UNIVERSITE DE LORRAINE
École Nationale Supérieure d'Agronomie et des Industries Alimentaires (ENSAIA)
Ecole Doctorale Sciences et Ingénierie des Ressources, Procédés, Produits, Environnement (RP2E)
Laboratoire d'Ingénierie des Biomolécules (LIBio)

THESE

Pour obtenir le grade de Docteur de l'Université de Lorraine

Spécialité : *Procédés Biotechnologiques et Alimentaires*

Nano-fonctionnalisation des hydrogels naturels bioactifs sous forme de matrice 3D

Présentée par

Rana KADRI

Soutenue publiquement le 9 Décembre 2015

Rapporteur	
Mr João MANO	Professeur, DEP, Université de Minho
Mr Bernard NYSTEN	Professeur, BSMA, Université catholique de Louvain
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Invité	
Mr Franck CLEYMAND	Maître de conférence - HDR, N2EV, Institut Jean Lamour

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A mes parents...

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Liste des abréviations

2D-3D	Two dimensional, three dimensional
AFM	Atomic force microscopy
Alg	Alginate
CNT	Carbon nanotubes
D2O	deuterium oxide
DLS	dynamic light scattering
DN	Double Network
ECM	Extracellular matrix
FTIR	Fourier transform infrared
G	Guluronic
G'	Elastic modulus
G''	Viscous modulus
GelMA	Gelatin methacrylate
H-NMR	H-nuclear magnetic resonance
IPN	Interpenetrating Polymer Network
lip	liposomes
LVE	Linear Viscoelastic
M	Mannuronic
MA	Methacrylic Anhydride
M_v	Molecule Weight
NP	Nanoparticles
PBS	Phosphate Buffered Saline
pH	Hydrogen Potential
PI	Photoinitiator
ppm	Particules per million
RGD	Arginine-Glycine-Aspartic
RIP	Réseaux interpénétrés de polymères
S	Siemens
SEM	Scanning Electron Microscopy
tan	tangent
UV	Ultraviolet
v/v	Volume/Volume
w/v	Weight/Volume
XPS	X-Ray Photoelectron Spectroscopy

Liste des figures hors publications

Liste des publications et communications

Articles scientifiques:

Kadri, R., Ben Massaoud, G., Tamayol, A., Aliakbarian, B., Zhang, H.Y., Sánchez-González, L., Arab-Tehrany, E., Preparation and characterization of nanofunctionalized alginate/gelatin methacrylate crosslinked hydrogels, RSC Adv. (soumise)

Kadri, R., Bacharouch, J., Ben Massaoud, G., Tamayol, A., Francius, G., Desobry, S., Linder, M., Sánchez-González, L., Arab-Tehrany, E., 3D Bioactive IPN hydrogel functionalized by nanoliposomes, Soft Matter. (soumise)

Kadri, R., Sánchez-González, L., Tamayol, A., Ben Massaoud, G., Arab-Tehrany, E., Physicochemical interactions in nanofunctionalized alginate/GelMA IPN hydrogels, Colloids and Surface B: Biointerfaces. (soumise)

Conférence internationale avec acte :

Kadri, R., Aliakbarian, B., Tamayol, A., Sanchez-Gonzalez, L., Desobry, S., Linder, M., Arab-Tehrany, E. (2015). 3D Construct Hydrogels Functionalized by Nanoliposomes for Tissue Engineering Application. Termis word congress, Boston, USA, 8-11 september, Tissue Engineering Part A, 21, S394-S395.

Communications Orales Internationales:

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Kadri, R., Aliakbarian, B., Sanchez-Gonzalez, L., Tamayol, A., Najafabadi, A.H., Khademhosseini, A., Arab-Tehrany, E., **2015.** "Nanofunctionalization of alginate and alginate/GelMA crosslinked hydrogels by nanoliposomes and 3D construct." 15-17 June, Paris, France.

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Kadri, R., Tamayol, A., Sanchez-Gonzalez, L., Desobry, S., Linder, M., Arab-Tehrany, E., **2015.** "Nanofunctionalization of 3D construct hydrogels for tissue engineering application" Eureka conferences, Drug Discovery and Therapy World Congress, 22-25 July, Boston, USA.

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I. Introduction & objectives

L'élaboration de nouveaux systèmes à base de biomatériaux est un axe de recherche important dans des domaines variés tels que le biomédical, la pharmaceutique, la cosmétique, la nutraceutique ou l'agroalimentaire. Les matrices polymériques sont largement utilisées de par leurs caractéristiques remarquables. Elles sont formées d'un réseau en trois dimensions (3D) constitué de polymères hydrophiles qui, une fois réticulés, procure à l'hydrogel son caractère insoluble. Ce réseau a la capacité d'absorber une quantité importante de solvant et de gonfler jusqu'à atteindre un certain degré de saturation. Ce degré dépend de la nature et de la densité des chaines polymériques. Suivant la méthode de gélification, des interactions chimiques où les molécules sont liées par des liaisons covalentes permettent l'établissement du réseau polymérique, ou des interactions physiques qui consistent en des interactions électrostatiques, ioniques ou de type Van der Walls.

Les hydrogels peuvent être préparés à partir de polymères naturels ou synthétiques. Dans ce travail, les polymères sélectionnés sont naturels. Ceux-ci présentent des propriétés intéressantes en termes de biocompatibilité et de biodégradabilité. Même si leur utilisation est limitée par certaines de leurs propriétés telles que leurs propriétés mécaniques, leur adhésion cellulaire ou leur stabilité physico-chimique et structurale. A ce jour il n'existe pas de polymère idéal d'origine naturelle qui puisse répondre à toutes les propriétés physiques, mécaniques et biologiques demandées.

Au vu de l'utilisation accrue des matrices 3D, il paraît donc essentiel de définir des pistes d'amélioration des propriétés de ces hydrogels. Une stratégie intéressante consiste en l'utilisation de nouvelles techniques plus complexes dans le but de renforcer et d'améliorer les caractéristiques des hydrogels afin d'élargir leurs champs d'utilisation. Ces techniques consistent en la formation d'hydrogels composites par l'association de polymères ou l'incorporation de nanoparticules dans la matrice 3D d'hydrogel. La méthode de préparation des hydrogels composites ainsi que le type de polymères ou de nanoparticules incorporées modifient le réseau polymérique.

Après mélange des différents polymères, l'hydrogel peut engendrer la formation de liaisons covalentes entre les différentes molécules ou la formation de réseaux interpénétrés de polymères (RIP). Les RIPs résultent de l'incorporation d'un polymère secondaire dans une matrice 3D déjà formée. Ce polymère subi par la suite une gélification et forme un réseau secondaire. Les RIPs ont été synthétisés pour la première fois par Aylsworth au début du 20ème siècle (Annabi et al., 2014)(Sperling, 2012)(Aylsworth, 1914). Cette technique permet

d'élaborer des hydrogels plus robustes ayant des propriétés physico-chimiques intéressantes notamment en termes de propriétés mécaniques.

La deuxième voie d'amélioration des caractéristiques des hydrogels consiste en l'incorporation de nanoparticules dans la matrice polymérique. Plusieurs types de nanoparticules naturelles ou synthétiques peuvent être incorporés, conférant ainsi des propriétés particulières aux hydrogels développés. Parmi les nanoparticules, les nanovecteurs tels que les nanoliposomes présentent l'avantage d'apporter une fonctionnalité supplémentaire à la matrice polymérique. . Les nanoliposomes sont des nanoparticules molles et naturelles qui, vu leur caractère amphiphile, présentent des propriétés d'auto-assemblage remarquables. Ces particules formées de bicouches lipidiques peuvent transporter et libérer des molécules hydrophobes, hydrophiles ou amphiphiles. Ces caractéristiques font des liposomes d'excellents vecteurs utilisables dans plusieurs domaines d'application. Ils sont biocompatibles, non toxiques et comprennent en grande partie des acides gras polyinsaturés. Les liposomes sont donc des systèmes intéressants pour des applications *in vivo.*

Ce travail de thèse consiste en l'élaboration d'hydrogels à base de polymères issus de deux catégories différentes : les polysaccharides et les protéines. L'alginate a été sélectionné pour ce travail car il présente plusieurs avantages en termes de biocompatibilité et de biodégradabilité. La méthode de gélification de ce polymère est simple, en effet il est possible d'obtenir un hydrogel par réticulation ionique en présence du cation calcium. Le second polymère utilisé pour cette étude est le GelMA. C'est une forme modifiée de la gélatine, qui présente une meilleure stabilité surtout vis-à-vis des changements thermiques. Ce polymère est réticulé lorsqu'il est soumis à des rayonnements ultraviolets.

Afin d'améliorer les propriétés des hydrogels d'alginate, l'association alginate – GelMA a été étudiée dans ce travail. Le GelMA et l'alginate sont mélangés selon trois concentrations d'alginate pour mettre en évidence l'influence de la concentration d'alginate sur les caractéristiques des hydrogels composites. . De plus, les hydrogels formés de polymères purs et de RIPs sont fonctionnalisés par des nanoliposomes à deux concentrations différentes.

Les modifications effectuées sur les matrices d'hydrogel soulèvent plusieurs questions sur le plan scientifiques :

- Quels sont les effets du mélange des deux polymères sur les propriétés de l'hydrogel et quelles sont les propriétés mécaniques obtenues? Comment ces propriétés varient avec le changement de concentration du polymère?

- Quelle est l'influence de l'incorporation d'une faible quantité de nanoliposomes sur les caractéristiques des hydrogels? Une variation de la concentration des liposomes entraine-t-elle des modifications significatives dans la matrice 3D malgré le faible ratio liposomes/polymère(s)?
- Existe-t-il des interactions entre les polymères ou/et entre les polymères et les nanoliposomes?

L'objectif de la thèse est en premier temps de comprendre et d'optimiser la méthode de préparation des hydrogels grâce à une étude bibliographique procurant de plus amples informations sur les gels envisageables. Cette revue met en relief l'importance de développer des hydrogels composites permettant d'obtenir de meilleures propriétés mécaniques et biologiques.

Une caractérisation physico-chimique multi-échelle a été effectuée par la suite dans le but d'étudier les propriétés des hydrogels préparés.

La seconde partie est consacrée à l'étude des caractéristiques de la surface et de l'épaisseur des hydrogels pour déterminer l'effet du mélange alginate-GelMA et de l'incorporation des nanoliposomes sur les propriétés des matrices polymériques 3D. L'analyse de la surface des hydrogels est essentielle pour déterminer l'hydrophobicité, les propriétés de mouillabilité et l'énergie de surface ainsi que pour contrôler leur porosité. Les tests physico-chimiques sont de même effectués dans l'épaisseur de l'hydrogel dans le but de déterminer la conductivité et les propriétés mécaniques de chaque matrice.

Le troisième chapitre est consacré à une étude structurale et mécanique des hydrogels suivant trois différentes concentrations du polymère d'alginate et deux concentrations de nanoliposomes dans le but de vérifier l'efficacité des systèmes préparés et de contrôler leurs propriétés physico-chimiques avant et après nanofonctionnalisation.

La formation des hydrogels composites entraine des modifications moléculaires et structurales au niveau de la matrice 3D. Une meilleure compréhension des interactions entre les composants de l'hydrogel assurera une meilleure maitrise des mécanismes mis en jeu, et donc une optimisation du système pour les différentes applications.

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II. Literature review

1. Introduction

Hydrogels, formed of crosslinked polymers chains, have been considered, in the past decades, as a promising biomaterial and were involved in many domains especially in biomedical applications due to their biocompatibility and their close resemblance with native extracellular matrix (Hoffman et al., 1972)(Ratner and Hoffman, 1976)(Kamath and Park, 1993)(Harland and Prud'homme, 1992)(Khademhosseini et al., 2006)(Ulbrich et al., 1995)(Khademhosseini and Langer, 2007)(Khademhosseini et al., 2006)(Annabi et al., 2014)(Peppas et al., 2000)(Falabella et al., 2010)(Aziz et al., 2015)(Samchenko et al., 2011)(Wichterle and Lím, 1960). Hydrogels contain hydrophilic groups which, in an aqueous medium absorb a large amount of water into its pores, and develop a superabsorbent material (Peppas et al., 2006)(Hoffman, 2002)(McClements, 2015). In the presence of a solvent, hydrogels swell and become soft with a degree of flexibility similar to living tissues (Rosiak and Yoshii, 1999)(Ahmed, 2015)(Prashan et al., 2011)(Peppas et al., 2000). Their shapes are maintained because of the crosslinked chains which prevent its solubilization (Langer and Peppas, 2003).

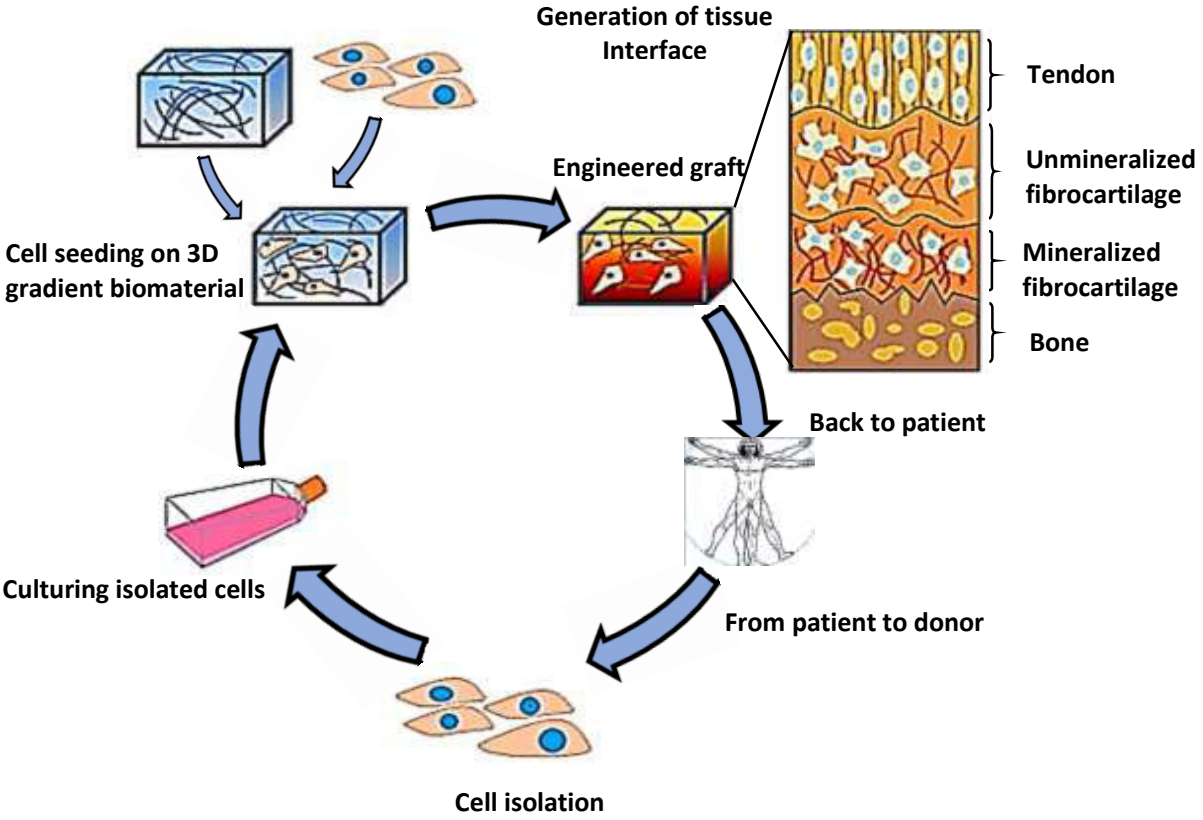

Fig.1. Representative scheme of the incorporation of 3D scaffold in tissue engineering (Seidi et al., 2011).

The architecture of hydrogels has revealed significant difference in the encapsulation process. 2D substrates do not provide the best development of the encapsulated cells and molecules because they present a different environment from that of living tissues. While 3D scaffold, mimic the extracellular matrix where the molecules and cells are immersed *in vivo* (Geckil et al., 2010)(Ni and Chen, 2009)(Porro et al., 2005)(Zhang et al., 2009). 3D hydrogels network are ideal materials for the encapsulation of molecules and tissue engineering applications (fig. 1). They have the potential to assure a better circulation and adhesion of the cells. They also allow the exchange between the cells and the environment, essential for cells growth(Geckil et al., 2010)(Peppas et al., 2006)(Ling et al., 2007)(Du et al., 2008).

Hydrogel scaffolds are formed of natural or synthetic crosslinked polymer chains (Samchenko et al., 2011). Synthetic polymers present strong mechanical properties and a powerful control of their microstructure, their degradability and their chemical properties. In contrast, natural crosslinked polymers are interesting biomaterials used essentially in biomedical and pharmaceutical sectors. They have potential advantages of biodegradability and biocompatibility. It presents a favorable environment for an adequate functioning of the cells and can present biological fragments which improve the cellular performance and activities (Annabi et al., 2014)(Lin and Metters, 2006)(Gunatillake et al., 2006)(Drury and Mooney, 2003). Alginate is a natural biocompatible anionic polymer which manipulation is simple as it has the ability to be stored, sterilized and chemically modified in an easy way. It is characterized for being mucoadhesive, nonimmunogenic and nontoxic (Pawar and Edgar, 2012)(Ingar Draget et al., 1990)(Martinsen et al., 1989)(Davidson, 1980)(Augst et al., 2006)(Li et al., 2012)(Draget and Taylor, 2011)(Eiselt et al., 2000)(Smidsrød and Skjak-Braek, 1990)(Karsa and Stephenson, 1993). Another natural polymer extensively used in biomedical application is gelatin. It is a natural cytocompatible protein (Barbetta et al., 2005) derived from either an acid or an alkaline hydrolysis of a natural product called collagen (Ward and Courts, 1977)(Veis, 1964). Gelatin is an interesting polymer due to the presence of many bioactive sequences such as Arg-Gly-Asp (RGD) providing cell attachment and growth of different type of active molecules (Galis and Khatri, 2002)(Van den Steen et al., 2002)(Nichol et al., 2010).

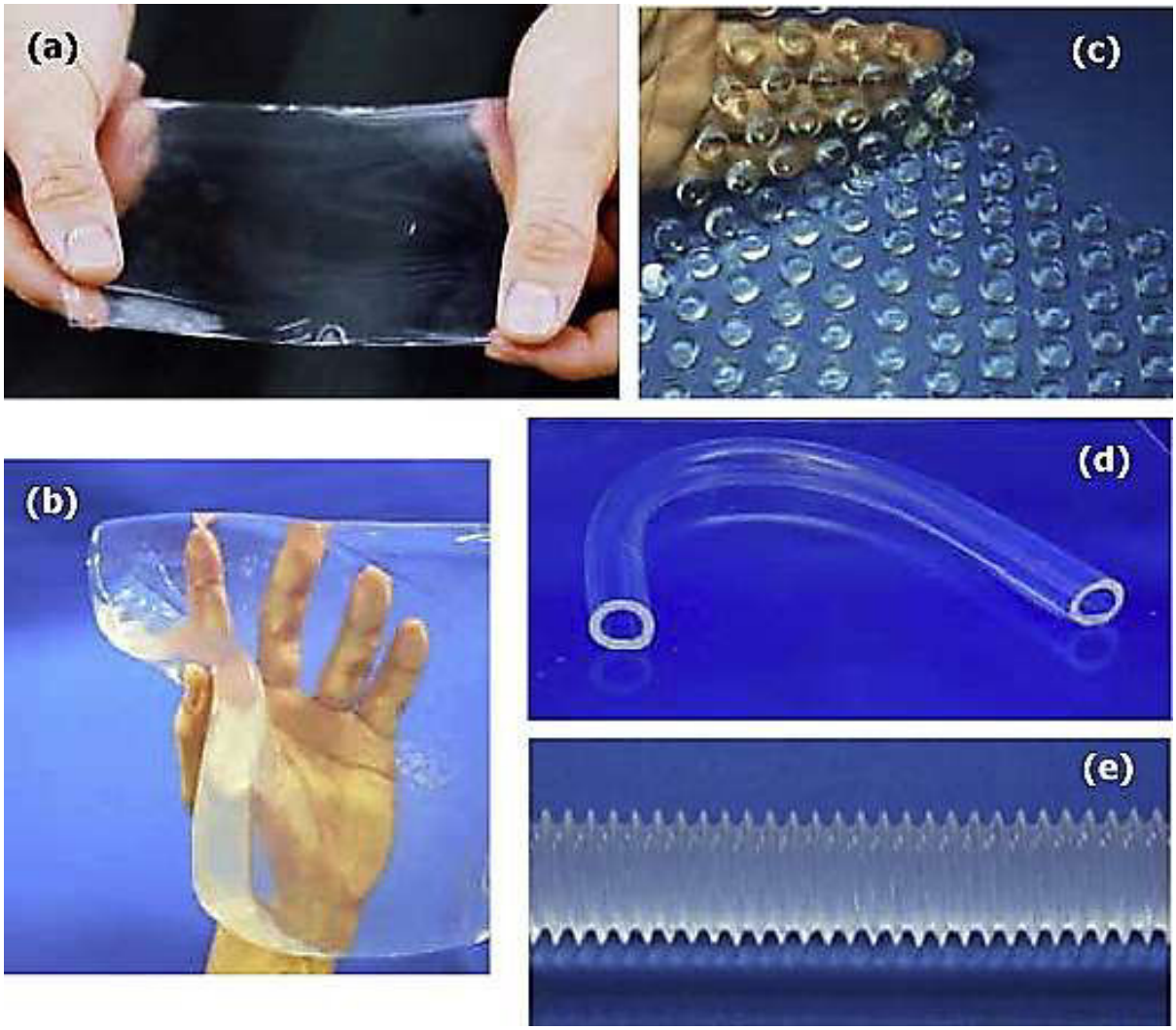

Fig.2. Hydrogel scaffolds with various shapes: (a) thin film, (b) sheet, (c) uneven sheet, (d) hollow tube and (e) bellow (Haraguchi, 2012).

Hydrogel scaffold, with their different shapes (fig. 2), has attracted much attention because of their unique properties and their potential applications. It can be used as bulking agent, for example against obesity when it swells in the stomach and gives a sensation of fullness (Sannino et al., 2006). It is used also as an adhesive agent for the treatment of wounds. The scaffold is utilized, in particular, to entrap and transport active molecules. It is an innovative structure which can isolate, protect and control the active molecule during the transport and ensure that it reaches the desired site. Thereby, side effects are minimized and the release of the bioactive molecules can be controlled depending on the body's need. The type of interaction between the polymer and the molecule must be taken into consideration since a strong interaction may delay or affect the release process (McClements, 2015). The isolation of a molecule limits the contact

with other systems and prolong the efficiency of the molecule until its liberation. It protects also the external environment if the molecule has undesirable effects on its surrounding (Vandamme et al., 2007). Hydrogels have been investigated also in tissue engineering field and are applicable for the majority of body tissues, because of their close resemblance with the ECM (Zhu and Marchant, 2011). The hydrogel 3D matrix exhibits large and accessible hydrate surface that improves the adhesion, growth, proliferation and differentiation of the cells as well as the interaction with the surrounding media. A high surface to volume ratio allows better placement of the cells that will restore or replace tissues or organ function. 3D hydrogel architecture assure a better mimic of natural tissues and thus obtain a more powerful system for *in vivo* utilization (Slaughter et al., 2009)(Drury and Mooney, 2003).

Biostability of the hydrogel is related to the mechanical properties of the hydrogel which include elasticity, viscosity, strength, material interface and chemical degradation. It depends specially on the type of polymer, the gelling conditions, the swelling behavior and the degradation rate (Slaughter et al., 2009)(Drury and Mooney, 2003)(Dhandayuthapani et al., 2011)(Anseth et al., 1996)(Hubbell, 1999)(Lee and Mooney, 2001). When the hydrogel carry an active molecule, it combines it with products essential for the survival and development of the molecule (nutrients, oxygen…) and in return generated waste products. The particles movement is related to the rate and size of pores. Polymeric scaffold with interconnected highly porous structure ensure the diffusion success and the growth and uniformity distribution of cells. Pore size must be adequate in order to permit cells diffusion, vascularization, and removal of waste products but in contrary restrict free diffusion of the encapsulated molecules out of the scaffold (Dhandayuthapani et al., 2011)(Drury and Mooney, 2003)(Lu and Anseth, 2000). The degradability is also essential in biomedical application for the release of the active molecule or for the regeneration of functional tissues. The degradation occurs by a physical or chemical process or it undergoes biodegradation in the presence of enzymes for example (Drury and Mooney, 2003)(Dhandayuthapani et al., 2011)(Hubbell, 1999)(Lee and Mooney, 2001)(Rowley et al., 1999)(Nuttelman et al., 2001)(Mann et al., 2001). Hydrogel scaffolds should be nontoxic to the molecules or organs and do not provoke inflammation or chronic immune response (Slaughter et al., 2009)(Dhandayuthapani et al., 2011)(Drury and Mooney, 2003). They also should provide attachment of the cells to the scaffold. In case of theabsence of cells adhesion, ECM protein or peptides (arginine-glycine-aspartic acid for example) can be incorporated to the surface or/and into the scaffold in order to increase the linkage between cells and scaffold (Slaughter et al., 2009)(Dhandayuthapani et al., 2011)(Hubbell, 1999)(Lee and

Mooney, 2001). Similarly, growth factors can be added to develop or control the cells functions in the scaffold (Tabata, 2003)(Drury and Mooney, 2003).

2. Network structure and crosslinking methods

Crosslinked hydrogel can be classified according to their sensitivity to the surrounding environment (Samchenko et al., 2011) Polymers can be crosslinked in two different ways. The first is physical which depend on environmental changes, hydrogel and protein interactions. It forms a network of polymers linked by hydrogen, ionic or hydrophobic bonds and present low mechanical properties. This type of interactions can be reversible by changing the physical conditions such as pH, temperature, ionic strength or even by applying a mechanical stress or adding a solute which compete with the crosslinking agent. The second type of crosslinking is based on chemical interaction which form stable hydrogels with higher mechanical properties. It consists on the formation of covalent bonds after a radical polymerization, chemical reactions, energy irradiation or enzymatic crosslinking (Annabi et al., 2014)(Hoffman, 2002)(Vlierberghe et al., 2008).

2.1 Ionically crosslinkable hydrogel

Ionically crosslinked hydrogel is a physical interaction between charged polymer chains and multivalent ions of opposite charge in order to create a network formed of ionic bridges (Berger et al., 2004)(Tan and Marra, 2010)(Hennink and van Nostrum, 2002). This method of polymerization does not present, in general, toxic crosslinkers (Berger et al., 2004).

