecule but in a very small quantity. They proposed a mechanism for the oxidative degradation of epoxidized NR using H_5IO_6 . The double bond C=C of ELNR is oxidized, resulting in the formation of a *vic*-diol. The single bond of the vic-diol is cleaved with another molecule of H_5IO_6 , leading to the chain degradation (Scheme I-5)



Scheme I-5: Oxidative degradation mechanism of ELNR by using the H₅IO₆, ref.[94].

Inspired by these results, finally, our group decided to synthesize the CTNR oligomers *via* only *one-pot* method directly from NR using periodic acid [97]. The first step was eliminated in order to save time, energy, chemical consumption and improve the overall yield in view of the future scale-up of process. The presence of some epoxy rings in CTNR oligomers was noticed, but in a very small quantity (~1-2%).

I.3.1.2 Reactivity and reactions of telechelic liquid natural rubber

Several reactions can be carried out by modification of the C=C bond in the backbone of TLNR such as "ene" reaction with maleic anhydride, chlorination via addition of Cl₂, epoxidation, and grafting with other monomers [87] (Scheme I-6, Left).



Scheme I-6: Chemical modification of TLNR, ref. [87].

Possessing not only isoprene units in the main chains, but also reactive end-groups, TLNR terminal groups can be also modified to obtain other suitable functional groups as required for various chain extension reactions. In previous work, TLNR bearing ketone and aldehyde terminal groups was modified to obtain oligomers with polymerizable and biocide chain ends. Among these oligomers, the Hydroxyl Telechelic Oligomers, (Figure I-10, Left) and Acrylate Telechelic Oligomers, (Figure I-10, Right) were used as precursors, respectively for the synthesis of polyurethane films and foams [20, 36, 98-103] and polyacrylate films. N.Kébir *et al.*[20] and R.Jellali *et al.* [95, 96, 104] of our team reported the different methods for the synthesis of new TLNR with quaternary ammonium groups and new photocurable TLNR bearing one or two acrylate groups at the chain end (ACTNR) for antibacterial/antifouling applications. The details of these materials will be reported in Chapter II, Section II.2.3.



Figure I-10: Examples of chemical structure of different oligomers obtained by modifications of reactive terminal groups of CNTR.

I.3.2 Synthesis of acrylate telechelic natural rubber, optimization of reaction conditions for different targeted molecular weights.

Our first objective was to synthesize diacrylate telechelic natural rubber with targeted molecular weight of 6000 g/mol and 1500 g/mol in three steps, *via* the oxidative degradation using periodic acid to access directly CTNR. The choice of target molecular weight will be explained in the next Chapter (Section III, Chapter II). These oligomers were reduced in hydroxyl telechelic natural rubber oligomers then reacted with acryloyl chloride to introduce the acrylate functional groups at the chain ends, allowing to access to ACTNR oligomers (Scheme I-7).



Scheme I-7: Chemical pathway to synthesize ACNTR, reported by R.Jellali et al. [95, 96, 104] with molecular weight in the range of 1000-3000g/mol.

This procedure was developed by our laboratory, so in this research work only improvements of the reaction conditions were introduced and are described in this section. The corresponding experimental details and the characterization methods including ¹H NMR, ¹³C NMR, FTIR and SEC analysis of each oligomer are reported in Section-II.4-2.4 of Chapter II. The conditions reported by R. Jellali *et al.*[95, 96, 104], worked rather well wether low molecular weight oligomers (range of 1000-3000 g/mol were

targeted. Consequently ACTNR1500g/mol was synthetized using these conditions, but they had to be optimized for each step to obtain ACTNR6000g/mol.

I.3.2.1 Controlled degradation of natural rubber using periodic acid



Scheme I-8: Synthesis of carbonyl telechelic natural rubber.

The first step consisted in the controlled oxidative degradation of NR (Scheme I-8), generating CTNR bearing aldehyde and ketone ends with targeted \overline{M}_n of 6000g/mol and of 1500g/mol by using periodic acid (H₅IO₆) in THF at 30°C for 6h. The added amount of periodic acid (H₅IO₆) was calculated according to the targeted molecular weight, as shown in an example:

"In an oligomer of $M_n = 1500$ g/mol there are 22 isoprene repeating units (molecular weight 68 g/mol). One gram of NR contains 0.014 mol of double bonds so we imagine to be in one single chain, in which we have to cut a double bond every 22 to have the oligomer of the desired chain length, so we have to cut 0.014 mol/ $22 = 6.3 \times 10^{-4}$ mol ≈ 1.0 equivalent. Herein, 2.0 equivalents of H₅IO₆ are required to cut 1 equivalent of double bonds, so the number of moles of periodic acid is obtained and the corresponding weight to be used with 1 gram of NR."

However, if this theoretical amount is added, the chain length obtained is much bigger than the desired one because two molecules of H_5IO_6 are used to cut one double bond but other molecules react with various non-rubber substances such as amino acids, proteins, carbohydrates, neutral and polar lipids, etc... contained in the NR. As consequence, depending on the commercial lots of NR, more molecules of H_5IO_6 are required; the experience indicated a range between 3.2 or 3.6 equivalents. In this study, with the lot STR5LCV60, 3.2 equivalents of H_5IO_6 were used to generate our desired molecular weight.

The ¹H-NMR spectrum of CTNR (Figure I-11) showed the characteristic protons of the aldehyde moiety at 9.78 ppm and of the methyl group at the ketone chain end at 2.10 ppm. The four protons between 2.20 and 2.55 ppm correspond to the two CH₂ groups in α position of the carbonyl groups.


Figure I-11:1H NMR spectra of CNTR, example with $\overline{M}n=6000g/mol$.

The number-average molecular weight (\overline{M}_n) of the oligoisoprene was determined by the integral value of the peak of the proton n°6 in the isoprene repeating unit and the total mass of the rest of the molecule.

 \overline{M}_{n} of CTNR was calculated according to the equation (I.1):

$$\overline{M}_{n,NMR} = \left[\frac{I_{5.13ppm}}{(I_{2.47ppm}/4)} \times 68\right] + 100$$
(I.1)

 $I_{5.13ppm}$ and $I_{2.47ppm}$ were the integration values of the peaks at 5.13 ppm and at the range of 2.2-2.55 ppm, respectively. The masses 68 and 100 g/mol are respectively the molar mass of C₅H₈ motif and the chain ends (C₅H₈O₂) of CTNR respectively. $\overline{M}_{n,NMR}$ was used to calculate the molar concentration of the oligomers for the modification of the chains ends in the synthesis of hydroxy telechelic natural rubber and acrylate telechelic natural rubber.

There was a good relation between the aimed molecular weight and the experimental one: 5200 g/mol for the target molecular weight of 6000g/mol, and 1260 g/mol for 1500g/mol. Size exclusion chromatography (SEC) was also performed and confirmed that $\overline{M}n$ were 5200 and 1380 g/mol for CTNR6000 and CTNR1500 respectively, with a reasonable dispersity of 1.94 and 1.87, respectively (Figure I-12).



Figure I-12: SEC analyses of CTNR (Left) and comparison with HTNR and ACTNR (Right).

I.3.2.2 Synthesis of hydroxyl telechelic natural rubber



Scheme I-9 : Synthesis of Hydroxyl Telechelic Natural Rubber.

The synthesis started with the selective reduction of the carbonyl terminal groups at the chain-ends of CTNR using NaBH₄ as reducing agent, to generate HTNR oligomers (Scheme I-9). R. Jellali *et al.* [96] reported that in presence of 4 equivalents of NaBH₄, stirring for 6h at 60°C in THF (named HTNR₁₅₀₀#Ref condition) HTNR could be obtained with targeted molecular weight of 1740 g/mol. Nevertheless, these conditions were not satisfactory in our case for targeted molecular weight of 6000g/mol. After 6h, our reaction was incomplete, only 90% of carbonyl groups were converted to hydroxyl groups. In order to optimize this reaction and to obtain 100% of conversion, two parameters were varied, reaction time and ratio between reducing agent and oligomers (Table I-3).

	HTNR1500#Ref	HTNR6000#1	HTNR6000#2	HTNR6000#3
Ratio(mol) of NaBH ₄ /CNTR	4/1	4/1	4/1	10/1
Reaction Time (hours)	6	6	24	24
Total conversion (%)	100	86	90	100

Table I-3:Several conditions used for the synthesis of $HTNR_{6000}$; $HTNR_{1500}$ #Ref is the standard condition used by our laboratory, reported by R. Jellali et al. [96]. Reaction temperature was 60°C, [CTNR] in THF was 0.01 mol/L.

The conversion was monitored observing the remaining signal between 2.55 ppm and 2.1 ppm, corresponding to the–CH₂ groups in α -position of aldehyde and ketone (Figure I-13). The best conditions

to obtain 100% of conversion was $HTNR_{6000}$ #3 in the presence of 10 equivalents of NaBH₄ for 24h at 60°C. With these conditions, we obtained $HTNR_{6000}$ with 75% yield.



Figure I-13:¹H NMR spectra of HTNR with different conditions (Table I-3).

The ¹H NMR spectrum of HTNR₆₀₀₀#3 (Figure I-13) showed the total disappearance of signals at 9.7 ppm and between 2.55 ppm and 2.05 ppm, corresponding to the aldehyde proton and the $-CH_2$ group in α -position of the aldehyde and ketone proton respectively, confirming the total reduction of all carbonyl terminal groups. The apparition of the signal H-11 located at 3.6 ppm attested the formation of hydroxyl groups at the chain-end. Size exclusion chromatography (SEC) was also performed and confirmed that \overline{M} n of HTNR₆₀₀₀ oligomer was 5500 g/mol with a dispersity of 2.5.

I.3.2.3 Synthesis of Acrylate Telechelic Natural Rubber





The last step for the synthesis of ACNTR was an esterification reaction. To this end, hydroxyl terminal group was reacted with acryloyl chloride (AC), in presence of triethylamine (Et₃N), in dichloromethane (Scheme I-10). This process was also optimized (Table I-4).

	ACNTR1500#Ref	ACTNR6000#1	ACTNR6000#2	ACTNR6000#3
Ratio AC/HNTR	2.25/1	2.25/1	5/1	5/1
Ratio Et ₃ N/HTNR	2.5/1	2.5/1	5.5/1	5.5/1
Reaction Time (hours)	6	6	24	48
Total conversion	100	75	85	100

Table I-4: Different conditions performed to optimize the synthesis of ACNTR. ACTNR₁₅₀₀#Ref was the standard conditions used by our laboratory, reported by R. Jellali et al. [96] [HTNR] in THF was 0.01 mol/L, reactions were carried out at RT.

By ¹H NMR analysis, it was shown that the esterification of HTNR using conditions $ACNTR_{6000}$ #1 and $ACNTR_{6000}$ #2, was not complete, as confirmed by the presence of the signals located at 3.6 ppm and 3.8ppm, corresponding to $-CH_2$ in α position of the hydroxyl group (Figure II-.24). Contrarily, $ACNTR_{6000}$ #3 conditions allowed to generate ACTNR with 100% of conversion and a yield of 80%.



Figure I-14:¹H NMR spectra of ACTNR obtained with different conditions described in Table II-4.

After optimization (Figure I-14), full conversion was confirmed by the shift of signals at 3.6ppm and 3.8ppm corresponding to $-CH_2$ in α position of the hydroxyl group at the chain-end HTNR, becoming two new signals at 4.16ppm and 4.95ppm. It can be also observed in Figure I-14 the appearance of the characteristic signals of the acrylate function in the range from 5.9ppm to 6.5ppm. Size exclusion chromatography (SEC) gave a value of 5500 g/mol and 2.9 respectively \overline{M} n of HTNR₆₀₀₀ oligomers and their dispersity.

I.4 Conclusions

In the first part of this chapter, we reported a description of the main features of natural rubber, used as starting material in this research work. The biosynthesis of natural rubber in the complex lactiferous system located under *Hevea* bark was also recalled.

In the second part, a literature review of the different methods to obtain liquid natural rubber was highlighted, followed by the modifications that allowed the generation of new elastomeric materials. In this part, we reviewed firstly the advantages and drawbacks of each method to explain why periodic acid was chosen to perform the degradation of natural rubber to generate carbonyl telechelic natural rubber (CTNR) with controlled molecular weight.

Bearing an aldehyde and a ketone functional groups at the chain-end, CTNR oligomers were chemically modified to prepare ACTNR. After our optimization, ACTNR oligomers (6000g/mol) were successfully obtained *via* three steps from NR with an overall yield of 51%. These oligomers are used as common precursors in the coming chapters to prepare new antimicrobial/antifouling materials. The CTNR oligomers will be also used as starting reagents to prepare other functional oligomers, presented in Chapter IV.

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[104] R. Jellali, I. Campistron, A. Laguerre, P. Pasetto, L. Lecamp, C. Bunel, J.-L. Mouget, J.-F. Pilard, Synthesis of new photocurable oligoisoprenes and kinetic studies of their radical photopolymerization, Journal of Applied Polymer Science, 127 (2013) 1359-1368. CHAPTER II. Natural Rubber based materials integrating a new guanidine monomer for antimicrobial properties

II.1 Introduction

This chapter focus firstly on a brief summary about antimicrobial polymers, including impact of microbial adhesion and how to prevent and eradicate it. The second section consists in current research on antimicrobial polymers, focusing on cationic ones. The mode of action against microbes is discussed and the different factors influencing antimicrobial activity are also presented.

In the last section, the research paper submitted is presented. It concerns the preparation of new antimicrobial polymers based on natural rubber, integrating a new guanidine monomer in the cross-linked network formed by the diacrylate telechelic natural rubber, presented in chapter I. The kinetic study of the photopolymerization is described, as well as the leaching tests of the films immerged in water. Finally, the results of the antimicrobial activity against three strains of pathogenic bacteria are reported.

II.2 Bibliography: Antimicrobial polymers

II.2.1 Impact of microbial adhesion: why do we need Antimicrobial Polymers?

Nowadays, microbial contamination and its serious effects pose a significant challenge to modern society. The formation of biofilm causes major problems in numerous situations, such as biomedical implants and devices, hospital surfaces/furniture [1-3], biosensors, textiles [4], food packaging, food storage, water purification systems [5, 6], industrial and marine equipment [7]. One of the best known cases of social health problems is represented by the nosocomial infections, which pose a serious threat to patients' health, causing about 648,000 infected patients and 80,000 deaths in the US every year [8, 9]. In Europe, two-thirds of the 25,000 patients who died of nosocomial infections each year were infected by only Gram-negative bacteria [10]. About 8.7% of hospitalized patients worldwide develop nosocomial infections [8, 9].

There are two ways to stop microbes from infecting humans or deteriorating materials: disinfection and antimicrobial surfaces. The use of disinfectants is the most widespread; however, it can cause considerable environmental pollution and also support the development of resistant microbial strains. Therefore, the development of strategies to prevent or eliminate microbial contamination is urgently required. A promising strategy is based on the production of antimicrobial polymers, which have attracted much interest over the last years in both academic and industrial research, due to the low propensity of these materials to cause drug-resistant microorganisms.

II.2.1.1 Mechanisms of Biofilm formation, bacteria-surface interaction

In order to maintain the sterile state of surfaces, it is important to understand how they become contaminated. In fact, when some bacteria are in contact with surface, they form biofilms that cause up to 75% of all bacterial infections. The organization of bacteria in biofilms allowing the formation of a

barrier (as a protection) confers them access to nutrients, resistance to antimicrobial agents due to the poor diffusion, and the possibility of transgenic mutations beneficial for bacterial survival [11-15].

The steps in biofilm formation are summarized as follow (FigureII-1):

1) Planktonic (free-floating) initial attachment of bacterial cells to a surface

2) Multiplication of bacteria and excretion of extracellular matrix to encase the colonies

3) Formation and Maturation of biofilm, cells form multi-layered clusters

4) Three-dimensional growth and further maturation of the biofilm, providing protection against host defense mechanisms and antibiotics

5) Dispersion of cells through detached biofilm



Figure II-1: The five stages of biofilm development. **Step1**: Planktonic (free-floating), initial attachment of bacterial cells; **Step 2**: Multiplication of bacteria and excretion of extracellular matrix to encase the colonies; **Step 3**: Formation and Maturation of biofilm, cells form multi-layered clusters; **Step 4**: Three-dimensional growth and further maturation of the biofilm, providing protection against host defense mechanisms and antibiotics; **Step5**: Dispersion of cells through detached biofilm, ref. [14].

It should be emphasized that after step 1 the entire process is irreversible. Therefore, it is quite important to prevent biofilm formation. To be more specific, the prevention of initial bacterial attachment on surface is extremely recommendable.

II.2.1.2 Strategies for prevention and eradication of bacterial biofilm

Great efforts have been devoted to the developing of various strategies in order to eradicate formed biofilm or to prevent biofilm formation on surfaces. One most common way to prevent surface contamination is to keep the environment sterile by using sterilization methods including disinfectants (eg. hypochlorite, reactive oxygen species), autoclaving, UV and ionizing radiation that can quickly and effectively kill bacteria encapsulated in biofilms. However, these actions are all carried out after the initial attachment of bacteria. The sterile state does not last for long and the frequent use of such disinfectants poses a great environmental problem. It can be also hazardous to the users and the materials

[13, 16-18]. Once bacterial infections are contracted, they are treated with antibiotics; unfortunately, overuse of antibiotics may induce drug-resistance in bacterial strains, so that new drugs must be developed with the risk of becoming ineffective after a while [18]. Therefore, it is a necessary and pressing need to develop a new approach to keep surfaces sterile effectively and "permanently" against a broad-spectrum of bacteria before their permanent attachment. These materials should also be still functional after multiple applications/uses and have a low probability of inducing resistance. In the meantime, the appearance and functionality of the surface should also be maintained after any new procedure of sterilization.

As result, antimicrobial polymeric systems have been frequently reported as a promising approach to prevent biofilm formation [19-23]. Moreover, compared with the small molecular counterparts, antimicrobial polymers demonstrate superior efficacy, reduced toxicity, minimized environment problems and greater resistance [4, 24-26]. Some of antimicrobial polymeric systems are summarized in Figure II-2.



Figure II-2: Antimicrobial polymeric systems, ref. [26].

The purpose of this study was to synthesize new antibacterial and/or antifouling polymers in a facile manner for the prevention of bacterial adhesion. To better understand this concept, a detailed review on current antibacterial and antifouling coatings will be given in the following sections.

II.2.1.3 Concept and Mechanisms of action of antimicrobial polymers

In the literature, there are several ways to classify the antimicrobial polymer systems. They can be divided either by the working-principle or by the bioactive group that infers the antimicrobial properties. Antimicrobial polymers involved traditionally covalent linkages of groups with antimicrobial activity to a polymeric backbone. They have been known since 1965, when Cornell and Donaruma described polymers and copolymers prepared from 2-methacryloxytroponones that were able to kill bacteria [27]. Since that work, a great number of antimicrobial polymers has appeared, showing a good and broad spectrum of antibacterial/antifouling activity. Recently, several reviews have summarized the state-of-art of antimicrobial polymers [4, 25, 28]. The influence of different parameters is discussed, including

molecular weight, type and degree of alkylation, distribution of charge, hydrophobic/hydrophilic ratio. Their effects on the action mode of antimicrobial polymers are also evaluated [19-23, 29]. According to the mechanism of antimicrobial activity, it can be categorized as either passive action (known as antifouling activity) or active action (antimicrobial, Figure II-3)



Figure II-3: The schematic reaction mechanism of passive and active action of the antimicrobial polymer, ref. [28] [19].

A layer constituted by a passive polymer repels only but do not actively interact with or kill bacteria. It inhibits/reduces protein adsorption on its surface, thereby preventing the adhesion of bacteria to ensure protection of surface from colonization (antifouling activity). Due mainly to the hydrophobic and negatively charged cells walls of microbes, passive polymers should be either (1) hydrophilic, (2) negatively-charged or (3) have a low surface free energy. Conversely, active polymers kill bacteria that adhere to their surface. The mechanism of polymer killing depends on the active agents. The most widely used active antimicrobial polymers are functionalized with positively charged (quaternary ammonium groups), which interact with the cell wall and destroy the cytoplasmic membrane, resulting in the leakage of intracellular components and cell death.

The antimicrobial polymers are divided in three general types: polymeric biocides, biocidal polymers and biocide-releasing polymers [19, 28, 30], presented in Figure II-4.



Figure II-4: Representation of clarification of antimicrobial polymers: Polymeric biocides; biocidal Polymers and biocide-releasing Polymers, ref.[19].

The first class (Polymeric biocides, Figure II-4, Left) is based on the covalent linkage of bioactive repeating units with antimicrobial activity such as amino, carboxyl or hydroxyl groups. The polymers are just multiple interconnected biocides, which act similarly to the biocidal monomers. The polymerization process may either enhance or reduce the antimicrobial activity of bioactive functional group [28]. Due to the sterical hindrance excercised by polymer backbone, polymeric biocides may be often less active than the respective monomer.

Conversely, the antimicrobial site of biocidal polymers (Figure II-4, Centre) is linked to the entire macromolecule. It can be a cationic biocide such as quaternary ammonium, phosphonium, guanidinium, tertiary sulfonium, etc... The biocide-releasing polymers (Figure II-4, Right) containing nanoparticles, antibiotic and/or antiseptic compounds, exhibit antibacterial properties by releasing them in the environment.

II.2.2 Cationic antimicrobial Polymers

A large number of antimicrobial polymers have been synthesized to date and can also be classified according to the nature of the bioactive groups (Figure II-5)



Figure II-5: Classification of antimicrobial polymers on the basis of their active function.

Among them, cationic antimicrobial polymers (CAPs) have attracted attention because they decrease the potential for resistance development due to their positive charge. The most commonly used cationic moieties are quaternary ammonium groups, pyridinium, guanidinium, biguanide, quaternary phosphonium, and tertiary sulfonium groups (Figure II-6). Non-quaternised ammonium groups, such as primary and tertiary ammonium groups, can also kill germs once they are protonated in a certain pH range [31].



Figure II-6: Most commonly utilized cationic groups reported for antimicrobial polymers.

II.2.2.1 Synthesis and Mechanism of action of Cationic Antimicrobial Polymers

The first discovery of the antimicrobial properties of quaternary ammonium salts (QAS) was reported by Domagk in 1935 [32]. Since then, different QAS have been extensively investigated as disinfectants

[33-35]. With structures and antimicrobial activities similar to QAS, quaternary phosphonium salts (QPS) have been developed representing a new progress in cationic biocides. The mechanism of action of CAPs is not fully understood, but all agree that the positive charges interact electrostatically with the negative charges present on the bacterial cell walls, leading to its destabilization and ultimately to the cell death. Furthermore, polymeric materials may interact more effectively with the cells of Grampositive bacteria as their polyglycane outer layer is sufficiently loosely packed to facilitate deep penetration of the polymer chain inside the cell to interact with cytoplasmic membrane. [36-41]. Recently, Xiao and co-workers [41] have reported a comprehensive overview of the synthetic methods and antimicrobial action of polymers with quaternary ammonium/phosphonium salts. Two common synthetic strategies to prepare QAS/QPS are based on direct polymerization of monomers containing QAS/QPS groups, or on covalent incorporating QAS/QPS moieties within synthetic or natural polymers, through copolymerization or modification of reactive functional groups [41-46] (Figure II-7).



Figure II-7: Representation of two common methods to prepare Cationic Antimicrobial Polymers.

II.2.2.2 Factors Affecting the Behavior of Cationic Antimicrobial Polymers

It was also reported that the characteristics of polymeric CAPs affecting their antimicrobial activity are: molecular weight, charge distribution and density, nature of counter anion and cationic moieties, amphiphilic balance, including the length of the substituted alkyl chain, the alkyl spacers between neighboring cationic groups, and the ratio of hydrophobicity/hydrophilicity (Figure II-8).



Figure II-8: Several factors can influence the activity of Cationic Antimicrobial Polymers.

A growing number of cationic units in the polymer increases the activity; particularly, polymeric QAS/QPS with high positive charge density promote initial adsorption onto the negatively charged bacterial surfaces, and this results in significantly enhanced antimicrobial activity[41]. In the case of antimicrobial polymethacrylates (Figure II-9), Palermo and Kuroda *et al.* [47] [44] studied the effect of hydrophobic alkyl chain and the nature of cationic group on antimicrobial polymer properties. They proved that a short alkyl chain when R is methyl, instead of n-butyl (Figure II-9, Left), gave the highest selectivity for bacterial and human cells, whereas alkyl groups with a longer chain gave rise to hemolytic and antimicrobial activities[44].



MIC: Minimum inhibitory concentration

Figure II-9: Effect of hydrophobic alkyl group and effect of cationic group on the antimicrobial properties, example of methacrylate copolymer, ref.[48].

They demonstrated also that the antimicrobial activity of the polymers was strongly dependent on the chemical structure of the amine or ammonium groups. With the same hydrophobic alkyl chain (butyl), the protonated primary ammonium appeared to be more effective at conferring non-hemolytic antimicrobial action relative to QAS group (Figure II-9, Right) [47-50] [34]. Nevertheless, with four

aliphatic groups attached to the nitrogen, QASs remain cationic and effective in both aqueous and dry environments, while primary and tertiary amine groups are only effective when protonated and the extent of protonation is affected by the pH of the solution. Recently, Kuroda *et al.* [51] and Locok *et al.* [44] reported that for the case of polyacrylates and the polymers which mimic host defense peptides [48], one of the most important factor on antimicrobial activity profile was the amphiphilic balance (Figure II-10). If a polymer is too hydrophobic, it causes increased cytotoxicity to both human and bacteria cells because their hydrophobic nature enables binding and inserting into human cell membranes even without the aid of electrostatic attraction. Moreover, if a polymer has too many cationic groups, it may bind to bacterial cell surfaces by electrostatic condensation, but is unlike to insert into the hydrophobic core of the membrane, thus resulting in decreased antimicrobial potency and a loss of selectivity. Therefore, the best strategy is to find correct amphiphilic balance. It will give a potent and effective antimicrobial polymer [52-54] (Figure II-10). Some examples on current cationic antimicrobial polymers are described in Table II-1.



Figure II-10: Optimization of balance between cationic and hydrophobic groups is one of the pivotal design principles in the field of antimicrobial polymers, ref. [48].

S. N°	Polymer (antimicrobial substance)	Target	Remark	Ref
1	Polyurethane containing quaternary ammonium $\begin{array}{c} HN-R - \left(\begin{array}{c} O \\ - \end{array} \right) \left(\begin{array}{c} O \end{array} \right) \left(\begin{array}{c} O \\ - \end{array} \right) \left(\begin{array}{c} O \end{array} \right) \left(\begin{array}{c} O \\ - \end{array} \right) \left(\begin{array}{c} O \end{array} \right$	S.aureus, E. coli	Good antimicrobial activities against even at low concentrations (5 wt %)	[55]
2	Poly(n,n-diethylethylendiamine-coyrosol-based acrylic) (tertiary amine) * $(H_2C-CH)_x + C^2 - C^H_y + C^H_y$	S.epidermidis S.aureus	Combination of two active compounds provide a synergistic action against biofilms and suppress reactive species oxygen	[56]
3	Organosilicon quaternary ammonium chloride $\sim O - S_{i-(-CH_2)_3}^{O_{s'}} N_{-} C_{18}H_{37}$	S.aureus	Exerted long-lasting antimicrobial activity for at least four hours	[57]
4	Acrylamide polymers with quaternary ammonium $* \sim (H_2C - C) \times K + N_X + N_$	S.albus, E.coli, Rhizoctonia solani, Fusarium oxysporum	Benzyl group attached to nitrogen atom showed better inhibitory effect on bacteria and phytopathogenic fungi	[58]

Quaternary ammonium/ phosphonium modified epoxidized natural rubber S.aureus, E. coli Moderate growth inhibition of microbes 5 [59-61] HO Guanylated polymethacrylate S.epidermidis, Candida Guanidine copolymers were much more [62] 6 albicans active compared to the amine analogues $_{12}H_{25}$ NĆ NH_2 ŇΗ 7 Quaternary ammonium polyethyleneimine Gram-positive & n-alkylated polyethyleneimine has [63] Gram-negative bacteria effective antimicrobial activity, dependent on the hydrophobic and positively charged immobilized long polymeric chains Ammonium ethyl methacrylate homopolymers Methicillin-resistant Very little or no hemolytic activity and [64] 8 higher inhibitory effects against Gram-S.aureus, E. coli



Table II-1: Examples of cationic polymers for antimicrobial applications, ref. [28].

II.2.2.3 Why guanidine-based polymers are an attractive new class of cationic antimicrobial polymers?

As described in the previous sections, cationic antimicrobial polymers, particularly quaternary ammonium compounds (QAC), have been commonly used as antimicrobial agents. However, it has been observed that serious bacterial resistance is caused by a large number of QACs after their use for a period of time. Another disadvantage of QACs is their relatively narrow antimicrobial spectrum, as they are mainly bactericidal and are more effective against Gram-positive than Gram-negative bacteria [70-72]. Therefore, apart from ammonium and phosphonium, another source of cationic charge is the guanidinium moiety. Guanidine derivatives are found in a wide array of natural and synthetic bioactive compounds. They have pharmaceutical importance because they exert a therapeutic activity including cardiovascular, antihistamine, anti-inflammatory, antidiabetic, antibacterial, antihypertensive, antiviral, and antineoplastic effects [73-75]. Recently, guanidine-based polymers including polyhexamethylene guanidine hydrochloride (PHMG), Polyhexamethylene biguanidine hydrochloride (PHMB) and some derivates of polyguanidine have been increasingly used as antimicrobial disinfectant for medical, textile, plastic fields, etc... mainly due to their high water solubility, wide-spectrum antimicrobial activity, excellent biocide efficacy and nontoxicity. All of these polymers (Figure II-11) are obtained by the condensation polymerization of hexamethylenediamine and guanidine hydrochloride [26, 76, 77].



Polyhexamethylene Guanidine







Derivates of Polyguanidine

Figure II-11: Chemical structure of some examples of polyguanidine, including polyhexamethylene guanidine hydrochloride or biguanidine or derivates of polyguanidine hydrochloride.

Their minimum inhibitory concentration is around 200μ g/mL for low molecular weight chains of 1000-3000g/mol [78]. Therefore, the most effective method to enhance the antimicrobial activity is to increase the molecular weight of the polymer by copolymerization or graft co-polymerization [79]. The study of structure-activity relationships (SARs) showed that the guanidine polymers without exception, exhibited a high activity against the Gram-positive bacteria *S.epidermidis*, *S. aureus*, and the pathogenic fungi, *C.albicans*, while exhibiting a low level of human red blood cell toxicity, in comparison to the corresponding ammonium polymers (Figure II-12) [62, 80].



Figure II-12: Example of comparison of antimicrobial properties of polymethacrylates containing either guanidinium group or ammonium group, ref.[62].

These different effects may be explained by the fact that a guanidium group is capable of binding to the cytoplasmic membrane, thus forming multidendate bonds by electrostatic attractive forces with the anionic phosphate headgroups of phospholipids (Figure II-13), yielding a stronger bacterial membrane interaction than ammonium groups. This binding can change the phospholipids environment of the membrane and cause the loss of its biofunction or destroy the cytoplasmic membrane, leading to the death of the bacteria. Furthermore, the guanidium group has a higher acid dissociation constant (pKa) (e.g., pKa of arginine guanidinium group = 12.48) than amines (e.g., pKa of lysine amino group = 10.53), meaning that a higher proportion of guanidines would be charged at physiological pH (7.4), imparting a greater cationic character to the material [81-85]. A high concentration of guanidines can also assist the translocation across membranes. [83, 86-89] (Figure II-13)



Figure II-13: Mechanism of uptake (adaptive translocation and endocytosis). 1. The guanidium group forms a bidentate bond with negative phosphates, sulfates and carboxylates on the cell-surface. 2 and 3. The charge-neutralized species moves through the membrane, in a process termed 'adaptive translocation'; driven into the cell by the membrane potential. 4. In the reverse of 1, the oligoguanidium transporter dissociates from the membrane once inside the cell, ref.[87]

II.2.3 Natural Rubber based Cationic Antimicrobial Polymers

The expertise of our research group is the modification of natural rubber for the generation of new materials for applications. One of the research axes of the team focused on the preparation of antifouling coatings by chemical modification of natural rubber, containing quaternary ammonium groups [90][61][91-93]. These biocidal functions were covalently incorporated at the chain-ends of Telechelic Liquid Natural Rubber.

N.Kébir *et al.*[90] reported the synthesis of 1,4-polyisoprene cationomers bearing one or more ammonium groups (Figure II-14), which were used as diols in the preparation of antibacterial ionic polyurethanes and ionic copolyurethanes, using a classical one-shot polyaddition polymerization *via* a reaction between a monomer containing at least two isocyanate functional groups (-N=C=O) and another monomer containing at least two hydroxyl groups (-OH) in the presence of dibutyltin dilaurate (DBTL) catalyst.



Figure II-14:1,4-polyisoprene cationomers bearing ammonium groups, used as precursors for the preparation of antibacterial ionic polyurethanes and copolyurethanes, ref. [90].

The coatings were tested against *Pseudomonas aeruginosa* and *S.epidermidis* and it was observed that after 8h of contact, the number of bacteria on most of these materials was constant, including polyurethanes (using cationomer <u>1b</u>, <u>2</u>) and copolyurethanes (using cationomer <u>3</u>), showing that they were not antibacterial. Conversely, the ionic copolyurethane using cationomer <u>1b</u> exhibited a significant decrease of living adherent bacteria. This effect could be explained by the proximity of the quaternary ammonium groups to the network junctions, making them inaccessible for the contact with the bacterial wall in the case of ionic polyurethanes. However, it was also proved that biocidal moieties from films containing cationomer <u>1a</u> bearing only one hydroxyl functional group diffused from these polymers, due to their weak intergrafting in the polymer network, yielding inactive polyurethane after leaching. Another drawback was that the quaternary ammonium groups not immobilized to surface cytotoxic once released. Hence the follow up of this first project was the preparation of non-leaching biocidal materials *via* a different polymerization mechanism.

R.Jellali *et al.* [91-93] synthetized another type of 1,4-polyisoprene cationomers bearing ammonium groups and also a photocurable group. These cationomers were used to prepare antimicrobial coatings by radical polymerization of acrylate groups and/or by cationic polymerization via epoxy groups inserted in the polyisoprene backbone. The procedure for the synthesis of both cationomers used in radical photopolymerization is presented in Scheme II-1.



Acrylate NR cationomer

Diacrylate NR cationomer

Scheme II-1: Synthetic route for the preparation of oligoisoprennes bearing quaternary ammonium groups and polymerizable chain ends, ref. [90-93].

Thick coatings were obtained from the photopolymerization of the acrylate oligomers presented in chapter I and different percentages of the cationomers (Scheme II-1). It was demonstrated that the presence of quaternary ammonium groups generated an effective antimicrobial activity against five strains of marine bacteria (*Pseudoalteromonas elyakovii, Vibrio aestuarianus, Polaribacter irgensii, Cobetia marina, Shewanella putrefaciens*). Five strains of marine fungi (*Halosphaeriopsis mediosetigera, Asteromyces cruciatus, Lulworthia uniseptata, Zalerion sp, Monodictys pelagica*) were also tested and only the coatings obtained by radical photopolymerization were active. All surfaces inhibited the growth of six strains of microalgae (*Navicula jeffreyi, Cylindrotheca closterium, Chlorarachnion globosum, Pleurochrysis roscoffensis, Exanthemachrysis Gayraliae, Amphora coffeaeformis*) and of spores of macroalgae *Enteromorpha intestinalis*. No release of biocidal molecules was detected for the coatings.



Table II-2: Summary of the tested coatings, their compositions and their antifouling activities, ref. [93].

These materials were designed to be active against marine microorganisms in view of their application as antifouling coatings for objects immerged in seawater. In the follow up, the collaboration with some colleagues from Akron University (USA) led to perform a study on films obtained from different formulations realized with the same acrylate oligomers and cationomers containing the ammonium groups but concerning their activity against pathogenic bacteria. This idea of having the same material active in two different applications against pathogenic bacteria and in marine antifouling was quite new at the time, as the two different communities working on the two topics were distinct. The hypothesis was that the same mechanisms leading to the formation of biofilms in sea water, may be similar to those used by pathogenic bacteria. So if a polymer has a biocide action toward the first type of strains it could work also with the other ones. The films were obtained from different formulations, containing from 0 to 100% weight of cationomers; as the oligomers bearing the ammonium groups require four steps for their synthesis, we wanted to optimize the formulation in order to insert the minimum amount to have a significant biological activity. The influence of the reactive diluent (HDDA, hexamethylenediacryate) was also studied; it was added to the prepolymerization mixture to reduce the viscosity and to help spreading it on the Teflon molds used to make the films by UV-irradiation. It was found that HDDA did not influence the biological activity (Badawy, H et al. [60, 61], and that it was not soluble with the NR oligomers, generating separate domains in the films after polymerization. Few experiments carried out in dynamic conditions (flowing water at 1 mL/min for 24 hours at RT, unpublished data) showed that the surface of the films changed morphology as if they had lost spherical domains. Hence, in the continuation of the work, the formulation was kept as simple as possible eliminating the reactive diluent.

