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Review

In situ forming polysaccharide-based 3D-hydrogels for cell delivery in regenerative medicine

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ABSTRACT

Regenerative medicine is evolving fast, in particular since the potential of stem cells has been assessed. This evolution process requires the development of new tools capable of meeting the needs of this field of investigation. Cell delivery is a crucial issue for the success of regenerative medicine as cells should be easily seeded, expanded and introduced on site with maintenance of their phenotype and their capability to develop into a neo tissue/organ. On a material standpoint, cell delivery system should meet the preceding needs but also permit an easy introduction at the site and remain without hampering tissue development. As is shown in this review, polysaccharide hydrogels, and in particular *in situ* forming ones, are materials with a high application potential in regenerative medicine.

Contents

1. Introduction

Regenerative medicine holds a lot of promises in health care as it can provide a solution for the repair of a damaged organ or tissue such as, for instance, the heart after a stroke and the skin after a severe wound. Moreover, regenerative medicine can help compensate the donor shortage and also substitute existing solutions to degenerative diseases by a simpler or less invasive treatment, like the suppression of the use of medical prosthetics for the replacement of damaged cartilage. Finally, the use of stem cells opens up new routes to more efficient and promising regeneration strategies thanks to the high potential of these cells to expand and differentiate into the appropriate cell type leading to a new tissue.

During embryo development, cellular interactions are controlled within the 3D-architectures defined as the Extra Cellular Matrix (ECM), mainly composed of glycosaminoglycans, proteins and water (Hynes, 2009). The local microenvironment strongly impacts cell growth via soluble factors (growth factors), steric or topological and mechanical constraints, cell–cell interactions as reviewed by various authors (Engler, Humbert, Wehrle-Haller, & Weaver, 2009; Lecuit & Le Goff, 2007; Nelson et al., 2005) and thus, cells should be viewed not isolated but in interaction with the extracellular matrix. As a consequence, the topography of the ECM and its mechanical properties like stiffness, elasticity or viscosity will directly impact tissue morphogenesis (Dvir, Timko, Kohane, & Langer, 2011).

Hydrogels from polysaccharides are interesting platforms for cell delivery due to their 3D structure and also because, like the ECM, hydrogels contain high water content, thus generating a favourable microenvironment for cell growth and/or

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differentiation (Varghese et al., 2008). *In situ* forming hydrogels are particularly attractive for cell delivery because they can be introduced on site by a minimally invasive procedure, and also perfectly fit the defect to be repaired. Designing such product is a challenge, as the network should form spontaneously chemically or physically, via weak but numerous interactions, and also allow the culture and expansion, both *in vitro* and *in vivo*, of the desired cells. Hence, a tremendous amount of research work is presently on going to design hydrogels that will meet these specifications, as a tool for the development of regenerative medicine. Polysaccharides are well adapted matrices on the basis of their biocompatibility and have found a widespread use in tissue engineering (Lee & Mooney, 2001). This is why this review focuses on the strategies under current investigation for achieving *in situ* forming hydrogels allowing the delivery of cells, based on polysaccharides as a polymer matrix.

2. Chemically *in situ* forming hydrogels

Hydrogels can be formed using well known cross-linking reactions (Liu et al., 2010) like photo-polymerization or Mickael addition onto (meth)acrylate derivatives of the polysaccharides, or by click chemistry or enzyme-catalysed coupling. But not all these well-described techniques can be applied *in situ*, like photopolymerization, as radiations, UVs for instance, cannot go deep into the tissues (Ruel-Gariépy & Leroux, 2004), without mentioning the cytotoxicity of some photosensitizers (Maia, Ferreira, Carvalho, Ramos, & Gil, 2005). Radical initiated polymerization could eventually be used by adding the (macro)monomer in a PBS solution containing an initiation system (Holland et al., 2005; Park, Temenoff, Tabata, Caplan, & Mikos, 2007) but the issue of removing the remaining initiator or initiator by-products will have to be addressed.

Hirakura et al. (2010) recently reported the gelation of hyaluronic acid by a Mickael addition reaction between the methacryloyl groups of HA derivatives and the sulfhydryl moieties of dithiothreitol under mild alkaline conditions. Though this strategy seems quite promising, to date it has not yet been used *in situ*.

Injectable cellular constructs based on gelatin microspheres were obtained via a cross-linking approach involving genipin as a non-toxic substitute of glutaraldehyde (Lau, Wang, & Wang, 2010). Genipin can cross-link aminated derivatives like gelatin but also chitosan a cationic polysaccharide extracted from shrimp/crab shells or fungi. Genipin cross-linked chitosan was used for the culture of bovine knee chondrocytes (Kuo & Lin, 2006), and again the genipin cross-linked chitosan-based delivery systems proved less toxic *in vivo* than their glutaraldehyde cross-linked counter-parts (Mi, Tan, Liang, & Sung, 2002) (Fig. 1).