But in reverse, the properties of the resulting product is limited due to the difficulty to control the gelation process (Tan and Marra, 2010). The use of ionical crosslinking is large and can occur for both natural and synthetic polymers, but the major problem of this method is the lack of stability in the final product. The swelling comportment of the hydrogel depends on the interaction between the charged polymer and its counter ions. In some cases, the presence of ions in a gel provoke an osmotic pressure leading to the amplification of the swelling characteristics of the ionically crosslinkable hydrogels (Okay, 2009).

Chitosan, a cationic polysaccharide, can be ionically gelled in the presence of the appropriate anion. The amine groups presents in a high density in the chitosan, are protonated in an acid solution. The anionic nontoxic (Lin et al., 2008) compound sodium tripolyphosphate provide the phosphate ions necessary to react with the protonated, positive charged, amine groups of the chitosan. This electrostatic interaction leads to the transformation of chitosan solution into

gel (Srinatha et al., 2008)(Oh et al., 2013)(Lee et al., 2010)(Gan and Wang, 2007)(Tsai et al., 2008)(Liu and Gao, 2009)(Shu and Zhu, 2002)(Honary and Hoseinzadeh, 2010). Chitosan polymer may be crosslinked with anions like citrate or phosphate. The biocompatible agent, trisodium citrate contains the functional group COO^- which reacts with the amino group $NH3^+$ of the polymer. The force formed between the two opposite charges create a network and form a gel (Chen et al., 2008)(Chen et al., 2008). Similarly, calcium phosphate can also react with the cationic chitosan polymer in the presence of NH_4OH and leads to the formation of a porous scaffold (Zhong et al., 2009).

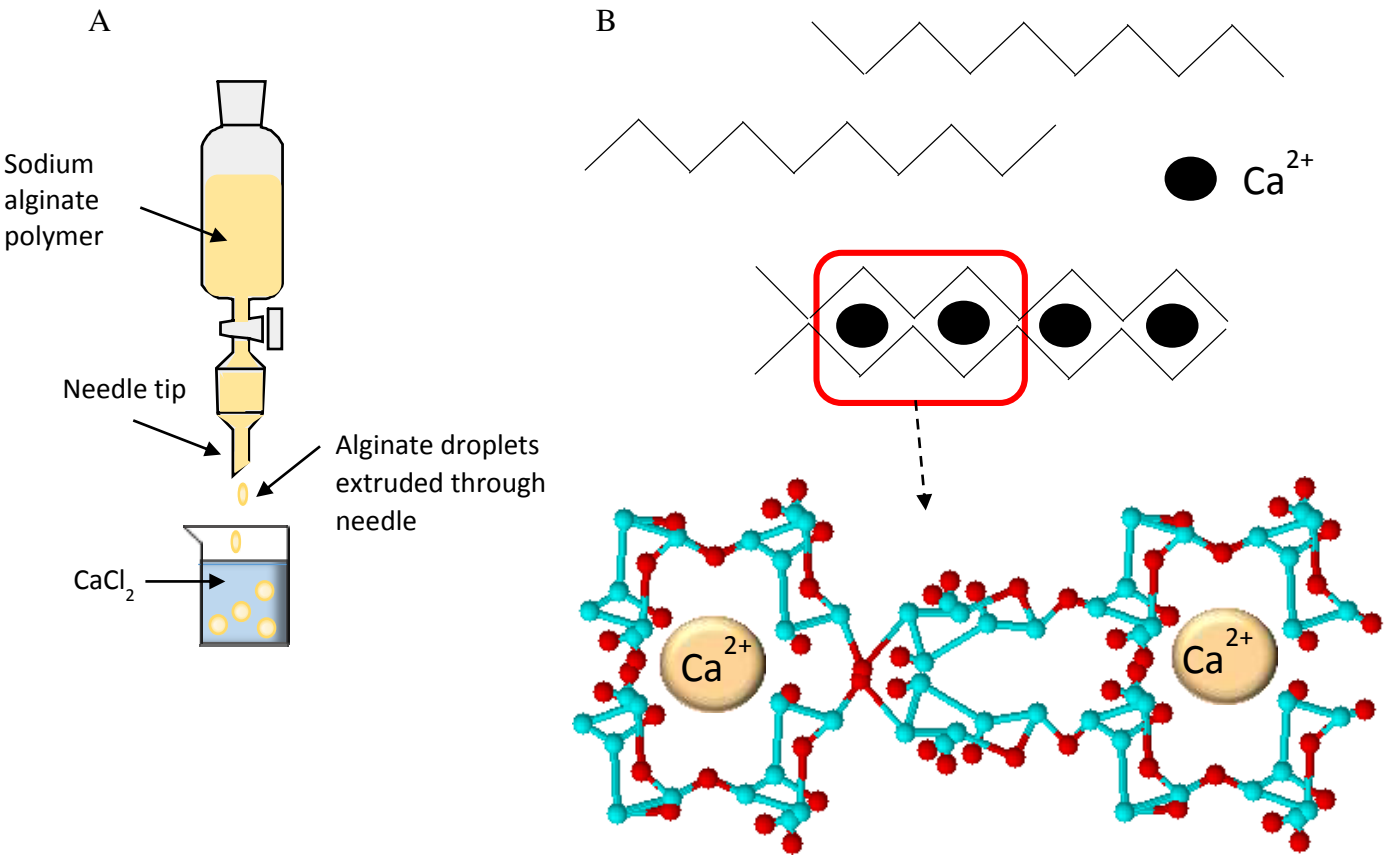

Fig.3. Alginate gel synthesis. A. Incorporation of the alginate solution into calcium chloride. B. Calcium associate alginate chains together and form a network.

The alginate polymers also react and form a complex with divalent cations. The obtained hydrogel is known as 'ionotropic hydrogel' (Donati and Paoletti, 2009). The polymer present different affinities towards divalent ions. Monovalent cations and Mg^{2+} do not induce gelation (Byrom, 1991) (Gombotz and Wee, 2012). However, Ca^{2+} is the most commonly used cation

for alginate crosslinking (fig. 3). This reaction depends on electrostatic forces, crosslinking density, as well as the composition and molecule weight of alginate. When the polymer is mixed with Ca^{2+}, G units of two different chains of alginate bind with the same divalent cation and forms a junction (Pawar and Edgar, 2012)(Sikorski et al., 2007)(Haug et al., 1970)(Smidsrød, 1974)(Grant et al., 1973) (Morris et al., 1978)(Palluault, 2010)(Lee and Mooney, 2012)(Paques et al., 2014)(Poojari and Srivastava, 2013)(McClements, 2015)(Matalanis et al., 2011). A minimum length of G sequence is required to form junctions during the gelation (about eight for calcium alginate gel at 20°C). MG blocks also interferes but forms weak junctions (Donati et al., 2005)(Pawar and Edgar, 2012). The network resulting from the association between the polymer and cations is called "egg-box" (Lee and Mooney, 2012)(Paques et al., 2014)(Poojari and Srivastava, 2013)(Grant et al., 1973)(Morris et al., 1978)(Palluault, 2010)(Rees, 1981)(Mørch et al., 2006)(Braccini and Pérez, 2001). Calcium-Alginate crosslinking process can be achieved in two methods. The diffusion method consist on the penetration of calcium ions from an outside reservoir into the dissolved alginate. The second method is the internal setting method, where the crosslinking agent is located in the alginate solution and the gelling process takes place when an agent triggers the mechanism of gelation (pH or calcium solubility) (Paques et al., 2014)(Skjåk-Bræk et al., 1989).

Hyaluronic acid is another polysaccharide widely used in cosmetics or medical applications. It is a linear polymer that ionically crosslink in the presence of Fe^{3+} and develop a highly soft gel (Vorvolakos, 2010).

The products derived from ionic reaction are generally biocompatible, but their major drawback is their low stability. Therefore, photocrosslinkable hydrogels can be more stable.

2.2 Photocrosslinkable hydrogel

Photocrosslinkable hydrogel are light sensitive hydrogel which can crosslink or degrade following a visible or UV light exposure (Annabi et al., 2014)(Slaughter et al., 2009)(Khademhosseini et al., 2006)(Nguyen and West, 2002). The UV irradiation is commonly used in biomaterial applications (Wang et al., 2012)(Fazel, 2011). It is an inexpensive, simple and rapid method which has gained increased attention due to the possibility of spatial and temporal control of the crosslinking process with a minimal heat production. (Annabi et al., 2014)(Slaughter et al., 2009)(Khademhosseini and Langer, 2007) It allows to produce complex form of gel suitable for tissue engineering applications (Annabi et al., 2014)(Rivest et al., 2007)(Liu and Bhatia, 2002)(Camci-Unal et al., 2013)(Gerecht et al., 2007)(Zhong et al.,

2010)(Wang et al., 2012)(Nguyen and West, 2002)(Decker, 1987). In order to initiate photocrosslinking process, it is required to generate free radicals in the hydrogel to react and form covalent bonds with the functional groups. These radicals are provided by the addition of photoinitiator, a high light-sensitive compound, that once exposed to visible or UV light breaks down into radicals (Annabi et al., 2014)(Slaughter et al., 2009)(Corrales et al., 2003)(Fazel, 2011)(Corrales et al., 2003)(Nguyen and West, 2002)(Scranton et al., 1997)(Fouassier, 1995). For a good encapsulation performance, the microstructure of the gel is controlled by varying many parameters: the monomer concentration, the photoinitiator concentration, light intensity and the hydrogel exposure time to light (Wang et al., 2012). The drawbacks of this method is the risk of affecting the performance of the encapsulated molecules and cells due to light exposure. This difficulty can be overcome in hydrogels with mild gelation conditions which are exposed to a brief duration of irradiation and a low intensity of light. Furthermore, for some types of hydrogels, the gelling process *in vivo* is not evident since UV light has difficulty to penetrate tissues. Long wavelength UV light can be transmitted through the skin. In this case, the hydrogel with the added photoinitiator are injected under the skin and are crosslinked once they receive the necessary irradiation. Even if, only a part of the radiation passes through the skin but it is sufficient to get the final gel. This method is used mostly for drug delivery or for cartilage tissue engineering (Annabi et al., 2014)(Slaughter et al., 2009)(Nguyen and West, 2002)(J. Elisseeff et al., 1999)(Jennifer Elisseeff et al., 1999)(Elisseeff et al., 2000).

The photocrosslinking of gelatin consist on the substitution of the free amine groups of the gelatin with the methacrylic anhydride (Nichol et al., 2010)(Barbetta et al., 2005)(Dubruel et al., 2007)(Galis and Khatri, 2002). The methacrylamide-modified gelatin called GelMA form a gel when it is exposed to UV irradiation (Nichol et al., 2010)(Van Den Bulcke et al., 2000). The remaining free methacrylate groups which have not reacted are removed by dialysis (Khan et al., 2006)(fig. 4). GelMA become an attractive polymer in particularly in tissue engineering applications due to the cells ability to proliferate, migrate, elongate and conserve a cellular alignment in the engineered tissue. In their study, Ramón-Azcón et al. showed the effectiveness of GelMA in the encapsulation of cell electropatterning due to the low viscosity and to the ion concentration of the gel. It allows the myoblast and endothelial cell patterns to grow and proliferate in the hydrogel and maintain their alignment during the culture period (Ramón-Azcón et al., 2012). GelMA is characterized by the presence of binding motifs such RGD and matrix metalloproteinase which facilitate the connection with the active molecules while for some polymers the addition of binding sites is essential for the encapsulation process (Galis and

Khatri, 2002)(Van den Steen et al., 2002)(Galis and Khatri, 2002). Depending on the type of gelatin used, GelMA can support different types of active molecules thereby the application field of the hydrogel is large (Nichol et al., 2010).

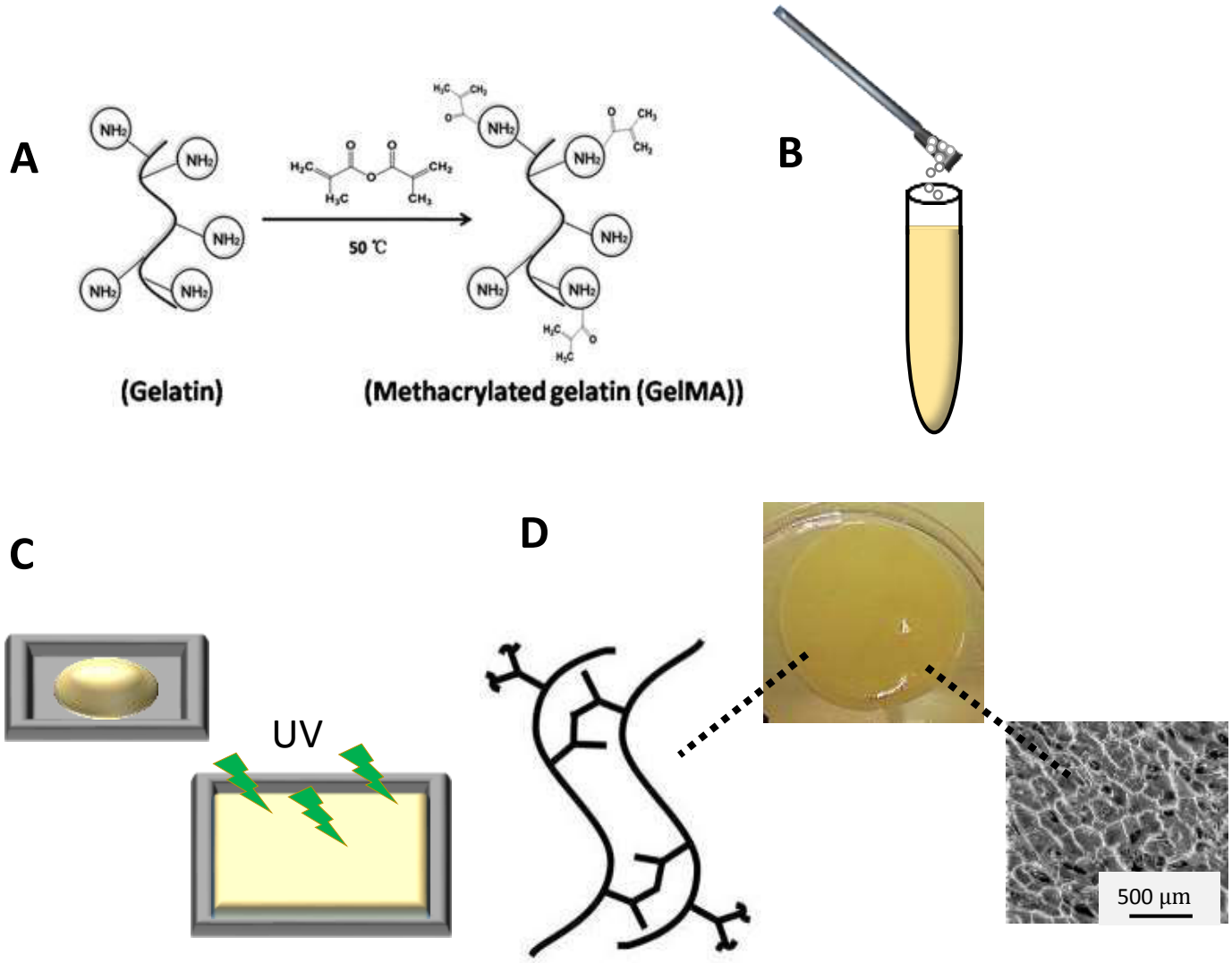

Fig.4. GelMA gel synthesis. A. Modification of gelatin with methacrylate anhydride (Xiao et al., 2011). B. Incorporation of a photoinitiator into the solution . C. Exposure of GelMA solution to UV light. D. transformation of GelMA to gel (Nichol et al., 2010).

Many studies on the crosslinkage of other natural polymers have been established. Chitosan, for example, is generally dissolved in the presence of acid. Even at low volume, the acid present undesirable effects on human tissue. The complete elimination of the acid is not possible due

to electrostatic forces between the polymer and the acid. Hence, the photocrosslinking method of a modified chitosan can be a solution to get over this problem. Therefore, the chitosan containing NH_2 react with C = C present in the acrylate. The obtained product is transformed into a crosslinked network, in the presence of a photoinitiator after its exposure to UV light. The modified photocrosslinked chitosan has large advantages especially in tissue engineering, in terms of degradability, biocompatibility and cell attachment and proliferation (Zhou et al., 2011)(Annabi et al., 2014).

Light sensitive hydrogels is not limited to natural hydrogels. Poly (ethylene glycol) diacrylate turns into gel after exposure to UV in the presence of photoinitiator. It is the result of the reaction between poly (ethylene glycol), a synthetic polymer known for its biomedical utilization, and acryloyl chloride. The 3D polymer network, can therefore, be effective in biomedical and tissue engineering due to its biocompatibility and the possibility of a spatial and temporal control. This method allows the formation of complex shapes and permit the control of the mechanical and biochemical properties of the gel (Annabi et al., 2014)(Koh et al., 2003)(Stephens-Altus et al., 2011)(Nemir et al., 2010).

3. Reinforced Composite Hydrogels

The use of a hydrogel in the biomedical field is a challenge. A single polymer cannot meet all the required chemical, physical and biological properties. This limitation can be overcome by the introduction of other polymers or nanoparticles into the basic hydrogel, in order to create a robust hydrogel with better properties and tunable characteristics (Liu et al., 2009)(Mogharabi et al., 2012).

3.1 Polymer-composite hydrogels

The use of hydrogels is becoming wider in the field of tissue engineering, biomedical and cosmetic. In order to extend their applications and overcome the difficulties faced during their utilization, polymer composite hydrogels are used to improve the characteristics and properties of hydrogels. It consists on a mixture of various synthetic or natural materials which can easily blend together and form a homogeneous phase due to their hydrophilic nature (Annabi et al., 2014). A phase separation may occur when the second polymer is introduced in an inappropriate way. Many composite hydrogels have been studied. Alginate is the best example because it is widely used and has several advantages especially in medical applications. Except that alginate polymer does not present the required interaction with the cells, thus, other polymers may be included to ensure the requested properties. The formation of alginate/silicate scaffold showed

an increase of gene expression of certain cells like osteoblasts cells which improve the application of hydrogels in tissue engineering (Schloßmacher et al., 2013). Another polymer composite hydrogel was prepared by Xiao & *al*. (Xiao et al., 2011) using GelMA and silk fibroin.

GelMA is mixed with Silk Fibroin solution and crosslinked following its exposure to UV light in the presence of a photoinitiator. 70% of methanol are then added and develop a GelMA/Silk

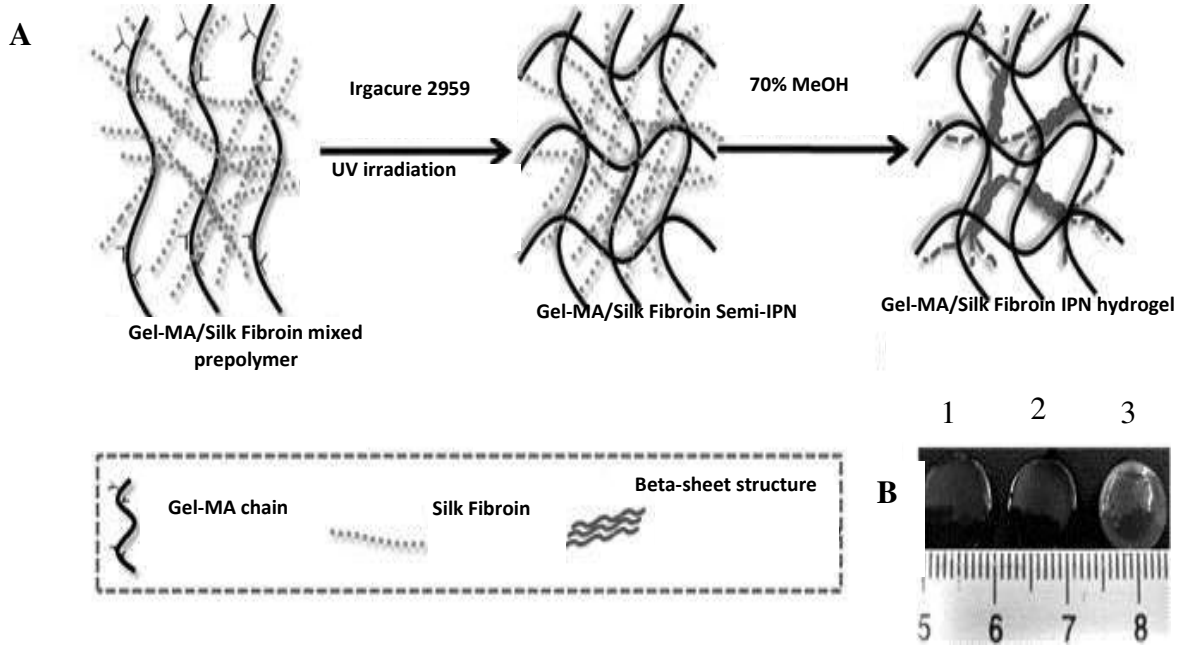

Fig.5A. Formation of polymer composite hydrogel: GelMA/ Silk Fibroin hydrogel. B. Optical representation of 1. GelMA, 2. GelMA/Silk Fibroin Semi-IPN, 3. GelMA/Silk Fibroin IPN hydrogel (Xiao et al., 2011).

Fibroin IPN hydrogel (fig. 5). The formed network present dense and elastic properties with a reduction of the degradation rate and swelling ratio. NIH-3T3 fibroblasts cells, cultured at 37°C in the hydrogel, are attached on the surface where they can spread and proliferate.

Polyvinyl alcohol has outstanding characteristics in tissue engineering, but presents also a deficit in cell attachment due to its highly hydrophilic character. The reinforced polyvinyl alcohol/gelatin hydrogel showed a remarkable improvement of the protein adsorption (Liu et al., 2009).

Similarly, alginate/gelatin hydrogel has the ability to entrap an enzyme and ensure its stability (Mogharabi et al., 2012). These two polymers react together and form a complex with regular structure and strong mechanical properties. This reaction consist on the combination of the

amino groups of gelatin and the carboxylic functions of alginate (Fadnavis et al., 2003). The composite hydrogel is biocompatible and ensures the attachment and the proliferation of cells within the hydrogel.

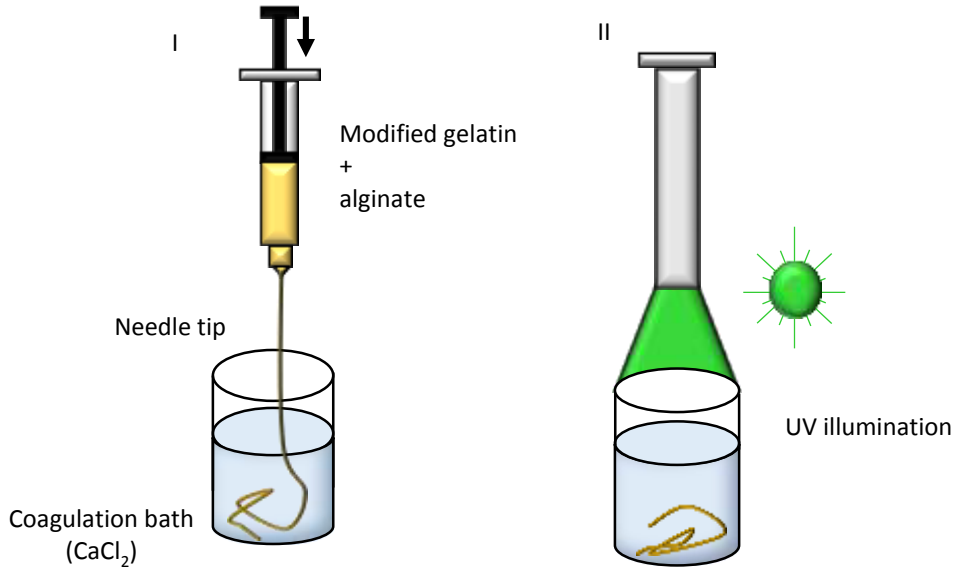

Fig.6. Synthesis of GelMA/Alginate hydrogel. I. Alginate mixed with GelMA and introduced into a solution of CaCl2 for alginate gelation. II. The process is followed by the exposure of the product to UV light inducing GelMA gelification (Tamayol et al., 2015).

Alginate can also be mixed with GelMA, the methacrylate modified (Tamayol et al., 2015) gelatin, in order to produce a gel with very interesting properties. The crosslinking process occurs in two stages. The mixture is firstly added to $CaCl_2$ solution to ensure the alginate gelification. The obtained hydrogel is than exposed to UV light to crosslink the GelMA polymer (fig. 6).

3.2 Nanocomposite hydrogels

In order to create reinforced nanocomposite hydrogels, nanoparticles from various sources and with different forms, can be incorporated into hydrogels. The added particles can undergo modifications to have remarkable physical and chemical properties. They can be used to encapsulate an active molecule to protect it from the surrounding environment or even protect the environment of undesirable effects of the molecules. In some cases, nanoparticles can

provide the desired physicochemical properties of the active molecule to make it more suitable for the system when it is released (Vandamme et al., 2007).