The films were immerged in presence of three strains of pathogenic bacteria (*S. aureus, S.epidermidis, P. aeruginosa*) and the number of colonies formed by cells which adhered to the surface or remained in the supernatant was counted. Most of the coatings reduced bacterial attachment, especially increasing the number of ammonium groups per oligomer (diacrylate NR cationomer better than monoacrylate NR cationomer) or increasing the percentage of cationomer Table II-2).

Another research group (Chun Li *et al.* [94]) prepared antimicrobial NR based polymers bearing phosphonium groups incorporated onto the molecular chains of epoxidized natural rubber (ELNR). Quaternary phosphonium salts were obtained by ring opening of epoxy groups with bromoacetic acid then reaction with triphenylphosphine (Scheme II-2). It was reported that PPh₃–ELNR presented better antimicrobial activity toward Gram-negative *E.Coli* than Gram-positive *S. aureus*.



Scheme II-2: The route to synthesize epoxidized and PPh₃-epoxidized NR chains, ref. [94].

II.3 Conclusions

In the first part of this chapter, we have accentuated the importance of antimicrobial polymers in everyday life, including the impact of microbial adhesion, the basic concept for prevention biofilms formation. We also reviewed the different structures and properties of cationic antimicrobial polymers, their methods of preparation and the key factors affecting their properties. The interest to use guanidinium as bioactive group was also discussed. In the last section of this bibliography analysis, we have highlighted the previous works of our group, on cationic antimicrobial polymers based on Natural Rubber, modified with quaternary ammonium.

In the final part of the chapter, we present a new type of antimicrobial coatings non-releasing and respectful of the environment have been successfully synthesized during this PhD. These results are presented under the format of a research paper (submitted). This material was prepared with a strategy that combined the industrial interests from Natural Rubber, explained in Chapter I and using a guanidium with high water solubility, wide-spectrum antimicrobial activity, excellent biocide efficacy and nontoxicity that was previously reviewed.

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II.4. Antibacterial activity of natural rubber based coatings containing a new guanidinium-monomer as active agent

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Abstract

This paper presents the synthesis and biological activity of a new antimicrobial material based on natural rubber derived building blocks and an organic antibacterial monomer, covalently bound to the polymer network. Our investigation has focused on the synthesis of an original acrylate monomer bearing an organic biologically active moiety (a guanidinium group) and in its co-polymerization in presence of telechelic acrylate oligomers, prepared from polyisoprene. The cross-linked films obtained have been characterized by InfraRed Spectroscopy, contact angle, and thermal analyses. We showed that no-leaching of the bioactive monomer occurred and that the material resists to long water immersions. Polyisoprene coatings prepared from pure acrylate oligoisoprenes also showed a weak antimicrobial activity that we drastically increased by integrating the guanidinium monomer. Biological tests carried out with three strains of pathogenic bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus*, and *Staphylococcus epidermidis*) proved the strong activity of the coatings.

Keywords

Natural rubber, guanidine and polyisoprene, guanidinium monomer, antibacterial and biocide polymers, antimicrobial non-leaching coatings, antifouling materials

1. Introduction

The search for new antibacterial/antifouling materials with applications in the medical and marine environments is at present the subject of numerous research works, as testified by the great number of publications and patents on this topic. However, miracle solutions have not been found yet.

The academic and industrial research effort in the synthesis of antimicrobial polymers is quite astonishing as demonstrated by the number of papers and patents published every year on this subject. The areas of application of the antibacterial polymers range from healthcare and biomedical devices, to drug delivery, dental

applications, food packaging, and textiles¹⁻⁴, just to name a few, as consequence, a wide number of new and different materials are tested depending on the application. So why new antimicrobial polymers would be needed since so many are already available on the market? One of the answers is that unfortunately pathogenic bacteria are extremely good at developing antibiotic resistance⁵ and there is a decline in the discovery of new antibiotics. The risk of catching dangerous or fatal⁶ infections is still considerably high. One possible solution to this problem is to reduce the probability of contamination by generating antimicrobial coatings, which could cover different material surfaces, minimizing the transfer of the bacteria themselves and consequently reducing the number of infections and the assumption of post-contamination drugs. Moreover, as the mechanism of action of polymeric materials differs from antibiotics, the resistance phenomenon could be thwarted. Public consciousness of environmental problems also pushes towards alternatives to petrol based polymers using biobased and sustainable resources instead and expecting no discard of nanoparticles or biocides in fresh or sea water.

The design of antimicrobial surfaces implies different mechanisms of action towards the bacteria. Thus the polymers are mainly divided in: biocidal polymers, which possess an intrinsic biocidal action (natural ,such as chitosan or synthetic, such as polyammonium); polymeric biocides (the biocide molecule is covalently attached to the backbone); biocide releasing polymers (with embedded nanoparticles and antibiotics in the polymeric matrix, to be released in the environment)². Different factors affect the biological activity such as the molecular architecture (homopolymer, copolymer; random, grafted, or di-block), the hydrophobic/hydrophilic balance (zwitterionic, amphiphilic polymers), the molecular weight, and the ionic / polar group effect (presence of ammonium, phosphonium, sulphonium groups or cationic metals).⁷ Original approaches are often proposed to avoid releasing biocides or nanoparticles in the environment, such as self-assembled nanofilms formed by the protein lysozyme from daily food, which showed durable *in vitro* and *in vivo* broad-spectrum antimicrobial efficacy against Gram-positive/negative and fungi. ⁸ Indeed many recent examples could be cited⁹, however we will focus more on cationic polymers.

The originality of the presented approach consists in the choice of the polymer substrate and in the synthesis of a new reactive monomer that will not be released. Natural rubber is composed by long polyisoprene chains with dead ends (aliphatic and pyrophosphate groups) and it is usually used without further modifications. In this work, functional oligoisoprenes have been obtained by breaking statistically a targeted number of carbon – carbon double bonds in the polyisoprene chain. In our group, two methodologies have been developed to obtain functional macromonomers from natural rubber. The first method allows the generation of telechelic oligomers with a ketone and an aldehyde groups at the extremities, via an oxidation reaction with periodic acid¹⁰, while the second one allows the introduction of two identical groups at the oligomer chain ends via a cross metathesis reaction¹¹. The first approach was used in this work as it allows the control of the oligomer chain length in the range 800-18000 g/mol with a dispersity around 2. Successively the carbonyl groups in the oligomers have been reduced to alcohols, and finally the reaction with acryloyl chloride generated acrylate chain ends, which could be polymerized by a classical radical polymerization mechanism. In our previous work, these acrylate oligomers have been photopolymerized to obtain cross-linked thick and thin films. The

bactericidal activity was due to the action of quaternary ammonium groups covalently bound to the chain ends of a second type of oligoisoprenes added to the formulation in various percentages. The films showed to be effective towards marine and pathogenic bacteria, as well as microalgae, marine fungi and macroalgae¹²⁻¹⁷. The effects achieved with these materials were satisfying, however studies about the danger represented by ammonium groups as endocrine disruptors started to appear, such as the list of 9 ammonium salts published by the United States Environmental Protection Agency¹⁸, or the French study carried out by INERIS back in 2012^{19} . Even if the ammonium groups were not supposed to be released by our surfaces, the leaking provoked by ageing or accidental disaggregation must be taken into account, so this pushed us to look for new active agents, in case these preliminary studies will be confirmed and the use of ammonium definitively forbidden. Our attention was attracted by the positively charged guanidinium group, which appears in the backbone of some antibacterial polymers²⁰⁻²¹. It is known that molecules with positive charges are able to kill bacteria and recently it has been shown that the same effect is obtained if the charges are covalently attached to a surface²². Furthermore, an evaluation of the surface charge density necessary to kill two strains of pathogenic bacteria, E.coli and S.epidermidis, has been estimated in the case of poly(4-vinyl-N-butylpyridinium) bromide brushes grafted on silicon wafers: it depended on the bacteria type and growth state²³. Both bacteria were killed or their membrane was seriously damaged by contact, both in the dividing and quiescent conditions. A threshold of positive charge was necessary to obtain an instant killing, depending on the bacteria metabolism. The explanation of the action of positive charges on both Gram-positive or negative bacteria was the electrostatic adhesion of the negative charges present on the microorganisms membranes to the opposite charged substrate. The consequence is that the bacteria lose their natural counter cations (such as Mg^{2+} and Ca^{2+}) when binding the positively charged polymers. The requirement of a minimum charge density for an immediate effect has been explained by the need of completely removing the counter cations to provoke the cell death.

As mentioned, in other studies the guanidinium group was in the backbone of the polymer chain; for instance, it was linked to norbornene monomers by ring opening metathesis to give polyguanidiniumoxanorbornene chains, which were active in solution against Gram-negative and Gram-positive bacteria, and were nonhemolytic against human red blood cells²⁰. In some cases the guanidinium group constituted the backbone itself, for example polyhexamethylene guanidine hydrochloride polymers were obtained from the reaction of guanidine hydrochloride and hexamethylene diamine and combined with bacterial cellulose to obtain a composite for wound healing²¹. The composite material showed excellent efficacy against multidrug resistant strains *Staphylococcus aureus* and *Klebsiella pneumonia* IMBG233, the phytopatogenic *Xanthomonas campestris* pv campestris IMBG299 and *Pseudomonas syringae* pv. tomato DC3000, as well as yeast. Recently, a guanidine-based polymer, namely poly(guanidine-hexamethylenediamine-Polyethyleneimine) was first synthesized by polycondensation and then covalently grafted to a polyamide membrane for reverse osmosis, showing antibacterial activity and antibiofouling property against *Escherichia coli* and *Bacillus subtilis*²⁴.

The strategy herein presented consisted in using the acrylate terminated oligoisoprenes to build an active polymer matrix and to increase the antimicrobial effect by co-polymerizing a novel acrylate monomer

containing a guanidinium group, in order to obtain cross-linked, elastic coatings, instead of following the strategy of the post-functionalization of amine groups on polymers repeating units to obtain the guanidinium groups²⁵. The results obtained so far concerning the optimization of the material formulation, the assessment of the non-leaching properties, and the action against three strains of the most dangerous pathogenic bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus*, and *Staphylococcus epidermidis*) are presented. The antifouling activity on marine bacteria strains and microalgae is currently tested and will be presented in a forthcoming paper.

2. Materials and methods

2.1 Materials

Natural rubber (NR, STR5LCV60) was produced by Jana Concentrated Latex Co., Songkhla, Thailand. Periodic acid (H₃IO₆, \geq 99%), triethylamine (Et₃N, \geq 99.0%), acryloyl chloride (\geq 97.0%), 2hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur 1173, \geq 97.0%), Pentaerythritol A technical grade, PETA), glutaraldehyde solution 2.5% in water, hexamethyldisilazane reagent plus 99.9%, were purchased from Sigma Aldrich and used without any further purification. Concentrated HCl and NaOH were also purchased by Sigma Aldrich solutions (research grade) were diluted to obtain the desired concentrations. Trifluoroacetic acid was purchased from Alfa Aesar. Sodium borohydride (NaBH₄, \geq 98%), 4-aminobutan-1ol (\geq 98%), *N*, *N'*-bis (*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (\geq 98%), cacodylic acid sodium salt trihydrate (98%) were purchased from Acros Organics. Sodium bicarbonate (NaHCO₃) technical reagent grade, anhydrous magnesium sulfate (MgSO₄) laboratory reagent grade, sodium thiosulfate pentahydrate (Na₂S₂O₃·5H₂O) and sodium chloride (NaCl) general purpose grade were purchased from Fisher Scientific. Dichloromethane (DCM), tetrahydrofuran (THF) were purchased from Aldrich and dried in a dry solvent station GT S100 (Glass Technology). Ethyl acetate (EtOAc) and cyclohexane were purchased from Fisher Scientific and used after distillation. Water was deionized using Ultrapure Milli-Q Reagent Water System Millipore and used at 18MΩ.

2.2. Physico-chemical characterization

Nuclear magnetic resonance (NMR) Analysis. ¹H-NMR and ¹³C-NMR spectra were respectively recorded at 400 and 100,6 MHz on a Bruker[®] spectrometer. The samples were dissolved in chloroform-*d* (CDCl₃) or methanol-*d*₄ (CD₃OD). The chemical shifts (δ) are expressed in part per million (ppm) relative to Me₄Si and the coupling constants (J) in Hertz.

Size exclusion chromatography analysis. Number-average molecular weight (\overline{M}_n) and dispersity (\overline{D}) were measured by size exclusion chromatography (SEC) in THF as eluent (flow 1 mL/min), at 35°C, on a Waters[®] chain 2707 autosampler equipped with a 1515 Isocratic Pump and a guard column (Styragel 30x4.6

mm) connected to a column (Styragel HR2 + HR4, 300x7.8nm). The Waters[®] 2996 PDA and Waters[®] 2414 Refractive Index Detector were used. Calibration was performed with polystyrene (PS) standards in the range from 580 g/mol to 483000 g/mol. The molecular weights of the oligoisoprenes were corrected according to the Benoit factor equal to 0.67^{26} . Generally, the molecular weight of all oligomers was determined by SEC and by ¹H-NMR. The ¹H-NMR spectrum for the carbonyl telechelic oligomers (CTNR) was exploited according to Eq. (1) and the relative number-average molecular weight was referred to as $\overline{M}_{n, NMR}$.

$$\overline{M}_{n,NMR} = \left[\frac{I_{5.13ppm}}{(I_{2.47ppm}/4)} \times 68\right] + 100$$
(1)

 $I_{5.13ppm}$ and $I_{2.47ppm}$ were the integration values of the peaks at 5.13 ppm and at 2.47 ppm, respectively. 68 and 100 were the molar masses of the isoprene repeating unit and the chain ends of CTNR, respectively (g/mol). $\overline{M}_{n,NMR}$ was used to calculate the molar concentration of the oligomers for the modification of the chains ends in the synthesis of hydroxy telechelic natural rubber (HTNR) and acrylate telechelic natural rubber (ACTNR).

LC-MS analysis. Liquid chromatography coupled to mass spectrometry experiments were performed using an UltiMateTM 3000 Rapid Separation Binary System (Thermo Fischer Scientific), interfaced with a mass spectrometer MicroTOF-QIII (Bruker[®]), equipped with an electrospray-ionization source operated in the positive and negative mode. The spectra were recorded in the range scan of 50 to 1000 m/z. Chromatographic separations were performed using a C18 HTec (2.0x250mm inner diameter, 5µm particle size, Fischer Scientific) at a column temperature of 35° C and at a flow rate of 0.3 mL/min. The mobile phase composition was a mixture of water and acetonitrile with gradient time period.

Photopolymerization equipment. The samples used for the biological tests were polymerized using a UV-curing equipment (UV Fusion System Corporation, Hanau, Germany), equipped with a hydrogen lamp (power 120 W/cm). The belt speed was 1.6 m/min, and the light intensity 0.313 J/cm². The prepolymerization mixture was deposited in TeflonTM molds of 1 cm diameter and 0.5 mm depth and flexible disks were obtained after irradiation. The kinetic study of photopolymerization was carried out irradiating with a UV lamp in the wavelength range 320-390 nm.

Fourier-Transform Infrared Spectroscopy (FTIR). The photo-crosslinking reaction kinetic was monitored by a FTIR Vertex 70V Bruker spectrometer with a deuterated triglycine sulfate detector, in ATR mode (attenuated total reflection). The absorption bands were recorded in the range of 400 - 4000 cm⁻¹ with 16 scans and a resolution of 2 cm⁻¹. The data were analyzed using OPUS software. The conversion of functional groups at (t) time is denoted as % and it is defined by Eq. (2) ¹²

$$\% = \frac{\left(\frac{A1405}{A1375}\right)0 - \left(\frac{A1405}{A1375}\right)t}{\left(\frac{A1405}{A1375}\right)0} * 100 \quad (2)$$

The areas under the absorbance peaks were calculated and reported at the initial time (A₀) and at different times (A_t). 1405 cm⁻¹ corresponds to the $\delta_{=CH2}$ of the acrylate chains ends and 1375 cm⁻¹ is the reference band which belongs to the $\delta_{=CH3}$ of the *cis*-1,4-polyisoprene repeating unit.

Microscopy

Scanning Electron Microscope: Micrographs were taken using a JEOL, JSM 6510 LV instrument. The samples were covered by a golden film before examination.

Optical microscope: Zeiss scope A.1., Axiovision program, equipped with a HB100 lamp.

Themogravimetric analysis

Thermogravimetric analysis (TGA) was performed on a TA Instrument (Hi-Res-Dynamic TGA Q 500) with an initial heating rate 10°C/min, under nitrogen or oxygen, in the range 25°C-800°C. The sample weight was 10 mg.

Contact angle measurement

Water contact angles (CAs) were measured at room temperature using a homemade setup with a halogen source and telecentric lens on the source and digital camera pathways. The thick films studied were deposited on microscopy glass slides, which were first extensively cleaned. Contact angles were measured with a precision of 0.1° . Several drops of 2 µL were deposited on different regions over the films surface to obtain an average value for each sample.

2.3. Synthesis of Guanidinium Monomer

The chemical structures of the compounds are displayed in Scheme 2.

2.3.1. Synthesis of carbamic acid, *N*,*N*'-[(4-hydroxybutyl)carbonimidoyl]bis-,C,C'-bis(1,1-dimethylethyl) ester (2)

To a solution of 4-aminobutan-1-ol (0.604 mL, 6.6 mmol, 1 equiv.) in dry THF (50 mL), under Argon, were successively added *N*, *N*'-bis (*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (2.04 g, 6.6 mmol, 1 equiv.) and Et₃N (0.92 mL, 6.6 mmol, 1 equiv.). After overnight stirring, the solvent was evaporated under reduced pressure and the crude product was taken up in ethyl acetate (40 mL). The organic layer was washed with HCl 0.25M aqueous solution (40 mL) and NaHCO₃ saturated aqueous solution (40 mL) then dried over MgSO₄ and concentrated under vacuum. The crude product was purified by flash chromatography (EtOAc/Cyclohexane: 1/2) to obtain the compound **2** (1.42 g, 4.3 mmol, 65%) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 11.46 (s, 1H, NH), 8.36 (t, *J* = 5.4 Hz, 1H, NH), 3.68 (t, *J* = 6.1 Hz, 2H, H-1), 3.43 (dt, *J* = 6.4 Hz, 5.4 Hz, H-4), 2.2 (s, 1H, OH), 1.85-1.52 (m, 4H, H-2, H-3), 1.48 (s, 9H, *t*Bu), 1.49 (s,9H, *t*Bu).

¹³C NMR (101 MHz, CDCl₃): δ 163.8 (C-11 or C6), 156.5 (C-5), 153.6 (C-6 or C11), 83.4 (C-12 or C7), 79.6 (C-7 or C12), 62.4(C-1), 40.7 (C-4), 29.8 (C-2), 28.6 (C-*t*Bu, Me), 28.4 (C-*t*Bu, Me), 25.9 (C-3) HRMS (ES⁺) calculated for C₁₅H₂₉N₃O₅ [M+Na]⁺, 354.1999 found 354.1991 and calculated for [M+H]⁺, 332.2180 found 332.2182

2.3.2. Synthesis of Carbamic acid, [(4-acryloxybutyl)carbonimidoyl]bis-,*C*,*C*'-bis(1,1-dimethylethyl) ester (3)

To a solution of <u>2</u> (301 mg, 0.91 mmol, 1.0 equiv.) in dry DCM (20 mL) was added under Ar at 0°C, Et₃N (0.22 mL, 1.59 mmol, 1.75 equiv) and acryloyl chloride (110 μ L, 1.35 mmol, 1.5 equiv.). The reaction mixture was stirred at RT for 24h then quenched by the addition of an HCl 0.25M aqueous solution (5 mL). The organic layer was washed with NaHCO₃ saturated aqueous solution (20 mL) then dried over MgSO₄ and concentrated under vacuum. The residue was purified by flash chromatography (EtOAc/Cyclohexane: 2/8) to provide the compound **3** (320 mg, 0.83 mmol, 91%) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ 11.49 (s, 1H, NH), 8.33 (t, 1H, *J* = 5.2 Hz, NH), 6.39 (dd, *J* = 17.4 Hz, 1.5 Hz, 1H, H-1*trans*), 6.10 (dd, *J* = 17.4 Hz, 10.5 Hz, 1H, H-2), 5.81 (dd, *J* = 10.5 Hz, 1.5 Hz, 1H, H-1*cis*), 4.16 (t, *J* = 6.0 Hz, 2H, H-4), 3.45 (dt, *J* = 5.4 Hz, 7.1Hz, 2H, H-7), 1.77-1.62 (m, 4H, H-5 and H-6), 1.49 (s, 9H, *t*Bu), 1.48 (s, 9H, *t*Bu).

¹³C NMR (101 MHz, CDCl₃) δ 166.5 (C-3), 163.9 (C-14 or C9), 156.5 (C-8), 153.7 (C-9 or C14), 131.0 (C-1), 128.8 (C-2), 83.4 (C-15 or C-10), 79.3 (C-10 or C15), 64.0 (C-4), 40.4 (C-7), 28.3 (C-*t*Bu, Me), 28.0 (C*t*Bu, Me), 25.98(C-5), 25.70 (C-6).

HRMS (ESI⁺) $C_8H_{16}N_3O_2$ calculated for [M+H] ⁺, 386.2213 found 386.2286

2.3.3. Synthesis of Guanidine, N-[4-(acryloxy)butyl]-,trifluorate (4)

To a solution of ($\underline{3}$) (3.1 g, 8.05 mmol, 1 equiv) in dry DCM (50mL) was added TFA (50mL) under Argon. The reaction mixture was stirred for 18h at room temperature then concentrated under vacuum. The crude product was taken up in DCM (30mL) and water (20mL) then the organic phase was extracted with water (2x10mL). The combined aqueous layers were washed with DCM (20mL) then lyophilized to give the expected compound (2.31g, 7.73 mmol, 96%) as a white solid.

¹H NMR (400 MHz, CD₃OD): δ 6.38 (dd, J = 17.3 Hz, 1.6 Hz, 1H, H-1*trans*), 6.16 (dd, J = 17.3, 10.4 Hz, 1H, H-2), 5.88 (dd, J = 10.4 Hz, 1.6 Hz, 1H, H-1*cis*), 4.20 (t, J = 6.3 Hz, 2H, H-4), 3.22 (t, J = 6.9 Hz, 2H, H-7), 1.79-1.63 (m, 4H, H-5 and H-6).

¹³C NMR (101 MHz, CD₃OD) δ 167.7 (C-3), 161.9 (C-2'), 161.5 (C-1'), 158.7(C-8), 131.6(C-1), 129.5(C-2), 65.0 (C-4), 42.0(C-7), 26.9 (C-6), 26.5(C-5).

 ^{19}F NMR (376 MHz, CD₃OD) δ -75.88

HRMS (ESI⁺) $C_8H_{16}N_3O_2$ calculated for [M+H]⁺, 186.1237 found 186.1235

HRMS (ESI⁻) C₂F₃O₂ calculated for [M-H]⁻, 112.9856 found 112.9857

2.4. Synthesis of acrylate oligomers

2.4.1. Synthesis of carbonyl telechelic oligomers (CTNR)

NR crumbs (100g, 0.167mol, 1 equiv.) were suspended in THF (3L, 0.56 M) for 18 h for dissolution, heating at 30°C, in a 5 L reactor, with mechanical stirring. H_5IO_6 (12.16g, 0.534mol, 3.2equiv.) was dissolved in 300

mL of dry THF and added slowly to the NR solution using a dropping funnel. The reaction mixture was stirred for 6 h at 30°C then filtered and the filtrate was concentrated under reduced pressure. The oligomers were dissolved in DCM (3L) and the solution was washed successively with a mixture (2*1L) of an aqueous solution of saturated NaHCO₃ and saturated NaCl (70/30, V/V) then a mixture of an aqueous solution of Na₂S₂O₃ (20% w/w) and saturated NaCl (2*1L, 50/50, V/V). The combined organic layers were dried over MgSO₄ for 2h then filtered and concentrated under vacuum (up to constant weight) to provide CTNR (85g, 85%) as an orange-yellow viscous liquid.

¹H-NMR(400 MHz, CDCl₃-*d*) δ 9.77 (s, 1H, CH₂C*H*O), 5.10 (m, nH, CH₃C=*CH*_{isoprene unit}), 2.52-2.45 (m, 2H, C*H*₂CHO), 2.43 (t, J=7.6 Hz, 2H, CH₃COC*H*₂), 2.34 (t, J=7.6 Hz, 2H, C*H*₂CH₂CHO), 2.24-2.30 (m, 2H, CH₃COCH₂C*H*₂), 2.13 (s, 3H, C*H*₃COCH₂), 2.00-2.11 (m, 4nH, C*H*_{2, isoprene unit), 1.68 (s, 3nH, C*H*₃CCH_{isoprene unit}).}

¹³C-NMR (101MHz, CDCl₃-*d*) δ (ppm): 208.6 (*C*=O_{ketone}), 202.1 (*C*=O_{aldehyde}), 135.2 (*C*=CH_{isoprene unit}), 125.1 (C=*C*H_{isoprene unit}), 44.0 (*C*H₂COCH₃), 42.4 (*C*H₂CHO), 32.2 (*C*H₂C=CH_{isoprene unit}), 26.4(*C*H₂CH=C_{isoprene unit}), 24.3 (*C*H₃CO), 23.5 (*C*H₃, isoprene unit). FTIR: v (cm⁻¹): 3035 (H-C=C), 2959-2726 (CH), 1721 (C=O), 1665 (C=C), 834 (C=C-H). $\overline{M}_{n, \text{ SEC}} = 5200 \text{ g/mol}, \text{ D}=1.94$ $\overline{M}_{n, \text{ RMN}} = 5200 \text{ g/mol}$

2.4.2. Synthesis of hydroxyl telechelic natural rubber (HTNR)

NaBH₄ (1.14 g, 30.0 mol, 10 equiv.) was added under Argon to a solution of CTNR ($\overline{M}_{n, NMR} = 5000$ g/mol, 15.00 g, 3.0 mmol, 1 equiv.) in dry THF (300 mL). The reaction mixture was stirred for 24 h at 60°C, hydrolyzed with 300mL of ice then concentrated under vacuum. The residue was dissolved in DCM (400 mL) and washed with an aqueous NaCl saturated solution (250 mL). The organic layer was dried over MgSO₄ for 1 h, filtered and concentrated under vacuum (up to constant weight) to provide HTNR (12.0g, 80%) as a yellow viscous liquid.

¹H-NMR (400 MHz, CDCl₃-*d*) δ 5.10 (m, nH, CH₃C=C*H*_{isoprene unit}), 3.69-3.82 (m, 1H, C*H*OH), 3.65 (t, 2H, C*H*₂OH), 1.96-2.11 (m, 4nH, C*H*₂ isoprene unit), 1.68 (s, 3nH, C*H*₃, isoprene unit), 1.18 (d, 3H, C*H*₃CHOH).

¹³C-NMR (101 MHz, CDCl₃-*d*): δ 135.2 (*C*=CH_{isoprene unit}), 125.1 (C=*C*H_{isoprene unit}), 68.0 (*C*HOH), 62.9 (*C*H₂OH), 32.2 (*C*H₂C=CH_{isoprene unit}), 26.4 (*C*H₂CH=C_{isoprene unit}), 23.5 (*C*H₃, isoprene unit), 22.3 (*C*H₃CHOH).

FTIR: v (cm⁻¹): 3374 (OH), 3035 (H-C=C), 2959-2726 (CH), 1665 (C=C), 834 (C=C-H).

 $\overline{M}_{n, \text{SEC}}$ = 5500g/mol, Đ=2.5

 $\overline{M}_{n, \text{RMN}} = 5200 \text{ g/mol}$

2.4.3. Synthesis of acrylate telechelic natural rubber (ACTNR)

To a solution of HTNR ($\overline{M}_{n, NMR}$ = 5000 g/mol) (10.0 g, 2.0 mmol, 1 equiv.) in dry DCM (200 mL) were added under Ar and at 0°C triethylamine (1.74 mL, 12.49 mmol, 6.25 equiv.) and acryloyl chloride (0.97 mL, 11.99 mmol, 6 equiv.). The reaction mixture was stirred at RT for 48h then DCM (300 mL) was added and quenched with an aqueous solution composed by 50% NaOH aqueous solution 0.25M and 50% of saturated NaCl solution (total volume 300 mL). After transfer in a separating funnel, the organic phase was washed with an aqueous solution composed by 50 % HCl aqueous solution 0.25M and 50 % of saturated NaCl (300 mL). The final washing was performed with an aqueous solution composed by 50% saturated NaHCO₃ (200 mL) and 50 % NaCl. Finally, the product was dried over MgSO₄ for 1 h and concentrated under vacuum. ACTNR was obtained as a yellowish viscous liquid in 80% yield (8.2 g).

¹H-NMR (400 MHz, CDCl₃-*d*) δ 6.38 (dd, *J* = 17.3 Hz, 1.5 Hz, 2H, CHC=C*H*_{2trans}), 6.12 (dd, *J* = 17.4 Hz, 10.5 Hz, 2H, CHC=CH₂), 5.8 (dd, J=10.2 Hz, J=1.5 Hz, 2H, CHC=C*H*_{2cis}), 5.10 (m, nH, CH₃C=C*H*), 4.96-4.92 (m,1H, CH₃CHOC(O)), 4.14 (t, 2H, C(O)OC*H*₂), 2.11-1.98 (m, 4nH, C*H*₂, isoprene unit), 1.68 (s, 3nH, C*H*₃, isoprene unit), 1.24 (d, 3H, C*H*₃CHO).

¹³C-NMR (101 MHz, CDCl₃-*d*): δ 166.2 (CHOCO), 165.9 (CHOCO), 135.2 (*C*=CH_{isoprene unit}), 130.5 (HC=*C*H₂, acrylate), 130.1(HC=CH₂, acrylate), 129.1(C=*C*H₂, acrylate), 128.6 (C=*C*H₂, acrylate), 125.1 (C=*C*H_{isoprene unit}), 70.9 (CHOCO), 64.3 (CH₂OCO), 32.2 (CH₂C=CH_{isoprene unit}), 26.4 (CH₂CH=C_{isoprene unit}), 23.5 (CH₃, isoprene unit).

FTIR: v (cm⁻¹): 3035 (H-C=C), 2959-2726 (CH), 1720 (C=O, ester), 1639 (C=C, isoprene), 1406(C=C, acrylate), 1126 (C-O), 834 (C=C-H).

 $\overline{M}_{n,\text{SEC}} = 5500 \text{ g/mol}, \text{ } \text{D}=2.9$ $\overline{M}_{n,\text{NMR}} = 5300 \text{ g/mol}$

2.5 Films preparation

Guanidinium monomer was dissolved in the reactive diluent (PETA), sonicated for 10 min at RT, and then added to the ACTNR oligomers and photoinitiator mixture (Scheme 1, Table 1). This suspension was mixed with a spatula then immerged for 5 min in an ultrasonic bath. The viscous solution was degassed under light vacuum and homogeneity of the dispersion was observed by optical microscope. On average, 50 mg of prepolymerization mixture were deposited on a Teflon mold (diameter 1 cm, thickness 0.5 mm) and irradiated by the UV lamp for the required amount of time. The yellowish elastic films were easily removed from the mold with tweezers to perform FTIR spectra and check the complete polymerization.



Scheme 1: General procedure for the synthesis of the antibacterial coatings.

2.6. Leaching tests protocol

The leaching tests were performed immerging the thick films in de-ionized water at 25°C for 14 days using two formulations: (F1) containing only oligoisoprenes without the antimicrobial group and (F2) containing the antimicrobial group (Table 1). Each film was introduced separately into a 10 mL glass flask containing 5mL of de-ionized water (18MQm⁻¹, Veolia water STI). These flasks were placed in an orbital shaker operated at 50 rpm. The solutions were sampled at different times and analyzed by UV spectrometry and High Performance Liquid Chromatography (HPLC) to detect the percentage of Darocur 1173 photoinitiator and Guanidinium monomer released. Following the same procedure, several assays with the two formulations were performed also at 37°C, analyzing the supernatants after 15 and 60 min.

Darocur 1173 release was monitored using a UV-3100PC Spectrophotometer (VWR) set at a wavelength of 247 nm. Quartz cells of 1 cm path length were used, with de-ionized water as a reference. A calibration range of Darocur 1173 was prepared in de-ionized water from 0.86 mg L⁻¹ to 17.23 mg L⁻¹. The calibration curve was linear with optical density ranging from 0.0 to 0.85. Supernatants after incubation were diluted before analysis to be within this range of absorbance²⁷. Benzaldehyde and benzoic acid, which have been reported to be the major products from Darocur 1173 photolysis ²⁸ absorb at the same wavelength as Darocur 1173 when analyzed by UV spectrophotometry. HPLC was therefore used to separate Darocur 1173 from its photoproducts, in order to quantify Darocur 1173 specifically. Benzaldehyde and Darocur 1173 were analyzed by injecting 20 μ L samples into a ThermoFisher Scientific series instrument equipped with a C18 column operated at 30°C (Alltima, 3 μ m, 2.1 x 150 mm) and a UV detector (Waters 996) set at 247 nm. The mobile phase was a water/acetonitrile (w/ac) solution with 0.1% TFA pumped at a flow rate of 0.2 mL/min, whose composition varied as follows: 85:15 w:ac (v/v) to 60:40 w:ac (v/v) for the first 12 min, followed by 5 min of stabilization

then 100% acetonitrile for 3 min, followed by 5 min of stabilization. Retention times were 16.1 min for benzaldehyde and 16.7 min for Darocur 1173. Calibration was performed between 0.86 and 17.23 mg.L⁻¹ with Darocur 1173 and from 1.66 to 16.64 mg.L⁻¹ for benzaldehyde.

Benzoic acid was analyzed in the same manner, setting UV detector at 230 nm. Retention time was 13.4 min. Calibration was done between 1.13 and 22.6 mg L^{-1} .

Guanidinium monomer release was monitored by HPLC as previously described and a UV detector set a 205 nm. Retention time was 3.7 min. The calibration quantified by peak area was linear between 25.65 mg.L⁻¹ to 513 mg.L⁻¹. The LOQ (Limit of Quantification) for Guanidinium Monomer was 20 mg.L⁻¹ (or percentage of Guanidinium monomer released < 3.9%).

2.7 Biological assays

Three pathogenic bacteria were used in this study: *Pseudomonas aeruginosa* (CIP: A22, **PA**, Gram negative), *Staphylococcus aureus* (CIP 52.16, **SA**, Gram positive), *Staphylococcus epidermidis* (CIP: 176.117, **SE**, Gram positive) from the Louis Pasteur Institute collection. Enzymatic catalase test was performed to check Grampositive strains integrity and oxidase test for Gram-negative ones, then three suspensions were prepared in culture media. The following concentrations were determined by comparing the suspension absorbance (at 600 nm for PA et SE, et 650 nm for SA) with the reference one from the literature: PA, $5.2 \cdot 10^6$ UFC/mL; SA, $9.75 \cdot 10^6$ UFC/mL; SE, $7.5 \cdot 10^6$ UFC/mL, respectively. Antibiograms were performed to determine the minimum inhibitory concentration (MIC) of the molecules that were used to make the films. Of each compound, 10 µL were deposited in 6 mm diameter holes made on the nutrient agar (BIOKAR diagnostics-solabia, Allonnes, France) contained in Petri dishes, on which the bacterial suspensions were spread (Figure 1 SI). Guanidinium monomer was dissolved in sterile water in a range of concentrations 0.8 – 40 mg/mL; Darocur 1173, PETA, and acrylate oligomers were deposited as pure samples and the inhibition halo diameter was measured after incubation at 37°C for 24h. A strain per Petri dish was used, and results were calculated from five replicates per strain.