Periodate oxidation of polysaccharides, whose mechanism is shown in Fig. 2 for dextran (Maia et al., 2005), leads to the formation of reactive aldehyde moieties on the polymer, that are used to form gels in mild conditions, compatible with the presence of biological moieties. The crosslinking of oxidized polysaccharides with adipic di-hydrazide, a non toxic and non mutagenic compound (Maia et al., 2005), is indeed a chemical cross-linking *per se*, via the formation of a stable acetylhydrazone-type adduct. What is interesting with this synthetic route is that it seems applicable to polysaccharides of different origins and chemical composition as long as they contain vicinal hydroxyl groups to let the oxidation reaction occur. Hence, chondroitin sulfate, hyaluronic acid, gum Arabic and sodium alginates were partially oxidized and transformed into a great variety of materials for biomedical applications (Liang et al., 2011). Instead of using a dihydrazide as a cross-linker, Tan et al. obtained injectable hydrogels by *in situ* cross-linking of N-succinyl chitosan, synthesized to increase the water solubility of chitosan, and oxidized hyaluronic acid. The amines of chitosan reacted with the aldehydes of oxidized hyaluronate and the resulting Schiff bases actually cross-linked the two counter parts. The gelation occurred in 1–5 min in PBS at 37 °C and could incorporate enzyme, insulin or chondrocytes (Tan, Chu, Payne, & Marra, 2009; Tan, Rubin, & Marra, 2010). Following a similar strategy, Nair et al. developed an injectable hydrogel based onto chitosan and oxidized hyaluronic acid, but they added some glycerophosphate (GP) to neutralize the protonated aminogroups of chitosan which could further react with oxidized hyaluronic acid to form Schiff bases. To neutralize a 2 wt% chitosan solution in 0.1 M hydrochloric acid, ca 6 wt% of GP was necessary. Chondrocytes could adhere on these porous scaffolds, proliferate, maintain their viability for over one month *in vitro* with a conservation of their phenotype and produce ECM components. The gelation took place within 2 min, which corresponds to the specifications of an *in situ* product (Nair, Remya, Remya, & Nair, 2011). Sodium alginate was oxidized by Liang et al. and reacted with a water-soluble derivative of chitosan (hydroxypropyl chitosan) to form a gel in less than 5 min. This mode of cross-linking decreased the toxicity of the materials and these gels were used for the encapsulation of corneal endothelial cells and successfully tested for the restoration of cornea in the New Zealand rabbit model (Liang et al., 2011). Biodegradable *in situ* gelling hydrogels were obtained by periodate oxidation of gellan gum a polysaccharide from microbial organisms, composed of tetra saccharide (Glc-GlcA-Glc-LRha) repeat units and FDA-approved as a food additive (Fan et al., 2010). Here, the chemical alteration of the polysaccharide was not for creating cross-links but to reduce the gelling temperature from 42 °C down to 28 °C, by cleaving the polymer chains, to allow chondrocyte culture and delivery.

3. Thermally responsive hydrogels

The more common situation with naturally occurring polymers is that they feature an upper critical solution temperature (UCST) above which the polymer is soluble and gelifies on cooling. For instance carrageenan, amylose or gellan gum (Coutinho et al., 2010) adopt a random coil conformation at high temperature, this is actually the way to solubilize them, and, on cooling, a 3D network forms via intermolecular hydrogen bonding, like gelatin does (Ruel-Gariépy & Leroux, 2004). To our knowledge, no application of UCST featuring polymers in cell delivery has been reported, probably because this solubilization temperature is too high to be compatible with cell viability.

On the other hand, the reverse process has attracted a lot of attention to obtain gels *in situ*. Heat can induce partial dehydration of some water-soluble polymers, leading to the formation of hydrogels, and this property can be tuned so as to occur at, or close to, body temperature. Hence, thermally induced gelation has long been an attractive route for the elaboration of rapid *in situ* gelling delivery systems, as this cross-linking strategy requires no potentially toxic chemical products, nor harsh physico-chemical conditions. A limited amount of polymers feature such a lower critical phase separation temperature (LCST) compatible with body temperature. The more commonly used ones are poly(N-isopropylacrylamide) (pNIPAM) whose LCST is around 32 °C (Ruel-Gariépy & Leroux, 2004), copolymers of ethylene oxide and propylene oxide (in particular Pluronic F127, LCST ca 30 °C, Higuchi et al., 2005), methyl cellulose (LCST ca 37 °C) which was used as an intercerebral construct after traumatic brain injury (Tate, Shear, Hoffman, Stein, & LaPlaca, 2001).

Since, naturally occurring polysaccharides do not feature thermal responsiveness, various strategies were envisioned to confer this physico-chemical property to polysaccharides such as (i) chemical modification, *e.g.* cellulose transformed into methyl cellulose, (ii) formation of interpenetrated networks (IPNs) with other polymers featuring thermo-responsiveness, and (iii) complexation with hydroxylated salts.