These nanoparticles strength the hydrogel and give it better properties which enlarge its field of application (fig. 7). For example, Shin et al. (Shin et al., 2012) have developed a biocompatible nanocomposite hydrogel by incorporating carbon nanotubes (CNTs) into gelatin

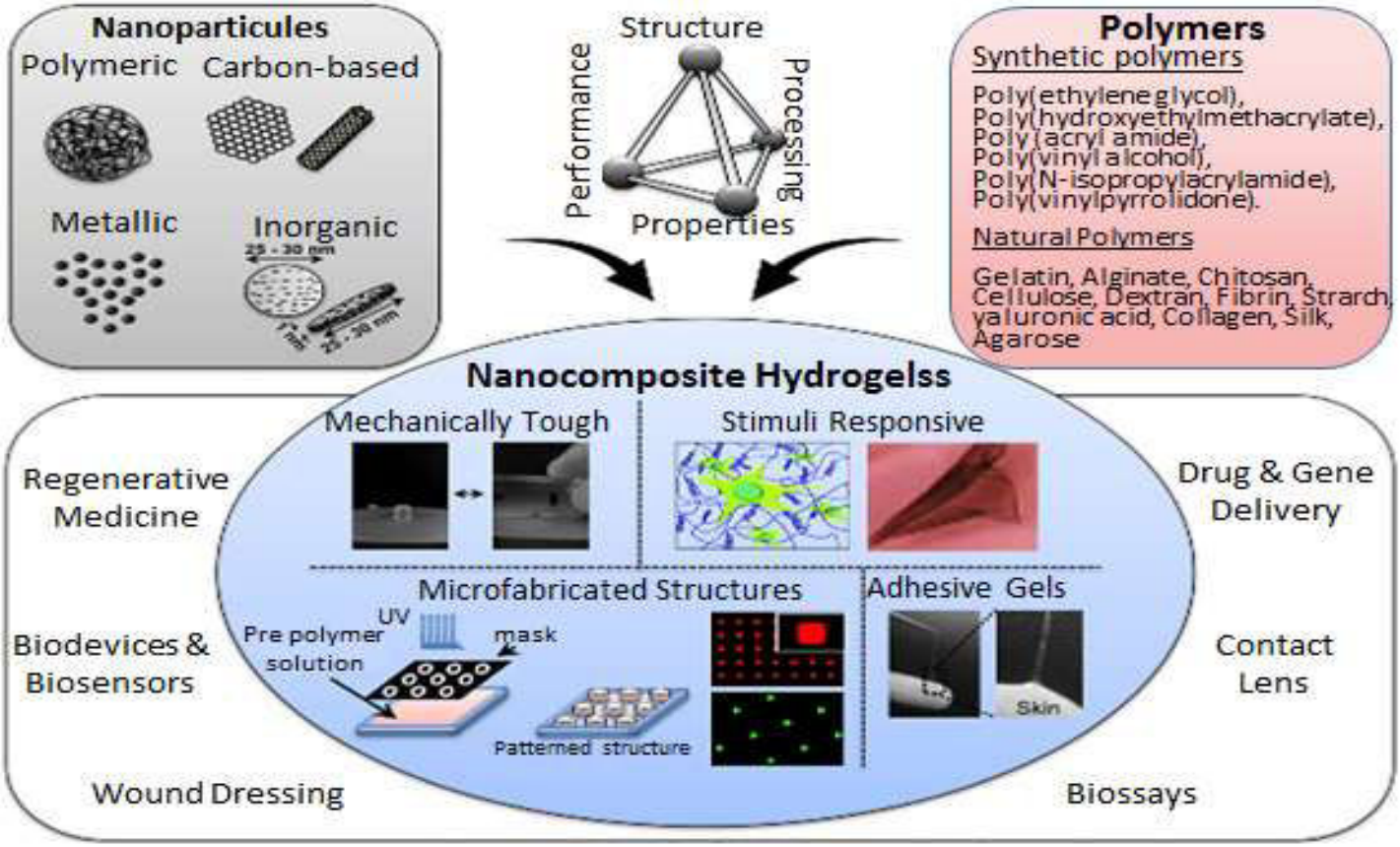

Fig.7. Different types of nanoparticles can be combined with natural or synthetic polymer to form, after the crosslinkage, nanocomposite hydrogels wich have various apllications (Gaharwar et al., 2014).

methacrylate (GelMA). The addition of the nanoparticles enhanced the mechanical properties of the hydrogel (fig. 8a) (Gaharwar et al., 2014). Nanocomposite hydrogels provide superior properties for the hydrogel network and allow a better control of the release of an active molecule specially in drug delivery (fig. 8b) (Gaharwar et al., 2013) (Merino et al., 2015).

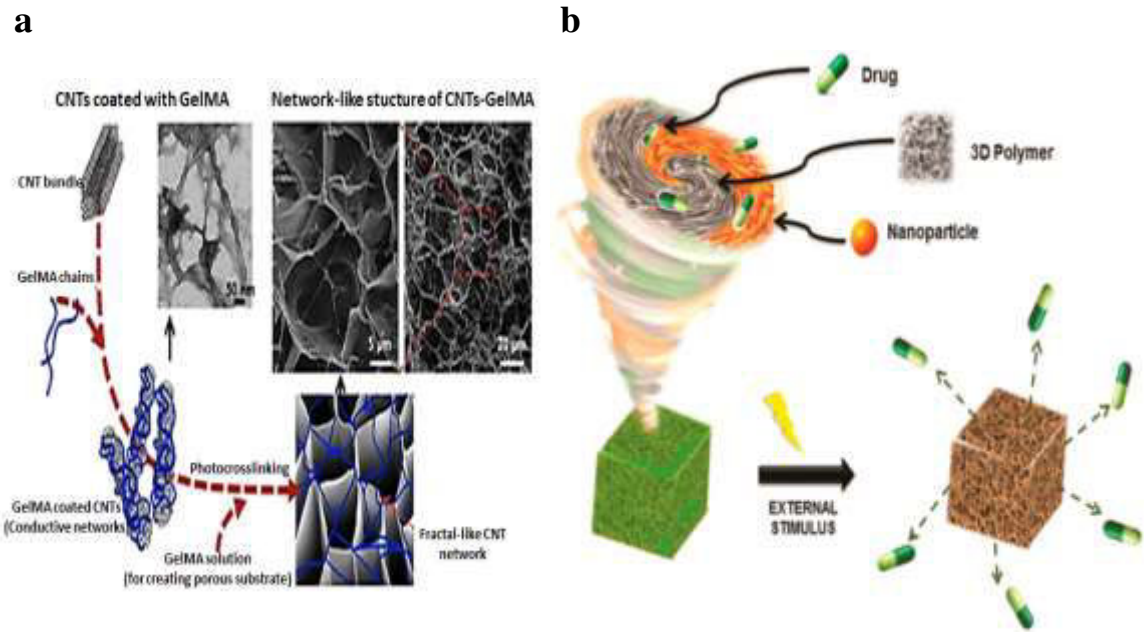

Fig.8. Example of use of nanocomposite hydrogels. (a) CNT-GelMA hydrogel to enhance the network properties (Gaharwar et al., 2014). (b) Nanocomposite hydrogel for drug delivery (Merino et al., 2015).

Liposomes are mainly composed of amphiphilic molecules (both hydrophilic and hydrophobic) called phospholipid (fig.9). Due to this character, in aqueous media, liposomes have a particular capacity of self-sealing, isolating subsequently an aqueous phase in the center. In fact, liposomes are organized in bilayers with apolar tails in the center in order to minimize their contact with water molecules due to their hydrophobic character. The polar hydrophilic heads are exposed to the interior and exterior aqueous phases (Bangham, 1961)(Bouarab et al., 2014).

According to DLS tests, liposomes are negatively charged. It may be due to the negative electrophoretic mobility of most of the phospholipids (Hasan et al., 2014)(Chansiri et al., 1999). Liposomes charge has an influence on the encapsulation technic. It can attract active molecules and create dipole-dipole bonds interaction (Bouarab et al., 2014).

Liposomal encapsulation technology is a technique consisting on the encapsulation of an active molecule in a liposome and transmit it to its target. The purpose of using liposomes is to create a non-toxic, protective environment that allows the survival of the molecule and prevent its degradation or oxidation. In this way, the molecule can be transported to its specific target and perform its work without being damaged (Hemanth kumar and Spandana, 2011)(Akbarzadeh et al., 2013). The encapsulation of an active molecule in a liposome may be either active

consisting on the introduction of the particles during the liposomes preparation or passively where the encapsulation is done after liposome formation.

Nowadays, liposomes are widely used due to several advantages, including their biocompatibility and biodegradability. Moreover, in some cases, liposomes reduce the toxicity of the encapsulated molecule or avoid its contact with a sensitive tissue or particle. Consequently, the immunological or non-desirable effects are reduced. In addition, liposomes create an effective environment for active molecules by increasing its stability and provide transport efficiency to its specific site, while avoiding any adverse effects from the outside environment or from the target site itself (Himanshu et al., 2011)(Akbarzadeh et al., 2013)(Gregoriadis and Florence, 1993)(Hasan et al., 2014). For example, the encapsulation of an enzyme allows a better conservation of its activity and assure a better functioning of the enzyme especially in a gastric environment (McClements, 2015)(Hsieh et al., 2002).

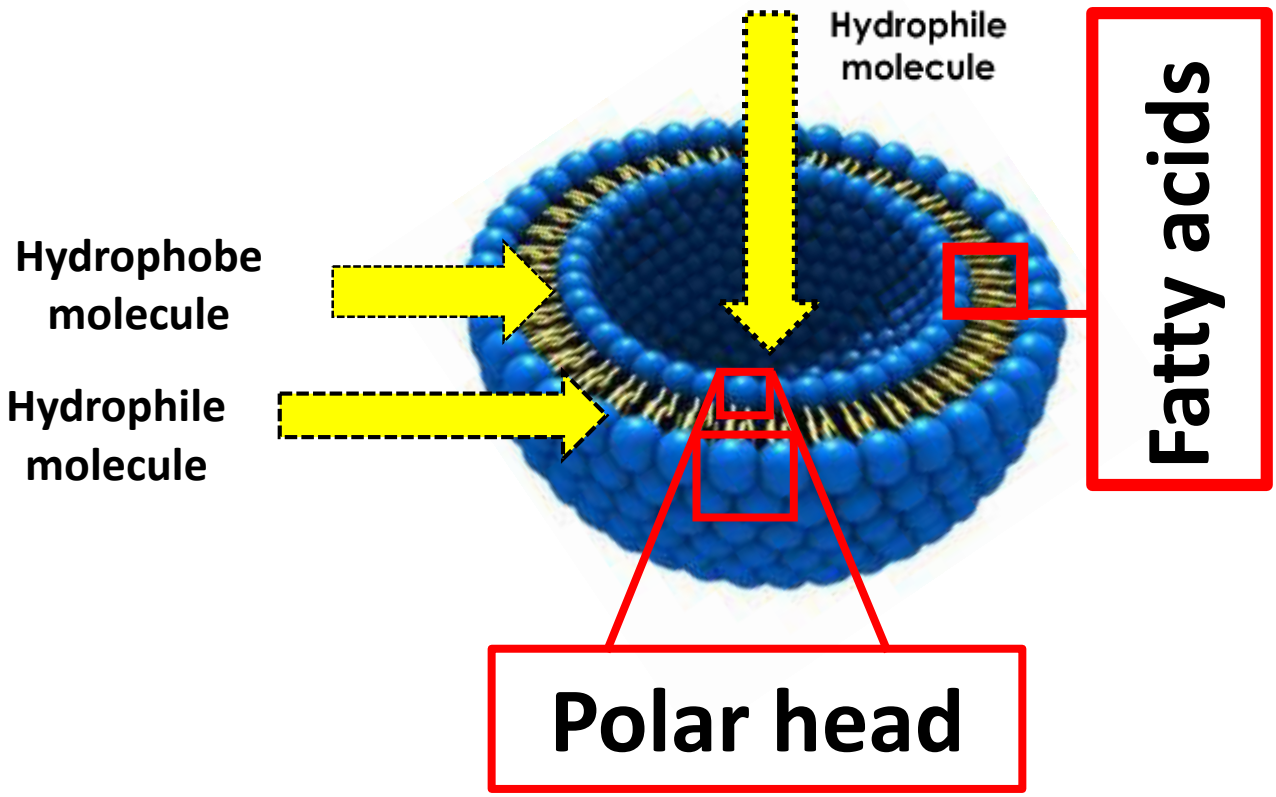

Fig.9. Structure of liposomes, organized in bilayer with hydrophilic polar heads and hydrophobic apolar tails.

Because of its amphipathic character, liposomes can transport hydrophobic or hydrophilic active molecules to their designated target (Atrooz, 2011)(Benech et al., 2002)(Shehata et al.,

2008)(Akbarzadeh et al., 2013)(Trotta et al., 1989)(Bouarab et al., 2014). The hydrophilic active molecules are encapsulated in this aqueous compartment, while lipophilic molecules are caughted by the lipid layers of the liposomes (Immordino et al., 2006)(Akbarzadeh et al., 2013). Hasan et al. showed in their study on curcumin encapsulation a high entrapment efficiency of liposomes (Hasan et al., 2014).

The purpose of using the liposome in nanoscale is to avoid sedimentation risk and avoid being noticed by the consumer (Acosta, 2009)(Zuidam and Nedovic, 2010). The creation of a specific environment for the molecule is not enough, it is essential to free rapidly the encapsulated molecule to exert the desired effect on the target site without losing its properties (Bergstrand et al., 2003)(Qualls and Thompson, 2001)(Maherani et al., 2011). The release of the entrapped molecule involves a change in the liposomes conditions in order to improve the sensitivity of the vesicles to release the charged molecule in response to a stimuli (Needham and Dewhirst, 2001)(Maherani et al., 2011).

Yuba at al. used a pH sensitive liposome as an antigen delivery system. The stable liposomes where modified with MGlu-Dex. MGlu-Dex is the result of the reaction of the dextran with 3-methyl-glutaric anhydride (Yuba et al., 2010). Similarly, yun et al. have developed a thermo-sensitive liposome to control the release of drugs towards tumors tissues (Cha et al., 2014).

In this work, polymer composite hydrogels have been prepared by mixing two polymers alginate which provides good mechanical properties and GelMA which facilitates cell adhesion and spreading. The gelation process occurs in two stages: It starts with the physical crosslinking of alginate consisting on ionic interaction and then a chemical crosslinking of GelMA which occurs under ultraviolet radiation. The prepared hydrogels are then functionalized with amphiphilic natural nanoparticules: nanoliposomes, which can ameliorate the physicochemical and mechanical properties of the formed hydrogels. Multiscale physicochemical analysis were performed on the surface of the different hydrogels by determining the contact angle, the surface energy, the morphology, the mechanical properties, the chemical structure, as well as in their thickness by studying the mechanical properties, the conductivity and the chemical structure (fig. 10). The aim of this study is to determine the interest in preparing polymer composite hydrogels and the effect of the functionalization on the properties hydrogel.

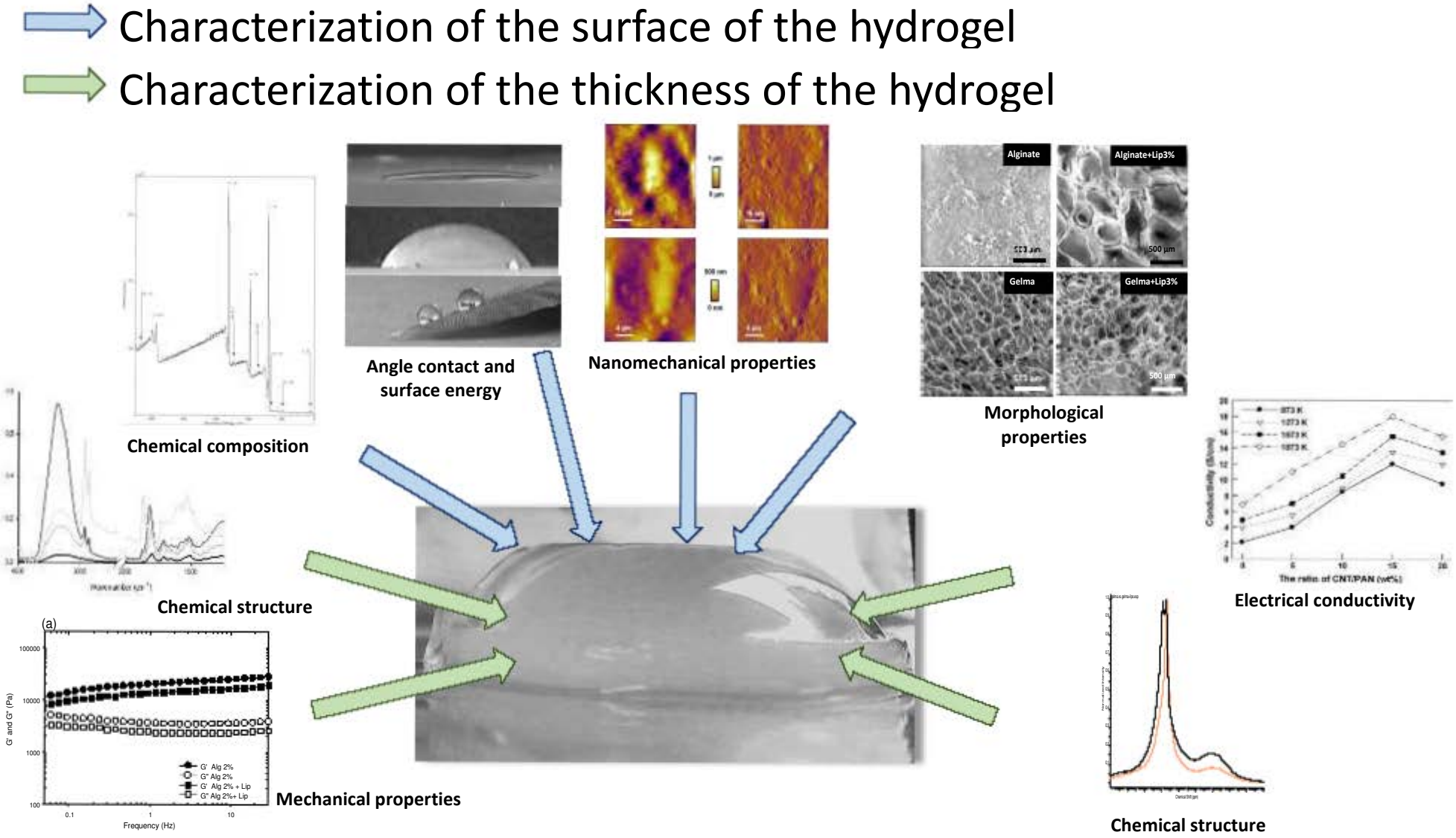

Fig.10. Multiscale characterization of Alginate/GelMA IPN hydrogels, before and after their functionnalization with nanoliposomes.

4. Conclusion

In the past decades, hydrogel has become an interesting material in various applications especially in biomedical, in cosmetic and in tissue engineering. Many of these fields requires determined properties in order to ensure the cells or molecules viability, proliferation and differentiation, without provoking undesirable side effects. To overcome the difficulty of reaching all these requests, advanced hydrogels have been formed to improve the hydrogel properties and make it appropriate for *in vivo* use. Polymer composite hydrogels and nanocomposite hydrogels have shown better mechanical characteristic, improvement in the porosity and degradation rate and ameliorate the cells or molecules conductivity in the hydrogel. These systems are increasingly used due to their usefulness and effectiveness.

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III. Experimental analysis

1. Material

Alginic acid sodium salt (SA) from brown algae (M/G ≈ 1.56). The average viscosity molecular weight M_v of 1.69 10^5 g.mol^{-1} was calculated using the Mark–Houwink–Sakurada correlation: $[\eta] = KM_v^{\alpha}$ Where α = 0.92 and k = 7.3 10^{-5} (Thu et al., 1996).

Calcium chloride dihydrate was obtained from VWR (International, Leuven, Belgium).

Gelatin (type A, 300 bloom from porcine skin), methacrylic anhydride (MA), photoinitiator (PI) 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone and phosphate buffered saline tablets (PBS) were purchased from Sigma-Aldrich (Chemie, Steinheim, Germany). Rapeseed lecithin were acquired from Solae Europe SA society (Geneva-Switzerland).

2. Nanoliposomes preparation

In the first time, we add 3 and 5g of rapeseed lecithin into 97 and 95 mL of water, the suspension was mixed for 5-6 hours under agitation at inert atmosphere (nitrogen). Then, the samples droplet size was decreased in first time by sonication at 40 KHz for 2 min (1s on, 1s stop) in an ice bath. Liposome samples were stored in glass bottle in the dark at 4°C. Then, 8.6% (v/v) of nanoliposomes were added to each solution.

3. Measurement of Liposome Size and Electrophoretic Mobility

The size distribution (Mean diameter and Polydispersity Index) and electrophoretic mobility (μE) of the liposome dispersions were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, UK). Prior to measuring size, the samples were diluted (1:400) into a distilled water ultra-filtrate which measures the mass distribution of particle size as well as the dispersed particles electrophoretic mobility that were carried out to evaluate the surface net charge around droplets. Measurements were made at 37°C with a fixed angle of 173° .Sizes quoted are the z-average mean (dz) for the liposomal hydrodynamic diameter (nm).Calculation of electrophoretic mobility (μm.cm/Vs) was done by the instrument special. Measurements were repeated three times.

4. Synthesis of GelMA

10% of gelatin was dispersed in PBS and stirred at 60ºC until fully dissolved. Then, 8 mL of MA was added very slowly and dropwisly under stirring. After 3h, the reaction is stopped following a dilution x5 using warm phosphate buffered saline. Diluted GelMA was then

dialysed at 40-50ºC for one week against distilled water using a dialysis membrane (Spectro/Por molecular porous membrane tubing, MWCO 12-14,000, SpectrumLabs, Inc., Rancho Dominguez, CA, USA). The solution was then lyophilized during 1 week.

5. Solutions preparation

To prepare alginate solution, 2 g of alginic acid sodium salt was dispersed into stirred 100 mL double distilled water. After complete solubilization, the alginate solution was degassed to remove air bubbles before its use.

GelMA solution (30%, m/v) was prepared by dissolving the freeze-dried powder into a PBS solution at 40 °C. Then, 1% of PI was added and the temperature was increased to 80ºC to allow its solubilization. The alginate/GelMA solution was prepared at 40 °C by mixing alginate (2%, 1%, 0.5%, m/v) with GelMA (30%, m/v), respectively. Then 1 % (m/v) of PI was added to the final mixture.

6. Hydrogels preparation

6.1 Alginate hydrogel

2 mL of alginate solution was poured carefully on 5 mL of calcium chloride solution (2% m/v) in petri dishes. The obtained hydrogel was incubated into the $CaCl_2$ during 24 h at 4ºC in order to complete the reticulation process and then washed with distilled water before use.

6.2 GelMA Hydrogel

1 mL of GelMA solution was poured on a specific mold with the controlled dimensions and then exposed to UV light (360-480 nm) for 240 s. The PI absorbs the UV light and transform the solution onto gel. The obtained gel was then washed with distilled water before use.

6.3 Alginate/GelMA IPN hydrogel

The crosslinking process occurs in two stages. First, 2 mL of the mixture was poured on 5 mL of $CaCl_2$ solution (2%, m/v), using a mold with thickness and diameter control, in order to allow alginate cross-linking. Then the obtained semi-IPN was exposed to UV light for 240 s to allow the free radicals photopolymerization of the GelMA and the formation of stable discs (with 2 diameter and 1.5 height). The final hydrogel was then rinsed with PBS to remove the excess of

$CaCl_2$. The size of the discs can be controlled by changing the size of the mold as well as the viscosity of the solution.

7. Characterization of the solutions

7.1 Zeta potential measurements

Zeta potential of the various hydrogels were measured with Zetasizer Nano ZS (Malvern Instruments Ltd., UK) using dynamic light scattering (DLS). The determined potential is an important parameter to analyze the effect of the nanoparticles in suspension. The samples were diluted (1:2) and introduced into disposable capillary cells equipped with gold electrodes designed to afford maximum zeta potential measurement capability. All measurements were carried out at 37 °C.

7.2 Surface tension

The surface tension of aqueous solutions of alginate, GelMA and the mixture of the two polymers, with and without liposomes was measured using a tensiometer Wilhelmy plate type (krüss GmbH, Hamburg, Germany) at a constant temperature of 37 °C. The platinum plate was cleaned with a flame treatment in order to remove any contaminating substances. The plate was then inserted into the solution placed in a circular glass vessel with an immersion depth of 2.0 mm. The surface tension was measured with a crosshead speed of 10 mm / min and a probe sensitivity of 0.005g and a duration of 60 seconds. The presented values of the surface tension are the mean of three measurements.

8. Characterization of the prepared hydrogels

8.1 Contact angle

The wettability of all samples is evaluated by the measurement of the contact angle formed on the surface of the hydrogel using the sessil drop method. Before starting the test, the gel is taken out of distilled water and dried superficially in an oven at body temperature of 37 °C. After the sample reach the temperature, uniform distilled water drops of 0.75 µl were deposited on the gels using a microsyringe. The liquid on the surface of the gel form a static angle measured with a goniometer (Digidrop, GBX instruments, France) equipped with an image analysis software (Windrop, France). All data presented the mean values of three replicates performed on the surface of each hydrogel.

8.2 Surface energy measurement

The surface energy of the hydrogel is calculated using the Owens-Wendt theory based on the contact angle measurements. It includes two constituants, the dispersion γ^d and the polar γ^p, composing the total of surface energy (Zhang et al., 2013)(Rotta et al., 2009).

$$\gamma^T = \gamma^d + \gamma^p \tag{1}$$

Owens and Wendt introduce the contact angle θ toward the equation and gives the following relation (eq.2):

$$\gamma_L\,(1 + cos\theta) = 2\sqrt{\gamma_S^d}\sqrt{\gamma_L^d} + 2\sqrt{\gamma_S^p}\sqrt{\gamma_L^p} \tag{2}$$

where γ_L is the liquide surface tension, γ_S^d and γ_S^p form respectively the dispersive and the polar components of the hydrogel, γ_L^d is the dispersive component of the liquid and γ_L^p is the polar component of the liquid. The total surface energy of each hydrogel is determined by measuring the contact angle of the hydrogels using two different liquids: the water and the diiodomethane before and after the nanofunctionalization of hydrogels.

8.3 Scanning Electron Microscopy

The surface morphology of the different hydrogels were characterized by Quanta 200 high resolution scanning electron microscope low vacuum mode (FEI-Japon). The use of "low vacuum" mode presents powerful tools for the observation of the surface topography of biological materials without sputter-coated. It also preserve the delicate samples from the electron beam damaging. The maximal resolution attained, employing an electron beam spot size of 7, could be lower than 5 nanometers. A large field detector (LFD) was used in order to execute this analysis. The squared shaped samples with dimensions 9mm x 9mm were inserted and maintained in a holder inside the SEM chamber and the tests were performed at laboratory temperature of 25°C with a relative humidity of 50%. A partial vacuum was created within the chamber and the air was evacuated using a pump which provide a regular pression of 60 mbar.

The images were taken from a distance of 10 mm with an acceleration voltage of 15 kV. The pictures were provided utilizing logical “xT microscope server”.

8.4 Conductivity

The conductivity measurements were performed with the use of a conductimeter (HD 2156.1, Delta OHM). The hydrogel is removed from the distilled water and then dried, because the presence of water can affect the result. The sample is then placed on a contact probe and the conductivity was displayed on a LCD display.

8.5 Viscoelastic measurements

Dynamic viscoelastic measurements were carried out using a kinexus pro rheometer (Malvern Instruments, Orsay, France) equipped with a plate-and-plate geometry (20 mm).

A dynamic frequency sweep test from 0.001 to 100 Hz was performed to determine the dynamic storage (G') and viscous (G") modulus, at a strain rate confirmed to be in the linear viscoelastic range for each type of hydrogel by a prior strain amplitude sweep. During the rheological experiments, the temperature was maintained at 37 °C and the measuring system was covered with a humidity chamber to minimize water evaporation. Three different hydrogel disks were tested for each type of hydrogel with the same experimental settings; average values are presented.