Attachment assays were performed using 4 films per bacterial strain; 3 films were used to enumerate viable bacteria and one treated to take SEM images (scanning electron microscopy). The bacterial suspension itself was used as growth control (after 3h of incubation at 37°C, without films) and pure ethanol was used as biocide agent. 250 μ L of bacterial suspension were deposited on the surface of each disk to completely cover it, and they were incubated in an oven at 37°C, for 3h. Successively, the 250 μ L of bacterial suspension were removed and the disks have been transferred in tubes containing 1 mL of nutrient broth and vortexed (Phoenix instrument RS-VF10) at the maximum speed for 30 s. The bacterial suspensions taken from the tubes (50 μ l) were injected in an automatic spiral plate (easySpiral, Interscience, Saint Nom, France); the appropriate dilutions of cultures were made and spirally plated on nutrient agar plates. The plates were incubated at 37°C, for 24h and the colonies were enumerated using the *easyspiralpro* program. For the reuse tests, the films after the first incubation, were decontaminated by washing extensively with ethanol, irradiating briefly with UV

lamp, and then they were incubated a second time following the same procedure described for the attachment assays.

The procedure to fix bacteria on the surfaces for SEM images was the following²⁹: after incubation and drying, the disks were washed twice with 1 mL of sodium cacodylate buffer (pH 7.4) and immersed for 30 min in a 2.5% glutaraldehyde solution in 0.05 M sodium cacodylate buffer (pH 7.4), at RT. The disks were washed again twice with sodium cacodylate buffer, immerged in a series of ethanol/water solutions (30/70, 50/50, 70/30, 90/10, 100) for 15 min in each one, and then immerged in pure hexamethyldisilazane for 30s. The films were coated with gold before observation.

3. Results and discussion

The aim of this work was to synthesize new environmentally friendly materials based on natural rubber and containing a novel guanidinium monomer displaying antibacterial properties. A photopolymerization between macromonomers (ACTNR) obtained from the controlled degradation of natural rubber and a monomer bearing the antibacterial group (guanidinium) was envisioned (Scheme 1). The main advantages of this strategy are to bind covalently the group responsible of the activity to the polymer network and so to avoid its leaching from the material. The synthesis of the macromonomers ACTNR was performed according to a procedure previously optimized ¹³. The results are discussed in four sections: the first one describes the synthesis of the active guanidinium monomer, the second one describes the studies of the film formulation and the photopolymerization of mixtures active monomer/ACTNR. The third part concerns the leaching tests results and the fourth part is dedicated to the antimicrobial activity.

3.1. Synthesis of guanidinium monomer

A new guanidine type monomer was synthesized, bearing a guanidinium group (known for its wide-spectrum antibacterial activity) and an acrylate group (polymerizable functional group with the same reactivity of the ACTNR). These monomers, which may co-polymerize with telechelic acrylate oligomers *via* UV-curing, appeared as an attractive process because these solvent-free mixtures can be cured within minutes at ambient temperature to yield highly resistant cross-linked polymers. The targeted guanidinium monomer **4** was prepared according to a strategy in three steps from 4-hydroxybutylamine **1** (Scheme 2). At first, the *N*,*N*'-di(BOc, *tert*-Butoxycarbonyle) protected guanidine function was introduced in mild conditions from the amine of the compound **1** and *N*, *N*'-bis(tert-butoxycarbonyl)-1H-pyrazole-1-carboxamidine^{30,31} with a good yield (65%). The compound **2** was then esterified by reaction with acryloyl chloride in order to introduce the acrylate function. The last step consisted in the deprotection of the guanidine function using trifluoroacetic acid. The final monomer **4** was obtained as a guanidinium salt with a counterion CF₃CO₂⁻ in 96% overall yield.



Scheme 2: General procedure for the synthesis of the Guanidinium Monomer <u>4.</u> Reagents and conditions: (i) N, N'bis(tert-butoxycarbonyl)-1H-pyrazole-1-carboxamidine, Et₃N, THF, rt, 24h, (ii) Acryloyl Chloride, DCM, Et₃N, rt, 24h, (iii) TFA, DCM, rt, 18h.

3.2. Films: formulation and photopolymerization kinetics

In view of future applications, the antimicrobial materials were prepared in the form of thick films. Concerning the composition of the polymer matrix, it was inspired by our previous work^{12, 16}. A noticeable antibacterial activity was generated by films obtained from acrylate oligomers alone without adding any biocide group, and it was shown that this activity was significantly enhanced by incorporating an increasing percentage of oligomers containing quaternary ammonium groups at one chain end^{17} . In the present work, the first objective was to determine the optimal conditions to obtain homogenous films as ACTNR oligomers are honey-like hydrophobic viscous liquids and the guanidinium monomer is a hydrophilic solid powder, so a miscibility problem was faced. The study of photopolymerization kinetics was carried out by FTIR-ATR (attenuated total reflection), using the acrylate oligomers alone as model to investigate the influence of thickness (Figure 1); successively, the combination with guanidinium monomer was studied. R.Jellali et al. worked on the photocrosslinking of the acrylate oligoisoprenes and showed that there was no significant difference in the presence or absence of oxygen¹³. The conversion of acrylate functions was nearly quantitative in the two conditions and the polymerization rates were almost identical, hence in the present work, the polymerization was carried out in presence of oxygen. The reaction rate increased only with the photo-initiator percentage (1-5% weight), as expected. ³²⁻³³ In our case, different photoinitiators were tested but Darocur 1173 was chosen again because it met the usual criteria of absorbing in the UV region, where the oligomers and guanidinium monomer do not absorb, where the UV lamp emits (Figure SI0) and also because, in comparison to other commonly used solid photo-initiators, it is liquid and it can be easily dispersed in the viscous polymerization mixture. The percentage retained for all formulations was 2.5% (weight) because it represented a good compromise between a lower amount (1%) that required longer polymerization times, and a higher amount (5%) that allowed shorter irradiation times but increased the amount of unreacted photoinitiator released in the environment (data not shown).

The first study was focused on the kinetic of the photo-polymerization, followed by FTIR in order to verify the conversion of acrylate groups in relation to the polymerization time and to the film thickness. It is important to polymerize the acrylate group without touching the carbon-carbon double bond in the polyisoprene repeating

unit to maintain the mechanical properties. Films dimensions were 2 cm diameter and 1.0 mm, 1.5 mm, or 2.0 mm thickness. IR spectra were performed of face A (the surface directly exposed to the UV-lamp) and face B, the surface in contact with the TeflonTM mold. The light intensity was kept constant at 29.4 mW/cm². To monitor the changes during irradiation, the band of acrylate group at 1405 cm⁻¹ was considered, as its absorbance diminishes with time, and the band at 1376 cm⁻¹ was taken as reference (Figure 1). The percentage of conversion was calculated with Eq. (2) (Section 2.2). Traditionally, the C–H out of plane band at 809 cm⁻¹ has been used to monitor acrylate polymerization. ^{34, 35} In the literature³⁶, the intensity of the =CH out-of-plane bending absorption band of the polyisoprene at 837cm⁻¹ was taken as reference because it is strong but in the present study, it was overlapped by the C–H out of plane band. Hence, the polymerization kinetics were monitored



Figure 1. The IR absorbance change of the acrylate group upon irradiation (peak at 1405 cm⁻¹). (Left) entire IR spectrum; (right) zoom of the 1800 – 1200 cm⁻¹ region.

following the =C-H₂ stretching band of the acrylate group at 1405cm⁻¹. It was noticed that the conversion rate was higher on face A than face B; this difference was due to the direct explosion of face A to the UV-light whereas face B was in contact with the TeflonTM molds. As the pre-polymerization mixture was not transparent, it was a viscous, yellowish honey-like mixture, it absorbed part of the light and the lower face received a less intense radiation; the effect was more remarkable when film thickness increased, as the conversion decreased (Figure 2).



Figure 2. Conversion of total number of acrylate functions versus irradiation time of films based on acrylate oligomers with different thickness (e) ($I = 29.4 \text{ mW/cm}^2$).

Successively, the guanidinium monomer was incorporated in the polymer network. The pre-polymerizationmixture of guanidinium monomer (white solid) and the acrylate oligomers was not homogeneous. Clumps of powder could be observed and they turned into separate domains in the cross-linked films (Figure SI1). To solve this problem, the idea of dissolving the monomer in a solvent in order to disperse it was discarded, because the guanidinium monomer is soluble only in water, alcohols, and dimethylsulfoxide, which are not miscible with the oligoisoprenes, and also because the solvent itself should have been removed in order to obtain non-leaching materials. Hence, the idea came of using the minimum amount of pentaerythritol triacrylate as a cross-linking agent, as it was able to dissolve the guanidinium monomer and to allow its dispersion in the pre-polymerization mixture, remaining covalently bound to the network. The photopolymerization of the diacrylate oligoisoprenes was carried out in presence of two different percentages



Figure 3. Influence of concentration of PETA on photopolymerization ($I= 29,4 \text{ mW/cm}^2$; e = thickness, d = diameter)

of pentaerythritol triacrylate, respectively, 0% and 10%, and the concentration of guanidinium monomer was kept constant at 5% (Figure 3).

Homogeneous and elastic films were obtained when the PETA percentage was 10% weight (Figure SI1). The photopolymerization rate was influenced by adding the PETA to the film formulations: the addition of the trifunctional crosslinker decreased the polymerization rate of the system composed by the guanidinium monomer and the acrylate oligomers, which are already a di-functional crosslinker. The degree of reticulation rapidly increased and the viscosity as well, limiting the diffusivity of the radicals and therefore reducing the conversion rate³⁷. For face B the influence of PETA in the polymerization rate was not relevant, and the same trend was observed in the difference between face A and B concerning the total conversion of double bonds at the end of the reaction. After these results, it was decided to prepare different formulations containing increasing percentages of Guanidinium monomer 4 (Table 1), in order to use them for testing the biological activity. Because a large number of replicates was needed, it was decided to prepare the films with only 0.5 mm thickness and 1cm diameter in order to use less pre-polymerization mixture. Another UV-equipment was used with a more powerful lamp to increase the polymerization rate (Light intensity 0.313J/cm²), and it was equipped with a sliding belt, so that all the films could be quickly prepared at the same time, in exactly the same conditions. The conditions to have the maximum conversion on the two faces had to be checked again by a kinetic study, with two formulations F1 and F2 (Table 1). It was decided to include 10% weight of guanidinium monomer, as the minimum amount that could give a noticeable antimicrobial activity, so that the percentage of PETA was raised to 20% to dissolve it.

Components	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)
Darocur 1173	2.5%	2.5%	2.5%
РЕТА	20%	20%	20%
Guanidinium Monomer	0%	10%	20%
ACTNR	77.5%	67.5%	57.5%

Table 1. General composition of films (% = weight percentage)

The conversion profile for this irradiation (Figure 4) clearly showed that after 45s, around 96% conversion of the total number of acrylate double bonds was reached on both faces of the films obtained by the two formulations, to form highly cross-linked networks. The length of reaction time was determined by the overlay of the films IR spectra, which indicated that after 45s the carbon-carbon double bond of the repeating unit started to disappear (Figure SI2). Therefore, the irradiation was stopped before the complete conversion of acrylate groups was reached. A difference between polymerization rate of face A and B was again observed for both formulation 1 and 2, however it was more relevant in absence of guanidinium monomer. In presence of guanidinium monomer (F2 vs F1), the polymerization rate decreased because of the increased opacity of the mixture, the increased viscosity of the system, and the consequent limited mobility of the radicals in a fixed network. ³⁸



Figure 4. Conversion of total number of acrylate functions versus irradiation time in two formulations: F1 (polyisoprene only) and F2 (with 10% guanidinium monomer). Light intensity I=0.313J.cm⁻².

3.3. Leaching tests

Among the potential applications of the antimicrobial materials, some could involve their contact or immersion in water, as well as repeated cleaning, so a study was carried out to detect if any of the film component was released in water. The main goal of this work was in fact to develop a new antimicrobial polymer between Guanidinium monomer and ACTNR with no release, by photopolymerization using Darocur 1173. The first compound to be analyzed was Darocur 1173, as it is known to be toxic to aquatic organisms when released in the environment²⁷, and also to ensure that the antibacterial activity of our films was due to the active monomer, rather than the photoinitiator. During the curing process, only part of the photo-initiator is actually consumed in the reaction of photopolymerization and integrated in the polymer chain ends. After splitting into two radicals, unfortunately, depending on the polymerization conditions, a certain amount of it remains intact and can flow out of the cross-linked polymers if they swell in a solvent.^{39,28,40} Salma *et al.* ²⁷ have shown that the potential release of the residual amount of photoinitiator Darocur 1173 remaining within commercial silicone coatings was approximately 20 - 90%, depending on the film thickness and the pH of the medium. The role of photoinitiators is to create the active forms that initiate the chain reaction⁴¹. However, during the fragmentation processes, new compounds may be formed which in turn may contribute to pollution. Benzaldehyde and benzoic acid are released from Darocur 1173 photo-degradation²⁸. As benzaldehyde and benzoic acid absorb in the same UV region (230-247 nm), it was not possible to quantify them separately by UV spectrometry, so HPLC was used for the quantification of Darocur 1173 and its possible photo-products.



Figure 5. Time course of Darocur 1173 (left) and benzoic acid (right) leaching into de-ionized water from thick films (0.5mm thickness x1.0mm diameter) at 25°C, detected by HPLC and UV-vis) methods.

The films were immerged in water, under mild agitation in a thermostated environment at 25°C, and the results obtained with two formulations, F1 and F2 (Figure 5), showed that after 14 days, 35% of Darocur 1173 from Formulation 1 was released and 60% from Formulation 2. A very small fraction of Darocur gave the photodegradation products, as only 3.2 % of the initial amount of benzoic acid from F1, and 1.7% from F2 were detected. Concerning the release of benzaldehyde, the values observed were not significant, less than 1%. The FTIR spectra of the films used for the tests showed that the conversion of the total number of acrylate groups was 95.5%, so the films were almost completely cross-linked materials. A possible explanation of these data is that F1 is a very hydrophobic material, being composed of polyisoprene only, and Darocur 1173 is also a hydrophobic molecule, so that once it is entrapped in the three-dimensional network, it is not likely to be expulsed in water. When the guanidinium monomer **4** is added to the formulation, the material becomes more hydrophilic and probably swells enough in water to allow the Darocur 1173 molecule to reach the surface and be released. It was observed that the leaking occurred essentially during the first two days and then a plateau was reached.

A second leaching test was carried out for disks obtained with F1 and F2, at 37°C, the same temperature at which the bacteria are grown, and analyzing the supernatants after 15 and 60 min, in order to monitor what happens in the first period of immersion. As in the biological tests the bacteria suspensions are in contact with

the surfaces for 15 minutes, it was important to assess what amount of Darocur 1173 was released during this time to determine if it was sufficient to kill some of the bacteria. It was found that around 2.5 % of the molecules were released after 15 minutes and 6-8% after 1 h, and there was not a relevant difference between the formulations in this case (Figure 6). This amount was not enough to kill a significant number of bacteria.



Figure 6. Time course of Darocur 1173 leaching into de-ionized water from thick films (0.5mmx1.0mm) at 37°C, detected by HPLC and UV-vis methods

The influence of temperature and presence of PETA on Darocur leaching were also studied using only the formulation without guanidinium monomer. We observed that the release of Darocur increased with in the presence of PETA (Figure 7, left) and with increasing the temperature (Figure 7, right). The first observation can be explained by the fact that the photocrosslinking was more effective by adding PETA, which is a tri-functional crosslinker and gives a more rigid porous structure in comparison to the case of ACTNR alone. At the same time the films become more hydrophilic and the two combined factors favored the release of Darocur. No release of guanidinium monomer, PETA, or oligomers was detected by HPLC (Figure SI9). The leaching studies pointed out that the photoinitiator was the only component of the coatings that could be released in water, as entire molecule or as decomposition products, depending on the temperature and the formulation.

Figure 7. Influence of PETA presence (left) and of temperature (right) on time course of Darocur 1173 leaching





The release of a percentage of unreacted photoinitiator is a known phenomenon⁴². Even if coatings are made industrially in large scale by photopolymerization and some photoinitiators are known to be toxic, few in depth

studies have been carried out so far about the leaching of the initiators and of the products of their degradation. Our work was in fact inspired by Ouali *et al.*⁴³ who studied the leaching of Darocur from commercial films produced by Bluestar Silicones. To solve the release problem, a solution would be to use a mixture of two different photoinitiators, to increase the curing performance. Light stabilizers, (molecules that contain highly conjugated systems which absorb in the UV region) and radical scavengers, such as hindered amines, have been used to improve films stability, or a combination of both that could remain covalently grafted to the material ⁴⁴. Other photoinitiators have also appeared in the literature for which a lower toxicity is claimed, such as curcumin based ones⁴⁵. The use of such systems will be explored in the follow up of this research work; in the meanwhile, the option was to simply immerge the coatings in water (once) before their use, and it was showed that after two days of immersion, the quantity of Darocur released was negligible (Figure 5). For industrial production, this "purification" procedure might be possible but, for the moment, it is considered not feasible, and the great majority of manufactured elastomers comes to the market with this problem and without a washing step.

3.4 Coatings thermal properties and surface contact angle

The antimicrobial disks were analyzed by IR, as mentioned, to calculate the percentage of polymerization, they were observed by optical microscope to assess the homogeneity of the monomer dispersion, and the leaching tests showed their behavior in water. Further characterization was carried out: the thermal properties and the effect of water absorption on thermal behavior were investigated using thermogravimetric analysis, after placing the films obtained by F1 and F2 formulations in water for 351 days at room temperature and comparing them with samples of the same films kept in dry conditions. The thermograms are presented in Figure 8 (the weight loss at a certain temperature from the thermograms is also listed in Table 2). The results show an increase in the decomposition temperature of our films at different decomposition levels. The initial decomposition temperature is used to predict the thermal stability of different films. T₅ was the temperature at which the sample weight lost was 5% that derived from the TGA curves and T_{max} was the maximum decomposition temperature derived from the maximum peak of the DTG curves. From previous work⁴⁶, films obtained from photoreticulation of ACTNR showed a thermal stability up to 200°C and a single degradation step, after which at 650°C the material was completely decomposed. The insertion of PETA seemed not to affect the thermal behavior, as one single degradation step was observed between 350°C and 500 °C. A slight difference in the thermal stability between the films F1 and F2 was observed; the decomposition of films F2 seemed to start a little earlier than that of F1. The films obtained without guanidinium monomer (F1) had higher thermal stability than films F2 (with guanidinium monomer), whereas the maximum decomposition temperatures of all samples were similar, around 374°C as they contain the same type of elastomer. Polyguanidines synthesized by polycondensation of guanidine hydrochloride in the range of molar masses 800-3500 g/mol showed a single decomposition step between 270-370 °C.47 The first decomposition step observed in F2 films concerns probably the degradation of the guanidinium moiety.

There was an insignificant influence of water immersion in the thermal stability as thermograms for F1 before and after immersion are overlaying, as well as for F2 before and after. The thermograms show a little amount of water desorbing around 100°C as expected, due to the presence of charges and of the non-hydrophobic character of the matrix. These results showing a good thermal stability are encouraging for future applications in which the coatings should require a long period in contact with water.



Figure 8. Thermograms of films with and without guanidinium monomer, before and after immersion in water for 351 days.

	Dec	Decomposition temperature (°C)				Contesteral
Sample	at the weight loss				I_{max} (°C)	Contact angle
	T ₅ "	T_{10} "	T_{50}^{a}	T ₉₀ "		(°)
F1	329	348	392	457	374	79
F1 in water	324	347	393	457	374	72
F2	235	322	387	450	374	64
F2 in water	233	319	388	451	375	56

 T_x^a : Decomposition temperature at the x% weight loss: a) data obtained from TGA curve, b) data obtained from DTG curve. Table 2. TGA and contact angle data of different films

The wettability of the four types of films used for TGA analysis was also determined by measurement of the contact angle. Figure SI3 shows a drop of water on a film surface. For all samples, contact angles are less than 80° (Table 2). However, a significant difference was observed between films obtained with guanidinium monomer (F2) which reach a higher level of hydrophilicity (64° versus 79° respectively) than without guanidinium (F1). These values are explained by the presence of the hydrophilic guanidinium monomer on the surface of F2 and were important to predict the antimicrobial effects of the films, which should be mainly due to the presence of the cationic groups. For both formulations, films immerged in water for 351 days were more hydrophilic than that non-immerged ones. This difference may reflect that all hydrophilic functional groups, particularly, guanidium groups buried deeply away from the surface, during water immersion could partially

migrate or orient towards the surface to form Hydrogen bonding with water molecules. PETA has a hydroxyl group that could also orient towards the surface after immersion of the F1 films; this effect in the F2 films could be added to the role played by the guanidinium.



Figure 9. Infrared spectra of disks containing different amounts of guanidinium monomer, showing the presence of guanidinium groups at the surface.

Raman spectrometry was used to attempt to detect the presence of guanidinium groups specifically at the surface, but the surface percentage of guanidinium groups could not be determined due to its presence in the bulk material homogenously and on purpose. The guanidinium –NH bonds being too polar to give noticeable peaks, we decided to use instead Infrared spectrometry, to compare the spectra of disks containing different percentages of guanidinium monomer, in the range 0 - 97.5% (Fig. 9). In the film containing 97.5% of guanidine monomer, two peaks are characteristic at 3183 and 3183 cm⁻¹, due to the –N-H bond stretching in the guanidinium group. These two peaks are still visible in the coatings containing 10% guanidine monomer (F2) but they are absent in disks containing acrylate oligomers and PETA only (F1), as expected. As ATR and not transmission mode was used, this demonstrate that a significant amount of guanidinium groups are at the interface, in line with the contact angle results.

The shown data would imply that the antibacterial action of the films increases with ageing in water, as more cationic groups are oriented to the surface, increasing the charge density, which appears to be related to the bactericide action as mentioned before. Moreover, as the guanidinium group is present in bulk, the antibacterial activity will be maintained if the material is damaged or cut.

3.5 Films activity against pathogenic bacteria

In order to test the biological activity of the films, three strains of pathogenic bacteria that pose threat to the human health and are ranked in the drug-resistant list⁶ were selected and studied in static conditions: *Pseudomonas aeruginosa* (CIP: A22, **PA**, Gram negative), *Staphylococcus aureus* (CIP 52.16, **SA**, Gram positive), *Staphylococcus epidermidis* (CIP: 176.117, **SE**, Gram positive). Antiobiograms were performed to

see the action of each component of the formulation alone, in case it was accidentally released from the films or, for the guanidinium monomer, to see if it had any activity of its own, which was maintained once inserted in the polymeric matrix. The results of the antibiograms (Figure SI10, table 1 SI) confirmed the toxicity of pure Darocur 1173 (BASF Safety data sheet⁴⁸), which inhibited all the three bacteria. The pure crosslinker PETA was active against SA and SE, while the acrylate oligomers inhibited only SE and had no effect on the two others. Trifluoroacetic acid was used as growth inhibition control and showed in all cases the largest inhibition halo. The guanidinium monomer being a solid powder, it was dissolved in water and a range of concentrations was used to calculate the minimum inhibitory concentration, which was 20 mg/mL on average considering different batches (but it was as low as 0.8 mg/ mL for the best batch).

After these qualitative assays, a procedure was established to incubate the films with the bacteria suspensions and to calculate the number of cells that remained attached to the surface. The first disks examined contained 10 % of guanidinium monomer and we could estimate that 0.002% of SA population, 1.038% of PA, and 0.638% of SE adhered to the disk surface. The previous studies performed on the simplest possible formulation in disks containing only acrylate oligomers showed the same trend concerning the sensitivity of the three bacteria; PA was less affected than SE and SA¹⁷. In that case the oligomers chain length was also varied (between 1700 and 4000 g mol⁻¹) and for SA and SE the longer one was more effective than for PA. The oligomers used in this study were of similar molecular weight (5000 g mol⁻¹) and the effect has been confirmed. Knowing that there was a noticeable antimicrobial action with surfaces formed by ACTNR alone, and having demonstrated the toxicity of the guanidinium monomer itself, it was finally found that the insertion of this new monomer generated a powerful effect, even more important than in the precedent studies, in which the oligomers containing ammonium chain ends were used.

Visual observation by electron scanning microscopy confirmed these results. Part of the incubated disks were treated to fix the bacteria on the surfaces and they were observed with the microscope to have complementary information. All the three bacteria grew on the surfaces containing only acrylate oligomers (Figure 10, a, b, c), but very few or none were found on those containing the guanidinium monomer (Figure 10, d, e, f).



Figure 10. Micrographs of disks not containing the guanidinium monomer after incubation with: a) PA b) SA c) SE. Disks containing 20% of guanidinium monomer (F2) incubated with d) PA e) SA f) SE.

The SEM images gave also information about the surface morphology. The aim of this work was not to obtain perfectly smooth films with nanometric roughness, for which other techniques of elaboration would have been

used such as spin coating, so the observed features were expected in materials obtained by spreading the liquid prepolymerization mixture on a TeflonTM mold. Atomic Force Microscopy technique was used to observe some selected samples (data not shown) however the roughness shown by SEM did not justify the use of atomic scale topology. Comparing films before and after immersion, for some formulations a slight change was observed, nevertheless the contact angles values observed here do not show the effect on topology, which was noticed only in ultrathin films.

Having obtained information from the leaching tests about the percentage of Darocur 1173 released at the same temperature and time used for the assays, and noticing from the antibiograms that to have an inhibition halo the pure compound was used, we concluded that the very low amount that could be released was not sufficient to have any bactericidal action, and that we could exclude any artefacts and killing of bacteria provoked by the small amount of unreacted photoinitiator.

We also wanted to verify if there was any difference in the activity of films used for the first time or used a second time after incubation. Generally, in the great majority of the publications, results are reported for materials without any purification step and without considering their reuse. We believed that, in order to assess the action in view of future real applications, the repetition of the analyses after a first immersion in water was necessary (Figure 11).



Figure 11. Effect of Darocur on the specific growth of four model bacteria (in each case, the first blue column on the left is the control suspension considered as 100% growth, the other columns correspond to the growing concentrations from 0.2 mg/mL to 5.4 mg/mL for the last one on the right).

It was found that, in order to have a significant effect, a concentration of 5.4 mg/mL was needed; for 0.2 mg/mL, only three bacteria saw their growth rate decrease of less than 5% in comparison to the control one. It was concluded that a similar effect should be obtained with the three pathogens tested in the present study; in fact among the four model bacteria, two were common ones and two marine ones, and had different characteristics. Calculations were made to estimate the amount of photoinitiator that could be released from the films. A typical disk weight in a biological assay was 50 mg and the percentage of Darocur contained = 2.5% in weight, which corresponds to 1.25 mg and represents the maximum amount of Darocur that could be released. The volume of bacterial suspension in contact with the film surface was 250μ L, so the maximum

value of Darocur concentration if 100% of it is released from the disk into the 250 μ L of bacterial suspension is 5 mg/mL. The leaching studies showed that only 2.3% of it is released (average value from Figure 6), corresponding to 0.028 mg, which would give a concentration of 0.112 mg/mL in the 250 μ L of bacterial suspension. This value indicates that the contribution of Darocur in the observed results is negligible.

As with the three pathogens (SA, SE and PA) only the inhibition halo test was carried out, it was decided to test the films twice, the first time just after their synthesis, and the second time after their first use, to see if there was a relevant difference in the efficiency that could be attributed to the action of the photoinitiator. Generally, in the great majority of the publications, results are reported for materials without any purification step and without considering their reuse. We believed that, in order to assess the action in view of future real applications, the repetition of the analyses after a first immersion in water was necessary (Figure 12, Table 3).



Figure 12. Growth curves at 37°C of PA, SE, SA, bacteria recovered on the surfaces after first incubation and second incubation with films (F3).

Strain	Initial	log(C) after	$\Delta \log(\mathbf{C})$	log(C) after	∆log(C')
	log(C)	first use		2nd use	
Pseudomonas a.	6.71	4.73	1.98	4.69	2.01
Staph. epidermidis	6.87	4.65	2.21	5.10	1.77
Staph. aureus	6.98	2.22	4.76	4.89	2.09

Table 3: recovered bacteria after incubation with disks (F3 20% guanidinium monomer, 20% PETA, 2.5% Darocur 1173), at 37°C, for 3h. $\Delta log(C') = initial log(C) - log(C_{30min, disks used second time})$.

The disks containing 20% weight of guanidinium monomer (F3) were incubated a first time, treated to determine the number of attached cells, and it was found that the effect of the films was strongly antiadherence for Staph. aureus, and less intense for Staph. epidermidis and Pseudomonas a., based on the value of $\Delta \log(C)$. It corresponds to the difference between the logarithm of the number of colony forming units (C, for the initial bacterial suspension) and the logarithm of the number of recovered bacteria from the surface (protocol detailed in section 2.7). The films were then decontaminated by washing extensively with ethanol, irradiating briefly with an UV lamp, and then they were incubated a second time following the same procedure described in the experimental section. A similar effect ($\Delta \log(C')$ value) was obtained for the PA and SE, whereas a diminution was observed for SA. Growths curves were also performed of the surviving bacteria rescued on the surfaces of the incubated films. The disks affected the growth of the three bacteria and the consequence was different between new and re-used ones. On Staphylococcus aureus the strongest effect was observed, the bacteria were deeply damaged by the new films, had a long inhibition period and then started to grow regularly; used disks had a lesser effect but the growth still diminished with time instead of increasing. The effect of the new films on Staphylococcus epidermidis was similar to the used ones, the growth slightly diminished with time. On *Pseudomonas* there was an effect at the beginning, which was more evident with the new films, then the growth curves were similar because of the effect of the de-structuration of the cell membrane operated by the guanidinium group.

As the surfaces were active against Gram-positive and Gram-negative bacteria, the effect can be explained by the role of the positive charge of the guanidinium groups present at the coatings surface in first binding the membranes via electrostatic interactions and then provoking the cell death by substituting the natural counter cations, as already observed for ammonium groups.^{49, 50} The coatings obtained showed an important antibacterial activity that was maintained with ageing. Concerning the potential marine antifouling activity of these guanidine based coatings, our experience with antimicrobial coatings indicated that they could also have the same activity against marine bacteria. One of the theories of formation of marine biofilms⁵¹ says that the bacteria are the first microorganisms that adhere to a surface and then all the others follow, up to the macroorganisms such as mussels and macroalgae. Therefore, the hypothesis was that if we avoid bacteria to attach to the surface in the first step, we also avoid the formation of the marine biofilm. In our previous work with quaternary ammonium groups¹⁶⁻¹⁷, this approach proved to work, and the same coatings and surfaces containing the guanidinium monomer will be also tested against microalgae and marine bacteria. Dedicated publications will address these questions that take time considering the tests planned in real marine immersion.

Conclusions

Antibacterial coatings were prepared using di-functionalized oligoisoprenes obtained from the renewable resource natural rubber as original macromonomers and a novel acrylate monomer containing a guanidinium group as active agent. The formulation to obtain thick, homogeneous and elastic films was optimized, studying the photopolymerization process by FTIR and the surface morphology by optical microscope. Leaching studies

demonstrated that no component of the coatings was released in water at different temperatures and after different times, except a fraction of the photoinitiator; this amount varied with monomer conversion and water temperature and was not enough to affect the results of the bacterial tests. Thermograms showed that the materials were stable up to 200°C, even after a long immersion in water; the contact angle values indicated that the surfaces were relatively hydrophilic, confirming the presence of the guanidinium groups at the surface and not only buried in the bulk matrix, as confirmed by IR spectra. The films were incubated with three strains of pathogenic bacteria, *Pseudomonas aeruginosa* (CIP: A22, **PA**, Gram negative), *Staphylococcus aureus* (CIP 52.16, **SA**, Gram positive), *Staphylococcus epidermidis* (CIP: 176.117, **SE**, Gram positive) in static conditions. Extremely low percentages of bacteria were found at the coatings surfaces after incubation showing and antiadherence effect towards the three microorganisms and the films retained their efficiency when washed, sterilized and used a second time. Observation of the coatings surface after incubation by scanning electron microscopy confirmed that the bacteria colonized the disks when the guanidinium monomer was absent, but very few or none were found when it was included, confirming the antimicrobial effect expected to come from the positive charge.

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Data availability statement

The raw data (supplementary information) required to reproduce these findings are available to download from: Pasetto, Pamela; Tran, Thi Nguyet Tran; Nourry, Arnaud; Brotons, Guillaume (2018), "Antibacterial activity of natural rubber based coatings containing a new guanidinium-monomer as active agent - Supporting information", Mendeley Data, v1 <u>http://dx.doi.org/10.17632/xysdyvzp7h.1</u>.

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Supporting Information

Antibacterial activity of natural rubber based coatings containing a new guanidinium-monomer as active agent

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Lampe Hg 4 ()**₩**'₩÷ 3,5 Darocur 1173 Oligomère 3 Absorbance 2,5 2 1,5 0,5 0 300 200 250 350 400 450 Wavenumber (cm⁻¹)

A. Films formulation

Figure SI0. UV absorption spectrum in dichloromethane of Darocur 1173 (5% by weight in oligomer), ACTNR oligomer (10⁻³mol.L-1) and main gray lines of emission of Hg lamp.



Figure SI1. Images observed using an optical microscope by focusing on two samples containing 10% of guanidinium monomer with or without PETA after photopolymerization. (a, b, c): films obtained with

acrylate oligomers, guanidinium monomer (10 %) without PETA and (d, e, f) corresponding films with PETA (20 %).



Figure SI2. The IR absorbance change of the acrylate group band at 1405 cm⁻¹ upon irradiation at 45s (Black line) and after 45s (Red line). It is clearly shown that the degradation of the carbon-carbon double bond of the repeating unit started.



B. Films characterization

Figure SI3. A drop of water on four films in contact angle measurement: (a) Film remained completely dry obtained with F1 formulation; (b)Film immerged for 351days in water with F1 formulation; (c)Film remained completely dry obtained with F2 formulation; (d)Film immerged for 351days in water with F2 formulation

C. Leaching tests



Figure SI4. Quantification of standard solutions of Darocur 1173 in water obtained by UV-visible absorbance at 247nm.



Figure SI5. The standard curve of Darocur 1173 was generated by plotting the peak areas obtained by HPLC method at 247nm, for different concentrations of analyte.



Figure SI6. Quantification of standard solutions of Benzoic acid, generated by plotting the peak areas obtained by HPLC method at 230nm.



Figure SI7. Quantification of standard solutions of Benzaldehyde, generated by plotting the peak areas, obtained by HPLC method at 247nm.



Figure SI8. Quantification of standard solutions of Guanidine monomer, generated by plotting the peak areas, obtained by HPLC method at 205nm.



Figure SI9. Peaks present on a HPLC chromatogram at 205nm obtained the leaching test of film with F2 formulation after 14 days in water, no peak at 3.7min and 24.9min corresponding to resp. Guanidine monomer and PETA released were detected

D. Biological assays



Figure SI10. Antibiograms: left) Pseudomonas aeruginosa; middle) Staphylococcus epidermidis; right) Staphylococcus aureus. (1) Guanidine monomer batch 1; (2) guanidine monomer batch 2; (3) Darocur 1173; (4) pentaerythritol triacrylate; (5) Acrylate oligomers; (6) Trifluoroacetic acid.

Compound	Concentration]	Inhibition halo (cm)		
Compound	(mg / ml)	SA	PA	SE	
Guanidine monomer batch 1	0.8	0.9	1,2	0.9	
Guanidine monomer batch 2	0.8	1.3	0	0	
Darocur 1173	pure	1.2	0.6	1.6	
pentaerythritol triacrylate	pure	1.3	0	1.6	
Acrylate oligomers	pure	0	0	1.5	
Trifluoroacetic acid	pure	3.6	4.8	4.6	

Table SI1. Inhibition halo for the three bacteria Pseudomonas aeruginosa (PA, gram negative), Staphylococcus aureus (SA, gram positive), Staphylococcus epidermidis (SE, gram positive). Guanidine monomer was dissolved in water.

CHAPTER III. Natural Rubber based films integrating a new monomer derived from Zosteric acid: New Approach to Reduce Biofouling

III.1 Introduction

Chapter II provided the necessary background and a literature review about the antimicrobial polymers including the impact of microbial adhesion, and how to prevent and eradicate it. It has been outlined that an innovative approach could be the incorporation of an anti-biofilm compound on the polymer matrix. Instead of using a bioactive group (guanidinium) to generate the antimicrobial activity as in the previous study, this chapter will focus on the use of bio-inspired molecules, in particularly Zosteric acid (ZA, Figure III-2), as an alternative biocide-free agent that plays a role in the shaping of biofilm architecture, in reducing biofilm and in extending the antimicrobial agents performance.

A general recall about antifouling properties of Zosteric acid and its analogs, including the mode of action against bacteria, will be reported in the first Section. The choice of tailor-made monomers (Zosteric acid analogues) will be discussed and the different strategies for their synthesis will also be presented before the incorporation of these monomers in natural rubber network. The kinetic study on photopolymerization, antibacterial activity and also leaching tests of these polymers will be also discussed in the last section.