8.6 Measurements of Elastic Modulus by AFM Nanoindentation

AFM experiments were carried out using a MFP3D-BIO instrument (Asylum Research Technology, Atomic Force F & E GmbH, Mannheim,Germany). The nanoindentation method provides the Young's modulus calculated from the force vs. indentation curve. Triangular cantilevers with colloidal probe (SiO_2 particle with radius of 10 μm) were purchased from Novascan (Novascan Technologies, Inc. Iowa State University Research Park, IA 50010 USA). The spring constants of the cantilevers measured using a thermal noise method were found to be 10 pN/nm. Experiments were performed in PBS saline buffer (pH 7.4) at 37°C. Maps of mechanical properties (FVI for Force Volume Image) were obtained by recording a grid map of 32-by-32 force curves at 3 different locations of the film surface. The maximal loading force was 6 nN. Elasticity maps and the corresponding histograms (statistic distribution) were estimated from the analysis of the approach curves according to the Dimitradis model (Dimitriadis et al., 2002) :

$$F = \frac{4E}{3\left(1-\nu^2\right)} R^{1/2}\,\delta^{3/2}\left[1+1{,}133\chi+1{,}283\chi^2+0{,}769\chi^3-0{,}0975\chi^4\right]$$

where δ is the indentation depth, ν the Poisson coefficient, *R* is the colloids radius and *h* the sample thickness. The Dimitriadis correction for finite thickness is defined by χ parameter:

$$\chi = \frac{\sqrt{R\delta}}{h}$$

FVI or force maps were analyzed by mean of an automatic Matlab algorithm described elsewhere (Polyakov et al., 2011).

8.7 X-Ray Photoelectron Spectroscopy

X-Ray Photoelectron Spectroscopy (XPS) spectra were carried out using KRATOS Axis Ultra X-Ray photoelectron spectrometer and performed with monochromatic incident radiation Al-K α X-Ray and a photon energy hv of 1486.6 ev. XPS is an effective technic to characterize the surface of the hydrogels and provide adequate information on the possible interactions in the hydrogel. The test was performed at 150 W and a base pressure of 10^{-9} mbar. Photoelectron data were recorded with survey scans at 1100-0 eV at an analyser pass energy of 160 eV and narrow scans at an analyzer pass energy of 20 eV. The binding energies were corrected by setting the C_{1s} spectral to 284.6 eV.

8.8 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of freeze-dried hydrogels were recorded with a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with an ATR accessory. 128 scans were used for both reference and samples between 4000 and 400 cm^{-1} at 4 cm^{-1} resolution. Spectral manipulations were then performed using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a 9 points smoothing function. After elastic baseline correction using 200 points, H2O/CO2 correction were then applied. Then, spectra were centered and normalized. All tests were run in triplicate.

8.9 H NMR

The chemical modifications are also determined using H-nuclear magnetic resonance (NMR) spectroscopy. The imaging were performed using Bruker Avance III 400 NMR spectrometer (Bruker, Karlsruhe, Germany). The polymers were dissolved, at the same concentration described above, in deuterium oxide (D2O) in order to stabilize the magnetic field and eliminate any perturbation in the spectrum. Hydrogels were introduced in tubes of 2 mm and measured with a stable temperature of 37 °C. The results were recorded with a chemical shift of hydrogen ranging from -4 to 16 ppm and treated with ACD/NMR processor program.

9. Statistical analysis

Results were analyzed by analysis of variance (ANOVA) with 95 % significance level using SigmaPlot software (SigmaPlot Version 11.0, Systat Software Inc., San Jose, CA, USA).

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IV. Results et discussion

IV.1 Preparation and characterization of nanofunctionalized alginate/gelatin methacrylate crosslinked hydrogels

Introduction

Les hydrogels composites présentent des caractéristiques intéressantes. Les propriétés de surface sont des paramètres importants sur le plan scientifique ainsi que sur le plan des applications pratiques. Les caractéristiques superficielles déterminent la sensibilité des hydrogels aux conditions environnantes ainsi que le comportement des matrices 3D. L'étude de l'extrême surface permettra de comprendre l'efficacité des systèmes développés pour différentes applications. En effet, les propriétés de l'extrême surface de l'hydrogel sont clés pour permettre l'adhésion interfaciale entre l'hydrogel et un autre matériau. .

Dans ce travail, les hydrogels composites sont préparés en mélangeant deux polymères, l'alginate et le GelMA. Ces hydrogels sont ensuite fonctionnalisés par des nanoliposomes. La démarche pour déterminer les propriétés de surface consiste en l'étude des systèmes purs formés par un seul polymère et des systèmes composites c'est-à-dire les RIPs, et ce, avant et après nanofonctionnalisation. La surface des différentes solutions présente une certaine force qui se traduit par la tension superficielle. Cette tension a été étudiée pour montrer l'effet des systèmes composites sur les caractéristiques d'adhésion et de cohésion. L'angle de contact et l'énergie de surface déterminés à la surface de l'hydrogel ont mis en relief l'effet de l'association des polymères dans les RIPs et de l'incorporation des nanoparticules molles sur l'hydrophobicité et les propriétés de mouillabilité des hydrogels.

Ce chapitre englobe aussi des paramètres étudiés au niveau de l'épaisseur des hydrogels : conductivité, propriétés rhéologiques. La conductivité est la capacité du matériau à laisser passer et conduire le courant électrique. De même un suivi visco-élastique des hydrogels a été établi dans le but de préciser les différents comportements et déformations pouvant subir les hydrogels face aux contraintes.

Preparation and characterization of nanofunctionalized alginate/gelatin methacrylate crosslinked hydrogels

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Abstract

We reported the preparation of alginate-gelatin methacrylate IPN hydrogels functionalized with nanoliposomes. The physicochemical properties of the polymer solutions and the resulting hydrogels were studied. The IPN hydrogels showed intresting mechanical and microstructure properties and could thus be an excellent candidate for biomedical applications.

1. Introduction

Hydrogels are polymeric networks with three-dimensional configuration capable of immobilizing high amounts of water or biological aqueous fluids (Hamidi et al., 2008).

These biomaterials possess interesting characteristics like swelling properties improving their mechanical flexibility and softness, ability to transport small species through the networks in microscopic environments, ease of functionalization, biocompatibility and facile control of their structure.

Due to these attractive properties, hydrogels are considered as a promising class of biomaterials with a large spectrum of applications in food (Farris et al., 2009), nutraceutical systems (Chen et al., 2006)**,** environment and water treatment (Crini, 2005), energy storage (Xu et al., 2013)(Wu and Xu, 2014) and biomedical applications (Langer and Tirrell, 2004).

The latter applications category covers several domains such as tissue engineering (Lutolf and Hubbell, 2005)(Drury and Mooney, 2003), drug delivery (Peppas, 1997) and microfluidics (Beebe et al., 2000)**.**

Hydrogels could be prepared with natural, semi-natural or synthetic polymers. The synthetic polymers strengthen the mechanical properties of the hydrogel and make its use more controllable in term of gelation, structure, chemical properties and degradation. In contrast, natural polymers have powerful biological advantages of biodegradability and biocompatibility due to their resemblance to extracellular matrix (Annabi et al., 2014).

Among natural polysaccharides, alginate is one of the most used polymer. It is a biocompatible anionic polysaccharide extracted from algae or bacterial biofilm. It is found abundantly and is characterized for being mucoadhesive, non-immunogenic and having low toxicity (Pawar and Edgar, 2012). Chemically, alginate is composed of two uronic acids monomers attached by glycosidic bonds: (1-4)-linked β-D-mannuronic acid (M unit) and C-5 epimer α-L-guluronic acid (G unit). These uronic acid units are distributed along the polymer chain in a pattern of blocks, consisting of homopolymeric blocks (G and M-blocks) and heteropolymeric blocks alternating sequence of M and G residues (MG-blocks) (Pawar and Edgar, 2012). The proportion and the sequential distribution of the M and G residues is closely related the origin from where alginate is extracted.

The gelling conditions of alginate are simple and can occur under mild conditions. It consists on a complexation of the G subunits with divalent cations such as calcium. MG blocks also participate, forming weaker junctions (Pawar and Edgar, 2012). The alginate hydrogel's crosslinks involve electrostatic, van der Waals forces and hydrogen interactions (Braccini and Pérez, 2001).

Gelatin is a cytocompatible protein which can naturally entrap cells due to the presence of bioactive sequences such as Arg-Gly-Asp (RGD) (Barbetta et al., 2005)(Nichol et al., 2010). However, gelatin produces a thermo-reversible physical hydrogel and therefore the chemical modification of this polymer is an interesting alternative to enhance its final physicochemical properties.

Among semi-natural polymers derived from this protein, gelatin methacrylate (GelMA) produced by the by the substitution of the free amine groups of the gelatin with methacrylate anhydride, is an interesting polymer for hydrogels preparation (Van Den Bulcke et al., 2000). It was also demonstrated that GelMA hydrogels constitute a cell-responsive platform for creating cell-laden microtissues and microfluidic devices (Nichol et al., 2010).

The preparation of IPN hydrogels containing both proteins and polysaccharides could lead to a promising system and may advantageously use distinct functional characteristics of each

compound. Many studies have combined alginate and gelatin to create a better environment for tissue engineering approaches (Luo et al., 2015)(Sarker et al., 2014)(Balakrishnan et al., 2005)(Duan et al., 2013)(Sakai et al., 2008).

Furthermore, hydrogel properties (swelling, strength, porosity ...) can be improved by the addition of nanoparticles within or/and on the surface of the matrix. The use of natural and soft nanoparticles is promising to mimic the natural tissues and overcome the body resistance and decrease the immunological effects.

Nanoliposomes are, as well, soft natural nanoparticles well known for their efficiency in the delivery of drugs and genes and in biomedical applications (Díaz and Vivas-Mejia, 2013). They are negatively charged spherical vesicles, composed of one or more lipid bilayer. Their amphiphilic character give them the ability of self-sealing, isolating an intravesicular medium in the center and preventing its contact with the external environment (Hasan et al., 2014).

The aim of this work was to investigate the influence of blending alginate/GelMA polymers as well as the impact of nanoliposomes incorporation into the matrix in order to study to prepare IPN hydrogels. The physicochemical properties of the polymers solution as well as the obtained IPN hydrogels were investigated.

2. Results and discussion

2.1. Solutions characterization

Zeta potential and surface tension of the pure solutions formed of a single polymer (alginate or GelMA) as well as the solutions composed of the mixture of the two polymers were determined before and after their functionalization with nanoliposomes (**Table 1**). Zeta potential tests were carried out at body temperature of 37°C, in order to measure the electrical charge of the particles suspended in the solvent. Alginate is well known to be negatively charged due to the carboxylic groups present in the polymer. After the addition of liposomes, the charge value of the polymer still low because the added soft nanoparticles are negatively charged. Zeta potential of GelMA was around -1.6 mV at pH 5. At this pH value, the zeta potential of the gelatin is normally positive. The methacrylation of gelatin consists on the addition of MA and therefore, the substitution of the NH_2 with COO^-. This reaction reduced the number of free amino groups and decreased the charge of gelatin. The small amount of the added liposomes, did not have an influence on the total charge of GelMA. It may be due to the low quantity of liposomes which did not affect the total charge of the hydrogel or the interaction between the liposomes and the

$NH3^+$ free amino-groups of the gelatin leading to the neutralization of the liposomes charge. Due to the amphoteric character of the GelMA, the positive charged part of the polymer can interact electrostatically with the negatively charged alginate when they are mixed together, and with the liposomes after the nanofunctionnalisation of the solutions. The surface tension of a solution determine the interface performance. Several methods can be used to determine the surface tension, however the method using the Wilhelmy plate remains the most reliable because it reduces the errors due to the viscous forces effects (Lee et al., 2012). The pure alginate had the highest surface tension of 53.1 ± 0.6 mN/m. The result obtained on the alginate solution may be due to several factors: the source of alginate, the presence of impurities or even the concentration of the polymer (Chan et al., 2009)(Kamaruddin et al., 2014). Indeed, an increase in the concentration of alginate provoke an increase of the G blocks which spread in the polymer and improves the activity of the alginate on the surface of the solution (Kamaruddin et al., 2014).

Table 1. Zeta potential (mV) and surface tension (mN / m) of alginate (2%), GelMA (30%) and alginate-GelMA with and without liposomes.

Solutions	Zeta Potential (mV)	Surface Tension (mN/m)
Alg	-82.4 ± 9.3[a]	53.1 ± 0.6[a]
Alg + Lip	-73.9 ± 3.9[a]	35.9 ± 0.4[b]
GelMA	-1.6 ± 0.5[b]	44.4 ± 0.9[c]
GelMA + Lip	-1.3 ± 0.1[b]	45.1 ± 0.9[c]
Alg - GelMA	-21.9 ± 2.5[c]	45.0 ± 1.4[c]
Alg- GelMA+ Lip	-21.1 ± 1.7[c]	40.2 ± 0.6[d]

[a,b,c] Different letters in the same column indicate significant differences among polymer solutions ($p < 0.05$).

The addition of nanoliposomes to the alginate solution decrease 1.4 times the value of the surface tension and affect significantly the behavior of the interface. In fact, the liposomes are rigid due to the low quantity of C=C bonds as well as the presence of saturated fatty acids and

fatty acid mono unsaturated. Moreover, the liposomes are amphiphilic surfactants which lower the surface tension (Kaci et al., 2014). Although added in small amount in the polymer, the nanoliposomes decrease the surface tension of the solution. Contrarily, the addition of liposomes in the GelMA's solution did not have considerable effect on its tension surface. GelMA based on gelatin may have different values of surface tension depending on the gelatin concentration and the pH of the solution (Liu et al., 2014). The gelatin present in the GelMA solution holds polar and nonpolar regions and allows a better diffusion of the amphiphilic nanoliposomes in the solution (Phillips and Williams, 2009)·(Sachithanadam and Joshi, 2013). The mixture of the two polymers showed values tending towards the GelMA curve. But contrarily, the impact of the liposomes on the surface were apparent and provided a significant decrease of the surface tension of the solution.

2.2. Hydrogels characterization

The contact angle measured on the various hydrogels demonstrates the hydrophilic properties of the polymers. Fig. 1.a presents the contact angle of alginate, GelMA and alginate-GelMA composite hydrogels.

The obtained static water contact angle values are comprised between 8.7°C and 33.4°C, showing the hydrophilic character of the various hydrogels which present a suitable characteristic for tissue engineering and drug delivery applications. Alginate presented a significantly lower contact angle than the GelMA illustrating the high affinity of the alginate polymer with water. The presence of carboxylate groups on mannuronic acid chains was responsible of the appearance of hydrophilic properties in alginate hydrogel. The difference of the contact angle between alginate and GelMA may be due to the difference of the charge of the polymers. The negatively charged alginate ensured a more favorable surface to the emplacement of the water molecules than the GelMA polymer (Yamanlar et al., 2011)·(Kolasińska and Warszyński, 2005). Although, the wettability of the GelMA hydrogel was improved by blending the polymer with alginate and therefore, the properties and performances of the hydrogel were enhanced.

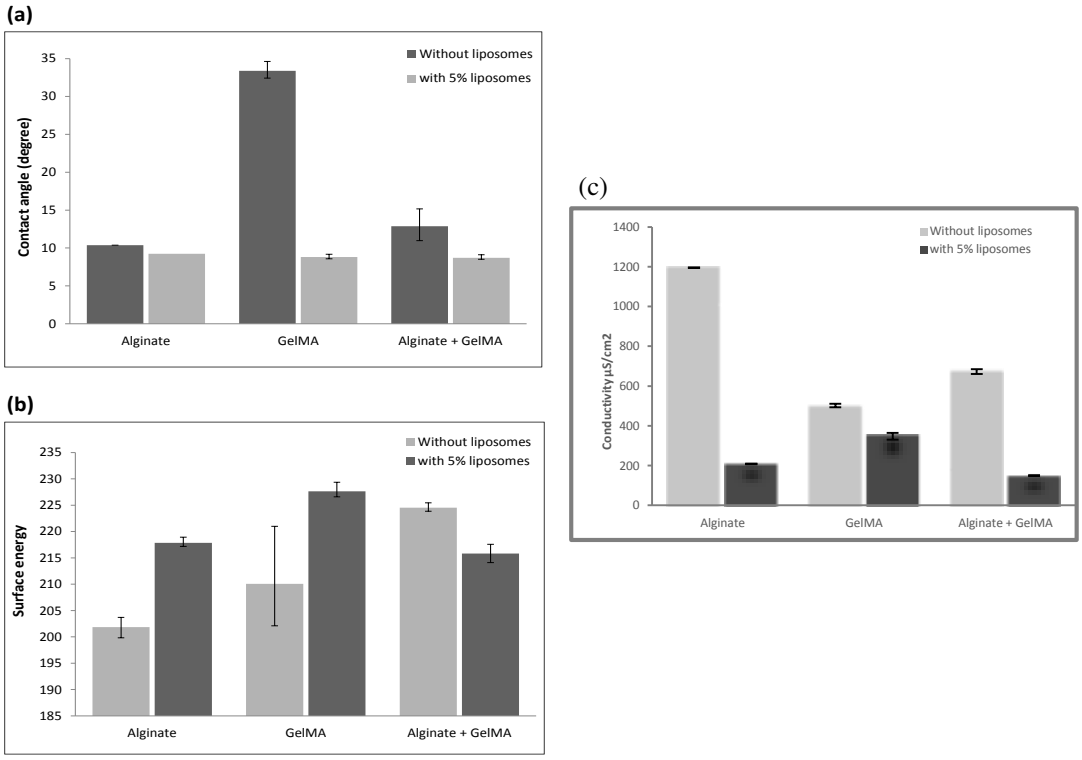

Fig.1. Water contact angle (a), surface energy (b) and conductivity (c) of alginate (2%), GelMA (30%) and alginate-GelMA with and without liposomes. *p<0.05 relative to each hydrogel without liposomes.

The addition of liposomes affected the surface properties of the hydrogels. The presence of liposomes reduced the height of the water droplet indicating an increase of the surface hydrophilic character of hydrogels. In fact, liposomes are organized in bilayers with non-polar

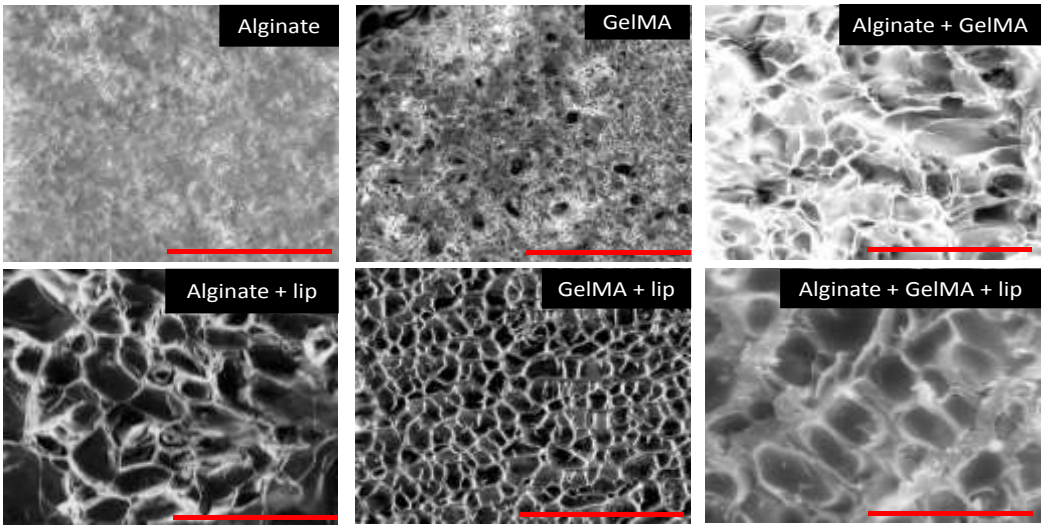

Fig.2. SEM micrographs of alginate, GelMA and alginate/GelMa IPN hydrogels with and without nanoliposomes (scale bar is 300 µm).

hydrophobic tails in the center to minimize the interaction with water molecules and polar hydrophilic heads exposed to the interior and exterior aqueous phases (Bangham, 1961),(Bouarab et al., 2014). This explained the decrease of the contact angle value with the addition of liposomes, since these nanoparticles present only their hydrophilic part in the surface of the hydrogel. Although, the proportion of liposomes in the hydrogel is low, their effect on the wettability of the hydrogels was noticed.

The surface energy of the different hydrogels is high (fig.1.b). It is related to the contact angle as well as the chemical composition on the surface of the hydrogel. The pure GelMA presented an important surface energy due to the presence of the amine groups essentially existing in the gelatin. These groups increased the polar component of the hydrogel and thus raise the total surface energy. The surface energy of the functionalized hydrogels differed significantly compared to the pure hydrogels, even with an addition of a small quantity of nanoliposomes. The polar head of the liposomes increased the polar component on the surface of the hydrogel and improved the surface energy of the hydrogel. It is remarkable that before the addition of liposomes, the IPN alginate/GelMA hydrogel had the highest surface energy showing that the mixture of the two polymers has improved the characteristics of the hydrogels. Contrarily, the addition of the liposomes decreased the surface energy of the hydrogel. It may be due to the aleatory position of the liposomes in the hydrogel.

The conductivity of the hydrogels (fig.1.c) becomes an attractive property especially in tissue engineering application. It improves the delivery of the electrical signal and ensures the growth, the viability and the differentiation of the cells (Shahini et al., 2013).

The obtained results showed a high conductivity of all the samples accompanied with a low resistance of the hydrogels. Even if the addition of the liposomes decreased the conductivity of the hydrogels, the conductivity remained high and the resistance was relatively low.

The SEM micrographs of the different studied hydrogels are shown in fig. 2. The images showed a difference in the surface architecture of pure alginate hydrogel compared to the functionalized alginate hydrogel with nanoliposomes. In fact, the addition of nanoparticles has transformed the rough surface of the alginate into a surface dominated by porous microstructures. The appearance of pores confirmed the dispersion of liposomes on the surface after gelation of alginate. In fact, an electrostatic interaction between the negatively charged liposomes and the divalent cations may cause firstly the aggregation of the liposomes and then disturb the formation of bonds between alginate and calcium during the gelation process. The

disruption of the crosslinking process as well as the position of the nanoparticles resulted in higher porosity. While for GelMA which initially showed pores, the addition of liposomes increased their size and changed their distribution on the surface area of the hydrogel. This confirmed the effect of the nanoliposomes on the control of the pore size in GelMA hydrogel.

As for the alginate/GelMA mixture (fig. 2), the addition of nanoliposomes in hydrogels presented a highly porous surface with more regular and well defined pores on the surface, giving more advantages in term of porosity to the hydrogel.

Dynamic shear oscillation measurements were used to characterize the mechanical properties of the cross-linked hydrogels and the influence of nanoliposomes incorporation on the viscoelastic properties of alginate, GelMA and IPN hydrogels at 37 °C.

Typical mechanical spectra are shown in Fig. 3 (a, b and c), where average G', G″ are presented over a frequency range of 0.05 - 30 Hz. The elastic modulus G' represents the elastic part, the loss modulus G" represents the viscous part of a material. One distinctive feature of all mechanical spectra is that G′ > G″, confirming that the alginate, GelMA and IPN materials have predominantly elastic rather than viscous character. This criterion distinguishes gels from

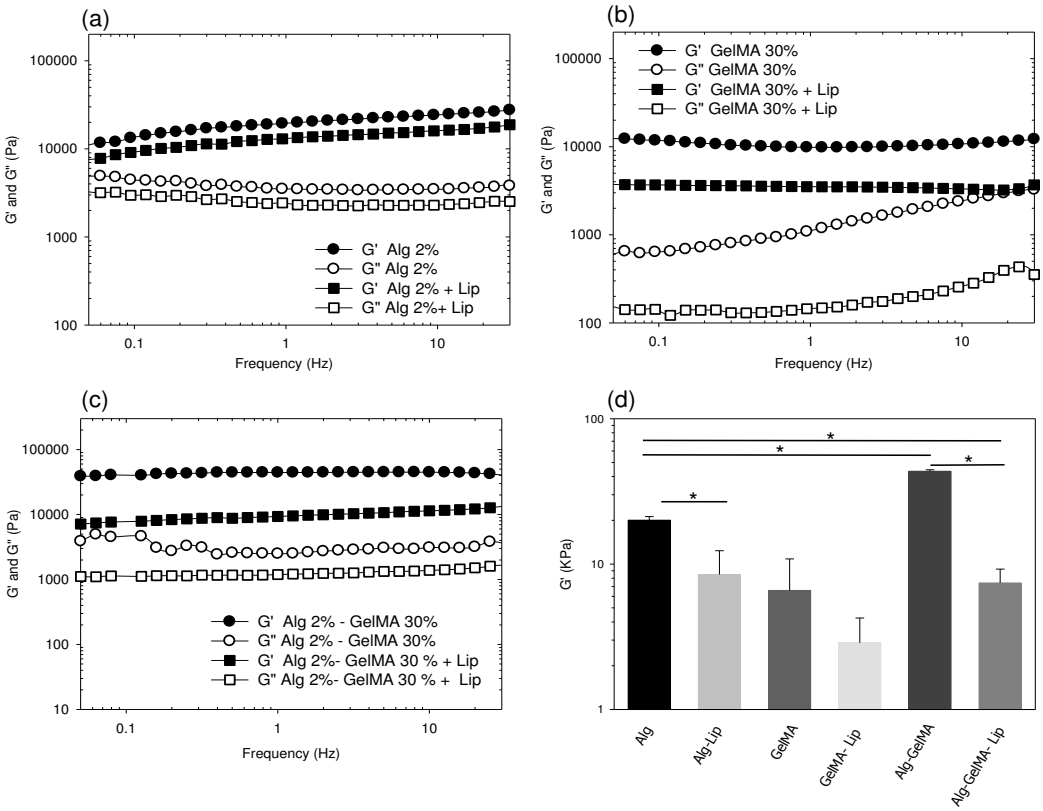

Fig.3. Rheological properties of the studied hydrogels with and without nanoliposomes. Frequency sweep tests of Alg 2% (a), GelMA (b), Alg 2%/GelMA IPN hydrogels (c) and storage modulus (G') at 1Hz for the different systems (d).

viscous liquids and specifies that the deformation energy is recovered in the elastic stretching of chemical bonds (Stendahl et al., 2006). The slow increase of alginate gels' storage modulus with frequency (Fig. 3a) indicated the existence of relaxation processes which could be induced by reversible release of the entrapped entanglements or by intermolecular junctions opening. These junctions resulted from coordination of Ca^{2+} cations to the alginate's interchain cavities made up of G and MG blocks, resulting in development of a so-called 'egg-box' (Grant et al., 1973). In general, the elastic modulus of an alginate gel depends on the number of cross-links and length and stiffness of the chains between cross-links.

The incorporation of liposomes decreased significantly the viscoelasticity of the alginate network where G' is lower than the elastic modulus of the pure alginate gel. In fact, the presence of liposomes could affect the activity of Ca^{2+} during alginate gelation due to potential electrostatic interaction between the divalent cations and the negatively charged phospholipids (Papahadjopoulos et al., 1990). The presence of liposomes and potentially fused or aggregated liposomes (due to calcium cations) with a large size could occasion spatial hindrance during crosslinking and consequently decreases G' (Liu et al., 2012).

For GelMA hydrogels the storage modulus was higher than the loss modulus (Fig. 3b). This proved that they were elastomeric materials at 37 °C. G' was reasonably constant with increasing frequency. In general, the mechanical stability of GelMa gels result from both chemical cross-linking and physical structuring (Van Den Bulcke et al., 2000).

However, at 37 °C GelMA Hydrogels structure is maintained essentially by the chemical crosslinking via C-C bond between GelMA macromolecules.

The incorporation of liposomes decreased but not significantly the elastic modulus of GelMA hydrogels which could be related to the soft nature of the liposomes membrane (Grassi et al., 2006)(Mourtas et al., 2007).

The mechanical spectrum of alginate 2% - GelMA IPN hydrogel (Fig. 3c) showed the highest G' and G'' with no significant frequency dependence. G' of the IPN gel is higher than G' of pure alginate and GelMA hydrogels.

However, the incorporation of liposomes results in a significant decrease of the G' and G". This observation could be directly related to the preparation process of the alginate/GelMA hydrogel.

In fact, the gelation procedure of the mixture starts by the preparation of a semi-IPN by alginate gelation with calcium followed by UV exposure. Therefore, during alginate reticulation, the

calcium cations could interact with liposomes and resulted in fused liposomes with larger size which will interfere not only with the formation of alginate gel (as shown in Fig. 2) but also with GelMA gelation. Therefore, the control of gelation conditions and liposomes amount is necessary for a better modulation of IPN-nanoliposomes final mechanical properties. For a better comparison between the different systems, the elastic modulus G' at 1 Hz of the studied hydrogels is plotted in Fig. 3d. The IPN alginate/GelMA hydrogel showed the highest mechanical stability compared to alginate and GelMA hydrogel, however the incorporation of nanoliposomes in the IPN hydrogels decreased significantly the mechanical strength of the final matrix. Therefore, a study of the influence of nanoliposomes concentration is essential to modulate the final physicochemical properties of the IPN hydrogels.

3.Conclusion

To conclude, we successfully synthetized IPN hydrogels based on alginate and gelatin methacrylate polymers. The obtained matrix was functionalized with nanoliposomes which could be used as nanovehicles to transport drugs, nutriments and active molecules. Finally, the association between GelMA and alginate might be a promising matrix in tissue engineering. Nevertheless, the efficiency of the obtained IPN hydrogels should be evaluated for mouse or human cells and properties like cells attachment, adhesion and proliferation must be investigated.

Acknowledgements

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Conclusion

Les études établies à la surface de l'hydrogel ont montré des matériaux ayant une affinité élevée pour l'eau. Malgré la faible quantité de nanoliposomes incorporés, leur influence sur les propriétés physico-chimiques des hydrogels a été significative. En effet, ces nanoparticules molles amphiphiles présentent des têtes polaires dirigées vers la surface et développent ainsi le caractère hydrophile des différents hydrogels. Les valeurs d'énergie de surface ont confirmé le rôle des liposomes dans l'amélioration des propriétés de mouillabilité. En effet, les liposomes augmentent la présence d'éléments polaires à la surface des hydrogels entrainant ainsi une élévation de l'énergie de surface du matériau. La tension superficielle des solutions diminue après fonctionnalisation des matrices 3D, ceci est dû au caractère tensioactif des liposomes.

La conductivité, un autre paramètre important, a été déterminée, avant et après la fonctionnalisation. Même si l'ajout des liposomes a entrainé une diminution de la conductivité, la résistance des hydrogels fonctionnalisés reste relativement basse.

L'étude des propriétés viscoélastiques des hydrogels fonctionnalisés a montré des modifications significatives notamment au niveau de l'alginate dont la gélification a été perturbée par la présence des nanoliposomes. Dans le but de comprendre ce phénomène, une étude plus approfondie a été menée dans le chapitre suivant de cette thèse doctorale.

IV.2 3D Bioactive IPN hydrogel functionalized by nanoliposomes

Introduction

Le chapitre précédent montre que les hydrogels composites (RIPs) et la nanofonctionnalisation par des nanoparticules molles présentent de nombreux intérêts. Dans le but de contrôler d'avantage les propriétés physico-chimiques, les hydrogels RIPs ont été préparés avec trois concentrations d'alginate (0,5% ; 1% et 2%) et ont été fonctionnalisés par deux concentrations de liposomes (3 % et 5 %).

La morphologie des hydrogels a été déterminée à l'échelle microscopique par microscopie à balayage électronique. Cette étude permet de visualiser l'effet de l'élaboration d'hydrogels composites ainsi que l'influence de la concentration des polymères et des nanoparticules molles sur la porosité de la matrice 3D. Ces analyses donnent la possibilité de contrôler la taille et la régularité des pores dans les hydrogels.

Le comportement mécanique des hydrogels RIPs, a été déterminé par caractérisation multi-échelle avant et après fonctionnalisation. Les propriétés viscoélastiques des hydrogels ont été étudiées, tout d'abord, à l'échelle mésoscopique par le rhéomètre. Les résultats ont montré les déformations des hydrogels en réponse à l'application des contraintes à différentes fréquences et ils ont permis d'évaluer la stabilité des hydrogels et de préciser la dominance du caractère élastique ou visqueux de chaque matrice 3D.En parallèle, les propriétés viscoélastiques des hydrogels sont déterminées à l'échelle nanoscopiques par observation atomique de la surface des hydrogels en utilisant l'AFM. Ces caractéristiques viscoélastiques sont obtenues suite à un balayage de la surface des hydrogels.

3D Bioactive IPN hydrogel functionalized by nanoliposomes

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Abstract

New innovative biomaterials with high effectiveness are prepared by the functionalization of IPN hydrogels with soft nanoparticles: the nanoliposomes. The polymeric network is formed basically from the combination of alginate and gelatin methacrylate. The physicochemical properties of the hydrogels are studied in multiscale on the surface and in the thickness of the 3D network. The obtained results showed interesting characteristic of the composite hydrogels including strong mechanical properties and high porous surface. The blend ratio as well as the concentration of nanoliposomes are factors controlling the properties of the hydrogels. These advantageous characteristics may open up many applications for hydrogels.

Hydrogels, from naturally occurring biopolymers, are an important class of biomaterials that are widely used in the pharmaceutical and biomedical sectors (Bouhadir et al., 1999)(Vieira et al., 2008). Among the naturally occurring biopolymers, alginate and gelatin are extensively used for many biomedical applications because of their biocompatibility and biodegradability (Boanini et al., 2010)(Vieira et al., 2008).

Formed by crosslinking of hydrophilic polymers, hydrogels are biomaterials which have been extensively used in tissue engineering, as scaffolds supporting cell attachment and growth as well as drug delivery systems (Boateng et al., 2008)(Peppas et al., 2006). Hydrogels form an ideal choice for tissue engineering owing to their close resemblance with native extracellular matrix (ECM), inherent cellular interactions, biocompatibility, water holding capacity and

fantabulous biological performance. The porosity in hydrogels permits local angiogenesis and fluid flux which is very crucial for neo organogenesis (Gerecht et al., 2007).
Hydrogels with their beneficial characteristics such as high water content and controllable biodegradability have been widely used as materials for generating 3D ECM scaffolds.
These biomaterials can effectively grow the cells onto their 3D network, traffic the nutrients, oxygen, and other aqueous metabolites. Because of their close resemblance with the ECM and similarity in the properties these are commonly used for the engineering of organ or organ parts (Drury and Mooney, 2003). The relatively weak mechanical properties of hydrogels remain a major limitation for their application as load-bearing tissue scaffolds. Several solutions have been proposed to improve the mechanical properties of hydrogels such as double-network (DN) hydrogels (Gong et al., 2003)(Nakayama et al., 2004)(Weng et al., 2008), nanocomposite hydrogels (Haraguchi and Takehisa, 2002), and ionically cross-linked triblock copolymer hydrogels (Henderson et al., 2010).

With the rapid development of nanotechnologies in recent years, extensive research efforts are being made to develop nanoparticles (NPs) for various biomedical applications. NPs can be engineered from a variety of sources (e.g., polymers, minerals, metals, and semiconductors) and into different shapes (e.g., spheres, rods, shells, wires, and tubes) (De et al., 2008)(Kestell and DeLorey, 2010)(Rotello, 2012). These nanoparticles are incorporated in hydrogels in order to create reinforced nanocomposite hydrogel to improve the physico-chemical, mechanical properties and cell adhesion, viability, and direct cells to self- assemble in three dimensions. Several studies have been reported the functionalization of polymers with gold and carbon nanotubes (CNTs) to enhance the electrical signal propagation (Dvir et al., 2011)(Shin et al., 2013)(You et al., 2011). Some of these nanoparticles like as Au can improve the electrical conductivity but not increase the elastic property of hydrogel (Dvir et al., 2011).

In this work, we developed a composite 3D scaffold by incorporation of active nanoliposomes into gelatin methacrylate (GelMA), which is a UV-crosslinkable and alginate that it is easily crosslinks through ion transfer upon exposure to calcium chloride ($CaCl_2$). GelMA is chemically modified with acrylic functional groups to render excellent photopatternable properties, allowing the fabrication of biocompatible microscale structures. The used nanoliposomes contain the micronutriments in their structure.

Free standing nanoliposomes-IPN hydrogels (alginate and GelMA) with two nanoliposomes concentrations were developed using a sonication-free nanoliposomes and hydrogel formation

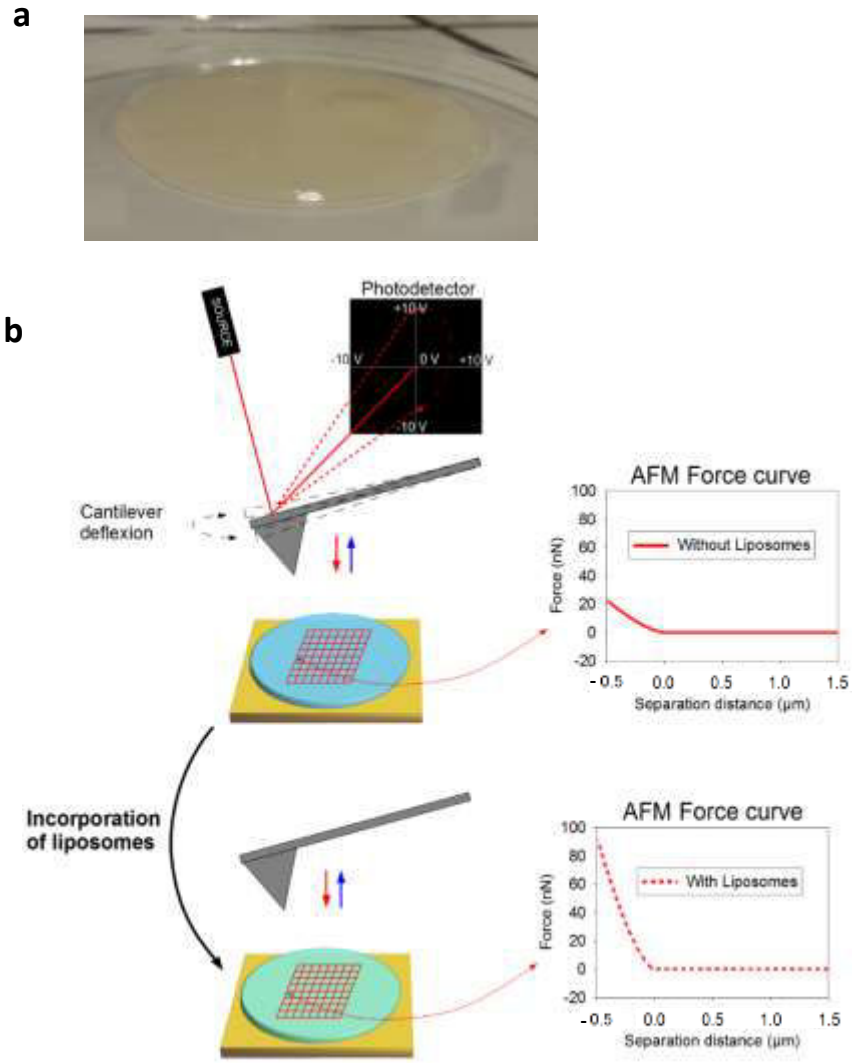
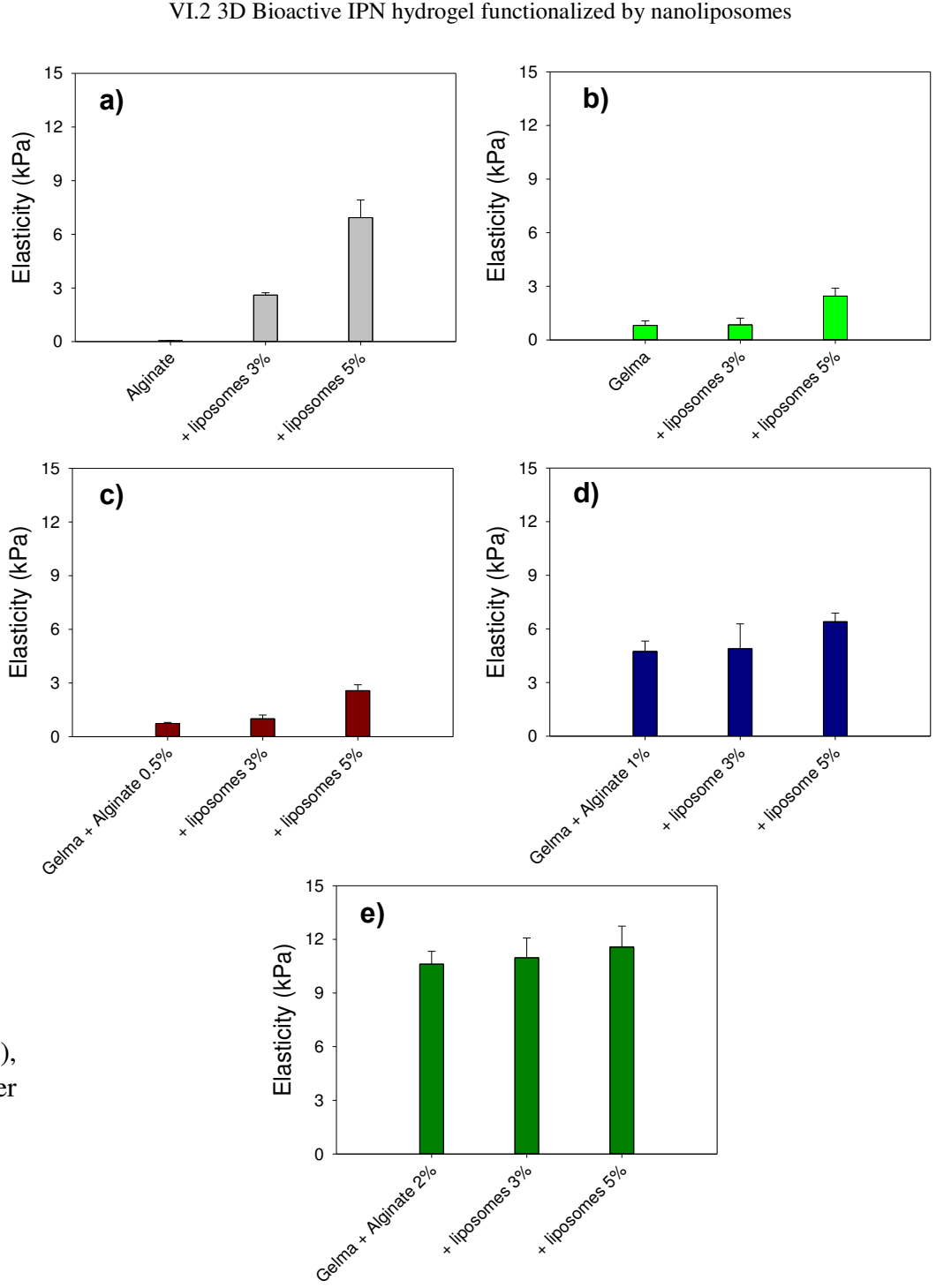


Fig.1. Image of 3D structure of hydrogel (a), AFM force curve (b), Evolution of the biomaterials elastic modulus before and after nanoliposomes functionalization (c).



by UV-crosslinking for GelMA and $CaCl_2$ for alginate under physiologically relevant conditions. The average particle size of nanoliposomes was 110 ± 0.2 nm with an electrophoretic mobility 3.41mV. We obtained free standing nanoliposome-hydrogels with an uniform surface and color beige where no evidence of aggregation was observed for each nanoliposome-loaded hydrogel composite a homogeneous distribution of nanoliposomes in hydrogel (Fig. 1a). The functionalize hydrogel presented an excellent mechanical properties at nanoscale for all of the systems.

Mechanical properties were measured by Atomic Force Mictoscopy (AFM) nanoindention technique using a colloidal probe (Fig. 1b). Each bar and corresponding errors derived from an average of 1024 values (Fig. 1c). Gels were based on a mixture of GelMA and alginate at 50:50 with various cross-linking rate of 0.5, 1 and 2% of the alginate compound. In addition, nanoliposomes were incorporated inside the mixed gels with two blending rate of 3 and 5%, respectively. Then, gels were analyzed by colloidal probe AFM and 1024 measurements were taken over 20µm×20µm surface area for each sample and each replicate (Table 1). The native and pure gels containing only alginate and GelMA are characterized by elastic moduli of about 0.05 ± 0.01 and 0.81± 0.25 kPa, respectively. This result indicates that alginate gel cross-linked at 2% is 16-times softer than the GelMA sample. When the alginate gel was blended with nanoliposomes at 3 and 5%, a drastic increase of the elastic modulus is observed. The elasticity increases from 0.05 kPa up to 2.60 kPa and 6.97 kPa for blending rate of 3 and 5%, respectively (Table 1). This increase emphasized that the nanoliposomes are involved in the gel reinforcement with stiffening factors of ca. 50 and 140, respectively. In the case of GelMA film, this effect is weaker with stiffening rate in range of only 1 to 3.

Table1. Evolution of the biomaterials elastic modulus before and after nanoliposomes functionalization.

Young modulus (kPa, n = 1024)				
Gel composition		**Nanoliposomes blending (%)**		
Alginate	**GelMA**	**0**	**3**	**5**
0.5%	-	0.05 ± 0.01	2.60 ± 0.13	6.93 ± 0.98
-	+	0.81 ± 0.25	0.85 ± 0.37	2.45 ± 0.44
0.5%	+	0.74 ± 0.05	0.79 ± 0.05	2.58 ± 0.31
1%	+	4.73 ± 0.58	4.90 ± 1.00	6.70 ± 0.45
2%	+	10.61 ± 0.72	10.97 ± 1.10	11.57 ± 1.16

The analysis of the mechanical properties of the mixed gel containing GelMA and alginate cross-linked at 0.5% shows the same stiffening effect i.e. a weak increase of the elasticity with stiffening rate in range of only 1 to 4. When the cross-linking rate of the alginate gel was increased during the mixed film build-up we systematically observed and increase of the Young modulus from 0.74 kPa up to 4.73 kPa and 10.61 kPa for the cross-linking rates of 0.5%, 1% and 2%, respectively. Here the stiffening seems to be driven by the alginate cross-linking with stiffening rates of about 6 and 14 for the cross-linking rate of 1% and 2%, respectively. Surprisingly, the blending of 3% and 5% of liposomes leads to a slight increase of the mixed gels elastic modulus' with stiffening rates in the range of 1.05-1.42 and 1.03-1.09 for the 1% and 2% cross-linked gels respectively. This result underlines that stiffer is the mixed gel weaker is the mechanical effect leaded by the nanoliposomes.

The surface morphology of alginate's and GelMA's discs before and after nanoliposomes functionalization, and at different ratio of alginate/GelMA were studied to evaluate pore distribution and their architecture by scanning electron microscopy (SEM) (Fig. 2). Alginate disc exhibit a folded surface structure in regular patterns. After adding the nanoliposomes into alginate, the disc surface at two concentrations of nanoliposomes show a totally homogeneous surface morphology without any regular patterns of folding with regular pores. However, the disc of GelMA showed the porous nature of scaffold with pore size ranging from 23 to 104 after nanofunctionalization.

The addition of nanoliposomes to alginate/GelMA matrix yielded heterogeneous and well defined pores (Fig. 2). Simultaneously, a highly interconnected porosity was also produced by the addition of nanoliposome. The interconnected feature of porosity suits the scaffolds for cell attachment, migration in to the deeper struts and neo-vascularization(Duarte et al., 2009)(Rowe et al., 2009). The results obtained from AFM and SEM presented that nanoliposomes modified the surface properties of hydrogels, in particular, the surface properties of alginate's discs.

The incorporated liposomes are non-fusogenic (the liposomes are stable during more than 2 months) however calcium cations could initiate their aggregation and the close contacts between them might therefore facilitate the lipid exchange and even the fusion of lipid bilayers (Tarahovsky et al., 2012).

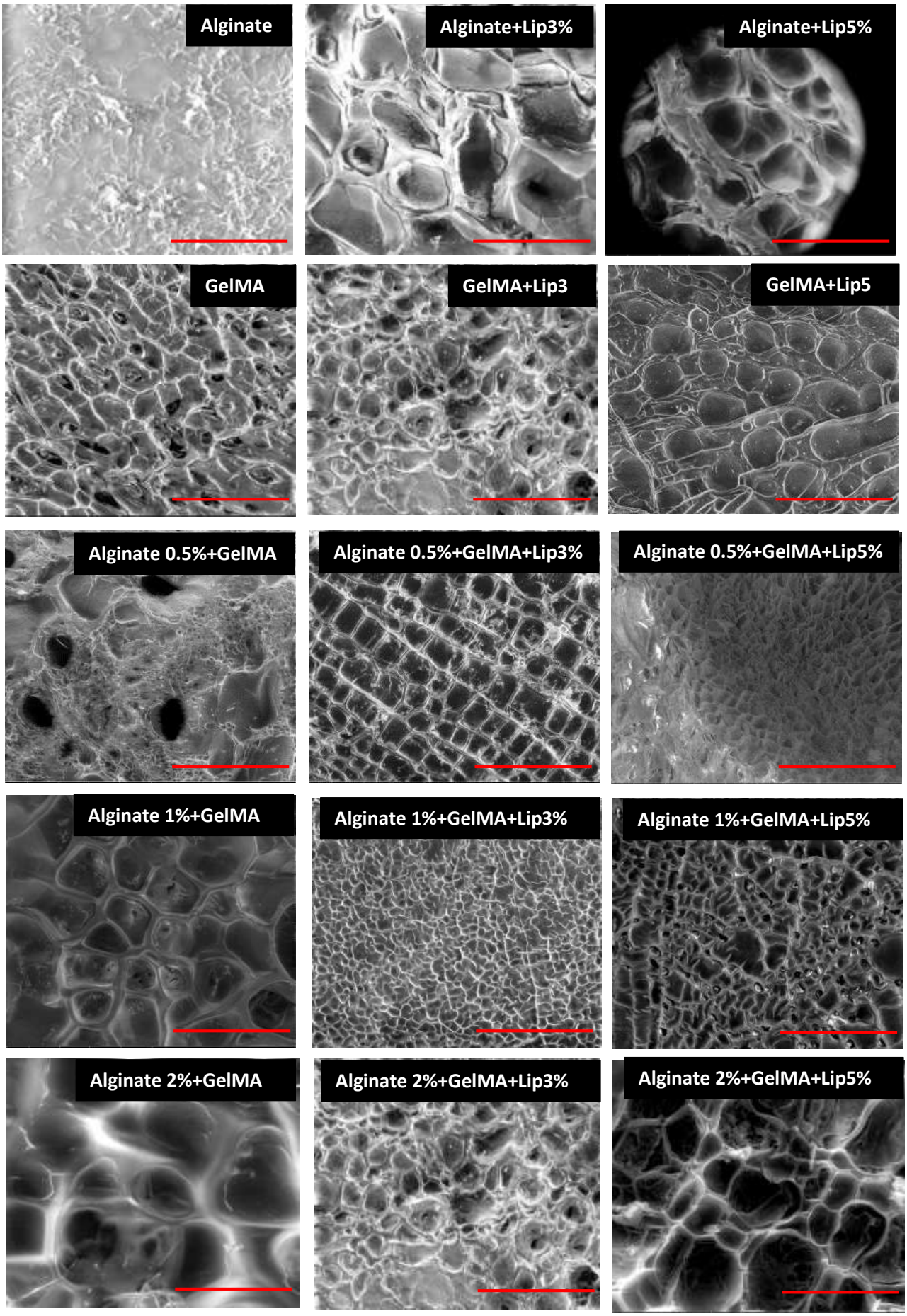


Fig.2. SEM images of 3D hydrogels of various systems before and after functionalization (scale bar is 300 µm).

The presence of nanoliposomes and especially fused liposomes with a large size could occasion spatial obstacle during crosslinking, which increases the porosity of the resulting gels (Fig. 2)(Liu et al., 2012).

Dynamic shear oscillation measurements were used to characterize the mechanical properties of the cross-linked hydrogels and the influence of nanoliposomes incoroporation on the viscoelastic properties of alginate, GelMA and IPN hydrogels at 37°C at mesoscopic scale.

Typical mechanical spectra are shown in Fig. 3, where average G', G″ are presented over a frequency range of 0.05 - 30 Hz. The storage (or elastic) modulus represents the elastic part, the loss (or viscous) modulus represents the viscous part of a material.

One distinctive feature of all mechanical spectra is that G′ > G″, confirming that the Alg, GelMA and IPN materials have predominantly elastic rather than viscous character. This criterion distinguishes gels from viscous liquids and specifies that the deformation energy is recovered in the elastic stretching of chemical bonds (Stendahl et al., 2006).