III.2 Bibliography: Zosteric acid, a new promising anti-biofilm compound coming from seagrass

III.2.1 Antifouling properties and toxicity of Zosteric Acid

Nowadays, the harmful effects of several biocides compounds to human health and the environment has been recognized, particularly, the use of heavy metals in antifouling polymers[1]. In this contest, the development of new alternative solutions able to replace the presently dominating products is becoming imperative. One of the most promising alternatives is offered by the development of antifouling polymers in which the active subtances are compounds naturally occurring in marine organisms and operating as natural anti-settlement agents [2, 3]. The great interest of these natural products antifoulant (NPAs) compounds for fouling control is through a non-toxic mechanism. For example, the antifoulant compounds bind to cell membranes or cell walls/extracellular matrices, thereby altering cell surface properties and affecting the ability of organisms to attach to a surface. Numerous extractions and isolation of natural products from marine environment have shown response as possible antifouling agent. Some of their origins are summarized in Figure III-1. Among them, Zosteric acid or *p*-(sulphooxy)cinnamic acid (Figure III-2), a secondary metabolite from the seagrass Zostera marina (violet part, Figure III-1), has proved to be a powerful antifouling compound against a broad range of organisms including bacteria, algae, barnacles and fungi [4-6]. The summary of its antifouling properties is listed in Table III-1.



Figure III-1: Phyletic distribution of marine species which have potential antifouling natural products, ref. [7]

The AF activity of ZA was evaluated for several species of fouling organisms, from micro- to macroorganisms. ZA was efficient in preventing the attachment of freshwater bacteria (*Pseudomonas putida* and Lake Erie bacteria), marine bacteria (*Acinetobacter sp.*, *Shewanella putrefaciens, Vibrio harveyi, V. natriegens, and V. parahaemolyticus*), Escherichia coli, Bacillus cereus, and fungi (*Aspergillus niger, Candida albicans, Colletotrichum lindemuthianum, Magnaporthe grisea, and Penicillium citrinum*) to various surfaces.

Xu *et al.*[8, 9] reported that after 14 days in fresh water, biofilm coverage from bacteria isolated from Lake Erie was reduced by 92.5% and 98.2%, respectively, with 50 mg/L and 500 mg/L of ZA. Further, Villa *et al.* [10] demonstrated that 500 mg/L of ZA decreased more than 90% *Escherichia coli* and *Bacillus cereus* cells adhesion. They also pointed out that with the same concentration, it caused a reduction of bacterial coverage of more than 57%, for *Aspergillus niger, Penicillium citrinum*. Later, they proved that 10 mg/L ZA reduced *Candida albicans* adhesion by at least 70 % leading cells to the inability of forming filamentous structures[4]. While Stanley *et al.*[11] observed that with 1000 mg/L of ZA, the fungal adhesion of *Colletotricum lindemuthianum* and *Magnaporthe grisea* on both abiotic and plant leaves surfaces was reduced by 40 %. Recently, it was proved by Polo *et al.* [12] that 200 mg/L of ZA cause a reduction of *P. aeruginosa* biofilm coverage by 80%.

Tested organisms	Conditions	Results	Ref.
Microfouling organisms Bacteria			
Acinetobacter sp.	ZA concentration varied from $10^{\text{-1}}$ to $10^3\mu\text{gcm}^{-2}$	Aggregate $EC_{50} = 10 \ \mu g cm^{-2}$	[13]
Vibrio harveyi and Shewanella putrefaciens	Coatings with silicone paint were prepared by mixing 5,10 and 25 wt% of ZA	At 25 wt%, coatings remained free of biofilm for more than seven days	[14]

Lake Erie bacteria and <i>Pseudomonas putida</i>	ZA concentration varied from 5 to 500 mgL ⁻¹	Lake Erie bacteria: Reduction of 92% at a concentration of 50 mgL ⁻¹ <i>P. putida</i> : surface coverage was 13, 1 and 0.4% respectively, for coatings immersed in 20, 50 and 500 mg L ⁻¹ of ZA	[15]
Vibrio natriegens and Vibrio parahaemolyticus	V. <i>natriegens</i> : ZA concentration 5 mgL ⁻¹ and 10 mgL ⁻¹	EC_{50} (V. <i>natriegens</i>) =7.4 mgL ⁻¹	[6, 9]
paranacinoryacias	<i>V. parahaemolyticus</i> : ZA concentration 10 mgL ⁻¹ , 20 mgL ⁻¹ , and 100 mgL ⁻¹	EC_{50} (V. <i>parahaemolyticus</i>) = 18.0 mgL ⁻¹	
Lake Erie bacteria and P. putida	ZA concentration varied from 5 to 500 mgL ⁻¹	Lake Erie bacteria: reduction of 92.5% at a concentration of 50 mgL ⁻¹ <i>P. putida</i> : reduction of 98.2% at a concentration of 500 mgL ⁻¹	[16]
		Reduction of 90% at a concentration of 50 mg L^{-1}	[10]
Escherichia coli and Bacillus cereus	ZA concentration varied from 5 to 500 mgL ^{-1}	Reduction of 90% at a concentration of 500 mgL ^{-1} and increase in bacterial motility by 40%	[12]
P. putida	ZA concentration varied from 5 to 600 mgL ^{-1}	Reduction of 80% at a concentration of 200 $\rm mgL^{-1}$	[5]
E. coli	ZA concentration varied from 0.183 to 1,830 µM	Anti-biofilm activity between 183 and 1,830 μM	[5]
Fungi			
Fungi Magnaporthe grisea and Colletotrichum lindemuthianum	ZA concentration varied from 0 to 2% (w v^{-1})	<i>M. grisea</i> : adhesion inhibition at concentrations as low as 0.01% (w v ⁻¹) <i>C. lindemuthianum</i> : adhesion inhibition at concentrations as low as 1% (w v ⁻¹)	[11]
Fungi Magnaporthe grisea and Colletotrichum lindemuthianum Aspergillus niger and Penicillium citrinum	ZA concentration varied from 0 to 2% (w v ⁻¹) ZA concentration varied from 0 to 25 g L^{-1}	<i>M. grisea</i> : adhesion inhibition at concentrations as low as 0.01% (w v ⁻¹) <i>C. lindemuthianum</i> : adhesion inhibition at concentrations as low as 1% (w v ⁻¹) Reduction of 57% at a concentration of 1,000 mg L ⁻¹	[11]
Fungi Magnaporthe grisea and Colletotrichum lindemuthianum Aspergillus niger and Penicillium citrinum Candida albicans	ZA concentration varied from 0 to 2% (w v ⁻¹) ZA concentration varied from 0 to 25 g L ⁻¹ 10 mgL ⁻¹ ZA was added at different times during biofilm development	<i>M. grisea</i> : adhesion inhibition at concentrations as low as 0.01% (w v ⁻¹) <i>C. lindemuthianum</i> : adhesion inhibition at concentrations as low as 1% (w v ⁻¹) Reduction of 57% at a concentration of 1,000 mg L ⁻¹ Reduction of 70% at a concentration of 10 mgL ⁻¹	[11] [10] [4]
Fungi Magnaporthe grisea and Colletotrichum lindemuthianum Aspergillus niger and Penicillium citrinum Candida albicans Macrofouling organisms	ZA concentration varied from 0 to 2% (w v ⁻¹) ZA concentration varied from 0 to 25 g L ⁻¹ 10 mgL ⁻¹ ZA was added at different times during biofilm development	<i>M. grisea</i> : adhesion inhibition at concentrations as low as 0.01% (w v ⁻¹) <i>C. lindemuthianum</i> : adhesion inhibition at concentrations as low as 1% (w v ⁻¹) Reduction of 57% at a concentration of 1,000 mg L ⁻¹ Reduction of 70% at a concentration of 10 mgL ⁻¹	[11] [10] [4]
FungiMagnaporthe grisea and Colletotrichum lindemuthianumAspergillus niger and Penicillium citrinumCandida albicansMacrofouling organismsEnteromorpha zoospores	ZA concentration varied from 0 to 2% (w v ⁻¹)ZA concentration varied from 0 to 25 g L ⁻¹ 10 mgL ⁻¹ ZA was added at different times during biofilm developmentZA concentration varied from 0 to 2.5 mM	 <i>M. grisea</i>: adhesion inhibition at concentrations as low as 0.01% (w v⁻¹) <i>C. lindemuthianum</i>: adhesion inhibition at concentrations as low as 1% (w v⁻¹) Reduction of 57% at a concentration of 1,000 mg L⁻¹ Reduction of 70% at a concentration of 10 mgL⁻¹ Reduction of 50% at concentrations up to 0.25 mM 	[11] [10] [4]
FungiMagnaporthe grisea and Colletotrichum lindemuthianumAspergillus niger and Penicillium citrinumCandida albicansCandida albicansbacrofouling organismsEnteromorpha zoosporesAscidians, barnacles bryozoa and tubeworm (Hydroides elegans)	ZA concentration varied from 0 to 2% (w v ⁻¹) ZA concentration varied from 0 to 25 g L ⁻¹ 10 mgL ⁻¹ ZA was added at different times during biofilm development ZA concentration varied from 0 to 2.5 mM Experiments consisted of three concentrations of ZA (data not show)	<i>M. grisea</i> : adhesion inhibition at concentrations as low as 0.01% (w v ⁻¹) <i>C. lindemuthianum</i> : adhesion inhibition at concentrations as low as 1% (w v ⁻¹) Reduction of 57% at a concentration of 1,000 mg L ⁻¹ Reduction of 70% at a concentration of 10 mgL ⁻¹ Reduction of 70% at a concentration of 10 mgL ⁻¹ Accidians: reduction of 80% at a flux of 500 $\mu g^{-1} cm^{-2} d^{-1}$ Barnacles and bryozoan: reduction of 50% at a flux of 500 $\mu g^{-1} cm^{-2} d^{-1}$ <i>H. elegans</i> : significant decrease at 50 $\mu g^{-1} cm^{-2} d^{-1}$	 [11] [10] [4] [17] [18]

Table III-1: The antifouling activity of ZA, Copyright, ref. [20]. EC₅₀ is the half-maximal effective concentration of a drug, which produces 50% of the maximum possible response.

In addition to the powerful antifouling activity, the potentiality of ZA is its very low toxicity towards the organisms and the environment. Xu *et al.*[8, 9] evaluated the toxicity of ZA to *P. putida* and aquatic bacteria. It was showed that the EC₅₀ of ZA was in the range of 7.4 to 375 mg/L(Table III-1). It was approximately five to six orders of magnitude smaller, i.e. less toxic, compared to the currently used antifoulants i.e. TBT (tributyltin) (EC₅₀=0.022mg/L) or SeaNine 211 (EC₅₀=0.036 mg/L) [21].

Organisms	EC ₅₀ of Zosteric Acid (mg/L)
P.putida	167±3.9
Lake Erie bacteria	375±10
V.natriegens	$7.4{\pm}0.1$
V.parahaemolyticus	18±0.6

Table III-2: Acute toxicity (EC50 values in mg/L) of Zosteric Acid, data taken from [6]

Zosteric Acid could be obtained either by natural extraction or by synthetic route from *p*-coumaric acid [13, 22, 23]. Both extracted and synthesized ZA were tested and showed similar antifouling activity using short attachment tests (less than 7 days). Nevertheless, Zimmerman *et al.*[22] reported that a yield of only 66 mg of ZA was obtained by natural extraction from 1700 g of dry biomass. Therefore, it was more convenient to produce ZA via chemical synthesis.

III.2.2 Mechanism of action and the structure-activity relationships (SARs) study of ZA and its analogs

The mode of ZA antifouling action has not yet been fully elucidated, however, it was supposed that ZA acts as an environmental cue, leading to global stress on cells and favoring the expression of various proteins necessary to escape from adverse conditions [24, 25]. It was recently demonstrated also that ZA might be a storage compound that is enzymatically converted to coumaric acid by sulfatase enzymes when environmental stress is present [26, 27]. The chemical structure of ZA (Figure III-2) contains a sulfate phenolic ester group at one end and a carboxylic acid group conjugated to a double bond (configuration *E*) at the other end (*para* position).



Figure III-2: Chemical structure of Zosteric Acid, 4-pentyphenyl sulfate and octyl sulfate.

In fact, the antifouling efficiency of ZA was firstly reported to be associated with sulfate ester group by Todd *et al.* [13]. They demonstrated that in general synthetic sulfate esters have similar antifouling (AF) properties. Indeed, non-sulfated phenolic acids were ineffective even at concentration 60-fold higher than the EC_{50} of sulfate esters. This result was confirmed again by Zimmerman *et al.*[14, 28] when they studied AF activity for two analogs of ZA, 4-pentylphenyl acid sulfate and octyl sulfate (Figure III-2). They observed that these compounds were more effective than native ZA in preventing biofilm formation in the case of *Vibrio harveyi*.

Later, Villa *et al.* [4] showed for the first time that the AF activity of ZA may be also due to the carboxylic acid group. Depending on the specific bacteria, this effect could be less or more significant. For instance, methyl zosterate was inactive against *C.Albicans*, while had antiadhesive effects on *Staphylococcus aureus*. While Guzman *et al.*[29] pointed out that a natural phenol *para*-coumaric acid (*p*-CA) provided more potent bacterial growth inhibitor compared to ZA and cinnamic acid, becoming an interesting scaffold for the development of new antimicrobial compounds.

Nevertheless, a study published in 2015 [26, 27] called in question certain points of the structure-activity relationship. In fact, the authors followed the sample preparation methods and thus synthesized several Zosteric acid or their salts. They showed that some traces of para-coumaric acid were still present on the most of synthesized ZA compounds. According to them, the activity associated with Zosteric acid was in fact linked to the presence of these impurities. This hypothesis was recently confirmed by C. Cattò et al. [5] when they studied the structure-activity relationships of ZA analogs. Several modifications based on ZA-scaffold were performed, such as modifications of the double bond conjugated with the aromatic ring, the sulphonic ester part or the acid carboxylic acid. A library of 43membre molecules was tested for antifouling activity against E.Coli. They demonstrated that the presence of the sulfate function was not necessary because p-CA was more active than ZA itself. The presence of the carboxylic acid (-COOH) was also important because if this acid is esterified (methyl or ethyl) or replaced with other functional groups as an aldehyde or alcohol, the biological activity also generally failed. Nevertheless, sometimes, the presence of a hydroxyl group in the 4-position allowed to maintain an interesting activity with esters. The replacement of a saturated alkyl chain or Zconfiguration in place of the double bond showed a loss of antifouling activity, confirming that it was highly necessary to have a double bond (C=C) with E-configuration, conjugated with RCOOH group for generating an antifouling activity.

III.3 Natural Rubber integrating a new monomer issued from Zosteric acid

The aim of this work was to covalently integrate a new antifouling monomer into a modified natural rubber matrix in order to generate new non-toxic, ecofriendly antifouling materials. The strategy used was to insert two acrylate groups in the chain-end of oligomer structures, prepared by chemical

modification of NR. This allowed performing the radical co-polymerization (*via* UV-curing) with a new monomer bearing one acrylate group and the bioactive functional group. This approach appeared as an attractive alternative because these solvent-free mixtures can be cured within seconds at ambient temperature to yield highly resistant cross-linked polymers. In the following sections, firstly the choice of the target monomer including advantages and drawbacks will be discussed. Then the synthesis of these monomers and some thick films will be presented and their properties as well. To conclude, the results of the biological assays will be shown.

III.3.1 How to design and synthesize new monomers derivated from Zosteric acid for antifouling properties

As mentioned in the previous section, the investigation of ZA toxicity and its analogs including structure activity relationships studies confirmed the potential of this compounds family as bacterial growth inhibitors, becoming interesting scaffolds for the development of new antibiofouling agents.

Our target monomers had to satisfy two criteria: the first one is to provide a ZA-scaffold, maintaining specifically the double bond (C=C) with *E*-configuration, conjugated with the aromatic ring. The second one is that the monomer has to bear an acrylate polymerizable group at one end to assure a covalent link to the matrix. We decided also to remove the sulfate part as it was mentioned in the literature that this group is not necessary for the biological activity[5]. One of the key-points for the targeted monomers is the choice of the anchor site for the acrylate function. Two targeted monomers p-CAA and p-CAB were designed for this project and their structures are presented in Scheme III-1.



Scheme III-1: Chemical structure of the two target monomers.

For the first monomer (*p*-CAA), the acrylate function was bound from the carboxylic acid since it was shown that a *para*-substituted cinnamic acid ester with a hydroxyl group presented some anti-biofilm activities[5]. For the second monomer (*p*-CAB), the acrylate function was linked from the phenol since it appeared that the presence of the carboxylic acid conjugated to the double bond was important as well

for the biological activity[5]. The synthesis strategies of each monomer will be discussed in the next section.

III.3.1.1 Synthesis of p-CAA Monomer <u>1</u>

The target *p*-CAA monomer $\underline{1}$ could be obtained according the following retrosynthetic scheme (Scheme III-2). The key step was the coupling between the compound $\underline{4}$ (acrylate part) from the primary alcohol and the coumaric acid from the carboxylic acid. To carry out this task, it was necessary to protect the phenol group of the coumaric acid and two types of protecting groups were envisioned. The first one tested was an ether group (methoxy) and its use will be described in the first approach. The second protecting group was a silylated ether (TBS) and its use will be commented in the second approach.



Scheme III-2: Retrosynthetic analysis of p-CAA monomer via two strategies.

III.3.1.1.1 First approach for the synthesis of *p*-CAA Monomer 1

At first, the synthesis started with the preparation of compound <u>5a</u> via a malonic reaction [30] between the 4-methoxylbenzaldehyde and the malonic acid. The reaction was conducted in pyridine for 12h under reflux, with a yield of 83% (scheme III-3). Then, the esterification of 4-methoxy cinnamic acid <u>5a</u> (1.2 equiv.) with 4-hydroxylbutylacrylate <u>4</u> (1.0 equiv.) in DCM under reflux with the presence of 1.5 equivalents of DMAP, 1.2 equivalents of DCC, and a spatula tipful of *para*-benzoquinone as radical inhibitor gave product <u>3a</u> in 65% yield. For the last step, the deprotection of methoxy group was performed with two types of reaction conditions including TMSI/NaI system and BBr₃ system. Unfortunately, only side-products were obtained. In the case of the use of BBr₃ system, the double C-C bond was degraded by bromination. No presence of *p*-CAA monomer was detected by ¹H NMR analysis. These unsatisfactory results of the preliminary screening of deprotection conditions with methoxy group encouraged us to investigate other procedures such as the use of *tert*-butyldimethysilyl ether (TBS) as a protecting phenolic group.



Scheme III-3 : Synthesis pathways to preparation of **p-CAA** monomer, using methoxy as protecting phenol group.

III.3.1.1.2 Second approach for the synthesis of *p*-CAA Monomer

The targeted *p*-CAA Monomer $\underline{1}$ could be prepared according to a strategy in three steps from of *para*coumaric acid (Scheme III-2, Right). The synthesis pathways was started firstly by the protection of alcohol function with *tert*-butyldimethylsilyl chloride (TBSCl) generating compound $\underline{5}$ that was then esterified with 4-hydroxybutylacrylate $\underline{4}$ via a Steglish or Mitsunobu coupling, in order to introduce the acrylate function. The last step consisted in the deprotection of the silylated ether. Screening conditions for each step will be detailed in the following section.

III.3.1.1.2.1 Protection of phenol group as silylated ether

The protection of the phenol group was necessary to avoid any secondary reactions during the next coupling step. A silylated *tert*-butyldimethylsilyl ether was chosen for its ability to be cleaved easily, in comparison to methoxy protecting group.



Scheme III-4: Protection of phenol group with a tert-butyldimethylsilyl ether

The summarization of screening protection conditions is presented in Table III-3.

Entry	Conditions	Post-treatment	Results
1	a) TBSCl, Imidazole, DMF, rt, 3h	-	<u>5</u> (54%)
2	a) TBSCl, Imidazole, DMF, rt, 20h	Acidification	<u>5</u> (33%) +
	b) LiOH, THF/H ₂ O, rt, 0,5h	(pH = 1)	p-coumaric Acid (40%)
3	a) TBSCl, Imidazole, DMF, rt, 3h	Acidification	<u>5</u> (95%)
	b) LiOH, THF/H ₂ O, rt, 1h	(pH = 5)	

Table III-3: Screening conditions to protect phenolic function.

It was reported in the literature [31, 32] that the reaction with 2.5 equivalents of TBSC1 in presence of 2.5 equivalents of imidazole in DMF generated protected alcohol with almost quantitative yields. However, in our case, using the same conditions, the expected product $\underline{5}$ was isolated with only a yield of 54% (Entry 1, Table III-3). This difference could be explained by the presence of a side-reaction taking place with the carboxylic acid group at the same time of phenolic group. That is why the reaction was tested again but adding LiOH as a second step [33, 34] in order to make a saponification of the previous formed silylated ester and generate the carboxylic acid corresponding (Entry 2, Table III-3). It was also demonstrated that the pH must be well controlled during the treatment. If the pH was too acidic (pH = 1), the silylated ether was partially degraded, returning to *para*-coumaric acid. The best yield of protected compound $\underline{5}$ was 95% with a pH of 5 (Entry 3, Table III-3).

The coupling reaction between protected coumaric acid $\underline{5}$ and 4-hydroxylbutyl acrylate $\underline{4}$ was firstly performed using Steglish conditions [30, 35] in chloroform under reflux with the system DCC, DMAP (Scheme III-5). A spatula tipful of *para*-benzoquinone was added as radical inhibitor but only unidentified products were obtained.





Due to this problem, another kind of coupling reaction was used and Mitsunobu conditions were carried out (Scheme III-5). When 2.0 equivalents of protected coumaric acid $\underline{5}$ were mixed with 1.0 equivalent of $\underline{4}$ in presence of PPh₃ and DIAD, in THF, at room temperature for 2.5 days, the expected product $\underline{3}$ was obtained with a yield of 54% (Entry 1, Table III-4). It was observed that a longer time could lead to a better yield. After three days of reaction, compound $\underline{3}$ was isolated with a yield of 85%. Moreover, these reaction conditions are mild and it was not necessary to add radical inhibitor.

Entry	Reaction time	Results
1	2,5 days	<u>3</u> (54%)
2	3 days	<u>3</u> (85%)
3	4 days	<u>3</u> (85%)

Table III-4: Effect of time reaction in Mitsunobu conditions at RT using 2equiv. of compound <u>5</u>; 1equiv. 4hydroxybutyl acrylate <u>4</u>, 2 equiv. DIAD/PPh₃.

III.3.1.1.2.2 Deprotection of TBS and obtention *p*-CAA Monomer

The last step of this synthesis strategy consisted in the deprotection of the silyl ester in order to regenerate the phenolic group (Scheme III-6). In fact, the most common method for the deprotection of silylated ether is the use of TBAF in THF [35-38]. The reaction was carried out in anhydrous THF in the presence of acetic acid, at room temperature for 16 hours. Finally, *p*-CAA Monomer <u>1</u> was obtained with a yield of 93%.



Scheme III-6: Deprotection of the silylated ether, generating p-CAA Monomer 1

III.3.1.2 Synthesis of p-CAB Monomer 2

The *p*-CAB monomer $\underline{2}$ could be prepared according to the following retrosynthetic analysis Scheme III-7.



Scheme III-7: Retrosynthetic approaches for the synthesis of *p*-CAB monomer (2)

The setting up of the carboxylic acid function conjugated to the carbon-carbon double bond for the monomer $\underline{2}$ could be obtained either by malonic synthesis or by a Wittig reaction from the aldehyde

function of the molecule $\underline{6}$. For the preparation of this precursor $\underline{6}$, two approaches were envisioned. The first one consisted in a Mitsunobu coupling between the 4-hydroxybenzaldehyde and the 4-hydroxybutyl acrylate $\underline{4}$. The second one would be based on the conversion of the alcohol function of the compound $\underline{4}$ in mesylate $\underline{7}$ and this compound could promote a nucleophilic substitution with the 4-hydroxybenzaldehyde to provide $\underline{6}$. (Scheme III-7). The different steps will be discussed in the following section.

III.3.1.2.1 Preparation of the precursor <u>6</u>

III.3.1.2.1.1 Route 1

A one-step method was used at first for the synthesis of <u>6</u>, a Mitsunobu reaction. So, the 4hydroxybenzaldehyde (2.0 quiv.) was reacted with 4-hydroxybutyl acrylate <u>4</u> (1.0 equiv.) in the presence of DIAD (2.0 equiv.) and PPh₃ (2.0 equiv.), in anhydrous THF at room temperature (Scheme III-8). A summary of the reaction conditions is listed in Table III-5.



Scheme III-8: Chemical pathways for the synthesis of precursor 6.

Entry	Reaction time	Conversions (%)	Yields (%)
1	4 days	35%	-
2	6 days	57%	-
3	7 days	70%	47%

Table III-5: Time reaction performed for Mitsunobu reaction, using 2.0 equiv. of 4-hydroxybenzaldehyde; 1.0 equiv. 4-hydroxybutyl acrylate <u>4</u>, 2.0 equiv.DIAD, at RT.

The reaction was performed firstly during four days but a conversion of only 35 % was observed (Entry 1, Table III-5). A second try was thus carried out in more concentrated medium by decreasing the quantity of anhydrous THF and by increasing the reaction time of two days allowing to reach a 57 % conversion (Entry 2, Table III-5). The third try was done again in more concentrated medium than during the first try and after a week, a conversion improved by 70 % was obtained (Entry 3, Table III-5). After chromatography on silica gel, the pure product $\underline{6}$ was isolated with a 47 % yield. The poor yield and the very long reaction time incited us to use another approach, the one using a mesylate group as leaving group in order to activate the nucleophilic substitution.

III.3.1.2.1.2 Route 2

For this approach, the first step was the introduction of a mesylate group from the alcohol function of $\underline{4}$. The mesylation reaction was performed in the presence of 2.0 equivalents of triethylamine and mesyl chloride (MsCl) in anhydrous THF at room temperature for 2 hours. After chromatography on silica gel, the product $\underline{7}$ was obtained with a yield of 99%. (Scheme III-9).



Scheme III-9: Mesylation of compound 4.

The next step was the coupling of compound $\underline{7}$ with 4-hydroxybenzaldehyde (Scheme III-10). This synthesis was carried out in anhydrous DMF in the presence of potassium carbonate at 60°C for 15h. The expected product $\underline{6}$ was obtained after purification on silica gel with a yield of 94%. For the synthesis of the compound $\underline{6}$, the route 2 was much more effective than the route 1. Indeed, a 93 % overall yield was obtained for the route 2 in comparison to 47 % from route 1. Moreover, reaction times are widely more favorable for the route 2 (17 hours) compared to the route 2 (7 days).



Scheme III-10: Nucleophilic substitution between mesylate compound $\underline{4}$ and 4-hydroxybenzaldehyde.

III.3.1.2.2 Final steps for the synthesis of monomer $\underline{2}$

III.3.1.2.2.1 Malonic synthesis

The last step to obtain monomer $\underline{2}$ consisted in a malonic synthesis from the aldehyde function of the molecule $\underline{6}$ and the carboxylic acid function. Thus, compound $\underline{6}$ was reacted with malonic acid (Scheme III-11). Various reaction conditions described in the literature using a basic[39, 40] or acidic[41] medium for this type of synthesis were tested and are summarized in Table III-6.



Scheme III-11: Preparation p-CAB monomer 2 via malonic synthesis.

Basic conditions were first tested. The first coupling reaction was carried out in anhydrous DMF and pyridine (Entry 1, Table III-6). The temperature of reaction was set at 90°C for 5h. The ¹H NMR analysis of the crude product showed the presence of the starting material in a mixture with another compound,

which was not the expected *p*-CAB monomer $\underline{2}$ since the signals of the *E*-configuration of the carboncarbon double bond were not present. The use of piperidine in solution in pyridine was then tested (Entry 2, Table III-6). The reaction was performed at 90°C for 5h but only degradation of reagents was observed. These results demonstrated that basic conditions were not the good strategy to prepare $\underline{6}$, encouraging us to perform malonic reaction in acidic conditions.

Entr	Reagent system	Solvent	T(°C)	Time	Products	Conversion
У				(11)		(70)
1	Pyridine	DMF	90	5	$\underline{6}$ + by-product	-
2	Piperidine	Pyridine	90	5	Degradation	-
3	CH ₃ SO ₃ H	AcOH	60	7	<u>6</u>	-
4	CH ₃ SO ₃ H	AcOH	80	20	<u>6</u> + <u>2</u>	50
5	CH ₃ SO ₃ H/ 4-methoxylphenol	AcOH	80	36	$\underline{2}$ + by-product	-
6	CH ₃ SO ₃ H/ 4-methoxyphenol	DMF	90	96	<u>6</u>	-
7	CH ₃ SO ₃ H/ 4-methoxylphenol	AcOH	90	7	<u>2</u>	75
8	CH ₃ SO ₃ H/ 4-methoxylphenol	AcOH	90	7	$\underline{2}$ + by-product	50
9	CH ₃ SO ₃ H/ 4-methoxylphenol	AcOH	90	15	$\underline{2}$ + by-product	75

Table III-6: Operating malonic reaction conditions, using 1.0 equiv. of compound <u>4</u>, 1.20 equiv. 4-*methoxyphenol and 0.5 equiv. of methylsulfonic acid.*

The first synthesis was carried out in acetic acid for 7 h at 60°C (Entry 3, Table III-6), however, only the starting material $\underline{2}$ was isolated. A second test was carried out by increasing the reaction temperature at 80°C for 20h and adding 0.5 equivalents of methylsulfonic acid (Entry 4, Table III-6). A mixture of starting material and another compound, which could be the expected product, was observed. Because of the small recovered quantity, the reaction was restarted in the same conditions but adding also 4-methoxyphenol (radical inhibitor), to avoid the degradation of the starting material at 80°C. After 32h of reaction, TLC monitoring showed very little evolution so the temperature was increased of 10°C and the solution was stirred at this temperature for 14h (Entry 5, Table III-6). After work-up and purification, an inseparable mixture of the expected monomer $\underline{2}$ and another by-product was obtained. On the ¹H NMR spectrum, it was observed that the proton integration of the acrylate system was lower than expected and a singlet signal at 2.05ppm and a triplet at 4.15ppm were also present. By mass spectrometric analysis, two signals were observed, the first one with a m/z of 313.1052 corresponding to *p*-CAB monomer, and another one with a m/z of 301.1056. This side-product has been identified and it corresponded to product $\underline{9}$ (Figure III-3), having a CH₃C=O in place of the double bond of the acrylate, due to a transesterification reaction of *p*-CAB monomer $\underline{2}$ with acetic acid (reaction solvent).



Figure III-3: Chemical structure of the secondary product, obtained by the transesterification reaction of p-CAB monomer $\underline{2}$ with acetic acid.

In order to avoid the formation of this side-product, DMF was used to replace acetic acid. The amount of malonic acid was also increased (2equiv. Entry 6, Table III-6). No conversion was observed after 96h of reaction. We concluded that the presence of acetic acid was highly necessary to form *p*-CAB monomer <u>2</u>. The malonic synthesis was therefore repeated in acetic acid at 90°C and decreasing the reaction time to 7h, always in the presence of methylsulfonic acid and of the radical inhibitor (Entry 7, Table III-6). An incomplete conversion (only 78%) was observed, giving 49% of *p*-CAB monomer <u>2</u> but always with some traces of side-product. In order to confirm the experimental conditions, a test was repeated on a larger scale at 90°C for 7h (Entry 8, Table III-6). However, the ¹H NMR analysis of the crude product showed a lower conversion (only 50%). A last attempt was performed in the same conditions but increasing the reaction time (Entry 9, Table III-6). With these conditions, we managed to increase the conversion (75% instead of 50%). Nevertheless, *p*-CAB monomer <u>2</u> was always obtained in the presence of side-product <u>9</u> with a 90:10 ratio. Bearing in mind the difficulties encountered with malonic synthesis, another route was considered to synthesize *p*-CAB monomer <u>2</u>, the one using a Wittig reaction.

III.3.1.2.2.2 Wittig reaction

Wittig reaction is a famous method to prepare olefins from aldehydes or ketones. Compound $\underline{6}$ was therefore reacted with a phosphonium ylide bearing a *tert*-butyl ester (commercial reagent). This reaction may allow obtaining the expected alkene conjugated to the aromatic ring and to the ester $\underline{8}$ (Scheme III-12)



Scheme III-12: Chemical pathways to synthesis **p-CAB** monomer <u>2</u> via Wittig reaction.

The reaction was carried out in dichloromethane at room temperature for 24 hours and compound **<u>8</u>** was isolated after purification with a yield of 78% (Scheme III-12). The *E* configuration of carbon-carbon double bond was confirmed since a coupling constant (${}^{3}J = 16 \text{ Hz}$) was observed for the two alkenic

protons on the ¹H NMR spectrum. The last step consisted in the hydrolysis of the *tert*-butyl ester to generate *p*-CAB monomer <u>2</u>. Bearing two ester functions, the compound <u>8</u> must be selectively hydrolyzed. The choice of the *tert*-butyl ester as a protecting group for the carboxylic acid was therefore justified since it could be cleaved in acidic medium. Finally, *p*-CAB monomer <u>2</u> with 89% of yield was successfully obtained by deprotection of *tert*-butyl ester, using trifluoroacetic acid at room temperature for one hour (Scheme III-12).

III.3.1.3 Conclusions

For the synthesis of *p*-CAA monomer $\underline{1}$, the use of a silvlated ether as protecting group of the phenol function was compared to the use of a methoxy group. The targeted *p*-CAA monomer $\underline{1}$ was obtained in three steps from *para*-coumaric acid with an overall yield of 75%.

Concerning the synthesis of the *p*-CAB monomer, the preparation of the compound <u>6</u> was optimized and it was shown that the pathway using a mesylate, then reaction of this mesylate in basic conditions, was the best alternative (93 % overall yield), while Mitsunobu coupling gave a yield equal to only 47 % after very a long reaction time. The different attempts of malonic synthesis from <u>6</u>, allowed the exclusion of the basic conditions, because for every essay the acrylate system was modified (probably by saponification). The conditions in acidic medium allowed us to obtain the monomer <u>2</u> with a 49 % yield but always with a side-product. Another approach allowed the elimination of the formation of the side-product, the one using a Wittig reaction then a selective acidic hydrolysis of the *tert*-butyl ester. The *p*-CAB monomer was generated in overall yield of 65% in four steps.

These two target monomers were integrated in the Natural Rubber network, as detailed in the next Section.

III.3.2 Preparation of thick films: formulations and kinetic study of the photopolymerization

In a previous work [42-46], it was demonstrated that the films obtained from ACNTR oligomers without adding any biocide group presented a low but noticeable antibiofouling activity, which was significantly enhanced by incorporating a bioactive function on the NR network, such as the quaternary ammonium groups at the oligomers chain end.

In the case of the integration of the guanidine monomer to the ACTNR₆₀₀₀ network for conferring the antimicrobial activity (Chapter II), it was needed to add PETA as reactive diluent in the formulation to obtain homogenous films. Indeed, ACTNR are liquid, hydrophobic, viscous oligomers (honey-like), and the guanidine monomer is a hydrophilic solid powder. Furthermore, it was observed that 10% weight of guanidine monomer presented the best value to generate antibacterial activity, and maintain interesting properties of natural rubber.

Knowing the data relative to the guanidine thick films, in the present work, two families of antifouling polymer materials based on natural rubber were envisioned, containing 10% weight of *p*-CAA or *p*-CAB monomer, in presence of PETA, to prepare thick films in view of future applications. This molecule could be able to dissolve *p*-CAA and *p*-CAB monomer and to allow its dispersion in the prepolymerization mixture, remaining covalently bound to the network at the same time. Darocur 1173 was chosen again as a photoinitiator with the same reason, reported in the previous chapter. It meets the usual criteria of absorbing in the UV region, where the ACTNR₆₀₀₀ oligomers and *p*-CAA or *p*-CAB monomer do not absorb, where the UV lamps emit. Furthermore, in comparison to other commonly used solid photo-initiators, it is liquid and it can be easily dispersed in the viscous polymerization mixture. The percentage retained for all formulations was 2.5% (weight) because it represented a good compromise between a lower amount (1%) that required longer polymerization times, and a higher amount (5%)[47, 48] that allowed shorter irradiation times but increased the amount of unreacted photoinitiator released in the environment. The general procedure for synthesis of the coating is presented in Figure III-4 (detailed protocol, see Section III.3.6.3).



Figure III-4: General procedure for the synthesis of the coatings.

The kinetic study of the photo-polymerization, monitored by FTIR-ATR, was carried out, using the formulations described in Table III-7, in order to verify the conversion of acrylate groups in relation to the polymerization time. Again, the idea is to polymerize only the acrylate group without touching the carbon-carbon double bond in the polyisoprene repeating units to maintain the mechanical properties.