The slow increase of alginate gels' storage modulus with frequency (Fig. 3a) indicated the existence of relaxation processes which could be induced by reversible release of the entrapped entanglements or by intermolecular junctions opening. These junctions resulted from coordination of Ca^{2+} cations to the alginate's interchain cavities made up of G and MG blocks, resulting in development of a so-called 'egg-box'(Grant et al., 1973). In general, the elastic modulus of an alginate gel depends on the number of cross-links and length and stiffness of the chains between cross-links.

The incorporation of liposomes decrease the viscoelasticity of the alginate network where G' of alginate / nanoliposomes is lower than the elastic modulus of the pure alginate gel. In fact, the presence of liposomes could affect the activity of Ca^{2+} during alginate gelation (at room temperature wherein liposomes are in the gel state which enhances binding of Ca^{2+} due to potential electrostatic interaction between the divalent cations and the negatively charged phospholipids (Papahadjopoulos et al., 1990). The evolution of the elastic modulus of alginate biomaterials after nanoliposomes incorporation is in a good agreement with hydrogels microstructure investigated by SEM.

For all GelMA hydrogels the storage modulus was higher than the loss modulus (Fig. 3b). This proved that they were elastomeric materials at 37 °C. G' was reasonably constant with increasing frequency. In general, the mechanical stability of GelMA gels results from both chemical cross-linking and physical structuring (Van Den Bulcke et al., 2000).

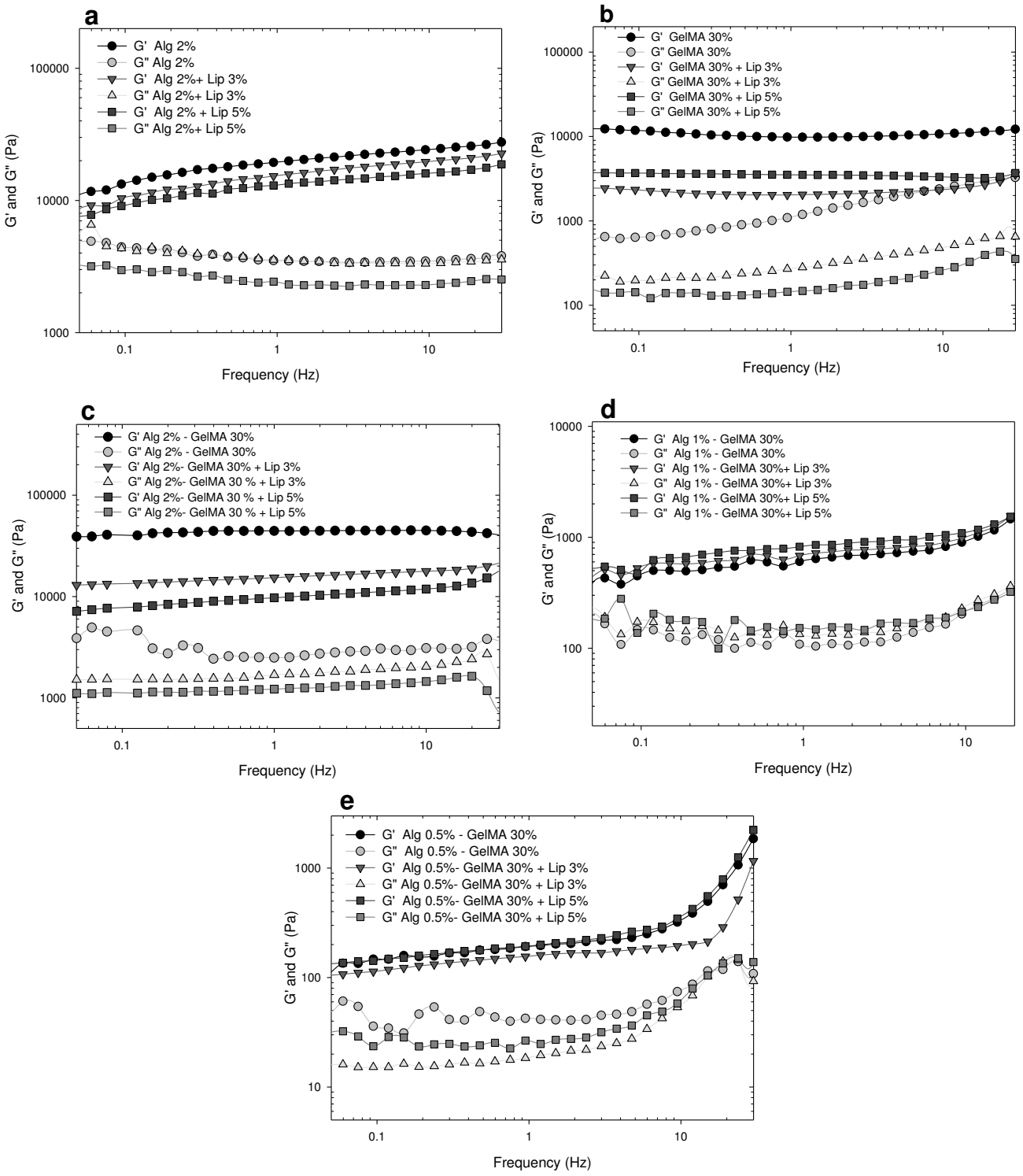


Fig.3. Rheological properties of the various hydrogels with and without nanoliposomes. Frequency sweep tests of (a) Alg 2%, (b) GelMA, (c) IPN of Alg 2% and GelMA 30%, (d) IPN of Alg 1% and GelMA 30% and (e) IPN of Alg 0.5% and GelMA 30%.

The physical gelation is known to result from a thermo-reversible conformation change from the triple helix to individual polypeptide coils at above approximately 40 °C. Upon cooling below 35 °C, the random coils join locally and associate into helix which grows, interconnect and form larger domains until the whole volume is percolated (Joly-Duhamel et al., 2002).
While the chemical cross-linking results through photo-polymerization of vinyl groups after UV initiation (Van Den Bulcke et al., 2000).
At 37 °C GelMA Hydrogels structure is maintained essentially by the chemical crosslinking via C-C bond between GelMA macromolecules.
The incorporation of liposomes decreased but not significantly the elastic modulus of GelMA hydrogels which could be related to the soft nature of the liposomes membrane (Samal et al., 2012a) (Linder et al., 2002).
The mechanical spectrum of Alg 2% - GelMA IPN hydrogel (Fig. 3c) showed the highest G' and G'' with no significant frequency dependence. G' of the IPN gel is higher than G' of pure Alg and GelMA hydrogels.
Gelatin is composed of 18 non-uniformly distributed amino acids with both positive and negative charges and its cationic property is basically due to lysine and arginine residues (Samal et al., 2012b).
Therefore, the stability of the IPN reinforcement role of liposomes could be attributed to intermolecular forces through electrostatic interaction between the unmodified protonated GelMA amino-groups and the negatively charged alginate.
However, the incorporation of liposomes results in a significant decrease of the G' and G". This observation could be directly related to the preparation process of the Alg/GelMA hydrogel. In fact, the gelation procedure of the mixture starts by the preparation of a semi-IPN by alginate gelation with calcium followed by UV exposure. Considering that alginate hydrogel provide the majority of mechanical stability of the IPN hydrogel, the incorporation of nanoliposomes interfere with the egg-box formation and therefore decrease the elastic modulus of the IPN hydrogels.
In contrast, for IPN hydrogels prepared with 1 and 0.5% wt alginate (Fig. 3d and e), the incorporation of nanoliposomes didn't affect the mechanical stability of the final 3D structures. In fact, the decrease of alginate polymer amounts on the final hydrogels could lead to higher chain mobility and therefore to easier macromolecular arrangement which will reduce the spatial hindrance effect of the incorporated nanoliposomes.
In the other hand, at lower alginate concentration (1 and 0.5% wt), the final mechanical stiffness of the IPN gels is given especially by GelMA. As nanoliposomes incorporation didn't affect

the pure GelMA hydrogel, the inclusion of nanoliposomes would not affect the mechanical stability of the IPN hydrogel.

From the rheological investigation of the different hydrogels systems, we can conclude that the IPN alginate 2%-GelMA showed the highest mechanical stability. The variation of alginate concentration highlighted the major role of the alginate in the preservation of the mechanical stability of the final composite structure. The influence of nanoliposomes incorporation was closely related to the matrix composition and to the gelation procedure. In fact, the addition of Ca^{2+} during alginate gelation may accentuate their contribution on the final matrix stability due to size increase as a result of nanoliposomal fusion and aggregation.

In summary, the robust approach for fabrication the functionalize 3D structure from hydrogel was proposed. The used of soft nanoparticle (nanoliposome) improved the physico-chemical and mechanical properties of hydrogels. In addition, we confirm the presence of alginate with highly mechanical properties cab be increase the mechanical properties of the engineering constructs from protein-based hydrogel with low mechanical properties.

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Conclusion

Dans un premier temps, l'étude de l'architecture interfaciale montre l'effet de la nanofonctionnalisation sur les propriétés des hydrogels. Après incorporation des nanoliposomes, des pores apparaissent au sein de la matrice d'alginate. Ceci est certainement dû à l'établissement d'interactions électrostatiques entre le cation divalent (calcium) et les nanoparticules molles chargées négativement qui perturbent la cinétique de gélification ionotropique de l'alginate. L'ajout des liposomes dans les hydrogels formés de GelMA ou les hydrogels RIPs permet le contrôle de la taille des pores ainsi que leur régularité à la surface de l'hydrogel.

Les propriétés viscoélastiques étudiées à l'échelle mésoscopique montrent que tous les hydrogels présentent un comportement plus élastique que visqueux. La nanofonctionnalisation des hydrogels par incorporation de nanoliposomes entraine une diminution des propriétés élastiques par modification de la cinétique de gélification de l'alginate. En revanche, les nanoliposomes n'affectent que légèrement l'élasticité du GelMA.

L'effet de la nanofonctionnalisation sur les propriétés élastiques des différents hydrogels a été également étudié dans ce travail à l'échelle nanoscopique. Les résultats ont montré une atténuation des modifications apportées par l'incorporation des nanoliposomes pour les matrices polymériques présentant une rigidité élevée.

Supplementary Information

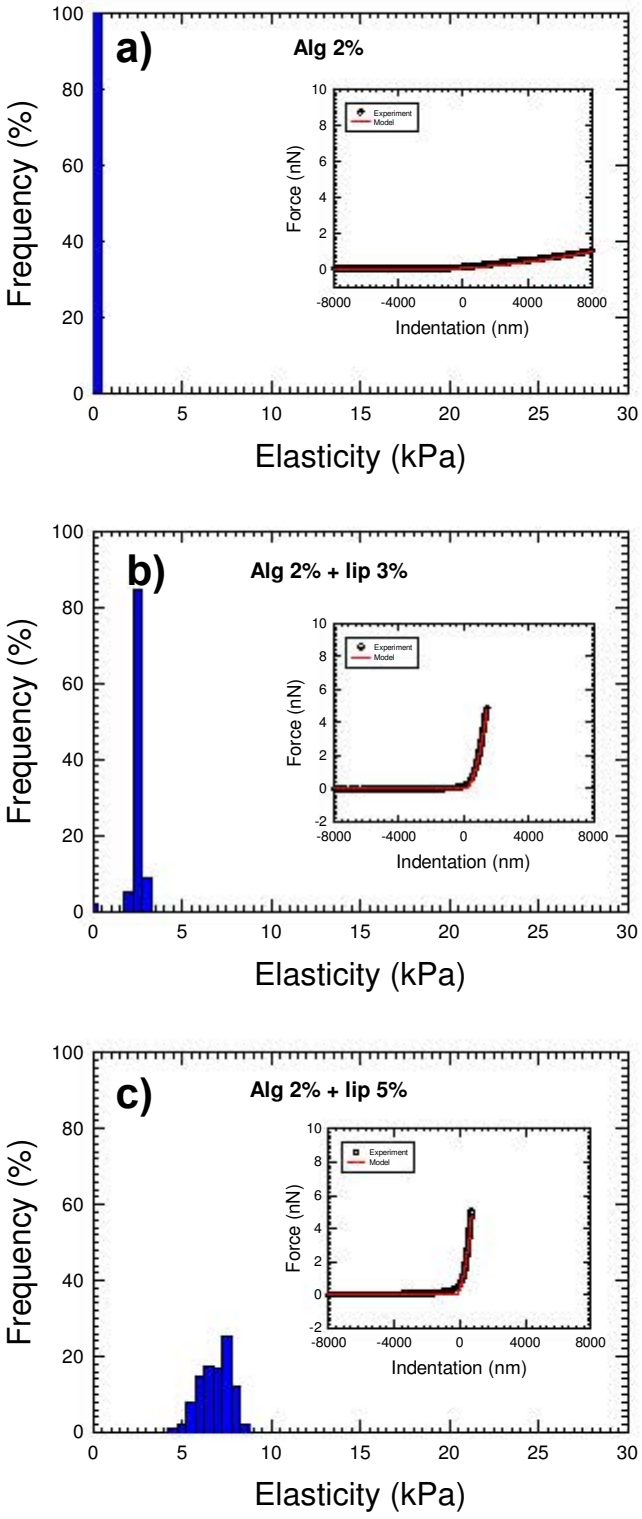

Fig.1. Statistic distribution of elastic modulus of alginate gels before (a) and after liposomes blending at 3% (b) and 5% (c). The inset corresponds to experimental force curve (white circle) and theoretical fitting (red line) for the most frequent elastic modulus value. Each distribution derived from the fitting of 1024 force curves.

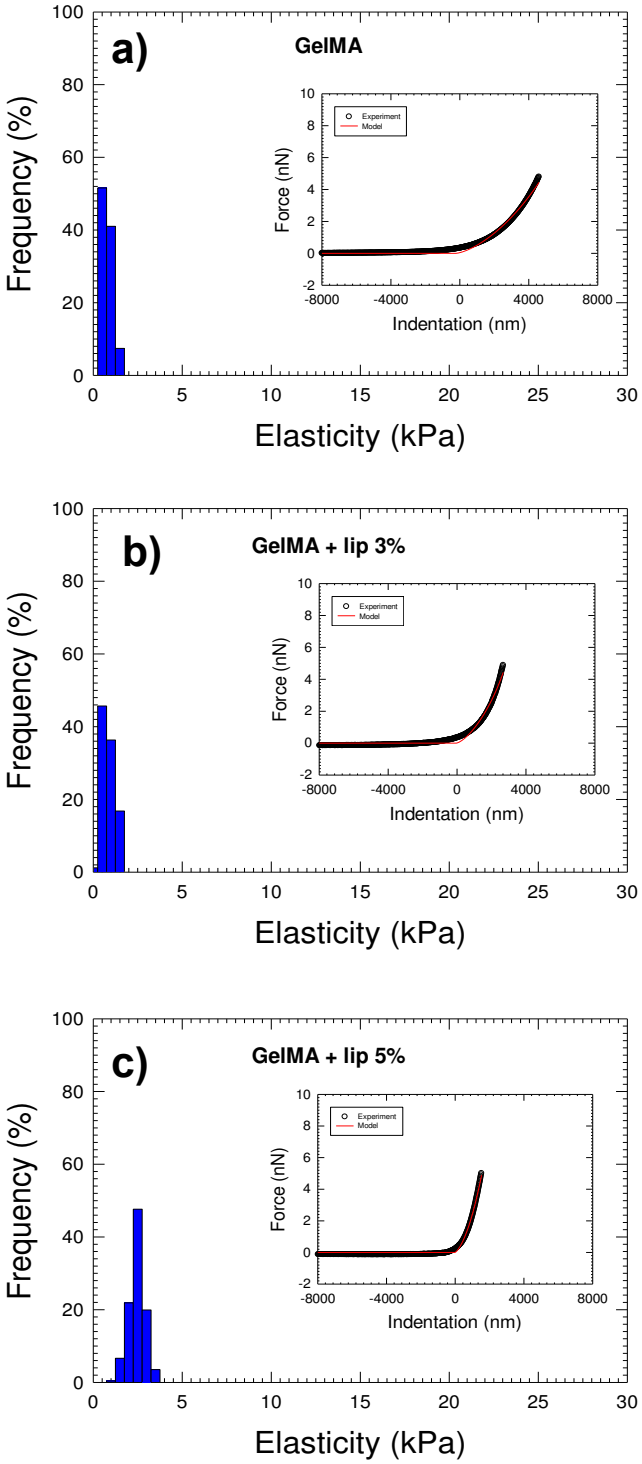

Fig.2. Statistic distribution of elastic modulus of GelMA films before (a) and after liposomes blending at 3% (b) and 5% (c). The inset corresponds to experimental force curve (white circle) and theoretical fitting (red line) for the most frequent elastic modulus value. Each distribution derived from the fitting of 1024 force curves.

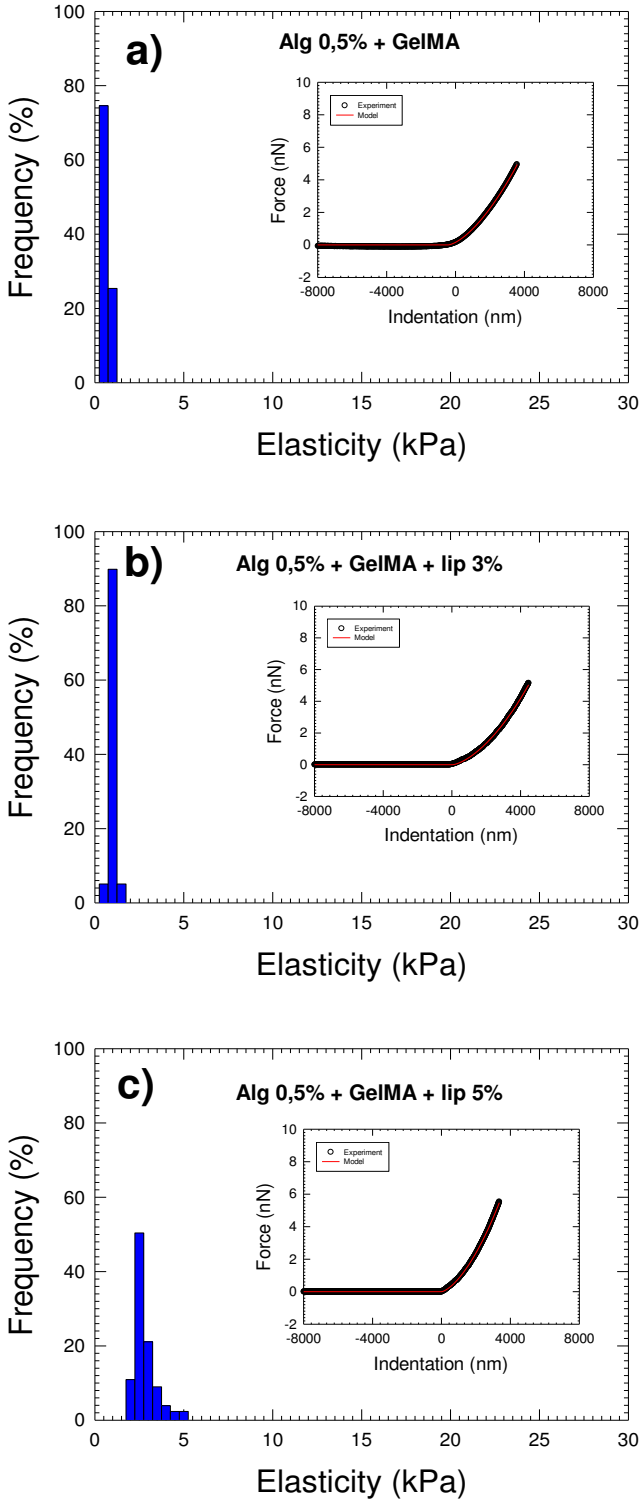

Fig.3. Statistic distribution of elastic modulus of mixed gel of GelMA and alginate at 0.5% before (a) and after liposomes blending at 3% (b) and 5% (c). The inset corresponds to experimental force curve (white circle) and theoretical fitting (red line) for the most frequent elastic modulus value. Each distribution derived from the fitting of 1024 force curves.

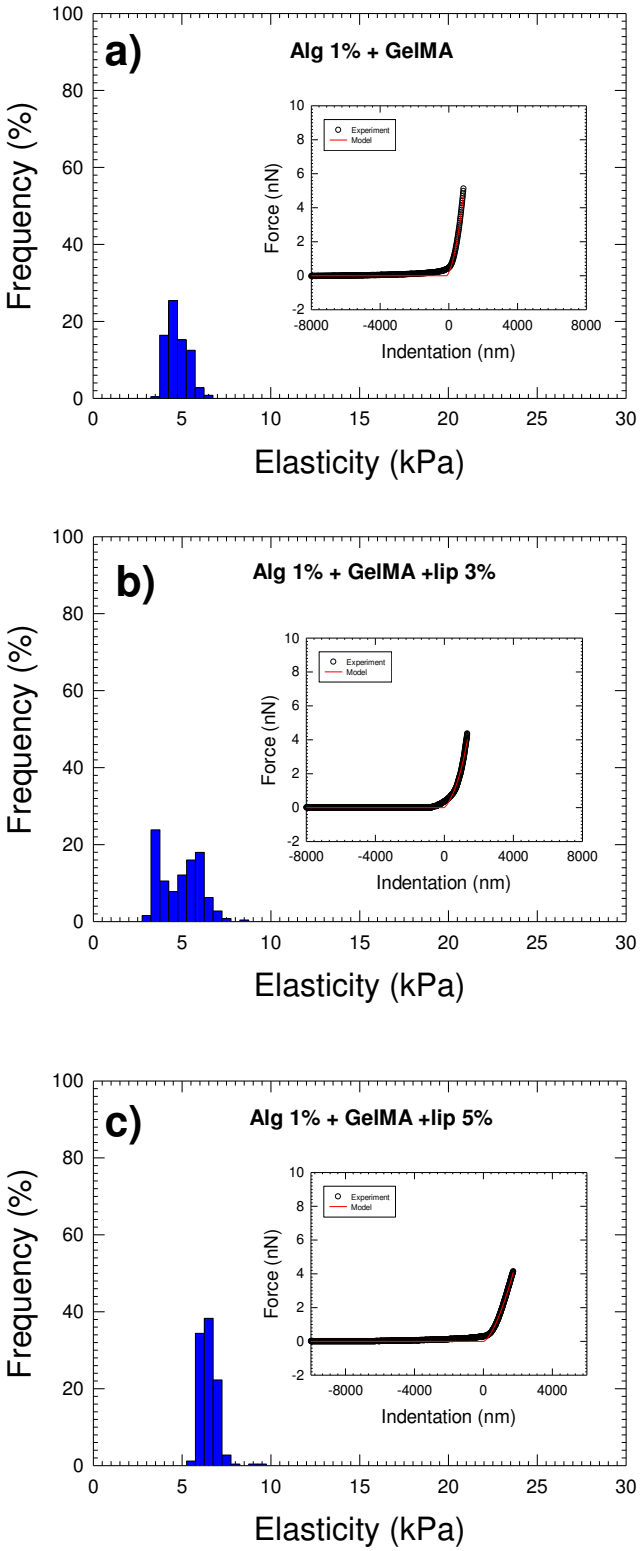

Fig.4. Statistic distribution of elastic modulus of mixed gel of GelMA and alginate at 1% before (a) and after liposomes blending at 3% (b) and 5% (c). The inset corresponds to experimental force curve (white circle) and theoretical fitting (red line) for the most frequent elastic modulus value. Each distribution derived from the fitting of 1024 force curves.

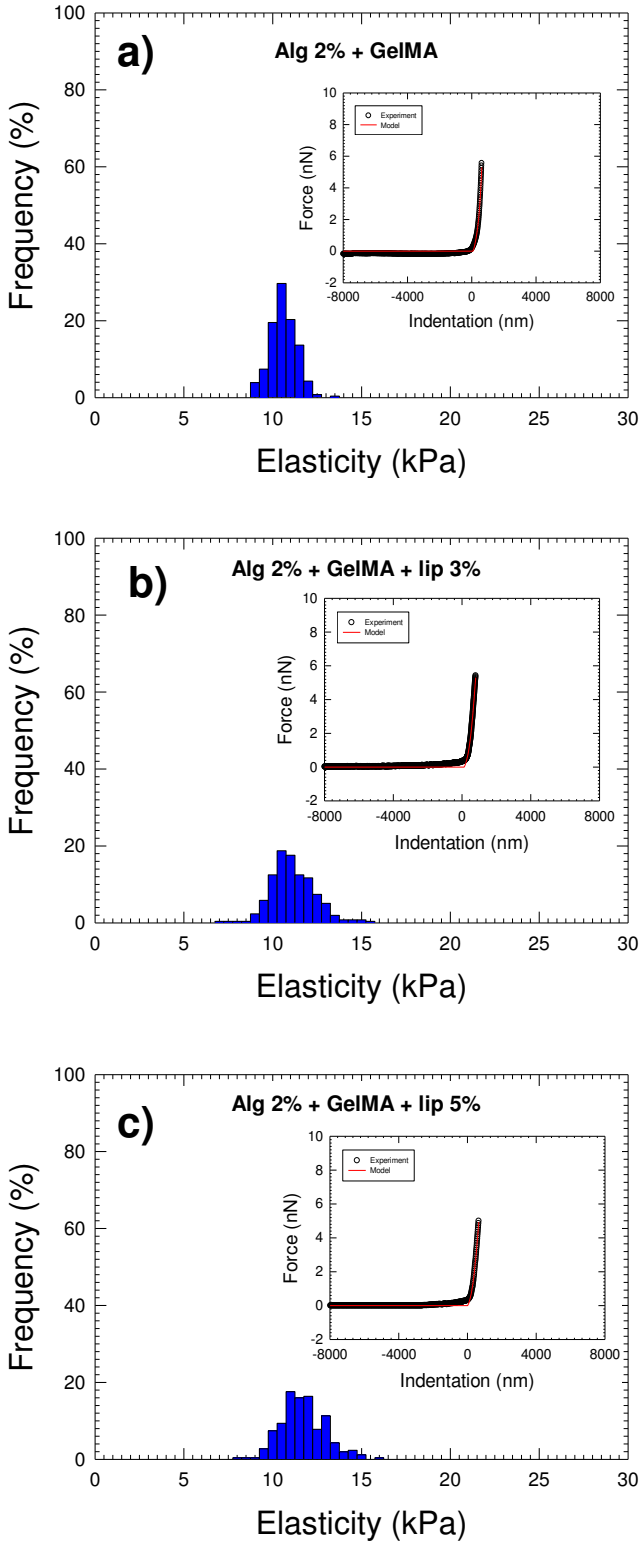

Fig.5. Statistic distribution of elastic modulus of mixed gel of GelMA and alginate at 2% before (a) and after liposomes blending at 3% (b) and 5% (c). The inset corresponds to experimental force curve (white circle) and theoretical fitting (red line) for the most frequent elastic modulus value. Each distribution derived from the fitting of 1024 force curves.

IV.3 Physicochemical interactions in nanofunctionalized alginate/GelMA IPN hydrogels

Introduction

Les hydrogels composites sont donc des réseaux de polymères réticulés dont les propriétés sont fonction du type de chaines formées entre les molécules ainsi que de la dispersion des nanoparticules molles dans l'épaisseur de l'hydrogel.

Il est ainsi intéressant d'étudier la nature des interactions existant dans la matrice 3D pour comprendre les mécanismes ayant induit l'amélioration des caractéristiques physico-chimiques décrite dans les chapitres précédents. En outre, l'étude de ces interactions entre les polymères d'une part et entre le(s) polymère(s) et les nanoparticules molles d'autre part, permettra une meilleure maîtrise des caractéristiques finales des hydrogels.

Dans ce travail, la composition chimique de chaque échantillon est déterminée à la surface et les interactions physico-chimiques sont étudiées dans l'épaisseur de la matrice 3D. Cette étude est établie dans le but d'analyser les groupements chimiques de chaque hydrogel et d'évaluer les nouveaux groupements présents dans les hydrogels RIPs et les hydrogels fonctionnalisés. Ces résultats sont ensuite corrélés avec les interactions possibles dans la matrice 3D.

La spectroscopie de photoélectrons induits par rayon X (XPS) a permis de déterminer la composition et la structure chimique de la surface. Pour compléter l'étude sur les différents groupements chimiques présents dans l'épaisseur de l'hydrogel, la structure chimique de la matrice a été de même évaluée par Spectroscopie Infrarouge à Transformée de Fourier (FTIR) basée sur l'absorption des hydrogels d'un rayonnement infrarouge. La résonnance magnétique nucléaire (RMN) a permis d'analyser la matrice 3D à l'échelle nanométrique et d'identifier les fonctions chimiques existantes.

La corrélation de ces différentes analyses spectroscopiques permet la formulation d'hypothèses possibles quant aux interactions entre les différents composants de l'hydrogel.

Physicochemical interactions in nanofunctionalized alginate/GelMA IPN hydrogels

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Abstract

Polymeric hydrogels are the center of researches due to their particular characteristics. They have tunable physical, chemical and biological properties making them a material of choice for a large range of applications. In order to optimize the hydrogels characteristics, polymer-composite and nano-composite hydrogels are formed to reinforce their properties and include numerous functionalities. The hydrogels characteristics depend on their component and the type of chains formed in the polymer network. In this work, pure hydrogels formed of a single polymer: alginate and GelMA and IPN hydrogels prepared with different alginate concentrations are investigated before and after the functionalization with nanoliposomes. The multiscale analysis were obtained thanks to XPS, FTIR and 1H RMN. The results showed interactions between two polymers as well as between the nanoliposomes and polymer.

1. Introduction

Recently, hydrogels have received considerable interests and are becoming more widespread because of their potential applications especially in food, tissue engineering, cosmetics and drug delivery. Hydrogels are three dimensional network consisting of crosslinked polymers chains. In the presence of a solvent, hydrogel swell and absorb a high amount of water due to the hydrophilic character of the polymers. They are prevented of dissolving and are able to maintain their shapes due to the physical or chemical crosslinking chains. The swelling properties provide to the hydrogels a significant degree of flexibility and a soft consistency. They are relatively deformable and are able to fit surface shape in which they are applied.

The various types of polymers including natural, synthetic and semi-natural polymers, used for hydrogels formation, define the characteristics of the network. The synthetic polymers present networks with strong mechanical characteristics and grant a better control over the properties.

While natural polymers have outstanding characteristics for biological applications such as biocompatibility, biodegradability and cell adhesion.

Among the most studied natural polymers, alginate, a natural polysaccharide extracted from algae or bacterial biofilm is well-known due to its large applications. It presents remarkable properties as biocompatibility, nonimmunogenicity, biodegradability and low toxicity (Pawar and Edgar, 2012)(Li et al., 2012)(Draget and Taylor, 2011). Alginate hydrogels are physically crosslinked in the presence of divalent cations. Calcium is the most commonly used cations for alginate crosslinking. Two units of two different chains of alginate bind with the same calcium and forms a junction. This association result in the formation of "egg box" which forms eventually the network.

Another interesting polymer is Gelatin methacrylate (GelMA). It is the result of the modification of gelatin, a natural and cytocompatible protein. Gelatin is an interesting polymer due to the presence of Arg-Gly-Asp (RGD) bioactive sequences which are effective in cells entrapment and allow active molecules to bind to the polymeric network. It is a semi-natural polymer derived from the substitution of the amine groups of the gelatin with methacrylate anhydride (Nichol et al., 2010)(Barbetta et al., 2005)(Dubruel et al., 2007)(Galis and Khatri, 2002). GelMA is a photocrosslinkable hydrogel which once exposed to UV light form covalent bond. The crosslinking process requires the addition of a photo-initiator that breaks down into radicals in the presence of UV irradiations. The aim objective of the gelatin modification is to get an irreversible and stable hydrogel with temperature variations (Xiao et al., 2011a).

In recent years, there have been many important advances in the field of materials to develop potentially applicable hydrogels utilized in various domains. New complex networks with valuable properties are required to overcome the limitations and challenges of a single polymer. Reinforced composite hydrogels are strong hydrogels in which other entities, polymers or nanoparticles are incorporated to enhance and optimize the network properties and expand their field of use (Baniasadi and Minary-Jolandan, 2015) (Thoniyot et al., 2015) (Annabi et al., 2014).

Polymer composite hydrogels are hydrogels formed by the mixture of several polymers with different characteristics. Each polymer combine with the other and generate innovative and interesting properties. Polymer composite hydrogels can form interpenetrating polymer network (IPN) when the added polymer forms a secondary network within a formed hydrogel

matrice. IPN hydrogels present strong mechanical properties and add physical characteristics to the 3D structure (Ullah et al., 2015) (Annabi et al., 2014) (Xiao et al., 2011b).

Another way to create a strong network are nanocomposite hydrogels. It consists on the incorporation of nanoparticles in the polymeric network (Thoniyot et al., 2015) (Faghihi et al., 2014) (Gaharwar et al., 2014) (Annabi et al., 2014). This combination can add advantageous chemical, physical and biological properties and provide superior functionality to an effective application of hydrogels in many fields. Nanoparticles can enhance the transportation and release of active molecules and preserve them from the premature degradation or deterioration.

Nanoliposomes, are soft and natural nanoparticles, composed essentially of phospholipids. They are negatively charged amphiphilic molecules, organized in bilayer with hydrophobic tails in the center and polar hydrophilic heads facing the solution (Bangham, 1961)(Bouarab et al., 2014). This particular character make liposome an effective system for the delivery of different types of encapsulated molecules. A powerful advantage of liposomes is their ability to transport and release materials with different solubility simultaneously.

The purpose of this study was to form IPN hydrogels with the combination of alginate and GelMA, and to study the possible interactions taking place in the 3D network. As well, nanoliposomes are incorporated in hydrogels formed of a single polymer (alginate or GelMA) and the IPN hydrogels in order to analyze the interaction of the polymers with the soft nanoparticles.

2. Results and discussion

2.1 Electrophoretic mobility determination

Alginate polymer contains carboxylic groups which are responsible of the negative charge of the polymer. Liposomes are also negatively charged (-3.41 ± 0.05 μmcm Vs^{-1}) due to the presence of phospholipids (Hasan et al., 2014). Therefore, the addition of nanoliposomes to alginate present a negative charge. The isoelectric charge of GelMA, measured at pH 5, is -1.6 μmcm Vs^{-1}. The modification of gelatin with methacrylate anhydride leads to the replacement of the amine groups by carboxylic acid groups, giving a negative charge to the polymer. The addition of liposomes affect slightly the charge of GelMA. In fact, GelMA has an amphoteric character and present both negative and positive charges. The nanoliposomes added in a small quantity may have reacted with the free amine groups of GelMA and their charges have been neutralized.

Table 1: Mean values of electrophoretic mobility (µmcm/Vs) of the pure polymers and their mixture, before and after addition of nanoliposomes.

	Without liposomes	With liposomes
Alginate	-82.4	-73.87
GelMA	-1.59	-1.26
Alginate + GelMA	-21.83	-21.07

In the blended polymers system, the presence of alginate decreases the charge of GelMA to reach -21.83 µmcm Vs^{-1}. The presence of the positive charge of amine groups in the GelMA, gives the possibility for alginate polymer and nanoliposomes to interact with GelMA.

2.2 Surface characterization using X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) based on the distribution of the electrons energy yield information about the surface composition of a material and characterizes possible interactions between the hydrogel components. Table 2 characterize the surface of the different hydrogels. The XPS analysis on alginate hydrogel shows characteristic peaks of Na_{1s} at 1071.55 eV and O_{1s} at 532.45 eV. The C peak can be fitted to three components C-(C,H), C-O and O-C=O. These elements are generated from alginate polymer. The addition of $CaCl_2$ during the crosslinking and the formation of the alginate hydrogel is confirmed by the presence of Ca_{2p} and Cl_{2p} pics at 347.25 eV and 198.55 eV, respectively. The incorporation of nanoliposomes into the alginate hydrogel shows similar peaks as the spectrum alginate but the atomic concentration percentage of the carbon increases from 54.63% to 78.11%. However a new peak characteristic of the liposomes appears at 133.06 eV representing P_{2P}. The spectrum of alginate with liposomes does not present any new elements and doesn't show any interaction between the polymer and the nanoparticles. Even though the atomic concentration of the calcium decreases to 50% with the addition of nanoliposomes. In fact, the changes seen on the spectrum may be due to an interaction between the soft nanoparticles and the chelating agent Ca^{2+} which had an impact on the alginate and calcium crosslinking.

Table 2. XPS analysis of the surface elemental composition of pure alginate and GelMA, IPN hydrogels, before and after functionalization with nanoliposomes.

	% O 1s	% C 1s	% N 1s	% O 1s O=C	% O 1s O-C	% N 1s C-(NH,NH2)	% C 1s C-(C,H)	% C 1s C-O	% C 1s C=O	% P 2p
Alg 2%	26.35	54.63	1.49	58.8	33.33	100	37.24	38.46	24.3	-
Alg 2% + lip 5%	17.47	78.11	-	27.96	65.83	-	68.44	22.85	4.47	0.62
GelMA	17.39	68.05	14.41	61.56	27.46	100	44.29	32.65	19.69	-
GelMA + lip 5%	16.21	74.33	9.09	49.64	35.61	100	55.01	27.87	13.05	0.38
Alg 2% + G	19.18	65.87	14.95	64.57	25.93	95.05	39.22	34.5	21.98	-
Alg 2% + G + lip 5%	17.48	72.40	9.24	47.41	38.71	93.97	53.40	28.22	13.97	0.3
Alg 1% + G	17.88	61.06	16.66	67.87	23.67	95.24	39.83	35.98	20.20	-
Alg 1% + G + lip 5%	20.86	77.8	-	44.28	44.48	-	55.20	27.76	12.34	0.51
Alg 0.5% + G	20.98	78.24	-	66.09	24.57	-	42.28	33.99	19.86	-
Alg 0.5% + G + lip 5%	20.76	78.77	-	59.21	29.10	-	43.60	30.87	20.84	-

While the spectrum of GelMA represents three essential component of the polymer: O_{1s} (531.150 eV), N_{1s} (399.650) and C_{1s} (284.650 eV). The ratio N/C is low confirming the methacrylation of the GelMA and replacement of the amine groups by methacrylamide. The addition of nanoliposomes shows also the same elements as the GelMA spectrum. Although the nitrogen concentration decreases of 63% after the incorporation of the soft nanoparticles. The P_{2P} peak of the liposomes appears with a lower concentration in compared to alginate polymer. It is possible that the phosphore of the liposomes have reacted with the amine groups of GelMA.

The IPN hydrogels containing 2% of alginate showed a new pic representing $C\text{-}NH^{3+}$ confirming the interaction between two polymers alginate and GelMA. After the addition of nanoliposomes, the phosphorus element appears, assuming the presence of liposomes in the solutions. It is remarkable that with the decreasing of alginate concentration, the nitrogen element as well as the phosphorus disappeared. The obtained result highlights that the alginate concentration affect the interaction between the different components leading to hydrogels with different properties. In fact, alginate and liposomes compete to react electrostatically with

GelMA. The incorporation of nanoliposomes occurs after the mixture of the two polymers. Since alginate reacts first with GelMA, a decreasing of the alginate concentration can increase the possibility for liposomes to interact with the free positive charges of GelMA.

2.3 Fourier Transform infrared spectroscopy results

Fourier Transform infrared (FTIR) spectroscopy is used essentially to characterize the presence of specific chemical groups in the hydrogels and to study the interaction between the blended polymers and the effect of the added nanoliposomes in the polymers. Fig. 1a shows the spectrum of pure alginate hydrogel and alginate functionalized with nanoliposomes. The spectra of alginate present a peak at 1608 cm^{-1} due to the stretching of COO asymmetric elongation carboxylate indicating the content of uronic acid in the polymer. While at 1415 cm^{-1}, the peak is attributed to the symmetric stretching vibration of COO^{-}. The CO stretching band appears at 1030 cm^{-1}. The peak between 3250 and 3386 cm^{-1} having a high intensity represent OH stretching vibration. The stretching vibration of aliphatic CH appears at 2920-2850 cm^{-1} due to the addition of liposomes (Fan et al., 2005) (Li et al., 2008) (Daemi and Barikani, 2012a). The FTIR spectra of the alginate with nanoliposomes covers the peaks of the alginate and the liposomes spectrum and does not show any interaction between the soft nanoparticles and the polymer. The peak at 1735 cm^{-1} which appears in the functionalized hydrogel is assigned to the C=O stretching band of the liposomes. The absorbance intensity of the two peaks at 1620 and 3380 cm^{-1} increases with the addition of nanoliposomes in the alginate hydrogel. The increased percentage is relatively related to the amount of the added nanoparticles. The hydroxyl and carboxylate groups representing this two pics are the responsible elements of the interaction of alginate with calcium (Daemi and Barikani, 2012). These elements presented narrow absorption region and the peaks are shifted to lower wavenumber after the addition of the soft nanoparticles. The result confirms that the incorporation of nanoliposomes in the hydrogels affect the gelation process of alginate due to an interaction between the calcium and the nanoliposomes.

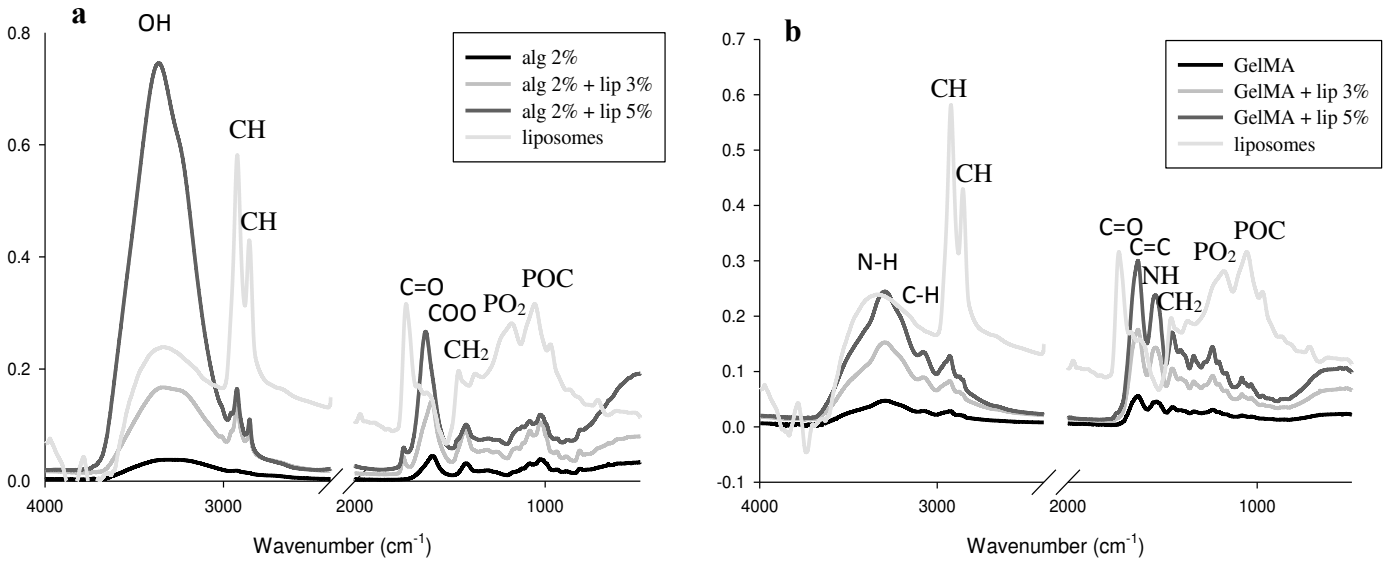

Fig.1. Surface analysis by FTIR of (a) alginate and (b) GelMA before and after functionalization with liposomes (3% and 5%, m/v).

Fig. 1b shows the spectrum of GelMA hydrogel derived from the modification of the gelatin with the methacrylate anhydride with and without liposomes. A strong peak appears at 1650 cm^{-1} related to amide I primarily C=O stretching groups. The band at 1500-1570 cm^{-1} corresponds to C-N-H bending while the band at 3200-3400 cm^{-1} indicates the presence of peptide bond (mainly N-H stretching). The peak at 3062 cm^{-1} represents the C-H stretching groups (Sandra Hermanto, 2013)(Sadeghi and Heidari, 2011).

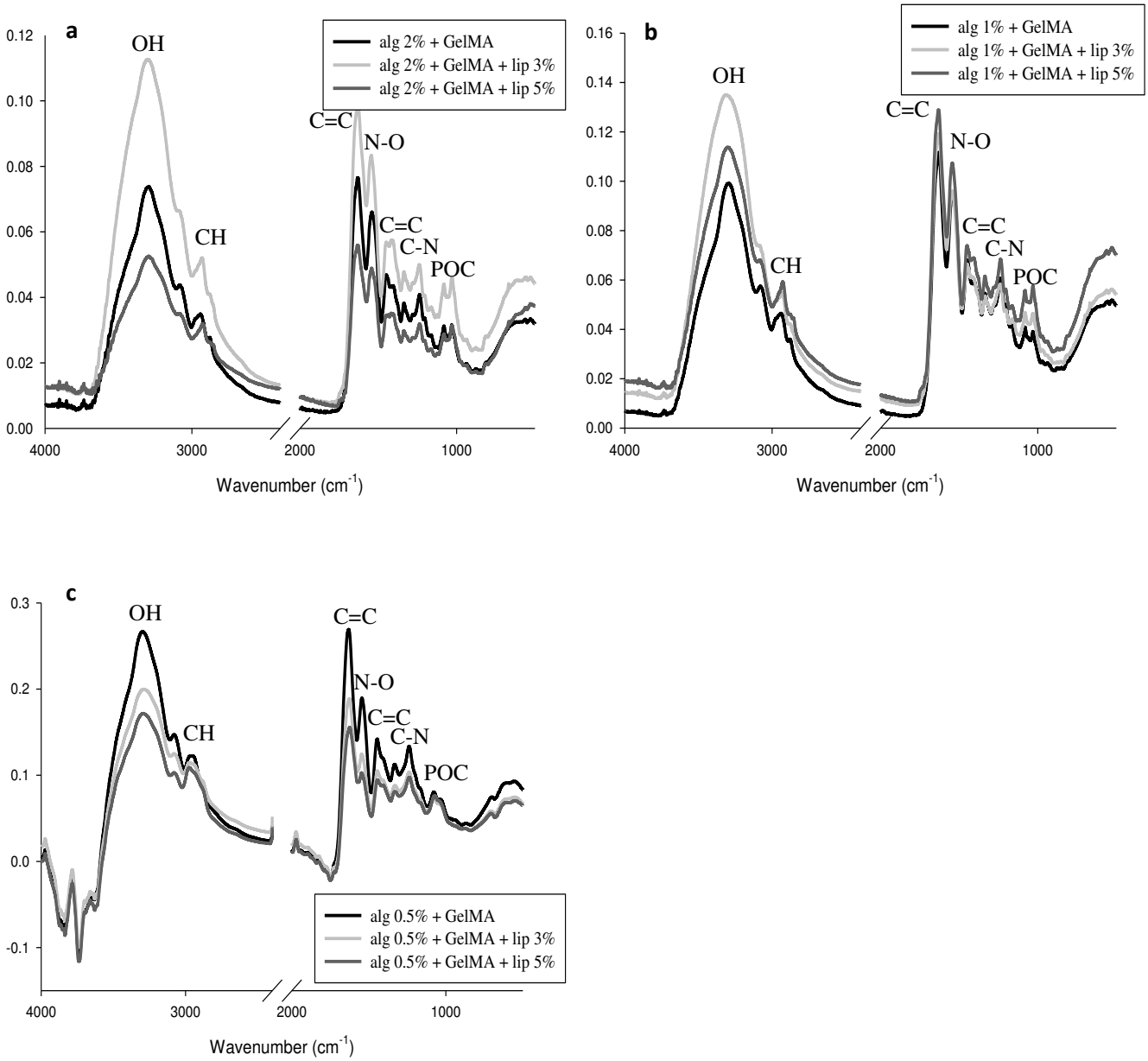

Fig.2. Surface analysis by FTIR of the mixture of GelMA and alginate with different concentration of alginate. (a) Spectrum of the mixture with 2% (m/v) alginate, (b) Spectrum of the mixture with 1% (m/v) alginate, (c) Spectrum of the mixture with 0.5% (m/v) alginate.

GelMA presents a peak at 1640 cm^{-1} related to the carbon double bond that presents in the methacrylate approving the interaction between gelatin and methacrylate anhydride (Coutinho et al., 2010)(Shin et al., 2012). The spectra of the hydrogel with the nanoparticles does not show the peaks corresponding to the liposomes spectra but it presents an increasing of the intensity

of certain peaks specially around 3300 cm^{-1}. This result shows an interaction between the GelMA and the nanoparticles.

The FTIR spectrum of the double network hydrogels (fig. 2) shows new peaks, a sign of the presence of interconnection between the two networks. The alginate concentration varies between 0.5%, 1% and 2% has a considerable effect on the position of the peaks as well as their intensity.

The added nanoliposomes react also with the mixture of the polymers and its impact on the hydrogel is influenced by the concentration of alginate. In fact, the presence of the alginate disturb the normal interaction between GelMA and the nanoliposomes due to the interaction which occur firstly between the two polymers.

2.4 Interaction study using H-NMR

However, it is possible to perform a full characterization of the hydrogels using NMR high resolution.

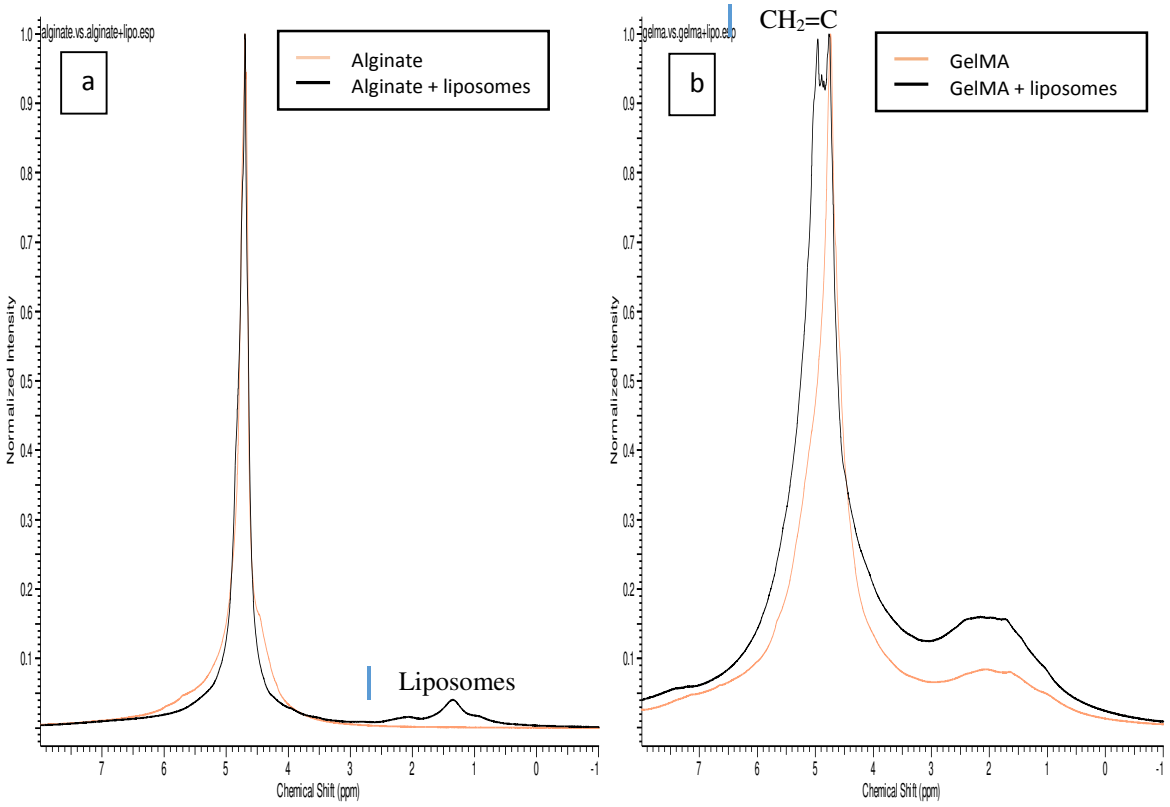

Fig.3. ^{1}HNMR spectra of (a) alginate and (b) GelMA before and after nanofunctionalization.

Fig. 3a shows the results of NMR obtained for alginate hydrogel and alginate with liposomes hydrogel. These results present any interaction between the alginate polymer and the nanoliposomes confirming the FTIR analysis. In fact, the spectrum shows a broad peak at 4.6-4.8 ppm representing the alginate polymer and the D2O solution in which it is solubilized. After the addition of liposomes, this peak does not change while a new peak attributed to the liposomes appears at 1.35 ppm.