Composition	F ₁₀ <i>p</i> -CAA	F10 <i>p</i> -CAB
ACTNR6000	67.5 %	67.5 %
Darocur 1173	2.5 %	2.5 %
РЕТА	20 %	20 %
<i>p</i> -CAA	10 %	0 %
p-CAB	0 %	10 %

Table III-7: General composition of films containing the two tailor-made monomer from Zosteric acid (% = weight percentage)

In fact, R.Jellali *et al.* [42, 43] revealed firstly that polymerization kinetics of the mixture ACTNR and photoinitiator Darocur 1173 without bioactive molecules could be monitored following the = $C-H_2$ stretching band of the acrylate group at 1405 cm⁻¹, considering as reference band the peak at 1376 cm⁻¹, whose intensity remains constant during irradiation.

A comparison of the FTIR spectra of *p*-CAA, and *p*-CAB monomers, *para*-coumaric acid and ACNTR₆₀₀₀ was performed and presented in Figure III-5, D. This data enabled to make sure that adding *p*-CAA or *p*-CAB monomers on ACTNR matrix did not cover the two bands used for conversion calculation. It was also confirmed that the main aromatic bands δ (CC) ar were located at 1601 and 1509 cm⁻¹ (Figure III-5, C). Both monomers *p*-CAA, *p*-CAB presented the band at 1630 cm⁻¹, characteristic of the C=C double bond conjugated with the carboxylic group (Figure III-5, B). These assignments agreed with the data reported by R. Świsłocka *et al.*[49] concerning spectroscopic (FT-IR, FT-Raman, ¹H and ¹³C-NMR) and theoretical studies of *p*-coumaric acid and alkali metal *p*-coumarates.



Figure III-5: Comparison of FTIR spectra of *p*-CAA, *p*-CAB monomer, para-coumaric acid and ACNTR₆₀₀₀, (A): entire IR spectrum ;(**B**, **C**, **D**): zoom, respectively for $1800 - 1610 \text{ cm}^{-1}$ region, $1620-1450 \text{ cm}^{-1}$ region and $1450-1300 \text{ cm}^{-1}$ region. Light intensity I=29.4 mW/cm².

Thick films were prepared, using two formulations $F_{10} p$ -CAA and $F_{10} p$ -CAB with dimensions of 1cm of diameter and 0.5mm of thickness. IR spectra were performed of face A (the surface directly exposed to the UV-lamp) and face B, the surface in contact with the TeflonTM mold. The light intensity was kept constant at 29.4 mW/cm². The percentage of conversion of the polymerization mixture was calculated with equation (2) (see details in Section II.3 of Chapter II), using the two bands at 1405cm⁻¹ and at 1376 cm⁻¹.

With formulation $F_{10}p$ -CAA, containing 10% of *p*-CAA monomer, we observed that the photopolymerization reaction was not complete after 25 min, for both two faces, confirmed by the presence of the band of the acrylate group at 1405 cm⁻¹ (Figure III-6). Conversely, the intensity of the characteristic bands of ACNTR ₆₀₀₀ including the reference band at 1376 cm⁻¹, and in the range of 3010-2775 cm⁻¹, decreased, suggesting a degradation of the polyisoprene motif that was not our objective. This slowing down of the reaction rate could be explained by the presence of the phenol group (-C₆H₄-OH) on the structure of the *p*-CAA monomer. Phenols are known for their role of polymerization inhibitors [50, 51], in particular, R.A. Bird *et al.*[52] studied the effect of phenols on the polymerization of vinyl acetate. They demonstrated that phenols acted as fairly strong retarders in radical

polymerization due to the attack of polymer radicals to phenols group by hydrogen abstraction. Nevertheless, this problem was not occurring with the $F_{10}p$ -CAB formulation, due to the absence of the phenol group. In the following section, we will focus only on $F_{10}p$ -CAB formulations.



Figure III-6: The IR absorbance change of the acrylate group upon irradiation in F_{10} p-CAA formulation (peak at 1405 cm⁻¹). Left: entire IR spectrum; **Right**: zoom of the 1800 – 1200 cm⁻¹ region. Light intensity I=29.4 mW/cm². Films dimensions of 1cm diameter and 0.5mm thickness.

It was noticed that the conversion was higher on face A than face B; similar to the case of guanidinium monomer, Chapter II. This difference was due to the direct exposition of face A to the UV-light whereas face B was in contact with the TeflonTM molds. As the pre-polymerization mixture was not transparent, it was a viscous, yellowish mixture, it absorbed part of the light and the lower face received a less intense radiation (conversion profile upon irradiation, using $F_{10}p$ -CAB formulation is reported in Figure III-7). It was clearly shown that after 10 min, roughly 93% conversion of the total number of acrylate double bonds was reached on face A and around 80% for face B.



Figure III-7: Conversion of total number of acrylate functions versus irradiation time in $F_{10}p$ -CAB formulation, containing 10% of *p*-CAB monomer (Light intensity I=29.4 mW/cm²).

A great number of replicates was needed to test antifouling properties, so it was decided to prepare the films with another UV-lamp equipped with a sliding belt, having more powerful light intensity of 0.313J/cm². However, with this equipment, it was demonstrated on previous studies that after 47s upon irradiation, the carbon-carbon double bond of the repeating unit started to disappear (Figure III-8). The deformation of spectrum profile in the range of 3100-2800 cm⁻¹ was observed. Therefore, the irradiation was stopped before the complete conversion of acrylate groups was reached. For these reasons, the first type of thick films using **F**₁₀*p*-**CAB** formulation were obtained upon 47s irradiation with 96% conversion on the A and around 80% for face B.



Figure III-8: The IR spectrum profile change completely upon irradiation after 47s (*Red line*), compared with that at 0s (*Back line*), at 47s (*Blue line*). It is clearly shown that the degradation of the carbon-carbon double bond of the repeating unit started.

In order to improve the conversion of face B and study the effect of crosslinking rate of bioactive monomer on leaching and biological tests, a second series of thick films was performed by submitting again face B Type I upon 4min with light intensity of 29.4 mW/cm². By FTIR analysis, we obtained the thick films Type II with 96% conversion on the face A and around 92% for face B without degradation C=C bond of isoprene unit.

Thick Films obtained by	Type I	Туре II
F10p-CAB formulation		
Conversion Face A (%)	96	96
Conversion Face B (%)	80	92

Table III-8: Characteristic of thick films, Type I and Type II, Conversion (%) was calculated with equation (2). III.3.3 Leaching test results

In previous section, we prepared successfully a new polymer film by incorporating p-CAB monomer on ACTNR₆₀₀₀ network *via* photopolymerization in presence of Darocur1173 as photoinitiator. As mentioned in introduction, the main goal of this work was to develop new antifouling materials nonreleasing, ecofriendly and nontoxic. Therefore, this section will be focused on the leaching test of films components from our materials when it is immersed in water. In order to make sure that in the conditions of the biological tests no leaching was occurring from the polymers, and to ensure that the antibacterial activity was due to the active monomer rather than the photoinitiator released, our films were immersed in water 24h before testing. 14 supernatant samples were taken to analyze the released percentage of Darocur 1173, its photo-degradation products benzaldehyde and benzoic acid, [53] and *p*-CAB monomer. The results, presented in Figure III-9, showed that after 24h of immersion in water, around 70% of Darocur 1173 from films Type I was released and 40% from films Type II. A very small fraction of Darocur gave the photodegradation products, as only around 3.0% of the maximum amount of benzoic acid that could be released from films Type II, and no significant values (< 0.4%) from films Type I, were detected. No release of benzaldehyde was detected. Concerning the release of *p*-CAB monomer (Figure III-9, C), around 0.5% was found for films Type I, comparing 0.2% for the films Type II. These difference can be explain by a greater conversion of acrylate group during photopolymerization process on the films Type II and so a lower release films components.



Figure III-9:Time course of Darocur 1173 (A) and benzoic acid (B) and p-CAB monomer (C) leaching into deionized water after 24h from thick films (0.5mm thickness x1.0mm diameter) using F10p-CAB at 25°C, detected by HPLC. Benzoic acid released for films type $I \le 0.4\%$ (few of sample out of calibration curve).

III.3.4 Coatings thermal properties and surface contact angle.

In order to determine the thermal stability (degradation temperature) of the films and thus studying the effect of adding *p*-CAB monomer on $ACNTR_{6000}$ matrix, TGA analysis was performed on three categories of films: films type I, type II and reference films that contained only 77.5% of ACTNR6000, 2.5% of Darocur and 20% of PETA without *p*-CAB monomer (Films Ref).

The TGA curves are presented in Figure III-10. They were obtained at a heating rate of 10° C min⁻¹, from 40° C to 800° C, under nitrogen atmosphere. No difference between the films of Type I and Type II was observed. The thermograms (Figure III-10) indicated that all films remained stable up to 200° C and decomposed completely at 374° C. Nevertheless, two degradation steps located at 250° C and at 374° C were present for both films containing 10% of *p*-CAB monomer, in comparison to only one step for the film Ref. The weight lost for the first and second step were 3.3% and 72.0% w/w, respectively. The previous studies performed on the same formulation but using Guanidine monomer instead of *p*-CAB monomer, showed the same trend for thermal stability profile.

Sathish *et al.* [54] demonstrated that when the decomposition of *p*-coumaric acid took place, for the first step, at 181°C there was a 56.89% mass fraction loss, which was attributed to the decomposition of - OH phenolic group. In the second stage the decomposition started at 181°C and ended at 266°C, corresponding to the breakage of the carboxylic group from the phenyl ring. Furthermore, Ouimet *et al.* [55] pointed out that by chemically incorporating *p*-coumaric acid into the backbone of a poly(anhydride-ester), the obtained polymer exhibited a decomposition temperature of 302 °C and T_g at 57 °C for a 26.7 kDa mass. Therefore, the first decomposition step observed at 250°C in our case concerns probably the degradation of the *p*-CAB monomer moiety and the other steps correspond to polyisoprene degradation.

Table III-9 presents the degradation temperatures and corresponding weight loss of each type of films, compared with film Ref.



Figure III-10: Thermograms of two type of films containing 10% of **p-CAB** monomer, compared to films without it (Films Ref).

Decomposition temperature (°C)							
Sample		At the we	eight loss		T_{max}^{b} (°C)	Contact angle	
_	T_5^a	$T_{10}{}^a$	$T_{50}{}^{a}$	$T_{90}{}^{a}$		(°)	
Films Ref	329	348	392	457	374	79	
Films Type I and II	309	346	400	460	374	53	

 T_x^a : Decomposition temperature at the x% weight loss: a) data obtained from TGA curve, b) data obtained from DTG curve.

Table III-9: TGA and contact angle data of different films.

The wettability of the three types of films used for TGA analysis was also determined by measurement of the contact angle (Figure III-11). The obtained data are presented in Table III-9. As for TGA results, no difference of wettability for films Type I and Type II was observed. For all samples, a value of contact angle less than 80° was found, that often denoted a rather hydrophobic character. However, a significant decrease of contact angle was observed by adding 10% of *p*-CAB monomer in the polymer matrix (53° versus 79°), indicating a higher level of hydrophilicity. This difference could be explained by the presence of the carboxylic group (-COOH) of the *p*-CAB at the films surface, as well as in bulk.



Figure III-11: A drop of water on three films in contact angle measurement: (A) Reference films without **p-CAB** *monomer, (B, C) films containing 10% of* **p-CAB** *monomer, Type I and Type II.*

III.3.5 Films activity against pathogenic bacteria

The films containing the monomers from Zosteric acid were submitted to similar assays performed with coatings containing the guanidine monomer, in order to evaluate the biological activity. Five strains of pathogenic bacteria that pose threat to the human health and are ranked in the drug-resistant list [56] were selected and studied in static conditions in contact with the films: *Pseudomonas aeruginosa* (CIP: A22, **PA**, Gram negative), *Staphylococcus aureus* (CIP 52.16, **SA**, Gram positive), *Staphylococcus epidermidis* (CIP: 176.117, **SE**, Gram positive), *Bacillus subtilis* (CIP, 67.7, Gram positive), *Escherichia coli* (**E.Coli**, CIP, 54.8, Gram negative).

Antiobiograms were performed to see the action of each target monomer alone before integration in the ACNTR₆₀₀₀ matrix. *p*-CAA and *p*-CAB monomers being solid powders, they were dissolved in DMSO (dimethylsulfoxide) to be deposited on the wells in the agar plates, in a range of concentrations from 2.5 mg/mL to 40.0 mg/mL. It was ensured that the presence of DMSO did not affect the microorganisms. The results of the antibiograms are presented in Figure III-12, reporting the inhibition halo radius observed. *p*-CAA exhibited a higher activity than *p*-CAB monomer against most of the five bacteria strains in all concentrations, especially Gram positive ones. *S.aureus* was particularly sensitive towards *p*-CAB even at 2.5 mg/mL.



Figure III-12:Antibiograms of *p-CAA* (*Left*) and *p-CAB* (*Right*) monomer on five strains of pathogenic bacteria including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus aureus in the range of concentration from 2.5 to 40 mg/mL in DMSO. No inhibition halo of DMSO solvent was observed (d = 0 cm).

Despite the inferior activity, due to the difficulty in obtaining a complete polymerization, coming from the phenol group in their structure, only thick films containing 10% of *p*-CAB (Type I, Table III-8) were prepared and tested.

A procedure was established to incubate the films with the bacteria suspensions and to calculate the number of surviving cells that remained attached to the surface after incubation for 3h at 37°C (Figure III-13). Being aware of the results of the leaching tests, it was decided to submit the disks to a washing step (one day immerged in de-ionized water under gentle stirring) before performing the assays, in order to eliminate all the Darocur photoinitiator and be sure that the observed activity was not an artefact.



Figure III-13:Antibacterial activity (A and B) of five stains of pathogenic bacteria including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, and Bacillus aureus on two types of films prepared with $F_{10}p$ -CAB formulation.

The bacterial suspension itself was used as growth control, and considering the appropriate dilutions, the number of colonies developed from the bacteria collected at the surface of the disks after the assays was compared to the one of the control. A considerable diminution of the suspension concentration was

observed for all bacteria. The most affected strains were the *S.epidermidis*, *S.Aureus* and *Bacillus subtilis*. These results enabled to confirm again the trend of bacterial attachment on ACNTR matrices, that was demonstrated on the previous studies performed either on the simplest formulation in disks containing only ACNTR₆₀₀₀ without any bioactive molecule [45] or in disks prepared by adding 10% of guanidine antimicrobial monomer (Chapter II). Moreover, these new coatings exhibited also a great antibacterial activity against *Bacillus subtilis*. It was reported in the literature that the *para*-coumaric acid and the 4-methoxyl cinnamic acid showed MIC (minimum inhibitory concentration) values of 2.0 mM [57, 58] and of 203µM [59], respectively, against *Bacillus subtilis*. Therefore, this effect can be attributed to the presence of *p*-CAB monomer at the surfaces and not only in the bulk of the material.

Based on the first results obtained, the films Type II containing also 10% of *p*-CAB monomer were tested with only three of the most effective pathogenic bacteria, including *S.epidermidis, S.Aureus* and *Bacillus subtilis*. The films Type II showed an excellent antibacterial property, higher than films Type I (Figure III-13). No *S.Aureus* was found on the surfaces after contact with films and only 0.01% of bacteria of the initial suspension of *S.epidermidis* and *Bacillus subtilis* remained attached. As previously demonstrated, after 24h, only 0.2% of the maximum amount of *p*-CAB monomer was released (~0.01mg).So during the biological tests, this release of *p*-CAB monomer could not affect the antibacterial activity. Observation of the bacteria attached to the surfaces was regularly made by scanning electron microscopy, on selected disks treated specifically to obtain the images (Figure III-14 for magnification of 500, and 1000, resp. for Figure III-15). The visual observation supported the quantitative data from the attachment assays; no bacteria were found on the surfaces, except for PA, for which few bacteria were observed (Figure III-14, C). In addition, the morphology of the surfaces was also monitored after immersion.



Figure III-14:Micrographs of disks containing 10% of p-CAB Type I monomer after incubation with: a) Bacillus b) E.coli c) PA, d) SA, e) SE. Zoom at 50µm with x500. No bacteria are visible.



Figure III-15:Micrographs of disks containing 10% of **p-CAB** monomer after incubation with: a) Bacillus b) E.coli c) PA, d) SA, e) SE. Zoom at $10\mu m$ with x1000.

Further studies will be carried out in the follow up of this preliminary work, in collaboration with microbiologists, to assess if the attached bacteria are dead or alive, if their growth is influenced, and to better understand the mechanism of action of the surface. Ongoing studies are dealing also with a formulation in which the two monomers guanidine and Zosteric acid are present, to investigate the impact against the same bacterial strains for comparison. Preliminary tests were also performed with three strains of marine bacteria to check the antifouling activity. It is necessary to repeat them with a higher number of replicates in order to assess and quantify precisely the efficiency observed in the initial assays. In a new collaboration with marine biologists, the attachment of microalgae, fungi and other macroorganisms will also be investigated, to monitor the materials activity during the entire process of aquatic biofilm formation.

III.3.6 Experimental part

III.3.6.1 Synthesis of p-CAA Monomer (<u>1</u>)

III.3.6.1.1 Preparation of (E) 3-(4-tert-butyldimethylsiloxy)phenyl acrylic acid, compound 5

To a solution of *p*-coumaric acid (5.000 g, 30.45 mmol, 1.0 equiv.) and imidazole (5.188 g, 76.13 mmol, 2.5 equiv.) in dry DMF (25 mL) was added under N₂ and at 0°C the TBSCl (11.47 g, 76.13 mmol, 2.5 equiv.). The reaction mixture was stirred at RT for 3 h then poured into distilled water (125 mL). The cloudy solution was extracted with EtOAc (3*125 mL) and the combined organic layers were washed with brine (125 mL), dried over MgSO₄ and concentrated under vacuum. The residue was dissolved in THF/water (100/25 mL) and LiOH (729 mg, 30.45 mmol, 1.0 equiv.) was added. The reaction mixture was stirred at RT for 30 minutes and quenched by the addition of an HCl aqueous solution (1N) up to pH = 5. The aqueous layer was extracted with EtOAc (2*125 mL) and the combined organic layers were washed with brine (70 mL), dried over MgSO₄ and concentrated under vacuum. The

residue was purified by chromatography on silicagel (cyclohexane / EtOAc 9:1 toward 0/1) to provide 5 (8.080 g, 29.02 mmol, 95%) as a white solid.

¹H NMR (200 MHz, CDCl₃) δ : 7.75 (d, J_{H3-H2} = 15.9 Hz, 1H, H-3), 7.45 (d, J_{H5-H6} = 8.6 Hz, 2H, H-5, H-9), 6.85 (d, J_{H6-H5} = 8.6 Hz, 2H, H-6, H-8), 6.32 (d, J_{H2-H3} = 15.9 Hz, 1H, H-2), 0.99 (s, 9H, *t*Bu), 0.23 (s, 6H, 2*Me).

III.3.6.1.2 Preparation of 4-(acryloxy)butyl(E)-3(4-((tert-butyldimethylsilyl)oxy)phenyl)acrylate compound <u>3</u> via Mitsunobu reaction

To a solution of 4-hydroxybutyl acrylate $\underline{4}$ (1.00 g, 6.93 mmol, 1.0 equiv.), protected *p*-coumaric acid $\underline{5}$ (3.87 g, 13.9 mmol, 2.0 equiv.), and PPh₃ (3.69 g, 13.9 mmol, 2.0 equiv.) in dry THF (110 mL), a solution of DIAD (2.7 mL, 2.81 g, 13.9 mmol, 2.0 equiv.) in dry THF (27 mL) was added under N₂ and at RT. The reaction mixture was stirred at RT for 3 days then concentrated under vacuum. The residue was purified by chromatography on silicagel (cyclohexane / EtOAc 95:5 toward 8/2) to provide $\underline{3}$ (375 mg, 0.927 mmol, 59%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃) & 7.63 (d, $J_{H10-H9} = 16.0$ Hz, 1H, H-10), 7.42 (d, $J_{H12-H13} = 8.5$ Hz, 2H, H-12, H-16), 6.84 (d, $J_{H13-H12} = 8.5$ Hz, 2H, H-13, H-15), 6.41 (dd, $J_{H1'-H1} = 1.5$ Hz, $J_{H1'-H2} = 17.3$ Hz, 1H, H-1'), 6.30 (d, $J_{H9-H10} = 16.0$ Hz, 1H, H-9), 6.13 (dd, $J_{H2-H1} = 10.4$ Hz, $J_{H2-H1'} = 17.3$ Hz, 1H, H-2), 5.83 (dd, $J_{H1-H1'} = 1.5$ Hz, $J_{H1-H2} = 10.4$ Hz, 1H, H-1), 4.25-4.21 (m, 4H, H-7, H-4), 1.83-1.79 (m, 4H, H-6, H-5) 0.98 (s, 9H, *t*Bu), 0.23 (s, 6H, 2*Me).

¹³C NMR (100 MHz, CDCl₃) δ: 167.3 (C-3 or C-8), 166.2 (C-3 or C-8), 157.8 (C-14), 144.5 (C-10), 130.7 (C-1), 129.6 (C-12, C-16), 128.4 (C-2), 127.7 (C-11), 120.5 (C-13, C-15), 115.6 (C-9), 64.1 (C-4 or C-7), 63.8 (C-4 or C-7), 25.6 ((*C*H₃)₃C), 25.5 (C-5 or C-6), 25.4 (C-5 or C-6), 18.2 ((CH₃)₃C), -4.4 (2**C*H₃).

HRMS (ESI) [M+Na]⁺ calculated for C₂₂H₃₂NaO₅Si: 427.1911, found: 427.1908.

III.3.6.1.3 Preparation of 4-(acryloxy)butyl (E)-3-(4-hydroxyphenyl)acrylate (Monomer <u>1</u>) by deproctection of TBS group

To a solution of $\underline{3}$ (940 mg, 2.32 mmol, 1.0 equiv.) in dry THF (17.5 mL), glacial AcOH (0.95mL) and TBAF (solution in THF (1M), 6.96 mmol, 3.0 equiv., 7.0 mL) were added under N₂ at RT. The reaction mixture was stirred at RT for 18h then THF was evaporated under vacuum. The residue was taken up in DCM (50 mL) and washed with a NaCl saturated aqueous solution (20 mL), then concentrated under vacuum. The organic layer was dried over MgSO₄ then concentrated under vacuum. The residue was purified by chromatography on silicagel (cyclohexane / EtOAc 4:1 toward 1/1) to provide $\underline{6}$ (627 mg, 2.16 mmol, 93%) as a colorless oil which crystallizes at room temperature.

¹H NMR (400 MHz, CDCl₃) & 7.63 (d, $J_{H10-H9} = 16.0$ Hz, 1H, H-10), 7.42 (d, $J_{H12-H13} = 8.6$ Hz, 2H, H-12, H-16), 6.86 (d, $J_{H13-H12} = 8.6$ Hz, 2H, H-13, H-15), 6.55 (bs, 1H, OH), 6.42 (dd, $J_{H1'-H1} = 1.4$ Hz, $J_{H1'-H1} = 17.3$ Hz, 1H, H-1'), 6.29 (d, $J_{H9-H10} = 16.0$ Hz, 1H, H-9), 6.13 (dd, $J_{H2-H1} = 10.3$ Hz, $J_{H2-H1'} = 17.4$ Hz, 1H, H-2), 5.84 (dd, $J_{H1-H1'} = 1.4$ Hz, $J_{H1-H2} = 10.3$ Hz, 1H, H-1), 4.26-4.22 (m, 4H, H-7, H-4), 1.83-1.80 (m, 4H, H-6, H-5).

¹³C NMR (100 MHz, CDCl₃) δ: 167.8 (C-3 or C-8), 166.6 (C-3 or C-8), 158.3 (C-14), 145.0 (C-9), 131.0 (C-1), 130.0 (C-12, C-16), 128.3 (C-2), 126.8 (C-11), 115.9 (C-13, C-15), 115.0 (C-10), 64.2 (C-4 or C-7), 64.0 (C-4 or C-7), 25.4 (C-5 or C-6), 25.3 (C-5 or C-6).

IR (ATR, cm⁻¹): 3371, 2961, 2875, 1699, 1633, 1601, 1517, 1441, 1412, 1325, 1309, 1278, 1197, 1164, 965, 843, 812.

HRMS (ESI) $[M+Na]^+$ calculated for $C_{16}H_{18}NaO_5$: 313.1046, found: 313.1048.

III.3.6.2 Synthesis of Monomer p-CAB (2)

III.3.6.2.1 Preparation of compound 4-((methylsulfonyl)oxy)butyl acrylate 7

To a solution of 4-hydroxybutyl acrylate (5.0 g, 34.7 mmol, 1.0 equiv.) in dry THF (7 mL) were added under N₂ and at 0°C triethylamine (9.7 mL, 69.4 mmol, 2.0 equiv.) and mesyl chloride (7.95 g, 69.4 mmol, 2.0 equiv.). The reaction mixture was stirred at RT for 2h then quenched by addition of distilled water (50 mL). The aqueous layer was extracted twice with EtOAc (2*100 mL). The combined organic layers were washed successively with NaCl aqueous saturated solution (200 mL), dried over MgSO₄ then concentrated under vacuum. The residue was purified by chromatography on silicagel (CH₂Cl₂ / EtOAc 1:0 toward 2/1) to provide <u>7</u> (7.685 g, 34.6 mmol, 99%) as a slightly brown oil.

¹H-RMN (400 MHz, CDCl₃), δ (ppm): 6.42 (dd, J_{H1'-H1} = 1.4 Hz, J_{H1'-H2} = 17.3 Hz, 1H, H-1'), 6.12 (dd, J_{H2-H1} = 10.4 Hz, J_{H2-H1'} = 17.3 Hz, 1H, H-2), 5.85 (dd, J_{H1-H1'} = 1.4 Hz, J_{H1-H2} = 10.4 Hz, 1H, H-1), 4.28 (t, J_{H4-H5} = 6.1 Hz, 2H, H-4), 4.21 (t, J_{H7-H6} = 5.9 Hz, 2H, H-7), 3.02 (s, 3H, H-8), 1.91-1.79 (m, 4H, H-5 H-6).

¹³C-RMN (100 MHz, CDCl₃), δ (ppm): 166.0 (C-3), 130.6 (C-1), 128.3 (C-2), 69.2 (C-7), 63.4 (C-4), 37.2 (C-8), 25.6 (C-5 ou C-6), 25.2 (C-5 ou C-6).

III.3.6.2.2 Preparation of precursor 4-(4-formylphenoxy)butyl acrylate 6

A. Via Mitsunobu reaction

To a solution of 4-hydroxybutyl acrylate (200mg, 1.38mmol, 1.0 equiv.) in dry THF (2.91 mL) were added under N_2 and at room temperature, *p*-benzaldehyde (304mg, 2.48mmol, 1.8 equiv.) and PPh₃ (656g, 2.5mmol, 1.8 equiv.). Then a solution of DIAD (451mg, 1.48mmol, 1.8eq) in dry THF (287µL)
was introduced within 10min. The reaction mixture was stirred at RT for 7 days then concentrated under vacuum. The residue was purified by chromatography on silicagel (cyclohexane / EtOAc 95:5 toward 85/15) to provide **6** (161.4 mg, 0.65mmol, 47%) as a colorless oil.

B. Route 2-Via Nucleophilic Substitution

To a solution of 7 (7.50 g, 33.4 mmol, 1.0 equiv.) in dry DMF (152 mL) were added under N₂ the *p*-hydroxybenzaldehyde (4.84 g, 39.7 mmol, 1.19 equiv.) and potassium carbonate (27.19 g, 197 mmol, 5.89 equiv.). The reaction mixture was stirred at 60°C for 16h then, after cooling down, quenched by addition of distilled water (300 mL). The aqueous layer was extracted twice with EtOAc (2*200 mL). The combined organic layers were washed successively with NaHCO₃ aqueous solution (200 mL) and NaCl aqueous saturated solution (200 mL), dried over MgSO₄ then concentrated under vacuum to provide **6** (7.81 g, 31.46 mmol, 94%) as a colorless oil.

¹H-RMN (200 MHz, CDCl₃), δ (ppm) : 9.86 (s, 1H, CHO), 7.84 (d, J_{H10 H9} = 8.8 Hz, 2H, H-10 H-12), 7.00 (d, J_{H9 H10} = 8.8 Hz, 2H, H-9 H-13), 6.42 (dd, J_{H1'-H1} = 1.7 Hz, J_{H1'-H2} = 17.2 Hz, 1H, H-1'), 6.12 (dd, J_{H2-H1} = 10.3 Hz, J_{H2-H1'} = 17.2 Hz, 1H, H-2), 5.84 (dd, J_{H1-H1'} = 1.7 Hz, J_{H1-H2} = 10.2 Hz, 1H, H-1), 4.25 (t, J_{H4-H5} = 6.1 Hz, 2H, H-4), 4.09 (t, J_{H7-H6} = 5.9 Hz, 2H, H-7), 1.96-1.87 (m, 4H, H-5 H-6).

¹³C-RMN (100 MHz, CDCl₃), δ: (ppm) 190.6 (CHO), 166.0 (C-3), 163.8 (C-8), 131.8 (C-10, C-12), 130.6 (C-1), 129.8 (C-11), 128.3 (C-2), 114.6 (C-9, C-13), 67.5 (C-7), 63.9 (C-4), 25.6 (C-5 ou C-6), 25.2 (C-5 ou C-6).

III.3.6.2.3 Preparation of Monomer2 either by Malonic synthesis or Wittig reaction

A. Via Malonic synthesis

To a solution of $\underline{4}$ (120 mg, 0.483 mmol, 1.0 equiv.) in acetic acid (1.5 mL) was added under N₂ the 4-Methoxyphenol (25 mg, 39.7 mmol, 1.19 equiv.) and then methylsulfonic acid (16 µL, 0.242 mmol, 0.5 equiv.). The reaction mixture was stirred at 90°C for 7h then, after cooling down, quenched by addition of distilled water (20 mL). The aqueous layer was extracted twice with EtOAc (2*25 mL). Combined organic layers were washed successively with NaCl aqueous saturated solution (60 mL), dried over MgSO₄ then concentrated under vacuum. The residue was purified by chromatography on silicagel (CH₂Cl₂ / EtOAc 1:0 toward 2/1) to provide <u>6</u> with some traces of by-products of transesterification (69.8 mg, 0.238 mmol, 49%) as a while solid.

B. Via Wittig reaction Preparation of compound (E)-3-(4-(4-(acryloxy)butyloxy)phenyl) acrylic acid terbutyl ester <u>8</u>

To a solution of <u>6</u> (1.213 g, 4.89 mmol, 1.0 equiv.) in dry DCM (28 mL) was added under N₂ at RT *t*-Bu-2-triphenylphosphoranylideneacetate (2.39 g, 6.36 mmol, 1.3 equiv.). The reaction mixture was stirred at RT for 24h then quenched by addition of distilled water (20 mL). Phases were separated and the organic layer was washed with brine (40 mL). Aqueous layers were extracted once with DCM (50 mL). Combined organic layers were dried over MgSO₄ then concentrated under vacuum. The residue was purified by chromatography on silicagel (cyclohexane / EtOAc 4:1) to provide $\underline{8}$ (1.326 g, 3.83 mmol, 78%) as a colorless oil.

¹H-RMN (400 MHz, CDCl₃), δ (ppm) : 7.54 (d, J_{H14-H15} = 15.9 Hz, 1H, H-14)), 7.44 (d, J_{H10 H9} = 8.7 Hz, 2H, H-10 H-12), 6.87 (d, J_{H9 H10} = 8.7 Hz, 2H, H-9 H-13), 6.40 (dd, J_{H1'-H1} = 1.5 Hz, J_{H1'-H2} = 17.3 Hz, 1H, H-1'), 6.24 (d, J_{H15-H14} = 15.9 Hz, 1H, H-15), 6.12 (dd, J_{H2-H1} = 10.4 Hz, J_{H2-H1'} = 17.3 Hz, 1H, H-2), 5.82 (dd, J_{H1-H1'} = 1.5 Hz, J_{H1-H2} = 10.4 Hz, 1H, H-1), 4.24 (t, J_{H4-H5} = 6.1 Hz, 2H, H-4), 4.02 (t, J_{H7-H6} = 5.8 Hz, 2H, H-7), 1.91-1.85 (m, 4H, H-5 H-6), 1.53 (s, 9H, *t*Bu).

¹³C-RMN (100 MHz, CDCl₃), δ: (ppm) 166.6 (C-16), 166.1 (C-3), 160.4 (C-8), 143.1 (C-14), 130.6 (C-1), 129.5 (C-10, C-12), 128.4 (C-2), 127.3 (C-11), 117.6 (C-15), 114.7 (C-9, C-13), 80.1 ((CH₃)₃C), 67.3 (C-7), 64.0 (C-4), 28.2 ((CH₃)₃C), 25.7 (C-5 ou C-6), 25.3 (C-5 ou C-6).

HRMS (ESI) [M+Na]⁺ calculated for C₂₀H₂₆NaO₅: 369.1672, found: 369.1665.

Preparation of (E)-3-(4-(4-(acryloxy)butyloxy)phenyl) acrylic acid (Monomer $\underline{2}$)from precursor $\underline{8}$

To a solution of <u>8</u> (1.288 g, 3.72 mmol, 1.0 equiv.) in dry DCM (20 mL) was added under N₂ at RT, TFA (0.28 mL, 3.46 mmol, 10 equiv.). The reaction mixture was stirred at RT for 1h (checked by TLC) then concentrated under vacuum. The residue was purified by chromatography on silicagel (Cyclohexane / EtOAc 1:1 toward 0/1) to provide <u>2</u> (959 mg, 3.30 mmol, 89%) as a white solid.

¹H-RMN (400 MHz, CDCl₃), δ (ppm) : 7.74 (d, J_{H14-H15} = 15.9 Hz, 1H, H-14)), 7.50 (d, J_{H10 H9} = 8.8 Hz, 2H, H-10 H-12), 6.90 (d, J_{H9 H10} = 8.8 Hz, 2H, H-9 H-13), 6.41 (dd, J_{H1'-H1} = 1.5 Hz, J_{H1'-H2} = 17.3 Hz, 1H, H-1'), 6.32 (d, J_{H15-H14} = 15.9 Hz, 1H, H-15), 6.12 (dd, J_{H2-H1} = 10.4 Hz, J_{H2-H1'} = 17.3 Hz, 1H, H-2), 5.83 (dd, J_{H1-H1'} = 1.5 Hz, J_{H1-H2} = 10.4 Hz, 1H, H-1), 4.25 (t, J_{H4-H5} = 6.1 Hz, 2H, H-4), 4.04 (t, J_{H7-H6} = 5.8 Hz, 2H, H-7), 1.93-1.86 (m, 4H, H-5 H-6),

¹³C-RMN (100 MHz, CDCl₃), δ: (ppm) 172.8 (C-16), 168.3 (C-3), 161.1 (C-8), 146.7 (C-14), 130.8 (C-1), 130.1 (C-10, C-12), 128.4 (C-2), 126.8 (C-11), 114.9 (C-9, C-13), 114.8 (C15), 67.5 (C-7), 64.1 (C-4), 25.8 (C-5 ou C-6), 25.4 (C-5 ou C-6).

IR (ATR, cm⁻¹): 2946, 2873, 1718, 1673, 1628, 1601, 1574, 1508, 1440, 1408, 1300, 1287, 1193, 972, 825, 810.

HRMS (ESI) $[M+Na]^+$ calculated for $C_{16}H_{18}NaO_5$: 313.1046, found: 313.1052.

III.3.6.3 Films preparation procedure

The thick films were prepared using the following procedure (Figure III-4):

- 1. The bioactive monomer either *p*-CAA or *p*-CAB was dispersed in the reactive diluent (PETA) for 10min in the *ultrasonic* bath, set at 30°C.
- 2. ACTNR oligomers were added mixing them with a spatula, then the solution was introduced for 15min in the *ultrasonic* bath.
- 3. Darocur photoinitiator was introduced in the previous obtained mixture, leaving it for other 5 min in the *ultrasonic* bath.

The viscous medium was degassed under light vacuum, and homogeneity of the dispersion was observed by optical microscope. On average 50 mg of pre-polymerization mixture were deposited on a Teflon mold (diameter 1 cm, thickness 0.5 mm) and irradiated by the UV lamp for the required amount of time. The yellowish elastic films were easily removed from the mold with tweezers to perform FTIR spectra and check the complete polymerization.

III.3.6.4 Leaching tests protocole

Before evaluating antimicrobial properties, the thick films containing 10% of *p*-CAB- monomer were immerged in de-ionized water at 25°C for 24h. Each film was introduced separately into a 5 mL glass flask containing 1mL of de-ionized water ($18M\Omega$ m⁻¹, Veolia water STI). These flasks were placed in an orbital shaker operated at 50 rpm. The obtained supernatants were analyzed by High Performance Liquid Chromatography (HPLC) to detect the percentage of Darocur 1173 photoinitiator and *p*-CAB monomer released. A calibration range of Darocur 1173 and it photodegradable products including Benzaldehyde and benzoic acid was prepared in the same manner of Section III, Chapter II. All samples were analyzed by injecting 20 µL into a ThermoFisher Scientific series instrument equipped with a C₁₈ column operated at 30°C (Alltima, 3 µm, 2.1 x 150 mm) and a UV detector (Waters 996) set at 247 nm.

p-CAB monomer released was monitored by HPLC as previously described and a UV detector set at 307.6 nm. Retention time was 24.8 min. The calibration quantified by peak area was linear between 0.213 mg.L^{-1} to 27.214 mg.L⁻¹ in acetonitrile (Figure III-16)



Figure III-16: Calibration curve obtained from known concentrations of solutions of **p-CAB** monomer. Peak areas obtained by HPLC at 307.6 nm are plotted against concentration

III.3.6.5 Biological assays

Same procedure, previous described in Section III, Chapter II, Biological assays.

III.4 Conclusions

In conclusion, the active monomers based on Coumaric acid analogues were successfully covalently incorporated in the natural rubber matrix.

The formulation to obtain thick, homogeneous and elastic films was optimized, studying the photopolymerization process by FTIR and the surface morphology by optical microscope. The excellent anti-biofilms properties of these materials were shown with three strains of pathogenic bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, in static conditions. No *S.Aureus* was found on the surfaces after contact with films and only 0.01% of bacteria of the initial suspension of *S.epidermidis* and *Bacillus subtilis* remained attached.

The effect of covalent integrating CA analogues into the structure of polymers guaranteed to the material a long life with a permanent efficacy against a broad-spectrum of bacteria.

Thermograms showed that the materials were stable up to 200°C and the contact angle values indicated that the surfaces were relatively hydrophilic, confirming the presence of the ZA moiety at the surface and not only buried in the bulk matrix. This information is very important for future industrial applications, where materials could be submitted to high temperatures. This stability will also avoid the emission of volatile organic compounds, and consequently limit toxicological impact.

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CHAPTER IV. Functionalization and covalent grafting of Polyisoprene Oligomers from Natural Rubber to Surfaces.

IV.1. Introduction

In the previous chapters, we described how we synthesized and evaluated the antibacterial properties of freestanding thick polyisoprene (PI) films from natural rubber. We demonstrated also that by incorporating active monomers, either Guanidine (Chapter II) or Zosteric acid (Chapter III) derivates, these films exhibited a much higher antimicrobial activity in comparison to films without active monomers.

Among the main goals of our work, we also wanted to covalently anchor such materials to surfaces for industrial applications. In the form of thin films, such layers might be used as protection coatings for microelectromechanical devices, thanks to the barrier properties of natural rubber toward water or they could be also used as an antibacterial and/or antifouling layers. To perform the grafting, we started with the synthesis of new oligomers from natural rubber, bearing a silane group at the chain-end that could be immobilized on silicon-based and other surfaces. The other end-group of these new oligomers would be converted in acrylate function, in order to carry out further radical polymerization of monomers (Guanidine or Zosteric acid ones) or to grow thicker films from the reaction of the acrylate oligomers. The objective of the first preliminary tests was to determine the essential wettability characteristics of each obtained surface and to observe if there was a significant difference if guanidine monomers were included. These first results of the grafting of amphiphilic polymer chains polyisoprene-copolyguanidine to glass surfaces were presented in a Poster format at the HYMA 2017 congress (5th international conference on "Multifunctional, Hybrid and Nanomaterials", which was held in Lisbon, on 6-10/03/2017, see Figure IV-1). A significant decrease of the contact angle in water was clearly shown after incorporating guanidine brushes on acrylate terminated oligoisoprene grafted brushes. This demonstrated the presence of the hydrophilic (guanidium) groups on the polymer surfaces. Performing such preliminary tests, we wanted to have the proof of concept and the feasibility of the strategy so, to save time, we only used fast characterization techniques such as contact angles, without performing time consuming surface characterization techniques such as X-ray reflectivity, XPS, etc. We successively concentrated our efforts in obtaining a robust covalent grafting with several experimental difficulties (described in the chapter) due to the first choices we made in terms of anchoring groups (see discussion on silanes stability of tri-aminosilanes).

In the current chapter, we first introduce the appropriate literature and specific techniques used for functionalizing surfaces. We then present, in the format of a scientific publication, the strategy elaborated to make ultra-thin PI coatings and the methodology built for anchoring the antibacterial films presented in the previous chapters to surfaces. Due to the fragile character of molecular layers, we favored a versatile approach with a pre-functionalization of surfaces with acrylates groups, which will be used to grow or to anchor thick films containing active groups in the bulk, in order to maintain the biological activity if the coating is abraded or damaged.

Hybrid materials based on natural rubber derived oligomers grafted to different inorganic supports. Application as antibacterial surfaces



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of the polymer brushes was measured by X-Reflectivity

100A

All surfaces were studied measuring their contact angle and the thickness

III. Surface properties

✓ Contact angle with water

I. Objectives

- ✓ Synthesize novel telechelic oligomers from natural rubber chains and successive modifications of the reactive chain ends
- ✓ Graft these oligoisoprenes covalently on the surface of different supports
 ✓ Polymerize a new monomer bearing a biocide group from the free extremity of the grafted oligoisoprene

II. Synthesis procedures

1. Synthesis of new telechelic oligomers with a mono, di, trialkoxy-silyl and a hydroxyl chain end is a three steps process starting from solid natural rubber. The oligomers have been grafted covalently on glass slides



Figure IV-1: Results obtained from preliminary experimental tests, in Poster format presented at HYMA 2017 congress, 5th international conference on "Multifunctional, Hybrid and Nanomaterials".

IV.2. Polymers at interfaces for coating applications

IV.1.1 State of the art

Among the methods for modifying surface properties, deposition of thin polymer layers tethered to the surface is an efficient way to generate new physical and chemical properties. For antimicrobial surfaces [1-4], the attachment of a surface polymer layer or brush onto a substrate can mitigate the formation of biofilms *via* two main mechanisms: either by providing a physical surface barrier, which resists to the adhesion of microbes, or by imparting microbiocidal effects to the surface with an effective chemical group exterminating pathogens upon contact [5-8].

IV.1.1.1 Physisorption versus Chemisorption

Tethering of polymer chains onto substrates could be a reversible (*physisorption*) or irreversible (*chemisorption*-covalent linkages) process [9] [10]. Physisorption [11] is often achieved by self-assembly of block copolymers in which at least one block has a preferential adsorption for the surface. The mechanism is driven by solvent-polymer interaction (solvent hydrophobic/solvent hydrophilic) and/or surface interaction with the polymer chains. The polymer chains are often reversibly attached to the substrate and can be cleaved off via solvent exchange, thermal treatment or competitive absorption. Note that robust physisorption may occur and satisfy some applications but for many others, the non-covalent attachment of the polymer chains strongly limits the stability of the assembly and restricts the use of such coatings. To reduce these limits, polymer chains are anchored on substrates *via* chemical covalent linkages [12, 13]. This is accomplished by two methods, the "grafting to" (Figure IV-2.A) and the "grafting from" approach (Figure IV-2.B). Each of these approaches presents advantages and disadvantages [9, 12, 14]



Figure IV-2: Two strategies to synthesize polymer coatings: (A): grafting-from and (B): grafting-to".

The "Grafting to" approach consists on a tethering process performed *via* end-functionalized polymers reacting with a suitable substrate surface under appropriate conditions to form a covalent bond, robust and resistant to common environmental conditions. The literature on grafting such brushes covers flat substrates [15], porous materials and structures [16], fibers/textiles [17] and nanoparticles [18]. It can provide tethered chains with precisely controlled molecular weight and molecular weight distributions. Nevertheless, only a small amount of polymers can be generally immobilized covalently onto the surface following the "grafting to" approach. Macromolecular chains must diffuse through the existing polymer film to reach the reactive sites on the surface and a repulsive barrier becomes more pronounced as the tethered polymer film thickness increases. For this reason, only low grafting densities and low film thickness are generated. To improve this limit, it is possible to increase the polymer concentration in the solution and the grafting time [19, 20]. Note that in some cases a favorable lateral interaction between molecules favors the formation of dense layers.

Other alternatives were used to obtain a high grafting density of polymers, among which the "Graftingfrom" approach in two steps. Initiators are first attached onto the substrate, often in the form of a selfassembled monolayer (SAM) and then chains are polymerized from the immobilized initiator layers, generating tethered polymers. Several conventional polymerization methods can be used in "graftingfrom" approach such as free radical polymerization, anionic polymerization, controlled radical polymerization, etc...[12, 13].

IV.1.1.2 Conformation of Oligomers and Polymers with a chain-end grafted at a surface

Polymers can adopt different conformations at an interface, depending mostly on the solvent and surface interactions, ending in a given surface grafting density (σ) (Figure IV-3).



Figure IV-3: Simplified scheme of three scaling law regimes for polymers at interfaces parameter. v depends on solvent quality. In moderate-density regime: v approaches 1/3 in a good solvent, approaches 0.8 in a poor solvent and 0.5 in a theta solvent, ref. [21]

When the chain density is high in comparison with the radius of gyration of the polymer chains (R_g) (Figure IV-3), individual polymer chains overlap with adjacent chains, inducing strong steric hindrance. It leads to increase in excluded volume polymer chains and finally, in a more extended and stretched conformation of chains brushes regime [22]. The polymer chains can also appear as mushroom like structures in good solvent. In the mushroom regime, the average distance (D) between chains is large enough so inter-chain interactions are not stretching the chains. The system moves to the "brush regime" with moderate-density or high-density of grafting. If the interaction with the substrate is stronger than with the solvent, polymer chains interact more strongly with neighboring molecules at high surface densities of grafting. The term **v** corresponds to the slope for the log (h) versus log (σ) plot, and its value in moderate-density regime, approaches 1/3 in a good solvent, ~ 0.8 in a poor solvent, and ~ 0.5 in a theta solvent. In this simplified description, the grafting density can be evaluated from the following equation (IV.1).

$$\sigma = \frac{h\rho N_A}{Mn} \quad (IV.1)$$

Where **h** is the brush thickness, which we can obtain from X-ray reflectivity for instance (see next section), ρ is the polymer bulk density, N_A is the Avogadro's number and M_n is the number average molecular weight of the grafted polymer chains.

IV.1.2 Bifunctional silane coupling agents for grafting polymer chains to surfaces

IV.1.2.1 Silanization and grafting of silane coupling agents to silicon-based material surfaces.

Anchoring covalently polymer chains to an inorganic solid surface can be difficult or impossible in absence of added chemical reactivity at the surface. This problem can be overcome by the introduction of coupling agents that bind to the surface and bring a reactive chemical group for subsequent reaction with the polymer [23]. Providing a bifunctional nature and a strong hybrid linkage between organic chain and other inorganic materials, silanization is a low-cost and effective covalent coating method to modify material surfaces that are rich in hydroxyl groups. These coating molecules are often engineered to form multiple inorganic bonds for retention at the interface and stable covalent bonds with polymers as well in a second step. Silane coupling agents are widely used [24] in improving the adhesion strength between pairs of materials such as metal and rubber [25], metal and resins [26], rubber and resins [27], glass and resins [28], and biomaterials [23, 29]. Many types of silane coupling agents are commercially available (several are shown in Table IV-1) among which, alkoxysilane are the most used and reported in the research literature as coupling agents between solid substrates and polymer matrices [30].

N°S	Structure	Functionality	Abb	Target matrix	Referenc e
1	(RO) ₃ Si-(CH ₂) ₃ -NH ₂ ^a	Amino	APS	Epoxy, Polyethylene Butyl rubber, Polyacrylate PVC	[31-34]
2	(RO) ₃ Si-CH=CH ₂	Vinyl	VTS	Polyethylene, Polypropylene Polyacrylate	[32, 35- 39]
3	(RO) ₃ Si-(CH ₂) ₃ -OOC(CH ₃)CH=CH ₂	Methacrylate	MPS	Polyethylene, Polyester	[32, 40- 42]
4	(RO) ₃ Si-(CH ₂) ₃ -SH	Mercapto	MRPS	Natural Rubber, PVC	[41, 43- 45]
5	(RO) ₃ Si-(CH ₂) ₁₅ -CH ₃	Alkyl	HDS	Natural Rubber, Polyethylene	[41, 46]
6	R ₂ -Si-Cl ₂	Chlorine	DCS	PVC, Polyethylene	[47, 48]
7	(RO) ₃ Si-R''-N ₃	Azide	ATS	Polypropylene, Polyethylene Polyethylene	[49, 50]

^aR=-methyl or ethyl, Abb=Abbreviation

Table IV-1:Some silane coupling agents used for the natural fiber/polymer composites: chemical structures, organofunctional and target polymer matrices, ref. [30].

The alkoxy group of silane coupling agents can react with silanols (Si-OH) groups on silicon-based substrates such as silica, glass, quartz, fibers, ... Then corresponding alcohols are liberated, generally methanol or ethanol, resulting in the formation of siloxane bonding (-Si-O-Si-, strong covalent bond). However, silane agents reactivity is greatly affected by their own stability, by the type of substrate composition and structure, as well as by the environmental conditions [51-55]. A basic mechanistic model for silane grafting on activated substrates was described by Arkles *et al.* [56] (see Figure IV-4) and follows 4 steps :



Figure IV-4: Mechanism of Silane grafting on an active substrate surface (so-called Silanization step).

• **Hydrolysis**: The alkoxysilane is first hydrolyzed to generate active silanol intermediates and the corresponding alcohols in presence of water and/or a catalyst (normally an acid or a base)

- **Condensation:** During the hydrolysis process, the concomitant condensation of silanol (aging) also takes place. The condensation should be minimized at this stage to leave the silanols free to approach the hydroxyl groups exposed on the active surface
- Adsorption: The reactive silanol monomer or oligomer (trimer shown in Figure IV-4) attach to the surface *via* hydrogen bonding. The free silanols also adsorb and react with each other thereby forming a rigid polysiloxane structure linked with stable Si-O-Si bonds
- Chemical grafting: Under heating conditions, hydrogen bonds between the silanols and the hydroxyl groups of the substrates are converted into stable Si-O-Si covalent bonds, liberating water

The reactions between silane coupling agents and activated substrates are somehow quite complex. The silane retention and self-organization on the surface are still under debate [51, 57-59]. Various interfacial processes may be involved such as covalent binding with the substrate, lateral polymerization of adsorbed silanes, three-dimensional polymerization, etc... Mainly two processes are involved, with or without requirement of a prehydrolysis step [30, 60]. In fact, the presence of water enhance the polymerization of alkoxy silanes, which can give rise to a number of possible surface structures, from uncontrolled-disordered multilayers to self-assembled ordered monolayers. Note that, anhydrous solvents with a trace amount of water are highly recommended for the preparation of silane monolayers [61] (detail in next Section IV.2.2.2, typically in the case of γ - aminopropylalkoxysilane). For all these reasons, we decided to test mono, di and tri ethoxy-silane grafting for our application (as presented in the form of a scientific publication in next section).

IV.1.2.2 Molecular structure of silane coupling agents to obtain reactive surface coatings.

The chemical nature of the reactive moieties bound to the silane silicon atom, typically alkoxy groups, affects the structure and stability of the silane layer formed, as well as its reactivity for further chemistry steps. R.Chambers *et al.* [62] demonstrated that the bulkiness of alkoxy groups would affect the rate of hydrolysis. Under the same hydrolytic conditions the methoxy groups of trimethoxysilane hydrolyzes more rapidly than the ethoxy groups of triethoxysilane [62, 63] [64]. This is probably due to more steric hindrance for ethoxy groups, in comparison to methoxyl groups. Furthermore, during the hydrolysis process, ethanol or methanol is released and due the toxicity of methanol for environment [65], the use of triethoxysilane is recommended. A.C. Miller *et al.* [66] demonstrated that di- and tri-alkoxy silanes produce a stronger adhesion strength than mono-alkoxy silanes because they form more binding sites after hydrolysis. Y.Xie *et al.* [30] reported that the α -methacryloxymethyltrimethoxy silane (MMS, α -silane) hydrolyzes 20 times faster than γ -methacryloxypropyltrimethoxy silane (MPS, γ -silane) in the same conditions. This has been attributed to the " α -effect", stronger electro-interactions between the functionality (X), and this particularly from the presence of free electron pairs from nitrogen and silicon atoms (Figure IV-4). W.Chen *et al.* [51] pointed out also that the primary amine group in the propyl (γ)

position (Figure IV-5, D) can bind to the silicon atom forming a stable five-membered cyclic intermediate for inter- or intra-molecular catalysis, which makes APS more reactive than corresponding alkylalkoxysilanes. This effect can cause uncontrolled polymerization/oligomerization of aminosilanes in solution.



Figure IV-5:Different length of the bridge connecting the silicon atom and functionalities: α -silane having a methylene spacer (**A**), γ -silane bearing a propylene spacer (**B**), and the " α -effect" in the aminomethylene silane (**C**); " γ -effect" in the aminopropyl silane (**D**) and "no-effect" in (**E**).

The 3-Aminopropylalkoxysilanes (APS or γ - aminopropylalkoxysilane) are widely used as coupling agents for silicon-based materials because of their bifunctional nature, useful to generate links between substrates and polymer chains [30]. The presence of a nucleophilic site from NH₂ groups in aminosilanes enables to promote adhesion in glass and resin composites. It can be also coupled with other functional groups like –COOH *via* esterification reaction, -CHO *via* reductive amination or epoxy resin [33], generating covalent bonding (Figure IV-6). Furthermore, in aqueous solution, with low to medium pH values, the NH₃⁺ group promote the attachment of negative charged species, such as DNA [67, 68], or PVC due to their acid-base characteristics [47].



Figure IV-6: Example of reactions on amino functional grafted silanes.

Nevertheless, the applications of APS in several industrial fields or in biology or environmental science was limited by its poor hydrolytic stability and loss of surface functionality from the aminosilane- layers. During exposure to elevated air humidity and temperature, the deposited APS layers might decompose, leading to reduction in coupling efficiency, thus decreasing the product quality and the mechanical strength of the polymer–inorganic interface. As described on section IV.2.2 (Mechanism interaction),

the alkoxysilane is first hydrolyzed to generate activated silanol intermediates. These intermediates partially oligomerize in solution at the active substrate interface, generating a stable Si-O-Si covalent bond. However, these processes are difficult to evidence and strongly depend on the hydration state. The major problem is associated with the ability of the precursor to copolymerize in the presence of traces of water, thus forming aggregates and disordered layers on the substrate. Conversely, in the conditions of very low water activity, grafting might occur through direct nucleophilic attack of the silanol surface.

In the case of APS, the presence of NH₂ functional groups offers unique properties. It also caused a major problem in presence of water traces. This problem is attributed to siloxane bond hydrolysis catalyzed by the NH₂ group. Various type of interactions between APS molecules and surface silanols can take place *via* hydrogen bonding, electrostatic attraction or covalent siloxane bonds (see Figure IV-7 [51, 61]). Covalently attached aminosilane layers typically have low grafting densities due to the presence of vertically (Figure IV-7, a) as well as horizontally (Figure IV-7, b) grafted silane molecules. Hydrogen bonding interactions with surface silanols alone result in weakly attached silane molecules on silica surfaces (Figure IV-7, b&c&d).





Depending on pH solution, NH_2 groups can be converted into NH_3^+ with a positive charge, that generates other types of interactions with silanol substrates (Figure IV-8).



Figure IV-8: In acid media, different types of bonding/interaction between aminopropylethoxylsilane molecules

and glass interface: (a') a covalently attached APS molecule with its amine group extending away from the interface, (a1') a covalently attached APS molecule with its amine group interacting with a surface silanol group, and (b'&c'&d') weakly bounded APS molecules via electrostatic attractions.

A.Y. Fadeev *et al.* [69] reported on the complexity of silane layers structures resulting from the multiplicity of reactive sites from the molecule, especially in the case of 3-aminopropyltriethoxylsilane (APTES). Possessing three ethoxyl groups, the polymerization increased in presence of water, thus generating APTES disordered multilayers (Figure IV-9): APTES molecules were covalently attached to the substrates. Among the covalent bonding, some bonded to surface silanol only (black system) and others bonded with neighboring silanes via horizontal or vertical reactions. Silanes can also be tethered to the surface in oligomeric structures (Red systems). APTES can be also physically absorbed via hydrogen bonding (Green system) or electrostatic forces (Blue and Orange systems).



Figure IV-9: APTES molecules with different structural organisations in the way they are linked to the substrate: (black) individual silane molecules can be incorporated via covalent bond (a, a'); (red) vertical and horizontal polymerization with neighboring silanes; (bleu &orange &pink) individual silane molecules adsorbed into layers via electrostatic attraction; (green) individual silane molecules adsorbed into layer via hydrogen bonding, ref. [69].

A significant effort has been made to optimize silanization conditions such as changing the solvent, amount of water present, silane concentration, temperature, time of immersion and drying/curing conditions to obtain aminosilane layers. E.T. Vandenberg *et al.* [70] demonstrated that monolayer films can be deposited from dry organic solvents, cured under mild conditions, and incubated in water before use so that thick films were cured in an oven and exposed to air atmosphere for several days. N. Aissaoui *et al.* [71] indicated also that working at higher temperatures reduces the formation of solution aggregates (in that reference optimum at 90°C, for 12h), probably from the increase of nucleation growth kinetics from the low-affinity sites, so that stable silane layers are formed for binding proteins applications. W.Chen *et al.* [51] pointed out that the silanization of APTES gave less hydrolytically stable silane layers due to the facile intramolecular catalyzed hydrolysis. Silane layers prepared in

anhydrous toluene at elevated temperature exhibited a higher packing density and a greater hydrolytic stability than those prepared in vapor phases at room temperature.

To summarize, all previous studied cases reported that aminosilane multilayers could be produced during the silanization in anhydrous solvents but also showed that repeatability could be significantly affected by many factors from experimental conditions. Working at high temperature promotes the generation of higher grafting surface densities and greater hydrolytic stability. In the following section, we report the comparison of several experimental conditions subsequently tested in order to optimize the grafting of silane coupling agents for surface functionalization of glass. Our goal was to covalently graft, on microscopy slides, PI from natural rubber and PI advanced materials with antibacterial properties inserted in the bulk polymer matrix.

IV.3. Thin coating characterization methods: X-ray reflectivity

	Methods							
Properties	XPS	XRR	IR	Raman	Contact Angle	AFM		
Chemical composition and	Х	-	Х	Х	-	-		
structure								
Thickness	-	Х	-	-	-	Х		
Grafting density	-	Х	-	-	-	х		
Topography and surface structure	х	-	-	-	-	х		
Wettability	-	-	-	-	Х	-		

The polymer characterization of ultra-thin coatings on flat surfaces can be a challenging task since many of the analytical methods in polymer science are bulk solution-based techniques. A selection of the most used techniques to study thin polymer coatings properties is listed in Table IV-2.

Table IV-2: Example of surface techniques that are available for the characterization of thin polymer films: **XPS**: X-Ray photoelectron spectroscopy; **XRR**: X-Ray reflectivity; **IR**: Infrared spectroscopy; **AFM**: Atomic Force microscopy.

Generally, IR spectroscopy or Raman spectroscopy can be used to qualitatively provide evidence for the presence of certain functional groups from their spectroscopic fingerprint. However, these two techniques are delicated to ultra-thin polymer films covalently grafted on glass surfaces, due to a very low signal detected. XPS can provide quantitative information on the chemical composition of the top part of a polymer layer. It can also give insight into the chemical structure of the analyzed material from the covalent links identified. Nevertheless, we did not use this method in this work since the technique is difficult to use when the signal studied comes specifically from the chain ends of a long molecule where most of the signal comes from repeated monomers in the chain (for C, O atoms). Although, XPS instruments are not available in our campus and "carbon-pollution" is often measured on such thin films

that travelled in contact with air. We took advantage of our laboratory instruments including surface sensitive techniques well adapted to the smaller film thicknesses obtained, namely X-ray reflectivity, as well as Atomic Force Microscopy (AFM, data not shown) and contact angles measurements to characterize thin coatings. Instead of discussing the technical details of all the analytical techniques, this section highlights specifically specular X-ray reflectivity (XRR) that is the most prominent used method for our study. Fundamental concepts of XRR are introduced and the experimental measurements and data analyses are briefly introduced. The description of contact angle method will be reported in the next Section IV.4.

IV.1.3 Basic concepts for XRR analysis

X-ray reflectivity (XRR) is a non-destructive and non-contact technique for characterising thin films and interfaces. This method is used here to determine the electron density, thickness and roughness of thin films including mono- or multi-layers with a high precision [72, 73]. The basic principle of XRR involves the reflection of a monochromatic X-ray beam (wavelength λ) at a known angle of incidence on a flat sample surface, and the measurement of the reflectivity as a function of the incident angle $\alpha_i =$ θ over a range starting from the critical angle for total reflection, $\alpha_{i,C}$. Above this particular angle, the specularly reflected intensity decreases, with a form that is dependent on the structural properties of the interface and more precisely on its electron density profile in depth, $\rho_e(z)$, expressed in electrons/Å³.



Figure IV-10: X-ray reflectivity principle: (1) X-rays impinge sample under incident angle α , at specular reflection occurs at the outgoing angle equal to α with a reflected intensity modulated by the interference of multiple interfaces reflections, (A) XRR of a single layer; (B) XRR of a multilayer, ref. [74],

Each time an X-ray beam meets an interface between two phases characterized by their different Xrays optical index (Figure IV-10), part of the beam is reflected and most of the remaining energy transmitted through the interface (refracted beam). The reflected beam intensity relative to the incident beam intensity is determined by the reflection coefficient that depends on the refractive indices (n) of the two materials (equation IV.2). They can be decomposed into two terms: δ (related to the electron density) and β that is used to include the X-ray absorption of the material [75].

$$n = 1 - \delta + i\beta$$
(IV.2)
 δ is the real part, describes electron density
and
$$\delta = \frac{\lambda}{2\pi} \left(\frac{b}{V}\right)$$
(IV.3)
(IV.3)
$$\beta$$
 is the imaginary part, describes the absorption
of the phase and
$$\beta = \frac{\mu\lambda}{4\pi}$$
(IV.4)
where b/V is the scattering length
density (SLD)
where μ is the linear absorption
coefficient

The scattering density length (SLD in Å⁻²) is proportional to electron density (ρ_e in electrons/Å³), which scales with the atomic number of elements of the material (Z) and their abundance (or mass density ρ in following equation IV.5).

SLD =
$$\left(\frac{b}{v}\right) = \rho_e r_e$$
 (IV.5) where $\rho_e = \frac{N_A \rho \Sigma Z_i}{M_n}$ (IV.6)
 r_e is the electron classical radius (=2.82*10^{-6} nm)
 M_n is the molecular weight of the characteristic repeating structure N_A is Avogadro's number ρ mass density
 Z_i number of elements i in the material

XRR measurements were performed in the specular reflection geometry (Figure IV-11). The X-ray beam impinges a flat interface between two media with refractive indices of n_1 ($n_{1=}$ 1 for air) and n_2 , respectively, at angle α_i . The refracted angle can be calculated according to Snell-Descartes's law (equation IV.7). For the specular reflection, the incident angle is always equal to the out-going angle, therefore the momentum transfer vector q, is perpendicular to the film interface ($q=q_z$), so that the material is mainly probed along the z-axis (Figure IV-10).



Figure IV-11 : Geometry of XRR, scheme of beam reflection and refraction at a sharp interface.

For a single interface, classical Fresnel's laws are used to calculate the complex reflection coefficient (r) in the form of equation IV.9 from the amplitude of wave-vectors projected along the z axis for X-ray waves propagating in the incidence medium ($k_{i,z}$) or in the transmission medium ($k_{t,z}$). The reflected intensity (R_F) is thus $R_F = r^2$ (equation IV.11). At grazing incidence angles below the critical angle of the interface (when $\alpha_i \leq \alpha_c$), X-rays are totally reflected so that $R_F=1$ (if the X-ray absorption is negligible). Above the critical angle of the interface (when $\alpha_i > \alpha_c$, that translates in a q_c value) the X-ray beam start penetrating through the interface and it is refracted. The single interface reflectivity R_F decreases rapidly (equation IV.12) following a q_z^{-4} law where $q_z = (4\pi/\lambda) \sin(\alpha_i)$.

$$\boxed{r = \frac{\mathbf{k}_{i,z} - \mathbf{k}_{t,z}}{\mathbf{k}_{i,z} + \mathbf{k}_{t,z}}} (IV.9) \quad \begin{cases} \mathbf{k}_{i,z} = -\mathbf{k}_0 \sin\alpha_i \\ \mathbf{k}_{t,z} = -\mathbf{k}_0 n \sin\alpha_i ; n \cos\alpha_t = \cos\alpha_i \end{cases}$$

when $\alpha_i \ll 1$, we obtained

 $\begin{cases} \sin\alpha_{i} \sim \alpha_{i} \\ \cos\alpha_{t} \sim 1 - \alpha_{i}^{2}/2 \\ n_{2} = 1 - 2\delta - 2i\beta \end{cases} \quad \text{(IV.10) and} \quad \boxed{\mathbf{R}_{F} = |\mathbf{r}^{2}| = \left| \frac{\alpha_{i} - \sqrt{\alpha_{i}^{2} - 2\delta - 2\beta}}{\alpha_{i} + \sqrt{\alpha_{i}^{2} - 2\delta - 2\beta}} \right|^{2} \\ \text{(IV.11)} \end{cases}$

• If $\alpha_i < \alpha_c \Rightarrow \mathbf{R_F} = 1$, corresponding au total reflection

• If
$$\alpha_i >> 3\alpha_c => \left[\mathbf{R}_F \sim \frac{q_c^4}{16q_z^4} \right]$$
 (IV.12), corresponding for an ideal sharp interface

If a thin film is tethered on a substrate with a thickness h, there are three media air/film/substrate and two interfaces (air/film and film/substrate). The beam reflected from each interface and its multiple reflections, interfere one with each other, generating interferences and oscillations clearly visible in the XRR data plots, so-called Kiessig fringes. The thickness *h* can thus be estimated from the distance between two consecutive oscillations (Δq_z)÷

$$\mathbf{h} \cong \frac{2\pi}{\Delta q_{z,\text{film}}} \sim \frac{2\pi}{\Delta q_{z,\text{air}}} \qquad \text{(IV.13) For } q_{z,\text{film}} \gg q_{z,\text{c}} \text{ where } q_{z,\text{c}} \text{ is the scattering vector magnitude at the critical angle}$$

IV.1.4 How to "read" X-ray reflectivity data

An example of X-ray reflectivity for a tethered PI layer on a silanized glass surface is shown in Figure IV-12. The XRR data show several typical features, including the visible Kiessig fringes with a period depending on thickness and an amplitude linked to the materials X-ray contrasts; a sudden drop in reflectivity at the value of the scattering vector ($q_{z,c}$), corresponding to the critical angle (α_c), and the power law decrease in the envelope of the total reflection when $q_{z,i} > q_{z,c}$. Due to the interfaces roughness, the oscillations are damped and this is more pronounced at larger incidence angles. To illustrate how XRR changes with some structural parameters, we calculated the reflectivity of an ideal oligoisoprene

layer (0.31 electrons/Å³) on a glass substrate (0.7 electrons/Å³ with 3Å roughness) at three different PI thicknesses: 7Å (red), 20Å (green) and 100Å (blue) in absence (solid curve) and presence (dashed curve) of a 5Å roughness at interfaces. We also plotted the XRR curve calculated for the bare substrate (grey curve, with or without roughness).



Figure IV-12: Left) X-ray reflectivity (XRR) data (blue dots) of a tethered CTNR film on a APMDES layer grafted to glass substrate and the best fit to the data plotted as a red line. The XRR data for the bare substrate is plotted with grey dots with corresponding best fit (grey solid line). The inset shows the electronic density profiles correspond to the fit calculations (same color codes). **Right**) XRR curves calculated for an idealized PI layer on glass for polymer thicknesses.

We can see that in the reflectivity raw data provides some simple information for thin films such as the thickness and interface roughness but more detailed information are obtained in the form of a SLD (or equivalently electron) profile that cannot be directly extracted and that is obtained performing a "fitting" procedure of the reflectivity data from a simulation model. In our work, the experimental reflectivity data was fitted from reflectivity calculations based on a structural slab model describing all layers and we finally obtained structural parameters corresponding to the "best fit" to the data measured. More details on the measurement of an analysis protocol are given in the publication inserted in this chapter.

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IV.4 Polyisoprene covalent coatings. Grafting bio-based oligoisoprene from Natural Rubber, from monolayers to thick films.

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Abstract:

This paper presents a new methodology to tether covalently polyisoprene (PI) from natural rubber (NR) on hydroxylated and silicon-containing surfaces. For this purpose, we synthesized a novel telechelic oligomer (Oligo-SiHTNR) from natural rubber, bearing a silane group at a chain-end that allows its immobilization on silicon-based and other materials. As an alternative, we also synthesized stable bifunctional carbonyl telechelic natural rubber oligomers (CTNR) with a ketone and an aldehyde end moieties. This CTNR, reacted selectively with surface amino groups, introduced via a prefunctionalization step using commercial amino-alkoxysilanes. We reached a glass surface coverage density and we converted the oligoisoprene free chain-end to acrylate function, so that we can possibly grow more polymer or engage a photopolymerisation with bio-based (acrylate-telechelic oligoisoprene, ACNTR) forming a thick PI film. Following these approaches, we formed thick oligoisoprene (PI) coatings from ultra-thin PI molecular layers, and we characterized them with sub-nanometer resolution by X-ray Reflectivity (XRR) experiments. A direct introduction on the surface of the meta-acrylate function was also obtained with a meta-acrylate alkoxysilane and this approach also resulted in a strong covalent grafting at the interface resisting to long immersions in water and partially to organic solvents such as tetrahydrofuran (THF) under strong magnetic stirring. Such ultrathin or thin coatings derivated of Natural Rubber, find many applications, as solvent protective barriers in microelectromechanical devices or as antibacterial-antifouling surface treatments, by inclusion of a bioactive monomer.

Keywords

Natural rubber, polyisoprene and oligoisoprene coatings, polymer brushes, bio-based elastomers, ultrathin polymer films

1 Introduction

Hybrid inorganic/organic materials are one of the most growing class of new materials, particularly when obtained by synergetic combination of inorganic and organic polymer components [1-3]. Nevertheless, one of the fundamental challenges is to increase their ability to adhere and stick together. This problem occurs repeatedly in numerous industrial and consumer applications. One of the best-known example in everyday life is the detachment of the rubber sole from the bottom of a pair of shoes, or one more serious example is the failure of Firestone tires, caused in part by adhesive failures within the high-stress areas of the tread and belt. To improve adhesion with rubber, new technologies appeared including surface treatments such as plasma etching (corona discharge) or chemical treatments such as bromination, fluorination, sulfonation and ozonolysis [4-6]. Since, as the surface treatment only usually affects the top few layers, changes in the bulk of the material are minimal; if films are just physically bound to their substrate, they still can be easily detachable by washing or calving. Therefore, the need to establish covalent anchoring protocols at the inorganic/polymer interface attractes a large interest [7, 8], particularly in the rubber industry.

Natural rubber possessing excellent properties including high elasticity, mechanical strength, fatigue resistance, wide range of operating temperatures, being bio-sourced and environmentally friendly is one of the most used elastomers in many applications including tires [9, 10]. Thin PI films play also an important role in modern technologies including in anti-fouling coatings and electronic applications [11, 12]. D. Liu et al. [12, 13] demonstrated that the adsorption of the protein bovine serum albumin was very low on surfaces of thin film polyisoprene (PI) and the patterning of adhesion proteins on PIcopolymer surfaces influenced the biological cell attachment and spreading. K. Shiju et al. [11] pointed out that a iodine doped thin film PI was used as active channel in the organic switching devices. K. Ayche et al.[14] reported that a thin film PI can be used as a protective layer for MEMS devices, for its barrier properties toward water. Nevertheless, in most of cases, PI thin films were just physically bound to their substrate by spin coating and dip deposition coating techniques. To our knowledge, no works have been reported on covalent anchoring directly PI on solid inorganic substrates. In this study, we developed a new methodology to tether covalently a thin film of PI on glass substrates via two approaches. The first one consisted on firstly activating the surfaces containing free hydroxyl groups with aminated alkoxysilane commercial molecules (APTES, APMDES or APDMES, Figure 1), to obtain the amino group (NH₂) functionalization; this allowed the reaction with Carbonyl Telechelic Natural Rubber (CTNR) via reductive amination reaction. Conversely, the second route consisted in the direct covalent grafting of a new oligomer Oligo-SiHTNR synthetized from PI (Figure 1) on the bare surface.