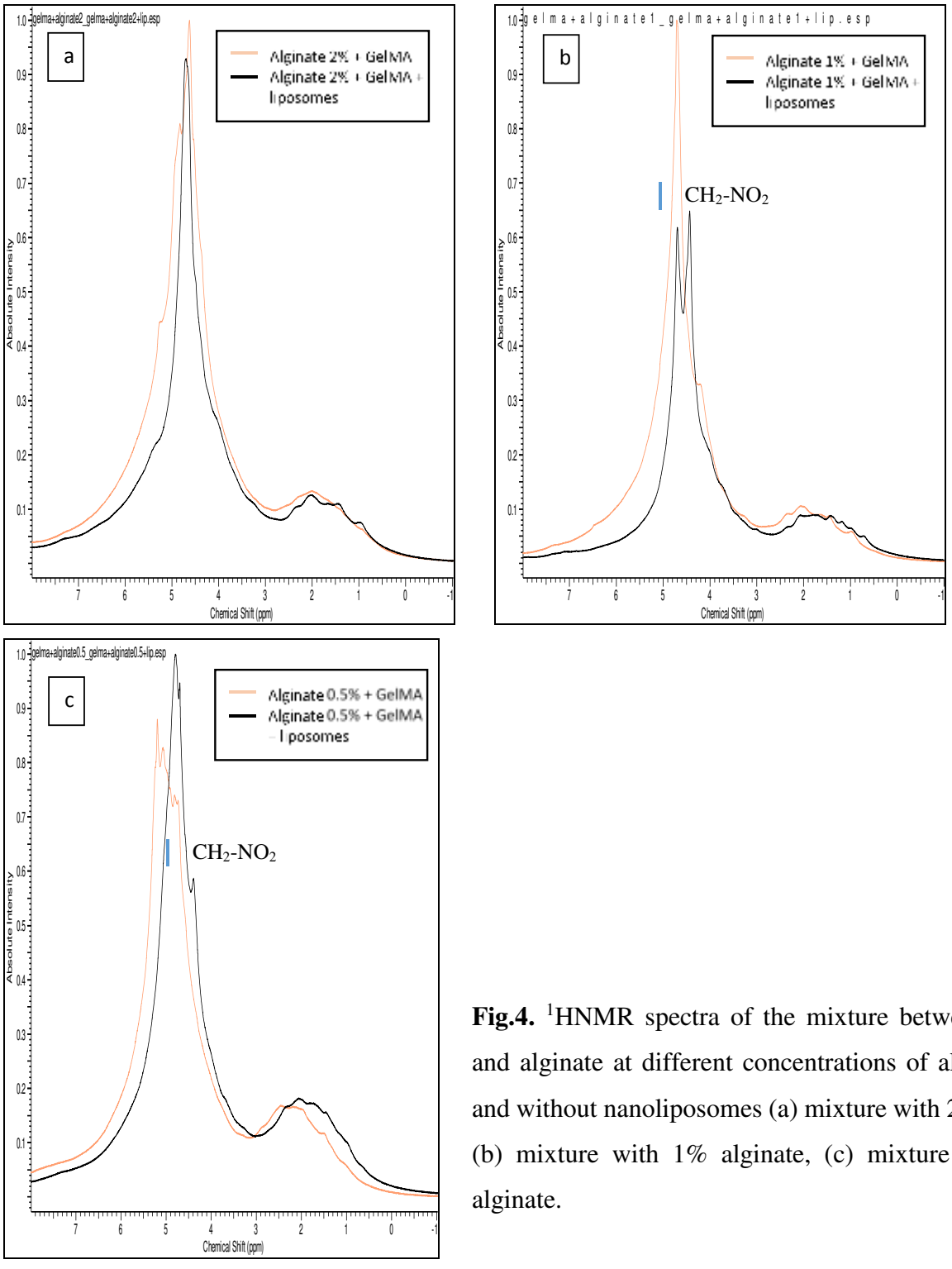

Fig.4. ^{1}HNMR spectra of the mixture between GelMA and alginate at different concentrations of alginate with and without nanoliposomes (a) mixture with 2% alginate, (b) mixture with 1% alginate, (c) mixture with 0.5% alginate.

GelMA hydrogel (fig. 3b) shows a large peak at 1.1-2.9 ppm assigned to methyl and methylene residues of amino acids present in the hydrogel. The present peak confirms also the reaction between the –COOH group of the methacrylate anhydride with the –NH2 of lysine (1.61 and 2.93 ppm) in the gelatin. The addition of methacrylate in the gelatin provokes the formation of H2C=C(CH3)- in the solution which leads to the apparition of two peaks at 5.64 and 5.36 ppm (Hu et al., 2009)(Bae et al., 2011). The spectra of GelMA does not show these peaks due to the polymerization of the double bonds during the hydrogel formation.

The mixture of the two polymers (fig. 4) before the incorporation of nanoliposomes shows new peaks resulting from an electrostatic interaction between alginate and GelMA. In fact, GelMA is classified as ampholytic polymer due to its composition in amino acids containing both negative and positive charges. The negatively charged alginate reacts with the positive charges present in GelMA creating new links in the network and leading to the apparition of new peaks. The functionalization of the hydrogels with soft nanoparticles provoke the perturbation of the reticulation process of alginate and disrupt the formation of egg-box due to an electrostatically interaction between the liposomes and the crosslinking agent Ca^{2+}. On the other hand, liposomes can also, like alginate, interact with the positive charges present in the GelMA polymer. These interactions are responsible for the modification shown on the spectra of IPN hydrogels after the functionalization. The comparison of the spectra of the IPN hydrogels shows that an increase of the alginate concentration involves a predominance of the peak representing alginate and decreases the effect of liposomes in the hydrogels. A decreasing in the concentration of alginate facilitates the reaction of the liposomes with GelMA.

3. Conclusion

In this work, we have demonstrated at two scales (micro and nano) that the functionalization of the alginate hydrogel with nanoliposomes does not affect the polymer while an interaction is observed between the soft nanoparticles and the calcium divalent cations. The incorporation of nanoliposomes in GelMA create a polymer-nanoparticles electrostatic interaction. These interactions occur also in the polymer-composite hydrogel. When the nanoparticles are added to the mixture, alginate and liposomes compete to react with the positive charges of GelMA. From this point of view, it was interesting to study these modifications which can improve the mechanical properties of the hydrogels.

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Conclusion

Cette étude a été menée sur les hydrogels préparés à partir de polymères seuls ainsi que sur les RIPs, avant et après leur fonctionnalisation par les nanoliposomes. Les spectres obtenus par les différentes techniques spectroscopiques à différentes échelles d'observation mettent en évidence l'existence d'interactions entre le GelMA et les nanoparticules molles. En effet, le GelMA présente un comportement amphotère due à la présence simultanée de groupements carboxyle et de groupements fonctionnels amine. La charge du GelMA varie selon son pH sauf que la présence des NH_3^+ dans ce polymère favorise sa réaction avec les liposomes qui sont chargés négativement. A l'inverse, l'alginate présentant un potentiel zêta négatif, n'interagit pas avec les nanoparticules molles. Des interactions entre les deux polymères ont été mises en évidence pour les hydrogels composites (RIPs). Ces interactions s'opposent à l'attraction électrostatique des liposomes par le GelMA. Par conséquent, une diminution de la concentration d'alginate dans les hydrogels RIPs entraine des interactions plus importantes entre le GelMA et les nanoliposomes.

Supplementary Information

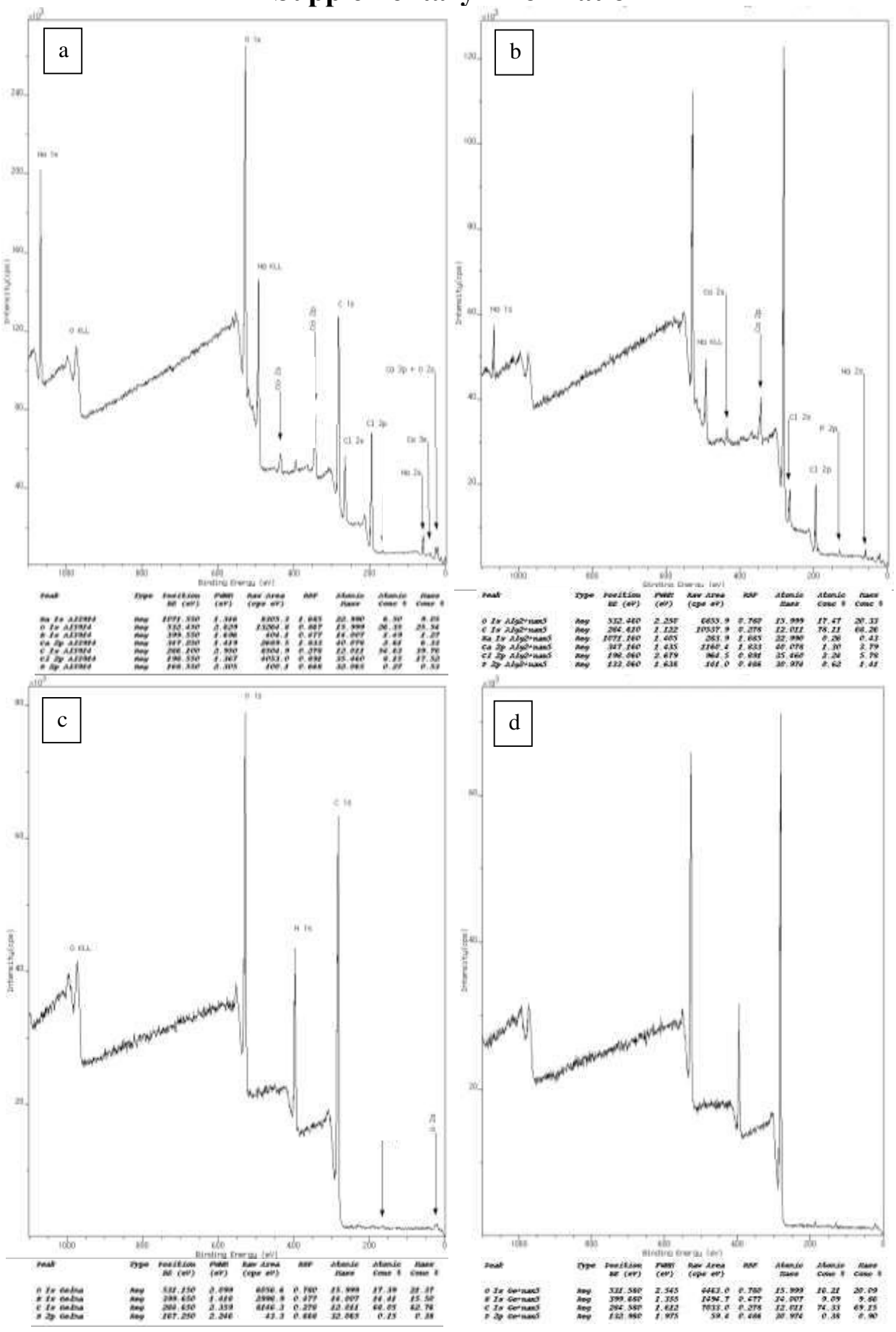

Fig.1. X-ray photoelectron spectroscopy of alginate hydrogel before (a) and after (b) nanofunctionalization and GelMA hydrogel before (c) and after (d) nanofunctionalization.

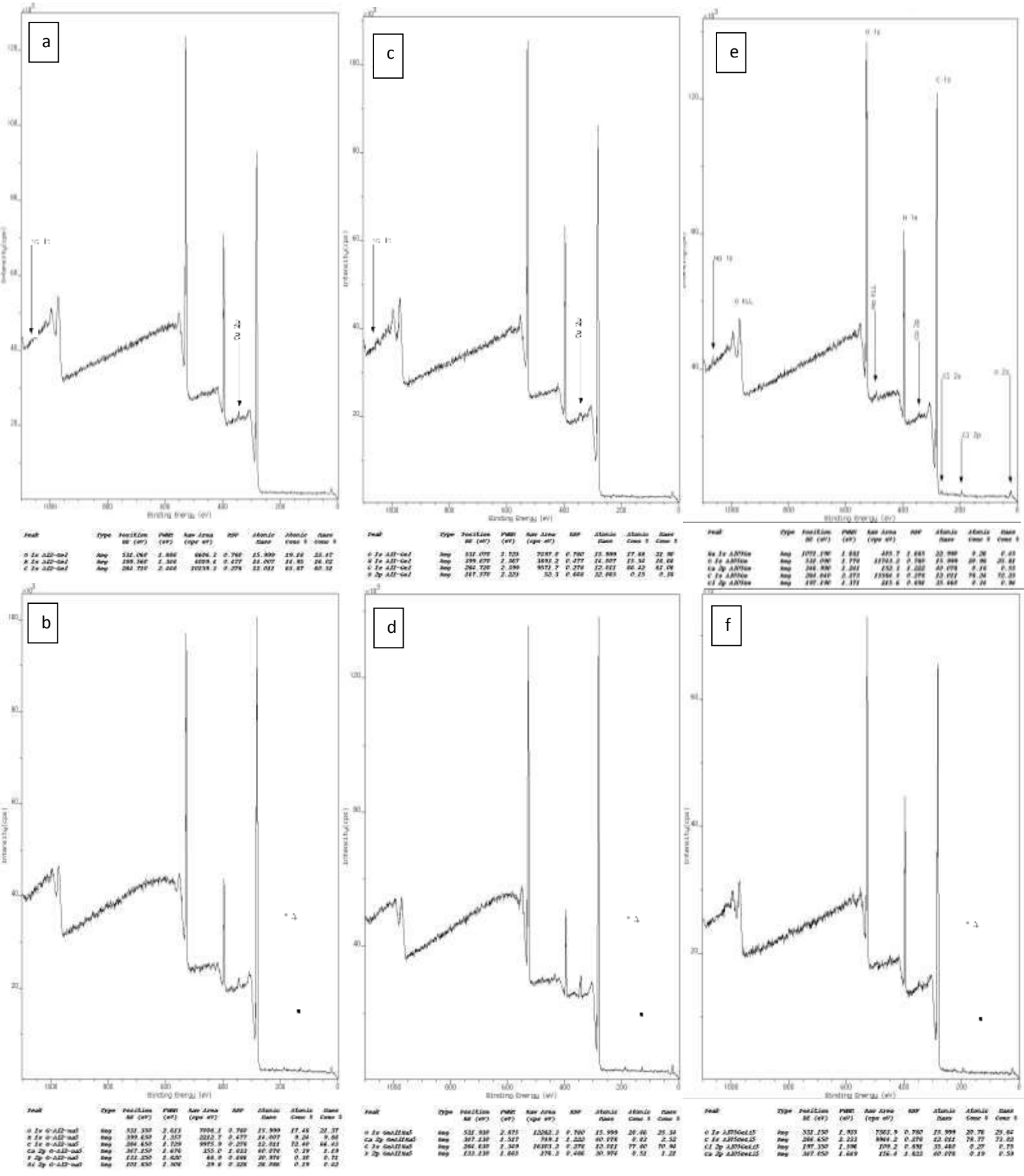

Fig.2. Surface composition of the IPN hydrogels before and after the addition of nanoliposomes by X-ray photoelectron spectroscopy. (a) XPS spectrum of alginate 2% + GelMA, (b) XPS spectrum of alginate 2% + GelMA + lip 5%, (c) XPS spectrum of alginate 1% + GelMA, (d) XPS spectrum of alginate 1% + GelMA + lip 5%, (e) XPS spectrum of alginate 0.5% + GelMA, (f) XPS spectrum of alginate 0.5% + GelMA + lip 5%.

V. Conclusion & perspectives

Chaque étape de préparation des hydrogels comporte des critères spécifiques permettant de contrôler les caractéristiques finales du produit pour créer un modèle optimal utilisé dans des domaines variés tels que l'alimentaire, la nutraceutique, la pharmaceutique ou le médical. Les hydrogels à base de polymères d'origine naturelle sont largement étudiés afin de développer des propriétés permettant d'élargir leur champ d'application et d'améliorer leurs caractéristiques.

Les propriétés physico-chimiques d'hydrogels à base de deux polymères de familles différentes ont été étudiées dans ce travail de thèse: l'alginate qui est un polysaccharide présentant des caractéristiques mécaniques remarquables et le GelMA qui dérive de la gélatine et constitue un polymère intéressant surtout pour des applications médicales (bonne adhésion cellulaire). De par les propriétés intéressantes de ces deux polymères, des hydrogels composites ont été formés par assemblage alginate/GelMA avec des concentrations différentes d'alginate (0.5% ; 1% et 2%) dans le but d'étudier le changement de comportement des hydrogels en fonction des différentes compositions.

En parallèle, des hydrogels nano-composites ont été élaborés grâce à l'incorporation de liposomes dans les matrices 3D. Les hydrogels formés d'un ou de plusieurs polymères ont été fonctionnalisés à partir de deux concentrations différentes de nanoliposomes (3% et 5%). Ces vecteurs amphiphiles de biomolécules lipidiques sont formés essentiellement d'acides gras mono et polyinsaturés et présentent des effets positifs pour la matrice 3D.

Les nanoliposomes ainsi que les composants polymériques des hydrogels affectent les caractéristiques des hydrogels d'où la nécessité d'une étude approfondie des propriétés de la matrice 3D et des interactions entre les différentes molécules.

La première partie de ce travail a consisté en l'optimisation de la préparation des hydrogels pour obtenir des matrices 3D convenables à l'exécution des analyses physicochimiques (surface lisse et propre, dimensions déterminées….). Une caractérisation multi-échelle des propriétés physico-chimiques des hydrogels élaborés a été menée de la surface jusqu'à l'épaisseur afin d'optimiser l'efficacité de ces systèmes et de comprendre leur comportement vis-à-vis du changement des composants de la matrice 3D. Les études ont montré que l'utilisation des hydrogels composites est une voie très prometteuse puisqu'ils présentent des propriétés interfaciales et mécaniques assez intéressantes.

Les propriétés de surface des hydrogels sont étudiées pour mieux comprendre le comportement des hydrogels en fonction des conditions externes environnantes et pour

déterminer leur force d'adhésion. Ce travail a mis en évidence l'importance de la nanofonctionnalisation dans l'amélioration de la mouillabilité des hydrogels et de leur énergie de surface tout en préservant une bonne tension superficielle. En effet les liposomes sont organisés en bicouches lipidiques avec des queues hydrophobes qui sont dirigées vers l'intérieur de la membrane et des têtes polaires hydrophiles orientées vers l'extérieur de la membrane. L'assemblage de deux polymères a de même augmenté les propriétés interfaciales de la matrice 3D.

Un autre paramètre important étudié à la surface des hydrogels est la porosité. La morphologie externe de l'hydrogel et la distribution des pores ont été déterminées par microscopie à balayage électronique. Les résultats obtenus montrent que la porosité de l'alginate a été améliorée considérablement après incorporation des nanoliposomes. Ces pores apparaissent à cause de la présence de nanoparticules molles qui perturbent le processus de gélification entre l'alginate en présence de cations calcium. L'incorporation des liposomes dans des hydrogels de GelMA a permis un meilleur control de la taille des pores présents dans l'hydrogel. La fonctionnalisation des hydrogels RIPs a développé des hydrogels avec une porosité régulière et bien définie.

L'effet des RIPs et des nanoliposomes à la surface de l'hydrogel a été étudié également à l'échelle nanoscopique par microscopie de force atomique. Les caractéristiques visco-élastiques des hydrogels ont été établies pour déterminer les propriétés nanomécaniques des hydrogels. Les résultats montrent que l'ajout des nanoliposomes augmente significativement le module Young de l'hydrogel d'alginate. Cette augmentation est moins importante pour le GelMA après nanofonctionnalisation.

La formation des hydrogels RIPs a augmenté le module de Young d'une part mais a diminué l'effet des nanoparticules molles sur les hydrogels d'autre part.

Les caractéristiques mécaniques des hydrogels, formés uniquement d'alginate ou de GelMA ainsi que des hydrogels RIPs, ont été étudiées dans l'épaisseur à l'échelle mésoscopique, avant et après fonctionnalisation par des nanoliposomes. Les résultats obtenus montrent une dominance élastique pour tous les hydrogels vu que le module élastique présente des valeurs plus élevées que le module visqueux. Ceci prouve la transformation des solutions en hydrogels. Les analyses ont montré l'influence des nanoliposomes sur la gélification de l'alginate confirmant le résultat obtenu à l'échelle microscopique par MEB. En effet la présence de ces vecteurs a perturbé le processus normal de réticulation de l'hydrogel à base d'alginate.

En revanche, le module élastique du GelMA, n'a pas été influencé par la présence de ces nanoparticules molles à cause de la méthode de gélification consistant en l'exposition de la solution à un rayonnement de type ultraviolet. Cependant, les hydrogels composites présentent des propriétés visco-élastiques les plus élevées.

L'analyse des interactions entre les deux polymères ainsi qu'entre les polymères et les nanoparticules est importante pour une meilleure compréhension des mécanismes à la base de ces modifications physicochimiques. Les liposomes dans l'hydrogel d'alginate n'interagissent pas directement avec le polymère, ceci est dû aux charges identiques entre ces deux composants. En revanche, le GelMA qui a un caractère amphotère entre en interaction avec les charges négatives de l'alginate d'une part et des liposomes d'autre part. Ces interactions sont plus ou moins faibles et dépendent des concentrations d'alginate ainsi que de la concentration de liposomes.

Pour conclure, ce travail de thèse a permis un meilleur contrôle des propriétés physico-chimiques des hydrogels à base d'alginate et de GelMA en formant des hydrogels composites à base de polymères et en incorporant des nanoliposomes dans la matrice 3D. La caractérisation multi-échelle des hydrogels confirme que l'élaboration de tels systèmes ouvre de nouvelles perspectives pour utiliser les hydrogels dans une plus large gamme d'applications. L'ensemble des résultats donne des pistes pour contrôler les caractéristiques du réseau polymérique et accroître l'efficacité de son application.

Il est possible d'envisager une étude sur le transfert moléculaire sur des molécules actives tels que la curcumine encapsulées dans les nanoliposomes afin de contrôler leur libération ainsi que leur orientation vers une cible.

Il serait aussi intéressant de déterminer le degré de saturation des hydrogels une fois le réseau polymérique placé dans un solvant et d'analyser l'effet des hydrogels composites sur les propriétés de gonflement. Suite à l'absorption du solvant, le réseau de polymère présente un allongement qui affecte les propriétés viscoélastiques de l'hydrogel. Une caractérisation viscoélastique de la matrice gonflée pourrait nous montrer de nouvelles propriétés mécaniques de l'hydrogel.

Dans le but de confirmer l'efficacité des systèmes composites, une étude biologique *in vitro* serait importante pour compléter les analyses faites sur le plan physico-chimique. Cette étude consisterait en un suivi de la prolifération et de la survie cellulaire dans la matrice

polymérique. Elle déterminerait la performance de l'utilisation des hydrogels RIPs et des hydrogels nano-composites dans les domaines cosmétique et biomédical.

Enfin après optimisation des caractéristiques des hydrogels, la préparation de microfibres à base de polymères par la méthode de wetspinning serait intéressante. Les microfibres seront, de même, formées de plusieurs polymères avec différentes concentrations pour étudier l'influence de chaque composant sur les propriétés des microfibres. Des nanoparticules peuvent également être incorporées au sein des matrices afin d'augmenter les caractéristiques des fibres.

Résumé

Des nouvelles méthodes de gélification avec association de différents composés permettent l'élaboration d'hydrogels sous forme de matrices 3D présentant des propriétés optimales et des fonctions intéressantes. Cette technique d'assemblage peut être effectuée par mélange de plusieurs polymères ou/et par incorporation de nanoparticules dans la matrice polymérique. Ce travail de thèse a montré l'intérêt de mettre en œuvre des réseaux interpénétrés de polymères à base d'alginate et de GelMA, et a mis en évidence l'effet de l'incorporation de nanoliposomes sur les propriétés physico-chimiques des hydrogels. Une caractérisation multi-échelle des hydrogels, a été complétée par une étude des interactions possibles au sein de la matrice 3D. Dans une première partie du travail, une analyse des propriétés de surface des matrices composites à différentes concentrations d'alginate, avant et après fonctionnalisation par des nanoparticules molles, a montré une amélioration de la mouillabilité et de l'énergie de surface des hydrogels. Les propriétés mécaniques des hydrogels ont été déterminées par une caractérisation multi-échelle incluant la microscopie à force atomique (nanoscopique) et le rhéomètre (mésoscopique). Ces analyses ont pris en compte les différentes concentrations d'alginate ainsi que les deux concentrations différentes de liposomes incorporés dans la matrice 3D. Les résultats obtenus ont montré l'intérêt de l'assemblage des deux polymères et l'effet des nanoliposomes sur le processus de gélification de l'alginate dû à une interaction entre les nanoparticules molles et l'agent réticulant (CaCl2). Une étude morphologique des hydrogels a montré la possibilité de contrôler la taille des pores en modifiant la concentration des différents composants des hydrogels ou en fonctionnalisant les matrices 3D par des nanoparticules molles. Les interactions physico-chimiques ont ensuite été étudiées par Spectroscopie de Photoélectrons X, spectroscopie de Résonance Magnétique Nucléaire et Spectroscopie Infrarouge à Transformée de Fourier.

Abstract

Novel crosslinking methods to design 3D hydrogels consist on an innovative combination of various components in order to create 3D structure with optimal properties and functionalities. This blending technic can be carried out by mixing several polymers or/and incorporation of nanoparticles into the polymer network. The present work showed the advantages of interpenetrating polymer networks forms composed of alginate and GelMA and highlighted the effect of the incorporation of nanoliposomes on the physico-chemical properties of the hydrogels. It consisted primarily on a multiscale characterization of the hydrogels and then on the study of the possible interactions in the 3D structure. At first, the surface characterization of the composite hydrogels at different alginate concentrations, before and after the functionalization with soft nanoparticles, showed an improvement of the wetting properties and the surface energy. The mechanical properties of the hydrogels were determined by multiscale analysis using the atomic force microscopy (nanoscopic) and the rheometer (mesoscopic). These analysis took into account the various concentrations of alginateas well as the two different concentrations of the liposomes added in the 3D structure. The results showed the effectiveness of mixing the polymers and the influence of the nanoliposomes on the alginate coagulation due to an interaction between the soft nanoparticules and the coagulation agent ($CaCl_2$). A morphological study of the hydrogels showed the possibility to control the size of the pores by the modification of concentration for each component of hydrogel or by functionalization the 3D structure. The physicochemical interactions were then studied thanks to the X-ray Photoelectron Spectroscopy, the Nuclear Magnetic Resonance Spectroscopy and the Fourier Transform Infrared spectroscopy.