Figure 1: Schematic presentation of the two routes for the covalent grafting of NR oligomers on different surfaces

1.1 Materials

Natural rubber (STR5LCV60) was produced by Jana Concentrated Latex Co., Songkhla, Thailand. Periodic acid (H₅IO₆, \geq 99%), triethylamine (Et₃N, \geq 99.0%), methacryloxylpropyl triethoxysilane (MAPTES, 98.0%), acryloyl chloride Toluene (dry, ≥99%), \geq (≥ 97.0%), (3aminopropyl)(ethoxy)dimethylsilane (APDMES, ≥97%), 3-aminopropyl(diethoxy)methylsilane (APMDES, ≥97%), 3-aminopropyl)triethoxysilane (APTES, ≥99%), 2-hydroxy-2-methyl-1-phenylpropan-1-one (Darocur 1173, \geq 97.0%), were purchased from Sigma Aldrich and used without any further purification. The purity information was provided by the manufacturer. Trifluoroacetic acid was purchased from Alfa Aesar. Sodium borohydride (NaBH₄, \geq 98%), 4-aminobutan-1-ol (\geq 98%), N, N'bis (*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (\geq 98%) were purchased from Acros Organics. Sodium bicarbonate (NaHCO₃) technical reagent grade, anhydrous magnesium sulfate (MgSO₄) laboratory reagent grade, sodium thiosulfate pentahydrate (Na₂S₂O₃·5H₂O) and sodium chloride (NaCl) general purpose grade were purchased from Fisher Scientific. Dichloromethane (DCM), tetrahydrofuran (THF), Toluene were purchased from Aldrich and dried in a dry solvent station GT S100 (Glass Technology). Ethyl acetate (EtOAc), Cyclohexane, Ethanol (EtOH), were purchased from Fisher Scientific and used after distillation. Water was deionized using Ultrapure Milli-Q Reagent Water System Millipore and used at 18MQ. Concentrated HCl and NaOH solutions (research grade; Aldrich Chem. Co.) were diluted to obtain the desired concentrations. Microscope Slides (76x26 mm, ground
edges 45°, white Glass, Thermo Scientific, two bathes used and named type 1 and 2) were purchased from Thermo Scientific

1.2 Chemical Characterization

1.2.1 Nuclear magnetic resonance (NMR) Analysis

¹H-NMR and ¹³C-NMR spectra were respectively recorded at 400 and 100,6 MHz on a Bruker[®] spectrometer. The samples were dissolved in chloroform-*d* (CDCl₃) or methanol-*d*₄ (CD₃OD). The chemical shifts (δ) are expressed in part per million (ppm) relative to Me₄Si and the coupling constants (J) in Hertz.

1.2.2 Size Exclusion Chromatography (SEC) Analysis

Number-average molecular weight (\overline{M}_n) and dispersity (\overline{D}) were measured by size exclusion chromatography in THF as eluent (flow 1 mL/min), at 35°C, on a Waters[®] chain 2707 autosampler equipped with a 1515 Isocratic Pump and a guard column (Styragel 30x4.6 mm) connected to a column (Styragel HR2 + HR4, 300x7.8nm). The Waters[®] 2996 PDA and Waters[®] 2414 Refractive Index Detector were used. Calibration was performed with polystyrene (PS) standards in the range from 580 g/mol to 483000 g/mol. The molecular weights of the oligoisoprenes were corrected according to the Benoit factor equal to 0.67[15]. Generally, the molecular weight of all oligomers was determined by SEC and by ¹H-NMR. The ¹H-NMR spectrum for the carbonyl telechelic oligomers was exploited according to equation (1) and the relative number-average molecular weight was referred to as $\overline{M}_{n.NMR}$.

$$\overline{M}_{n,NMR} = \left[\frac{I_{5.13ppm}}{(I_{2.47ppm}/4)} \times 68\right] + 100$$
(1)

 $I_{5.13ppm}$ and $I_{2.47ppm}$ were the integration values of the peaks at 5.13 ppm and at 2.47 ppm, respectively. 68 and 100 were the molar masses of the isoprene repeating unit and the chain ends of CTNR, respectively (g/mol). $\overline{M}_{n,NMR}$ was used to calculate the molar concentration of the oligomers for the modification of the chains ends in the synthesis of hydroxy telechelic natural rubber (HTNR) and acrylate telechelic natural rubber (ACTNR).

1.2.3 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra were carried out with a Vertex 70V Bruker spectrometer with a deuterated triglycine sulfate detector, in ATR mode (attenuated total reflection). The absorption bands were recorded in the range of 400 - 4000 cm⁻¹ with 16 scans and a resolution of 2 cm⁻¹. The data were analyzed using OPUS software.

1.3 Synthesis of derived oligomers from natural rubber

1.3.1 Synthesis of carbonyl telechelic oligomers (CTNR)

NR crumbs (50 g, 0.74 mol) were suspended in 1.250 L of THF (0.6 M) for 15 h for dissolution, heating at 30°C, in a 5 L reactor, with mechanical stirring. The H_5IO_6 (3.2 eq., 24.22 g, 0.11 mol) was dissolved in 275 mL of THF (0.4 M) and added slowly to the NR solution using a dropping funnel. The solution was stirred for 6 h at 30°C then the suspension was filtered. THF was eliminated under reduced pressure then the oligomers were dissolved in distilled dichloromethane (roughly the same volume as THF used to perform the reaction), they were transferred in a separating funnel, and washed twice with an aqueous solution composed by 70% vol of saturated NaHCO₃ (700 mL) and 30 vol% of saturated NaCl (300 mL). The second washing was performed twice with an aqueous solution composed by 50 vol% of Saturated NaCl (500 mL). Then the organic phase was dried over MgSO₄ for 2h, filtered and evaporated in a rotary evaporator. The product was vacuum dried and an orange-yellow viscous liquid in 85% yield (42.5 g) was obtained.

¹H-NMR(400 MHz, CDCl₃-*d*) δ 9.77 (s, 1H, CH₂C*H*O), 5.10 (m, yH, CH₃C=*CH*_{isoprene unit}), 2.52-2.45 (m, 2H, C*H*₂CHO), 2.43 (t, J=7.6 Hz, 2H, CH₃COC*H*₂), 2.34 (t, J=7.6 Hz, 2H, C*H*₂CH₂CHO), 2.24-2.30 (m, 2H, CH₃COCH₂C*H*₂), 2.13 (s, 3H, C*H*₃COCH₂), 2.00-2.11 (m, 4yH, C*H*₂, isoprene unit), 1.68 (s, 3yH, C*H*₃CCH_{isoprene unit}).

¹³C-NMR (101MHz, CDCl₃-d) δ (ppm): 208.6 (C=O_{ketone}), 202.1 (C=O_{aldehyde}), 135.2 (C=CH_{isoprene unit}),
125.1 (C=CH_{isoprene unit}), 44.0 (CH₂COCH₃), 42.4 (CH₂CHO), 32.2 (CH₂C=CH_{isoprene unit}),
26.4(CH₂CH=C_{isoprene unit}), 24.3 (CH₃CO), 23.5 (CH₃, isoprene unit).

FTIR: v (cm⁻¹): 3035 (H-C=C), 2959-2726 (CH), 1721 (C=O), 1665 (C=C), 834 (C=C-H).

 $\overline{M}_{n, \text{NMR}} = 1260 \text{ g/mol}$

 $M_{n, SEC} = 1600 \text{ g/mol}, D=1.89$

1.3.2 Synthesis of Oligo-SiHTNR

1.3.2.1 Preparation of Oligo-SiHTNR

To a solution of CTNR (1.30 g, 1 mmol) in anhydrous chloroform (20 mL) was added, under Argon the appropriate commercial coupling silane agent (1.0mmol, 1eq). The reaction mixture was stirred for 24h at room temperature, concentrated under vacuum to give a colorless oil, which was diluted three times with chloroform (15 mL) then concentrated again after each dilution. The residue was subsequently taken up in a mixture of anhydrous chloroform (20mL) and absolute ethanol (20mL) and reacted with sodium borohydride (2mmol, 2eq) at room temperature for 30min. Solvents were evaporated to give a

yellow-orange liquid. Excess borohydride was then hydrolyzed with water (60 mL). Oligo-SiHTNR were obtained by extraction with chloroform (40 mL). The organic phase was dried over MgSO₄ then concentrated to provide the expected oligosilane: Oligo-DiSiHTNR (1.18g, 80%) or Oligo-TriSiHTNR (1.23, 82%).

Compound Oligo-TriSiHTNR



¹H-NMR: (CDCl₃) δ (ppm)): δ 5.12 (t, yH, H-5), 3.89-3.75 (m, 7H, H-14, H-16, H-18 and H-2), 2.89-2.50 (m, 4H, H-10 and H-11), 2.11-1.98 (m, 4nH, H-8_{isoprene unit}, H-4_{isoprene unit} and 2H of H-9), 1.79-1.57 (m, 3nH of H-7_{isoprene unit} and 2H , H-12), 1.24-1.13 (m, 12H, H-15, H-17,H-19 and H-1), 0.65-0.50 (m, 2H, H-13).

¹³C NMR (101 MHz, CDCl₃-*d*) δ: 135.2 (C-6_{isoprene unit}), 125.0(C-5_{isoprene unit}), 67.9 (C-2), 64.5 (C-10 or C-11), 58.5 (C-14, C-16, C-18), 32.2 (C-8_{isoprene unit}), 26.4 (C-4_{isoprene unit}), 23.4 (C-7_{isoprene unit}), 18.3 (C-15, C-17, C-19).

FTIR: 3035 cm⁻¹(H-C=C), 2959-2726 cm⁻¹ (C-H), 1589-1516 cm⁻¹ (C-N), 910-1187 cm⁻¹ (Si-O-C), 834 cm⁻¹ (C=C-H).

 $\overline{M}_{n, \text{NMR}} = 1470 \text{ g/mol}$

 $M_{n, SEC} = 2200 \text{ g/mol}, D=1.84$

Compound Oligo-DiSiHTNR



¹H-NMR: (CDCl₃) δ (ppm)): δ 5.12 (t, nH, H-5), 3.89-3.64 (m, 5H, H-15, H-17 and H-2), 2.89 -2.48 (m, 4H, H-10 and H-11), 2.11-1.98 (m, 4nH, H-8_{isoprene unit}, H-4_{isoprene unit} and 2H of H-9), 1.79- 1.57 (m, 3nH of H-7_{isoprene unit} and 2H, H-12), 1.24- 1.13 (m, 9H, H-16, H-18 and H-1), 0.65-0.45 (m, 2H, H-13), 0.22- 0.03 (m, 3H, H-14).

¹³C NMR (101 MHz, CDCl₃-*d*) δ: 135.2 (C-6_{isoprene unit}), 125.0 (C-5_{isoprene unit}), 67.9 (C-2), 64.5 (C-10 or C-11), 58.1 (C-15, C-17), 32.2 (C-8_{isoprene unit}), 26.4 (C-4_{isoprene unit}), 23.40 (C-7_{isoprene unit}), 18.4 (C-16, C-18), -4.94 (C-14).

FTIR: 3035 cm⁻¹ (H-C=C), 2959-2726 cm⁻¹ (C-H), 1589-1516 cm⁻¹ (C-N), 910-1187 cm⁻¹ (Si-O-C), 834 cm⁻¹ (C=C-H).

 $\overline{M}_{n, \text{NMR}} = 1440 \text{ g/mol}$

 $M_{n, SEC} = 2300 \text{ g/mol}, D=1.86$

1.4 Experimental protocols to graft covalently oligoisoprene on glass.

The strategy based on the covalent grafting of NR, bearing an acrylate at the chain-end on the surface of different supports, followed two alternative routes (Figure 1): Route 1 and Route 2. The first one (Route1) consisted in the immobilization of NR on glass surface using commercial silan coupling agents (ComSiA) (APTES, APMDES or APDMES) to give the amino NH₂ groups exposed on the surface, to allow the reaction with CTNR via reductive amination. Route 2 consisted on the covalent grafting of a new oligomer Oligo-SiHTNR synthesized from PI directly on active surfaces (Figure 1), without using commercial silane coupling.

All chemical structures and purity of the reagents used (APTES, APMDES, APDMES) were checked by NMR analysis. All X-ray reflectivity (XRR) and contact angle measurements were carried out at room temperature. All glassware was cleaned in potassium hydroxide solution 0.1M then in ethanol, rinsed with distilled water (3 times), and stored in a clean oven at 110 °C until use. First, the glass surfaces (microscopy slides) were prepared through a series of cleaning procedures that removed contaminants. Freshly cleaned substrates were then activated in a Uv-O₃ plasma for 15min (UVOcleaner, Model N°42-420) and immediately placed in the reaction jar, which was closed and purged again extensively with Argon (over 10min).

1.4.1 Description of functionalization conditions for Route 1

Route 1 consisted in the following steps: the activated glass surfaces was aminosilanized, thereby coated with amine groups that covalently coupled with CNTR compound via reductive amination reaction. The ketone groups were expected to be exposed to the air. Ketone groups were then chemically modified to hydroxyl groups via a reduction reaction. In the last step, an acrylate group was then introduced via an esterification reaction (detailed below) to further polymerize with different compounds.

1.4.1.1 Step 1 of silanization, using commercial amino- silane coupling (R1St1)

To a 100mL round bottomed flask, closed and purged extensively under Argon, anhydrous toluene (40mL) was introduced and the appropriate volume of the reagent was added (APDMES, APMDES or APTES) to the desired concentration. After 30 minutes of stirring at room temperature, a syringe was used to transfer 35mL of this solution into a reaction vessel (a custom-designed slide-staining jar with plastic screw cap and kept under Argon, see Figure 10). Then, the vessel was placed on an orbital shaker operated at 50 rpm. Different reactions were carried out at 22°C, 40°C or 70°C for different times (all conditions tested are reported in Table 1). After this silanization step, the glass slides were rinsed individually with toluene (5min), water (5min), and ethanol (5min), all under ultrasonic agitation, and finally some were dried in an oven at 110°C for 15 min.

	APDEMS (MonoSi)		AP	MDES (I	DiSi)		APTES	(TriSi)	
	S1(*)	S1	S2	S3-5	S7-8	S9-16	S1	S2	S3-4
[C] mol/L	0.086	0.086	0.086	0.03	0.086	0.086	0.086	0.086	0.03
Immersion temperature (°C)	RT	RT	RT	RT	70	40	RT	RT	RT
Argon flush / Sealed with a Teflon ring.	Y / N	Y/ N	Y/ N	Y/ N	Y/ Y	Y/ Y	Y/N	Y/ N	Y/ N
Immersion time (hours)	72	48	72	72	24	24	24	72	72
Dry at 110°C in an oven (in air if No)	N	N	N	N	Y	Y	N	N	N

*Y=Yes, N=No

*Table 1: Different conditions tested for the silanization step on glass surfaces (R1St1); * S= Serie; S1: Serie 1; S3-5: from Serie 3 to Serie 5, RT for room temperature.*

1.4.1.2 Step 2, immobilization of CTNR layer on aminated-silane surface via reductive amination reaction

All pre-functionalized surfaces obtained from step 1 were used for grafting with CNTR via reductive amination reaction, following two different protocols (A and B) herein described. This step is the same for both protocols, a solution of different concentration (described in Table 2) of CTNR in dry DCM or Toluene was prepared in a 100mL round bottom flask, under Argon. Depending on different conditions, a desired volume of acetic acid was added. This mixture was stirred at room temperature for 10min. Five pre-functionalized microscope slides obtained from step 1 were placed in a custom-designed slide-staining jar, which was airtight closed and submitted to three cycles of *vacuum* and Argon. This slide-staining jar was flushed with argon for 15min before injecting 35mL of the mixture CTNR solution previously prepared *via* a syringe, and all was stirred for 10min. The next step followed protocol A or B (**)

Protocol A: 8mL of anhydrous EtOH was added via a syringe. This mixture was stirred for 5min before adding 2eq. of NaBH₄ (see Table 2)

Protocol B: Without adding anhydrous EtOH, 4eq. of reducing catalyst NaBH(OAc)₃) was immediately added.

Then final mixture obtained was stirred under Argon for 10min before being placed in an orbital shaker operated at 50rpm. The reaction was carried out at different temperatures (RT, 40°C) for a desired time (described in Table2). After reductive amination step, the microscope slides were rinsed individually in an *ultrasonic* bath using reaction solvent (5min), water (5min), ethanol (5min) and they were dried in an oven at 80 °C for 3 min.

		TriSi			DiSi				
	S2	S 3	S4	S1	S2	S 3	S4	S7-16	
$\overline{M}_{n, \text{ NMR}} \left(\mathbf{g}/\mathbf{mol} \right)$	1500	1256	1256	1500	1500	1256	1256	1256	
Anhydrous Solvent	Toluene/ EtOH	Toluene/ EtOH	Toluene/ EtOH	Toluene/ EtOH	Toluene/ EtOH	Toluene/ EtOH	CHCl ₃ / EtOH	CHCl ₃	
[CNTR] (mol/L)	0.010	0.030	0.030	0.010	0.010	0.015	0.015	0.030	
Ratio CH3COOH/ CNTR	0/1	20/1	20/1	0/1	0/1	0/1	20/1	2/1	
Type of reduting agent	a	b	b	а	а	a	а	b	
Ratio catalyst/CTNR	2/1	4/1	4/1	2/1	2/1	2/1	2/1	4/1	
Reaction temperature (°C)	RT	40							
Reaction time (h)	48	72	90	48	24	72	72	48	

*(**a**): NaBH₄; (**b**): NaBH(OAc)₃

Table 2: Different parameters used for reductive amination step 2 (R1St2).

1.4.1.3 Step 3 of chemical modification of ketone terminal group, exposed on surface via reductive reaction

The same manner, the microscope slide obtained from step 2 via reductive amination were placed in a custom-designed slide-staining jar, which was airtight closed and submitted to three cycles of *vacuum* and Argon. Then 25mL of anhydrous CHCl₃ and 10mL anhydrous EtOH were introduced, followed by adding 4eq. of NaBH₄. The mixture was stirred at room temperature for 10min under argon before placing it in an orbital shaker operated at 50rpm. The reaction was carried out at 40°C for a desired time (described in Table3). Once the reaction finished, the microscope slides were rinsed individually in an *ultrasonic* bath with CHCl₃ (5min), water (5min), ethanol (5min) and dried in an oven at 80 °C for 3 min.

		DiSi							
	S1&2	S 3	S4-10	S	S11 S12-16				
Ratio (*) CTNR/NaBH4	-	3	-	4	4	4			

Reaction Time (h)	-	72	-	72	48	48
Reaction temperature (°C)	-	40	-	40	40	40

Table 3: Different condition to carry out for selective reduction reaction -R1St3 (*) all calculus were based on the amount of CTNR used at the first step.

1.4.1.4 Step 4, introduction of acrylate group at the polymer chain-end on surface

At the same manner of the previous steps, to a custom-designed slide-staining jar, airtight closed and purged under *vacuum* then with Argon (3 times), were successively introduced at 0°C, 35mL of anhydrous CH_2Cl_2 , 1.75eq. Et₃N and 1.5 eq. acryloyl chloride (ACLC). The reaction mixture was stirred at RT for 48h, in an orbital shaker, operated at 50rpm (described in Table 4). Once the reaction was finished, these microscope slides were rinsed individually in an ultrasonic bath with CH_2Cl_2 (5min), water (5min), ethanol (5min) and dried in an oven at 80 °C for 3 min.

	Dis	Si
	S1-11	\$12-\$15
Ratio Et ₃ N/CTNR	-	1.75/1
Ratio ACLC/CTNR	-	1.5/1
Reaction Time (h)	-	48
Reaction temperature (°C)	-	RT

Table 4: Conditions carried out for esterification reaction -R1St4.

1.4.2 Description of functionalization conditions for Route 2

The method works using for Route 2 as follows: First, the glass substrate was cleaned and activated to remove contaminants and hydroxylate the surface. Next, the surface was directly silanized with different Oligo-SiHTNR compound, synthetized from PI, thereby coating the glass with monolayer PI containing hydroxyl group at the chain-end that that enable to modify successively, first via selective reduction, then via esterification reaction to obtain a exposed the surface-bound acrylates groups

1.4.2.1 Step 1 of silanization of Oligo-SiHTNR

The preparation of Oligo-SiHTNR solution was carried out at the same manner to Step 1, route 1 (R1St1), previously described at the Section 1.4.1.1. Several conditions were performed, presented in Table 5

		Oligo-DiSiHTNR			
	TTN111	TTN213	TTN215	TTN216	TTN217
[Oligo-SiHTNR] mol/L	0.01	0.03	0.03	0.03	0.03

Temperature of reaction solution (°C)	RT	40	40	40	40
Argon flush / Sealed with a Teflon ring	Y/N	Y/Y	Y/Y	Y/Y	Y/Y
Immersion time (hours)	24	48	72	120	48

*Y = Yes, N = No

Table 5: Experimental conditions for Step 1 Route 2 (R2St1), using anhydrous toluene as solvent.

1.4.3 Description of functionalization with MAPTES conditions

The MAPTES (methacryloxylpropyl triethoxysilane) solution for functionalization was prepared in the same way that we did in Step 1, route 1 (R1St1 for other aminosilanes as previously described in Section 1.4.1.1). Activated glass substrates were functionalized with MAPTES, using the optimal condition presented in Table 6. We used a custom-designed slide-staining jar that was airtight closed during reaction and that was previously extensively cleaned and purged with Argon.

	MAPTES
	S1-4
[C] mol/L	0.086
Temperature of reaction solution (°C)	40
Immersion time of reaction (h)	24
Dry at 110°C in an oven	Yes

Table 6: Experimental conditions for functionalization with MAPTES.

1.5 Surface and thin films characterization

1.5.1 Contact Angle measurements (CA)

Water contact angles were measured using a homemade set-up with a bright LED white source and a telecentric lens on the source and associated (CMOS) camera pathways. Contact angles were measured with a precision of 0.1° with a homemade software for image analysis. At least 5 drops of 1 µL were deposited on separated regions over the surface to obtain an average value for each sample. To obtain robust values for a surface state, we also calculated errors bars from the cumulate values obtained from several identical samples.

1.5.2 X-ray reflectivity (XRR) of interfaces, molecular and thin films

X-ray reflectivity (XRR) measurements were conducted using a PANalytical Empyrean X-Ray diffractometer running at 40 kV and 30 mA (Cu-Ka radiation reflected on a parallel beam x-ray optics, acting as a monochromator at 0.154 nm). Reflectivity scans, data treatment and fitting analyses are presented in the supporting information section: *X-ray reflectivity measurements*.

2 **Results and discussions**

Novel telechelic PI oligomers were synthesized from the oxidative controlled degradation of natural rubber chains. In order to graft PI chains to surfaces for many applications, the functionalization of silicon-based materials was studied, starting with silicon dioxide from glass microscopy slides, made from a widely used class of glass and presenting an excellent starting surface roughness and flatness. The first idea was to synthetize a bi-functional molecule that would be bound covalently to the material and that could be polymerized at the other end with PI oligomers or other molecules for coatings. In the next part, the new Oligo-SiHTNR synthesis for covalent binding to silicon-containing surfaces will be presented.

2.1 Synthesis of different Si-HTNR oligomers for surface functionalization

Several silano telechelic natural rubber oligomers (Oligo-SiHTNR) could be obtained following the synthesis routes displayed in Scheme 1.



Scheme 1: Chemical pathways to synthesize Oligo-SiHTNR. Oligo-MonoSiHTNR wasn't obtained.

The NR chains were cut by oxidative degradation to obtain of Carbonyl Telechelic Natural Rubber oligomers (CTNR) using periodic acid (H_5IO_6) [16, 17]. The molecular weight of CTNR was well controlled by choosing the concentration of periodic acid and adapted reaction conditions. Two functional groups at the chain-end of CTNR oligomers were first generated: a ketone and an aldehyde. According to the strategy, only one end has to be functionalized with a silane group, whereas the other end will be converted in acrylate function. Having a superior reactivity, the aldehyde function was the only one to selectively react with the commercial silane coupling agents (ComSiA) to obtain the designed Oligo-SiHTNR used for the glass surface functionalization (Figure 2).

As shown in the literature [18, 19] the reducing agent, $NaBH(OAc)_3$, allows the selective functionalization of the aldehyde CTNR chain-end without modification of the ketone moiety, via a

reductive amination reaction. Unfortunately, this reducing agent presents weak acidic properties and it can promote hydrolysis of ethoxyl groups at the chain-end of the commercial silane coupling agent, giving unwanted products. To confirm that, a simple test was carried out in the same manner of our reaction conditions. To a solution of commercial APMDES (0.5 mL, 1 equiv.) in dry DCM (10 mL) was added NaBH(OAc)₃ (2 equiv., Entry B, Figure SI-2) or NaBH₄ (2equiv., Entry A, Figure SI-1), or without reducing agent (Entry C, Figure SI-1). After treatment, the chemical structure of the obtained residue was analyzed by ¹H NMR. The spectra are presented in Supporting Informations (Figure SI-1). They show clearly the disappearing of the signal of ethoxyl groups in the case of NaBH(OAc)₃ but not in the case of NaBH₄, meaning that the NaBH(OAc)₃ can not be used as reductive agent in presence of aminosilane reagents. Therefore, to reduce this effect, the Oligo-SiHTNR was obtained by the reductive amination reaction of CTNR at room temperature using NaBH₄ as reductive agent. The oligomers CTNR were at first reacted with the appropriate aminosilane (one equivalent) for 24h in a mixture of CHCl₃/EtOH (for CTNR solubility) then two equivalents of NaBH₄ were added and the reaction was continued for 30min. In these conditions, Oligo-SiHTNR was generated with silane chain end (from the aldehyde) and a hydroxyl group by reduction of the ketone moiety as orange-yellow viscous liquid in 80% yield for Oligo-DiSiHTNR and 82% for Oligo-TriSiHTNR. In fact, APTES (tri-ethoxysilane) and APMDES (di-ethoxysilane) are much more stable silane coupling agents, in comparison with the APDMES (mono-ethoxysilane) [20]. In our case, we faced the same difficulty. Furthermore, some characteristic proton signals of mono-ethoxysilane moieties of Oligo-MonoSiHTNR were very low in term of integration, and were overlapped with the massifs signals of polyisoprene. The structure was not clearly established for 1, that is why, in this paper, we reported only the synthesis of the two silano oligomers 2 and 3 (Scheme 1)

The chemical structure of all oligomers (CTNR and Oligo-SiHTNR) was checked by ¹H-NMR, ¹³C-NMR, FTIR and SEC analysis. Depending on the NR batch used, we finally obtained \overline{M}_n =1256 and 1528 g/mol (respectively named CTNR₁₂₅₆ and CTNR₁₅₂₈), calculated by ¹H-NMR. For SEC analysis the \overline{M}_n were 1640 and 1770g/mol for CTNR₁₂₅₆ and CTNR₁₅₂₈ respectively. The ¹H-NMR spectrum of Oligo-SiHTNR oligomer showed the appearance of characteristic proton signals from the ethoxy silane moiety in the range of 3.75ppm-3.89ppm and 1.13ppm-1.24ppm, corresponding respectively to CH₂ and CH₃ ethoxy silane protons. The appearance of a signal between 2.50-2.89ppm was also observed corresponding to –CH₂ group in α -position of NH, confirming the amine presence between the silane coupling agent (ComSiA) and the CTNR. Furthermore, the total reaction of all carbonyl terminal groups was confirmed by the total disappearance of signals at 9.7 ppm and between 2.55ppm and 2.05ppm, corresponding to the protons from aldehyde and the –CH₂ group in α -position of hydroxyl group was overlapped with the one from ethoxyl silane moieties in the range of 3.75ppm-3.89ppm. The presence of correlation signals in 2D ¹H-¹³C HSQC NMR (Figure 2) between signals located at 67.9 ppm and proton signals in the

range of 3.75ppm-3.89ppm proved the formation of hydroxyl groups only from ketone chain-end (CH₂-C<u>H</u>-OH), generating secondary alcohol. This was also confirmed by the shift of proton signal from 2.13 to 1.20ppm corresponding to CH₃ in α -position of the ketone group.



Figure 2:¹H-¹³C HSQC NMR (in CDCl3) of Oligo-TriSiHTNR.

Furthermore, in order to confirm the coupling reaction between the aminosilane reagent and the oligoisoprene, 2D diffusion-ordered spectroscopy (2D DOSY) ¹H NMR analysis was performed with Oligo-TriSiHTNR oligomer (Figure 4) and also with the mixture of HTNR₁₅₀₀ and APTES (w/w:3/1) (Figure 3). In the case of this mixture, on 2D-DOSY spectrum (Figure 3), the presence of two diffusion coefficients was observed. The first one was only coupled with all the signals of the HTNR₁₅₀₀ whereas the second one was coupled only with the signals of APTES. No correlation between isoprene unit, ethoxyl silane moieties and a common diffusion coefficient was detected. Conversely, as expected, the 2D-DOSY spectrum of Oligo-TriSiHTNR in Figure 4 showed clearly a good linear correlation with the same diffusion coefficient between the protons signal of isoprene unit, located at 5.1ppm (CH₃C=*CH*_{isoprene unit}), CH₂ protons in α-position of -NH in the range of 2.50-2.89ppm, and ethoxyl silane protons moiety, located between 3.75-3.89ppm. Theses analyses allowed confirming the coupling between APTES and CNTR.



Figure 3:2D DOSY ¹H NMR spectrum of the mixture HTNR and APTES.



Figure 4:2D DOSY ¹H NMR of Oligo-TriSiHTNR.

The FTIR spectrum measured on the Oligo-DiSiHTNR (1500 g/mol) is shown in Figure 5, in comparison with the spectrum of APMDES (commercial silane coupling), $CTNR_{1500}$ and $HTNR_{1500}$. The FTIR spectrum of Oligo-DiSiHTNR1500 in Figure 5 showed the disappearance of the peaks at 1720 cm⁻¹, corresponding to C=O bonds of CTNR and the appearance of absorption bands in the range

of 1591 cm⁻¹ and 1514 cm⁻¹, assigned to N-H deformational vibration of Oligo-DiSiHTNR[21, 22]. In this figure, CTNR also showed absorption bands in the range of 1400-700 cm⁻¹, so it is not easy to identify the Si-O-C deformation of silane coupled with CNTR [23, 24]. However, the peaks intensity at 1064cm⁻¹ changed in comparison to the neighboring peaks at 1128 and 1037cm⁻¹ that corresponded to unchanged stretching vibration of the ethoxy-silane. Furthermore, it was also reported by Demjen *et al.* [23, 25] and Masmoudi *et al.* [23] that during the reaction or during the post-treatment, the hydrolysis of the alkoxy-groups generated a peak at 1010 cm⁻¹, assigned to the formation of Si-OH groups. A further crosslink and Si-O-Si formation, generate peaks at 1012 cm⁻¹ and 980 cm⁻¹. We do not have such strong signals and thus we do not expect that hydrolysis occurred. Nevertheless, in case a low amount of hydrolysis occurred, these peaks would be difficult to identify unambiguously since they are located nearby the strong Si-O-C absorption bands.



Figure 5: ATR-FTIR spectra of CTNR, HTNR and Oligo-DiSiHTNR, full spectra (Left); zoom (Right).

All these results demonstrated the generation of the desired Oligo-DiSiHTNR from silane coupling agent and CNTR oligomers.

2.2 Grafting PI derivates from NR to hydroxylated and silicon-containing surfaces

Two routes were envisioned to obtain PI coatings from NR derivates on glass. The first one in three steps used a silanization of glass for exposing NH₂ groups (R1St1, R1St2, R1St3), on which CTNR oligomers were post-grafted. The ketone end-moiety was then reduced to alcohol function. The second route was a direct functionalization of glass in *one pot* from the oligo-SiHTNR already bearing the hydroxyl end-moiety (R2St1). Depending on the CTNR (route 1) or on the oligo-SiHTNR (route 2) molecular weight used, we could expect thin oligoisoprene (PI) molecular layers of different thicknesses. In both cases, the alcohol functions were converted in acrylate for further reactions (R1St4

and R2St2), among which thicker PI coatings could be prepared by further polymerization of acrylate oligomers. To test the reactivity of CTNR on NH_2 surfaces (R1St2) and oligo-SiHTNR (R2St1) with silicon-containing surfaces, activated glass microscopy slides were used exposing a high density of silanol groups after solvent cleaning and UV plasma treatment (Section 1.4).

2.2.1 Grafting Oligo-SiHTNR on glass surfaces (Route 2)

All tested protocols used to graft Oligo-SiHTNR in *one pot* (route 2) by direct contact of the molecules solubilized in toluene are described in section 1.4.2. As a matter of fact, we found that the ethoxysilane group of Oligo-SIHTNR could be grafted to glass (see XRR data presented in Figure SI-13 and Table SI 2) but that it required that the molecule was synthetized shortly before use. Its solubility and stability evolved fast and consequently, samples reproducibility was poor. We thus decided to try an alternative method in two steps presented in the following section (Route 1), which turned out to be much easier and robust with the possible storing of compounds for weeks before use.

2.2.2 Grafting CTNR on aminosilanized glass surfaces (Route 1)

Aiming at obtaining a high grafting density of CTNR following the route 1 protocol, we tried successively three commercial silane coupling agents to get NH₂ groups at the surface of the glass substrates: APTES (tri-ethoxysilane), APMDES (di-ethoxysilane) and APDMES (mono-ethoxysilane). The most commonly used one, APTES (3-Aminopropyltriethoxysilane), is also the most reactive. Unfortunately, it is known that a possible bulk polymerization or vertical polymerization from the substrate might occur in presence of water traces and this results in the growth of solutions aggregates or multilayers with uncontrolled structures [26]. Note that even in the case of a 2D surface silane polymerization, some conformations appear with neighboring molecules and a weak binding to the substrate. We thus always conducted extensive rinsing procedures with several solvents and under *ultrasonic* agitation at each step to eliminate materials with weak "hang time". To measure the thickness, density and possible roughness of the aminosilane layer, we conducted specular x-ray reflectivity (XRR) experiments as well as water contact angle measurement, at each step of the protocols. Results are discussed herein for different pre-functionalization conditions (described in see Section 1.4.1.1).

To illustrate the difficulties encountered with APTES and more generally with tri-ethoxysilane, we first compared the aminosilane coatings obtained in ambient air (series S1 and S2) or under argon flushing (S3 and S4). Representative XRR data are shown in Figure 6 and their best fitting parameters are given in Table SI 3. All data could be well fitted using a slab model that describes the electron density profile in depth from air to substrate for reflectivity calculation in a fitting routine presented in the supporting information (section *X-ray reflectivity measurements and analysis*). For each layer of the profile, XRR fit parameters correspond to: layer thickness (d in nm) ; top root mean square roughness (σ_{RMS} in nm) ; electronic density (ρ^e in electrons/nm³ that translates in a real scattering length density Re(ρ b) after multiplication by the Thomson factor $r_0 = 2.81794.10^{-5}$ Å); linear attenuation coefficient (that translates

in the imaginary part of the scattering length density $Im(\rho b)$, which was fixed here to calculated values from material compositions). The formation of an ultra-thin coating on the glass substrate appears clearly on the reflectivity data (red curve) in the form of a slope break that is absent for bare glass reflectivity (grey curve). The first conditions in ambient air (S1) based on a 24h pre-functionalization step (or 72h for S2, not shown), corresponded to the thinnest APTES coatings obtained with only 1±0.3 nm thickness and an electron density equal to the one expected for dense APTES molecules (0.304 \pm $0.005 \text{ e}^{-}/\text{Å}^3$). The thickness is close to an APTES molecule length but with a roughness comparable to thickness, that indicates that we formed an incomplete monolayer. Many samples were prepared with different molecules concentration, contact times and humidity in the reaction vessel, leading to APTES layers with variable thicknesses and organization. Figure 6 shows the experimental XRR curves and their corresponding best fits for three representative APTES coatings (at R1St1) with thickness of 1 ±0.3nm (R1St1, red marks), 5.65 ±0.4nm (R1St1, green marks) and 12.19 ±0.2nm (R1St1, blue marks). Note that to improve the fit quality, thicker APTES layers were fitted using a two slab electron density profile with a lower electron density for the top layer in contact with air (up to 60% decrease from calculated APTES density) indicating a disordered top surface. We obtained the thickest sample by simply slowing down significantly the stirring speed during its formation (R1St1), illustrating how sensitive it can be to control the APTES layer growth. All samples were stable with an excellent coating resistance to the cleaning treatment that were conducted at each reaction step to eliminate non-covalently bound material. However, the XRR curve measured after step 2, consisting in a long contact with the CTNR molecule and sub-sequent cleaning (R1St2), did not change noticeably from the XRR curves measured on the APTES pre-functionalization, indicating that CTNR₁₅₀₀ oligomers did not react noticeably or bound robustly to APTES (one can compare plain and empty data marks for all samples). This is due to the disorder of APTES layers formed and to the lack of availability of embedded -NH₂ groups for reaction with CTNR aldehydes via NaBH₄ (S1-S2) or NaBH(OAc)₃ reduction, even after days of contact in solution.

To overcome this problem, we then tried to prefunctionalize glass prior to grafting CTNR oligomers with the mono-ethoxysilane (APDMES). Notwithstanding the fact that it is the best candidate for self-assembled monolayers, we never obtained one. The XRR data shows no evidence of a SAM formation and moreover, degradation of APDMES was evidenced in the solutions by NMR analysis, including dimer formation (the poor stability of this mono-ethoxysilane was already reported due to its reactivity in solution [20].



Figure 6: Left) X-ray reflectivity (XRR) data with best fits to the data (solid lines) of an activated glass microscopy slide (grey) and glass substrates prefunctionalized with APTES tri-aminosilanes, before and after reaction with CTNR oligomers (respectively in plain and empty marks). **Right**) XRR data from samples prepared with APMDES di-aminosilanes (see text for experimental conditions).

The same protocols were then optimized for APMDES, the di-ethoxysilane molecule. Several experimental conditions were first tested at room temperature on activated glass surfaces (R1St1, S1 to S3). The first conditions (series S1) based on a 48h pre-functionalization step, gave the thinner films obtained with only 1.15 nm in thickness and the electron density expected for pure APMDES. A single monolayer of APMDES should not exceed 0.9 nm in thickness [27-29], therefore this layer surely includes some disorder (R1St1_S1) and did not effectively tether CTNR molecules at the functionalization step (R1St2_S1). Increasing the contact time to 72h translated in an increase of the APMDES layer to 1.9 nm (R1St1 S2) and reducing the contact solution concentration did not give thinner films. The thicker layers obtained in this way resulted in a dense coating (2.5 nm for R1St2_S3) with an incomplete layer on top (1.3 nm and 34% of the compact layer density). All these layers were unreactive to CTNR grafting until we increased the reaction temperature to 40°C for the aminosilane prefunctionalization, obtaining also a better reproducibility. Note that at 70°C the reactivity was poor again (series S7 and S8, XRR not shown). Satisfying conditions for the aminosilane prefunctionalization (R1St1) were obtained following the protocol adopted for series S9 to 16 (see Table 1) and carrying out the CTNR functionalization step (R1St2) at room temperature, using NaBH(OAc)₃ in chloroform (conditions adopted for series S7 to 16 in Table 2). The Figure 7, Left shows representative XRR data and best fits obtained (Table SI 4 for S14) at pre and functionalization steps (R1St1 and R1St2, respectively) with the clear formation of a robust and dense 2.2 nm thick CTNR coating on top of a 3 nm dense APMDES reactive layer. The plotted electron density profiles (Figure 7, Right) from these fits also evidence a quite small roughness at air (0.46 nm) that is similar to the one measured for the bare substrate (0.35 nm) and for the APMDES pre-coating (0.36 nm).



Figure 7:Left) Specular X-ray reflectivity (XRR) data with best fits to the data (solid lines) of a prefunctionalized glass substrate with APMDES (di-aminosilanes), before and after reaction with CTNR oligomers (respectively in plain blue and empty red marks). **Right**) Electronic density depth profiles corresponding to the best fit to the XRR data (same color code, see text for experimental conditions).

Since the graft polymer flat layer exhibits the PI melt density ($\rho = 0.93 \text{ g/cm}^3$), one can estimate from its thickness (h = 2.2 nm) the grafting surface density ($\sigma_{PI} = h \rho N_A/M_n$) and the corresponding mean distance between grafting points ($D = 2/\text{sqrt}(\pi \sigma_{PI})$) where $M_n = 1242 \text{ g/mol}$ and N_A is the Avogadro number [30]. We obtained $\sigma_{PI} = 0.98 \text{ chains/nm}^2$ (*i.e.* $1.625 \text{ 10}^{-10} \text{ mol/cm}^2$) so a mean distance between anchoring points of 1.14 nm. We considered that the chain is made of $N \sim 20$ monomers, and we used a Kuhn length for 1,4-polyisoprene (PI) of b = 0.82 nm [31] that corresponds to the segment length in a freely jointed chain model. It is related to the real monomer length *a* (~0.382 nm) via the flexibility Flory' characteristic ratio equal to $(b/a)^2 = 4.6$. Following the Alexander-de Gennes model of graft polymer chains, the adimensional grafting density $(a/D)^2$ is here smaller than N^{-6/5}, so that each grafted oligomer can adopt the mushroom regime where other surrounding chains act like a good solvent with negligible inter-chain repulsions. In that case, the vertical extension of a grafted chain within the dense melt is equal to the Flory radius: $R_F = a N^{3/5} = 2.31 \text{ nm}$, that is here equal to the layer thickness measured with X-rays (2.2nm). The CTNR coating scales perfectly with an oligomers monolayer.

Contact angle measurements of water droplets (θ_c) were carried out as a simple and complementary method to check for surface chemical modifications. Figure 8 shows representative data for the different surface states with error bars including several measurements per sample and many samples. A high density of surface silanols was obtained after plasma treatment with θ_c values below 3° (a regime where it becomes difficult to measure precisely the angle). The silanization with amino ethoxysilanes (R1St1 prefunctionalization with a mono, di or tri-silane) always resulted in an increase up to 56° ± 2° [28, 32] well above the expected glass loss of hydrophilicity with time after activation and immersion in organic solvents for days. At the functionalization step (R1St2), it was found that grafted CTNR only increased θ_c of few degrees (~ 59°), since layers have similar molecular interactions with water. The transformation of the chain-end ketone group solely into a hydroxyl group (R1St3) resulted in a slightly lower hydrophobicity (53°). The contact angle increased to 61° with the transformation into acrylate

(R1St4). However, contact angles also depend on the thickness, roughness and organisation of the layer. Polyisoprene freestanding thick films with a high degree crosslinking gave the highest values with $\theta_c \sim 80$. A value, we never reached with oligoisoprene ultra-thin films, bearing (ketone, OH, acrylate chainend as expected)



Figure 8: Contact angle measurements of water droplets of $1\mu L$ deposited on bare glass, functionalized glass and polyisoprene thick films. The error bars come from measurements of many droplets per sample and several samples for each surface state.

We also prepared glass slides functionalized with the commercial MAPTES molecule (methacryloxylpropyl triethoxysilane) in order to generate the surface methacrylate function in *one pot*. The protocol optimized for APMDES was used in one step (see 1.4.3. for details) and we obtained stable MAPTES monolayers with a high reproducibility. XRR data representative of these samples are shown in Figure 9 with the best fitting parameters given in Table SI 5. We obtained a 0.9 nm layer that resisted to washing procedures and which exhibited an electron density equal to 90% of the value expected for a total coverage of glass. Such samples correspond to a high surface density of methacrylate functions on glass and have been compared to the CTNR grafted oligomers with methacrylate surface groups in the following section.



Figure 9:Left) Specular X-ray reflectivity (XRR) data with best fits to the data (solid lines) of a prefunctionalized glass substrate with APMDES (di-aminosilanes), before and after reaction with CTNR oligomers (respectively in plain blue and empty red marks). **Right**) Electronic density depth profiles corresponding to the best fit to the XRR data (same color code, see text for experimental conditions).

2.2.3 Robustness and immersion tests of covalently grafted PI thick films on glass

The CTNR grafted layer resisted to our extensive cleaning protocols under ultrasonic shacking and was not noticeably affected by subsequent chemistry steps (R1St3 and St4) used to transform ketone groups in hydroxyl groups and then in acrylate groups. To further test the reactivity of the oligoisoprene coatings bearing a reactive acrylate group (R1St4), we then studied the post grafting of a thick layer obtained from a mixture of acrylate oligomers from NR and photoinitiator. We compared the PI postgrafting on different reference surface states obtained at different steps of our protocols, including PI adhesion to bare glass surfaces. Figure 10 shows the different surfaces tested, namely: activated bare glass substrates, pre-functionalized surfaces with aminosilanes exhibiting NH₂ groups (R1St1), functionalized surfaces exhibiting ketone groups (R1St3) or acrylate groups (R1St4), as well as functionalized glass with MAPTES. On all these surfaces, a thick film of pre-polymerization mixture composed by reactive ACTNR (97.5% w/w of ACNTR₆₀₀₀ with acrylate groups prepared from NR as described in Section I.3, Chapter I) and by 2.5% w/w of Darocur (photoinitiator) was deposited. UV-Curing was then carried out for 3 minutes (light intensity $I = 29,4 \text{ mW/cm}^2$). Then all samples were either immersed in THF solvent (samples 1 to 5) or in water (samples 6 to 8), under strong magnetic stirring (500rppm) in sealed vials at room temperature, to demonstrate the formation of covalent bonds between the pre-functionalized surfaces and the thick cross-linked coating obtained after the photopolymerization of the acrylate oligomers. The surface attachment was checked regularly and pictures are shown after 24h, 48h, 20 days and 4 months immersion. After 24h in THF, a good organic solvent for CTNR and ACTNR, the thick coatings only remained on surfaces bearing acrylate groups (samples 3 and 5).In THF, two days were needed to degrade in the bulk films from grafted CTNR coatings (R1St4) and 4 months for MAPTES coatings. For many applications, immersion in water is more relevant and all PI thick films resisted much longer to water molecules, except on the highly hydrophilic activated glass surface. In this case, physisorption only was expected with surface silanol groups and it resisted several days in water before separation. The PI thick films on grafted CTNR coatings and on MAPTES monolayers resisted to vigorous stirring in water all of the four months of the immersion experiment (still going on). To our knowledge, it is not possible to experimentally measure the fraction of acrylate functions that reacted with the surface coating ones under UV exposition. Nevertheless, it was high enough to create a strong tethering of the PI thick deposition on grafted oligoisoprene coatings, comparable to the grafting to MAPTES monolayers known to expose a high surface density of methacrylate groups. All experiments and sample manipulations in air confirmed that grafted CNTR oligomer coatings on silicon-based surfaces, offer a good chemical reactivity to tether other oligoisoprene or to carry out new chemistries on them.



Surface obtained after polymerization with the mixture (Formulation 1), before immersed in water/THF

Figure 10: A prepolymerization mixture containing 97.5% w/w of ACNTR6000 and 2.5% w/w of Darocur was deposited on different functionalized glass surface: (1&6) Raw activated glass; (2&7) pre-functionalized surface with NH₂; (3) target surface exhibiting acrylate function; (4) functionalized surface exhibiting ketone group, (5&8) MAPTES monolayers exhibiting meta-acrylate functions. All surfaces were cured under strong UV exposure for 3 minutes and then immersed in THF (1 to 5) and in water (6 to 8) at room temperature for several days as indicated

3 Conclusions

This paper presents a new approach to covalently graft natural rubber (NR) derived coatings at materials interfaces, in the form of dense monolayers of polyisoprene (PI) oligomers (less than 20 monomers), or thin (sub-µm) to thick (mm) PI films, tethered by contact and UV-curing in presence of a photoinitiator. The chosen silane functions were tested on silicon-containing materials, including different types of commercial silica glasses (microscopy slides and cover-glasses) and silicon wafers. Silanization is a versatile surface chemistry that can be transposed to many other materials bearing hydroxyl groups for instance. For several applications, ultra-thin elastomeric molecular layers with solvent barrier properties are needed. For those, novel telechelic oligoisoprenes (Oligo-SiHTNR) were designed with a silane anchoring group at one end and a hydroxyl group at the other extremity. Since the stability of such molecules is weak over time due to their high reactivity, we also proposed alternative routes starting with a prefunctionalization of the support prior to PI immobilization. To avoid supplementary cumbersome organic synthesis steps, we optimized prefunctionalization conditions to obtain a reactive amino-silane for post functionalization with synthetized bifunctional carbonyl telechelic natural rubber oligomers (CTNR). Among the commercial amino-alkoxysilanes tested (APTES, APMDES or APDMES), the di-ethoxysilane gave the more reproducible conditions to generate reactive $-NH_2$ groups and to graft a 20 units CTNR oligomer forming an ultra-thin PI coating. The PI ketone chain-end was then transformed in hydroxyl and in acrylate for further surface reactions. Finally, dense PI derivate surfaces with a roughness smaller than 0.5 nm (conformational roughness with glass surface) were obtained. On such surfaces, different thicknesses of films from bio-based ACTNR (acrylate-TNR oligomers) could be grafted. Such films were also bound covalently to methacrylate surfaces obtained in one pot using commercially available molecules such as MAPTES (methacryloxylpropyl triethoxysilane). All these films showed excellent resistance to long exposures in water under strong stirring (up to 4 months) and a good resistance to organic solvents including THF (very aggressive for NR polymers), whereas physisorbed PI films detached rapidly from all surface states.

The protocols optimized herein to reinforce the mechanical attachment to interfaces open the route to many industrial applications for bio-based NR derivate building blocks and PI hybrid coatings. Indeed, we recently submitted how to incorporate covalently and homogeneously in the same PI matrix, antimicrobial and antibiofouling functions, as a possible way to generate bio-inspired and sourced elastomer coatings with smart properties in a non-leaching bioactive materials.

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Supporting Information

Polyisoprene covalent coatings. Grafting bio-based oligoisoprene from Natural Rubber, from monolayers to thick films.

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1 Degradation of APMDES when contacted with reducing agent NaBH(OAc)₃

Figure SI-1. ¹H NMR spectra of commercial APDMES (D) and different sample obtained, using the following procedure A to C, using procedure following: 0.5mL of commercial APMDES dissolved in, 10mL of dry DCM+ 2equiv. of: (B) NaBH(OAc)₃, (C) NABH₄, or (D) without reducing agent. Theses mixtures were stirred for 10 min then extracted with 10mL of water. Organic phases were dried, filtered and evaporated. The obtained residues were analyzed by ¹H NMR

Oligo-MonoSiHTNR

2 Synthesis of Oligo-SiHTNR oligomers



Figure SI-2. Chemical pathways to synthesize Oligo-SiHTNR

2.1 NMR spectra

2.1.1 Oligo-TriSiHTNR





Figure SI-5. HSQC- NMR spectra of Oligo- TriSiHTNR.



2.1.2 Oligo-DiSiHTNR



Figure SI-7. ¹H NMR spectrum of Oligo- DiSiHTNR.



2.1.3 Oligo-MonoSiHTNR



Figure SI-11. ¹³C NMR spectrum of Oligo- MonoSiHTNR.



Figure SI-12. HSQC- NMR spectrum of Oligo- MonoSiHTNR.

3 X-ray reflectivity (XRR) measurements and analysis.

For X-ray reflectivity measurements, three types of in-plane of incidence scans were collected on each sample using an Empyrean reflectometer from PANalyticalTM. The specular reflection was measured by keeping the incident angle, α_{in} , and the out-coming angle equal ($\alpha_{out}=\alpha_{in}$) by use of coupled ($\alpha_{in}/2\alpha_{in}$) reflectivity scans. Therefore, the momentum transfer vector q, was perpendicular to the film interface (q=qz). To record non specular intensities we performed standard longitudinal scans (offset scans) and rocking scans (transverse scans). The longitudinal scan was similar to the reflectivity scan except that the detection angle was intentionally offset by a fixed angle $\Delta \alpha_{offset}$. In the rocking scan, the detector did not move (i.e. the sum $2\theta=\alpha_{in}+\alpha_{out}$ was fixed) while the sample was rocked in the incident beam so that α_{in} varied from 0 to 2 θ . From the width of the specular peak measured from the rocking curves (not shown) we defined the incident angle offset for the longitudinal scans to $\Delta \alpha_{offset}=0.1^{\circ}$. These scans were subtracted from the specular scans to obtain the so-called true specular reflectivity curves that were

fitted using Fresnel optics. Without this subtraction, specular reflectivity was overestimated at large angles and the film roughness was underestimated of several angstroms. At small angles, the beam footprint over-spilled the film surface and a geometrical correction was used to compensate for this effect by integrating the incident flux reaching the surface at each angle of incidence. The primary Gaussian beam full width at half maximum was fixed to 0.08° from a monochromator mirror and a set of slits before the sample. The reflected beam was measured with 3 channels of a PIXcel-3D fast detector. X-ray reflectivity measurements (XRR), presented in the main manuscript, were used to determine the electron density profiles of the films. The best fits to the truth specular x-ray reflectivity data, corresponded to the electron density depth profiles. All interfaces could be well described with an error function profile and the pure material layers with constant density slabs. We obtained very good agreements from the fits with the volume densities calculated for pure materials, for the substrate medium (classical microscopy glass slides from different suppliers, noted type I and II) and for the aminosilanes or polymer layers (see Table SI1 below).

Molecule, molecule segment or material domain	M (g/mol)	Volume of molecule or group (nm³)	<i>d</i> , Volume density at 20°C (g.cm ⁻³) for calculation	ρb, x-ray Scattering length density (x10 ⁻⁶ Å ⁻²)	Pe, corresponding electron density (e ⁻ .Å ⁻³)
Water (from ambient air)	18.015	0.0300	0.998	9.452	0.3354
SiO ₂ glass (microscopy slides, type I and II)	60.085	0.0434 to 0.0356	2.3 to 2.8	19.72 to 24.01	0.7 to 0.852
Silicon (substrate)	14	0.02	2.33	20.13	0.714
APDMES (1xCH ₃ CH ₂ substracted, MonoEtO-aminosilane grafted)	132.14	0.256	0.857	8.083	0.287
hydrophilic segment	44.09	0.08542		7.360	0.2612
hydrophobic segments	88.06	0.17061		8.444	0.2997
APMDES (2xCH ₃ CH ₂ substracted, Di-aminosilane grafted)	133.13	0.2413	0.916	8.462	0.3003
hydrophilic segment	60.09	0.18333		7.855	0.2787
hydrophobic segments	73.04	0.10892		8.961	0.318
APTES (3xCH ₃ CH ₂ substracted, TriEtO-aminosilane grafted)	134.1	0.23542	0.946	8.559	0.3037
hydrophilic segment	76.1	0.13355		8.105	0.2876
hydrophobic segments	58.03	0.10187		9.1536	0.3248
CTNR₁₂₅₆ (surface grafted. Not counting NH group)	1242	2.218	0.93	8.828	0.3133
hydrophilic segment	28	0.05		9.033	0.3205
hydrophobic segments	2114	2.167		8.236	0.3131

Table SI 1 : Calculated scattering length densities (ρ b) and electron densities (ρ e) of the pure materials composing the coatings, from their chemical composition, atomic scattering factors for (λ X-Rays=1.54 Å) and materials volume densities at 20°C. The hydrophilic group of reacted aminosilanes grafted do not include the ethanol groups expected to leave in the form of ethanol, but it includes remaining oxygen atoms that formed covalent links with glass silicon. Also, graft CTNR oligomers density was calculated without counting the surface reacting $-NH_2$ group.

3.1 XRR results from Route 2 synthesis



Figure SI-13. **Route2, Left**) X-ray reflectivity (XRR) data with best fits to the data (solid lines) of an activated glass microscopy slide (grey) and glass substrates functionalized with Oligo-DiSiHTNR (preparation conditions given in section 1.4.2.). **Right**) Electronic density depth profiles corresponding to the best fit to the XRR data (same color code).

Layers from substrate to air:	Substrate			Layer 1				
Samples:	-	Re(<i>µ</i> b), X10⁻⁵ Å	<i>σ,</i> Å	d, Å	Re(<i>µ</i> b), X10⁻⁵ Å	<i>σ,</i> Å		
	Glass (type II)							
Bare glass substrate	-	21.6	3.6	-				
	Glass (type II)			Oligo-SiHTNR				
Oligo-SIHTINK	-	21.6	4.0	15.1	8.4	2.7		

Table SI 2:Best fit-parameters of X-ray reflectivity (XRR) data from Oligo-SiHTNR samples.

Layers from substrate to air:	Substrate			Layer 1			Layer 2			
Samples:	-	Re(<i>p</i> b), X10⁻⁵ Å⁻	σ,Å	d, Å	Re(<i>p</i> b), X10 ⁻⁶ Å ⁻	σ, Å	d, Å	Re(<i>p</i> b), X10⁻ ⁶ Å⁻²	σ, Å	
Baro glass substrate	Glass (type I)							•		
Bare glass substrate	-	19	3.6							
D10+1_01	Glass (type I)			APTES						
RISTI_SI	-	19	6.9	9.8	8.56	11				
D10+2_01	Glass (type I)		•	APTES	& CTNR					
RISTZ_SI	-	18.5	5.82	11.5	8.56	7]			
D10+1_04	Glass (type I)			APTES			Top APTES	Top APTES		
RISTI_54	-	19	5.8	17.1	8.56	3.8	39.4	op APTES 39.4 7.1	7	
B10:3.04	Glass (type I)	-		APTES			APTES	& CTNR		
RISTZ_54	-	18.5	5.7	16.8	8.57	6.4	42.3	5.7	6.9	
B10:1.04	Glass (type I)			APTES			Top APTES	•		
RISTI_54	-	19.4	4.4	99.1	8.55	8	22.7	5.45	6.2	
D1642 64	Glass (type I)			APTES			APTES	& CTNR		
K1512_54	-	19.5	4.5	98.5	8.56	10	24.6	5.2	6.5	

3.2 XRR results from Route 1 synthesis

Table SI3: Best fit-parameters of X-ray reflectivity data from APTES samples (tri-ethoxylaminosilane R1St1) that are functionalized with CTNR oligomers (R1St2). The layer thickness is d (in Å); RMS roughness is σ (in Å); optical index is given in the form of a complex scattering length density $\rho b = Re(\rho b)+i Im(\rho b)$ (in Å-2) from which the electron density ρe (in electrons/Å3) can be calculated and is equal to $Re(\rho b)$ divided by the scattering constant r0 = 2.81794.10-5 Å. The X-ray absorption is quantified here in the form of the imaginary part of the scattering length density $Im(\rho b)$ calculated from the atomic composition of materials.

Layers from substrate to air:	Substrate			Layer 1			Layer 2		
Samples:	-	Re(<i>µ</i> b), X10⁻⁵ Å	σ, Å	d, Å	Re(<i>µ</i> b), X10⁻⁵ Å	<i>σ,</i> Å	d, Å	Re(<i>p</i> b), X10⁻⁵ Å	<i>σ</i> , Å
David and a state of the state of the	Glass (type I)								
Bare glass substrate	-	19	3.6]					
D10.1_01	Glass (type I)			APMDES					
RISTI_SI	-	18.9	5.7	11.9	8.5	4.1]		
D16:2 61	Glass (type I)			APMDES	& CTNR				
RISTZ_SI	-	18.9	4.9	12	8.5	3.2			
D1C+1 C3	Glass (type I)			APMDES					
RISTI_SZ	-	19	4.6	19.6	8.6	4.7			
P10:2 C2	Glass (type I)			APMDES	& CTNR				
RISTZ_SZ	-	19	5.0	20.9	8.6	5.3]		
D10:1_02	Glass (type I)			APMDES			Top APMDES		
RISTI_53	-	19	5.5	24.6	8.72	4.3	13.4	2.5	6
D 10-0_00	Glass (type I)			APMDES			APMDES	& CTNR	
RISt2_53	-	19	5.5	25.5	8.6	7.5	16.7	3	8
B10+1_014	Glass (type II)			APMDES			Top APMDES		
RISTI_S14	-	21.9	3.5	6.3	8.3	3.0	24.1	9.3	3.6
B10:3 014	Glass (type II)			APMDES			APMDES	& CTNR	
K15t2_514	-	21.9	3.5	6.3	8.3	3.0	45.8	8.9	4.6

Table SI4: Best fit-parameters of X-ray reflectivity (XRR) data from APMDES samples (di-ethoxylaminosilane R1St1) that are functionalized with CTNR oligomers (R1St2).

Layers from substrate to air:	Substrate			Layer 1		
Samples:	-	Re(<i>µ</i> b), X10⁻ ⁶ Å	<i>σ,</i> Å	d, Å	Re(<i>µ</i> b), X10⁻ ⁶ Å	<i>σ</i> , Å
Bare glass substrate	Glass	(type I)			L	
	-	19.1	3.3	1		
MAPTES monolayer	Glass	(type I)		MAPTES		
	-	19.1	3.3	9.2	7.7	3.1

Table SI5: Best fit-parameters of X-ray reflectivity (XRR) data from MAPTES prefunctionalizations of glass microscopy slides (in one step).
General conclusions and outlooks

The common thread of this research work was to design bio-based elastomeric materials from natural rubber that kill and/or inhibit adhesion of harmful microorganisms and suppress the subsequent biofilm formation (antimicrobial/antifouling properties). Natural rubber was used as a starting renewable resource, since it is a bio-based ecofriendly material and an alternative to petroleum-based elastomers. We mainly tackled two key issues to envisage the industrial application of our materials: firstly, the synthesis of bulk polyisoprene films from NR integrating bioactive monomers covalently bound. This in order to limit the leaching of toxic molecules and to have a bulk property resisting to fracture of the material; and secondly, the covalent grafting of oligoisoprene from NR on different supports, in the form of robust, ultra-thin molecular layers, thin films and thick coatings.

Two categories of bioactive monomers were first synthetized prior to their integration in the NR matrix. One is a monomer containing a guanidinium group, an agent known for its antibacterial activity. This monomer was synthesized in three steps as a guanidinium salt with a trifluoroacetate counterion in 57% overall yield. The second monomer type designed was bio-inspired by an antifouling agent and two new tailor-made monomers were successfully prepared from Zosteric acid (ZA)-scaffold, *p*-CAA and *p*-CAB, with a 75% overall yield in three steps from *para*-coumaric acid and 65% in four steps from 4-hydroxyl benzaldehyde, respectively. Both were used as an alternative biocide-free agent to control biofilm formation with antimicrobial/antifouling properties.

Thick freestanding films were obtained from the covalent cross-linking performed during a photopolymerization process between di-functionalized acrylate oligoisoprenes (ACTNR) with a targeted molecular weight of 6000g/mol and bioactive co-monomers with an acrylate functional group (guanidinium monomer or tailor-made monomers issued from ZA). The formulation to obtain thick homogeneous elastomeric films was optimized, studying the photopolymerization process by FTIR and the surface morphology by optical microscopy. As guanidinium monomer and the two tailor-made monomers issued from CA are solid powders, while the acrylate matrix oligomers are liquid, they could not be mixed homogeneously, resulting in separate domains in the cross-linked films. Therefore, a minimum amount of pentaerythritol triacrylate (PETA) was added as a cross-linking agent and it dispersed homogeneously the bioactive monomers in the pre-polymerization mixture. We also demonstrated that 10% of bioactive monomer was the optimum quantity to obtain a good antibacterial/antifouling property and a good elasticity from the NR matrix. A high cross-linking degree was reached for thick freestanding films (discs of 1 cm diameter and 0.5 mm thickness), for two formulations F_{10} guanidine (containing 10% of guanidinium monomer) and F_{10} p-CAB (containing 10% of *p***-CAB**). On the opposite, the mixture containing 10% *p***-CAA** monomer ($\mathbf{F}_{10}\mathbf{p}$ -CAA) was not completely cross-linked even after a long UV-curing treatment (with 29.4 mW/cm² intensity). We attributed the poor reaction rate to the presence of the phenol groups (- C_6H_4 -OH) from the *p*-CAA monomer, which might inhibit the polymerization.

Natural rubber based coatings with guanidinium-monomers were incubated with three strains of pathogenic bacteria in bio-assays for antibacterial testing in static conditions: 1) *Pseudomonas aeruginosa*, **PA**, Gram negative; 2) *Staphylococcus aureus*, **SA**, Gram positive; 3) *Staphylococcus epidermidis*, **SE**, Gram positive. Less than 1% of the three bacteria remained on the film surfaces and this result was confirmed after extensive washing and sterilization of the films. Scanning electron microscopy confirmed the absence of bacteria on the films. The antimicrobial activity was caused by the positive charges of the guanidinium groups known to disrupt bacterial cell walls. A good thermal stability was determined up to 200°C, even after a long immersion in water (351 days). The water contact angle values (64° for \mathbf{F}_{10} guanidine versus 79° for the reference thick film without bioactive monomer) were attributed to the presence of the guanidinium groups in the bulk and at the surfaces. No component of the coatings was released in water at different temperatures and after different immersion times, except for a tiny fraction of the photoinitiator (Darocur 1173) and the associated photo-degradation products (benzaldehyde and benzoic acid). After 14 days in immersion, 60% of Darocur 1173, only 1.7% of benzoic acid and less than 1% of benzaldehyde were released. Nevertheless, these amounts were small enough and did not affect the bacteria bio-assays.

Due to the difficulty of incorporating *p***-CAA** monomer in the natural rubber matrix, we decided to perform the biological, thermal and wettability tests with thick films containing 10% of p-CAB monomer (formulations F_{10} p-CAB). These films displayed excellent antibacterial and antifouling activities against three strains of pathogenic bacteria: SA, SE, Bacillus aureus. No SA bacteria were found on the surfaces after contact with films and only 0.01% of bacteria of the initial suspension of S.epidermidis and Bacillus subtilis remained attached. A very few or no bacterial colonies were found on the surface of thick films. In order to confirm that this antibacterial activity was due to the active monomer rather than the photoinitiator released, our films were immersed in water for 24h before testing. Fourteen supernatant samples were taken to analyze the released percentage of Darocur 1173, its photo-degradation products benzaldehyde and benzoic acid and p-CAB monomer. We noticed that after 24h of immersion in water, around 40% of Darocur 1173 from thick films was released. Benzoic acid was less than 0.4% of the total amount of benzoic acid and no release of benzaldehyde was detected. Nevertheless, a minor amount of *p***-CAB** monomer was observed after 24h (around 0.2%). As for films containing 10% of guanidine monomer, these films remained stable up to 200°C and decomposed completely at 374°C. A significant decrease of contact angle was observed by adding 10% of *p*-CAB monomer in the polymer matrix (53° versus 79°), indicating a higher level of hydrophilicity. This difference could be explained by the presence of the carboxylic group (-COOH) of the *p*-CAB at the films surface, as well as in bulk.

In order to covalently graft such oligomers to other materials, we studied how to bind oligoisoprene from NR to silicon-containing interfaces. To perform that, a novel telechelic isoprene oligomer (**Oligo-SiHTNR**) also from natural rubber was designed with a silane anchoring group at one

end and a hydroxyl group at the other end. This approach presented some drawbacks due to the poor stability of Oligo-SiHTNR oligomers and poor reproducibility of samples prepared in the conditions we used (they had to be used immediately after synthesis). A more robust alternative method was defined in two steps. It started with a prefunctionalization of the support, using commercial amino-alkoxysilanes (APTES, APMDES or APDMES), prior to oligoisoprene immobilization. Among tested silane coupling agents, the di-ethoxysilane gave excellent and reproducible conditions to generate a reactive NH₂ coating. Then, CTNR oligomers with 20 isoprene units were grafted on it forming an ultra-thin coating characterized to angstrom resolution in thickness from X-ray Reflectivity (XRR) surface experiments. Dense CTNR coatings of few nanometers thickness and roughness below 0.5 nm were obtained, with grafted chains adopting a good solvent conformation at a surface grafting density near 1 chain/nm². On such oligomer coatings, thick films (from the mixture of ACTNR and photoinitiator) could be anchored via UV-curing. Similarly, thick films were formed and bound to methacrylate obtained *one pot* functionalization using commercial surfaces molecules (MAPTES, methacryloxylpropyl triethoxysilane). The thick films were particularly resistant to long water immersion, as well as to THF contact under mechanical agitation.

In summary, we successfully synthesized and evaluated the antibacterial and/or antifouling properties of freestanding thick films from natural rubber. We demonstrated also that by incorporating active monomers either Guanidine (Chapter II) or tailor-made Zosteric acid monomer (Chapter III), these films exhibited a much higher bio-activity. Furthermore, these materials have a high thermal stability. These findings allowed us to envisage possible industrial applications of elastomeric materials with no leaching in solution, emission of volatile organic compounds and absence of toxicological impact. Thick, thin and ultra-thin films were covalently grafted to glass and silicon substrates (Chapter IV) with a good resistance to solvents, opening the route to coatings applications as solvent protective barriers and antibacterial/antifouling surfaces.

The main outlook, in short terms of this PhD work is to evaluate the bio-activity of the grafted composites films. It would be also quite interesting for future applications to polymerize other monomers of interest, from the acrylate free extremity. The coatings also offer a perfect substrate to study more deeply the mechanisms taking place for bacteria and microorganisms interactions with bioactive monomers. Concerning the silane oligomers, they have been used in preliminary experiments to functionalize silica nanoparticles; the study will be pursued to obtain well defined hybrid particles to be used for instance, as inorganic charges in elastomeric films. All of this work is also sustained by the long term effort needed to replace fossil-based plastic products.



UNIVERSITE/MATIERE BRETAGNE MOLECULES LOIRE/ET MATERIAUX

E Mans Université

Titre : Elastomères composites biosourcés pour l'antibactérien/antisalissure. Méthodologie de synthèse et de greffage d'oligomères fonctionnalisés issus du caoutchouc naturel.

Mots clés : Caoutchouc naturel, polymères antibactériens, matériaux antimicrobiens sans relargage, films minces, surface hybride, greffage polyisoprene.

Résumé : Ce manuscrit présente la synthèse de élastomères nouveaux matériaux dérivés du caoutchouc naturel et de monomères organiques possédant des propriétés antifouling et/ou antibactériens, liés de manière covalente au réseau polymère. Des monomères acrylates originaux comportant un groupement bioactif (un groupe quanidinium ou des dérivés de l'acide Zostérique) ont été synthétisés et co-polymérisés avec des oligomères acrylates teléchéliques. Aucun relargage significatif des momonères bioactifs n'a eu lieu après immersion dans l'eau du matériau. Les films auto-portés synthétisés uniquement à partir des oligoisoprènes de type acrylate ont montré une faible activité antibactérienne qui a pu être drastiquement améliorée en intégrant aux films des monomères de type guanidinium ou dérivés de l'acide Zostérique. Les revêtements se sont montrés actifs contre plusieurs souches de bactéries pathogènes, parmi lesquelles

Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis et Staphylococcus epidermidis. Afin de greffer de manière covalente des brosses d' oligoisoprene sur des surfaces, de nouveaux oligoisoprènes bifonctionnels comportant un groupe terminal alkoxy-silane ont été préparés puis couplés avec des matériaux à base de silicium. Une autre approche a également été étudiée, consistant à préfonctionnaliser la surface avec des groupements amino puis à partir de ces fonctions à greffer des oligomères ayant des extrémités carbonyle. La postpolymérisation à partir des extrémités de chaîne libres à la surface a donné un revêtement épais d'oligoisoprène offrant une forte résistance à l'immersion dans des solvants (eau, THF). De cette manière, nous avons pu accéder à des couches moléculaires denses et attachées, à des films minces et à des revêtements épais.

Title: Bio-based elastomeric composites for antibacterial and antifouling applications. Methodology for the synthesis and grafting of functionalized oligomers issued from natural rubber.

Keywords : Natural rubber, antibacterial polymers, antimicrobial non-leaching materials, molecular and thin films, hybrid material, polyisoprene grafting

Abstract: This manuscript presents the synthesis of new elastomeric materials based on natural rubber derived building blocks and organic monomers having antifouling and/or antibacterial properties, covalently bound to the polymer network. Original acrylate monomers bearing an organic bioactive moiety (a Guanidinium group or Zosteric acid derivates) were synthesized and co-polymerized with telechelic acrylate oligomers from polyisoprene. No significant leaching of the bioactive monomers occurred and the material resisted to long water immersions. Freestanding films prepared from acrylate oligoisoprenes also showed a weak antibiofouling activity which was drastically increased by integrating the guanidinium and the Zosteric acid monomers. The coatings were active against several strains of

pathogenic bacteria among which Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis Staphylococcus epidermidis. In order to and covalently graft oligoisoprenes to surfaces, new bifunctional oligoisoprenes bearing an alkoxy-silane end moiety were designed and coupled with siliconcontaining materials. An alternative approach was followed by prefunctionalizing the surface with amino groups and by covalently grafting oligomers with carbonyl chain-ends. Post-polymerization from the surface free chain-ends resulted in a thick oligoisoprene coating with strong resistance to solvent immersion (water, THF). In this way, we were able to build dense and tethered molecular layers, thin films and thick coatings.