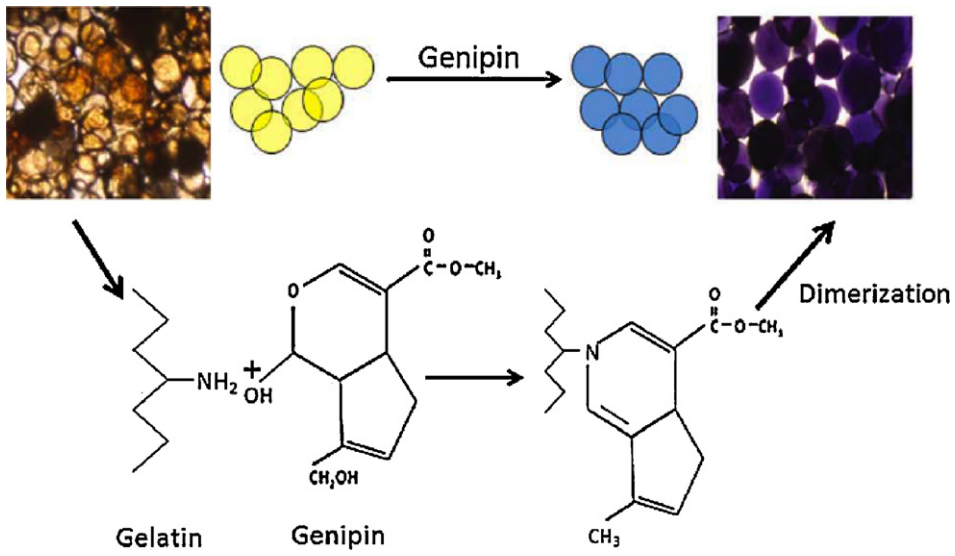

Fig. 1. Genipin cross-linking of materials.
From Lau et al. (2011) with permission.

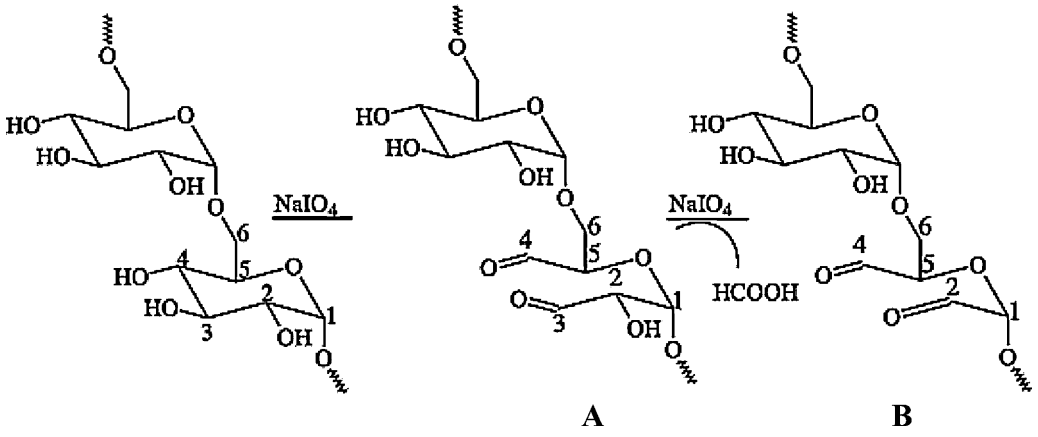

Fig. 2. Two step sodium periodate oxidation of dextran.
From Maia et al. (2005) with permission.

Besides methyl cellulose, other cellulose derivatives like hydroxypropyl cellulose and hydroxymethyl propyl cellulose also possess LCSTs (respectively LCST 45 °C and 55 °C, Kita, Kaku, Kubota, & Dobashi, 1999) as the incorporation of ether moieties create hydrophobic zones involved in the thermal dehydration leading to hydrogel formation. Chitosan was modified with hydroxybutyl groups, conferring to the macromolecules a thermo-responsiveness not observed with the parent polymer (LCST 21–26 °C depending on molar mass and degree of substitution). The thermo responsiveness of this chitosan derivative can be observed on the rheological properties of a 1 wt% solution that features a sol–gel transition temperature around 20 °C, with G' of 900 Pa at 37 °C (Fig. 3) (Zhu, Wei, Hou, Gu, & Chen, 2010). These derivatives were used to construct 3D structures loaded with disk cells or mesenchymal stem cells, that remained viable and proliferative for over two weeks without the addition of exogenous growth factors. Considering these results, with the fact that the sol–gel process took place in 60 s, hydroxybutyl chitosan-based hydrogels appear as potentially attractive cell delivery systems for the treatment of degenerative disk disease and potentially in numerous other contexts (Dang et al., 2006).

To confer thermo-responsiveness to polysaccharides without altering their chemical nature, i.e. no chemical modification,

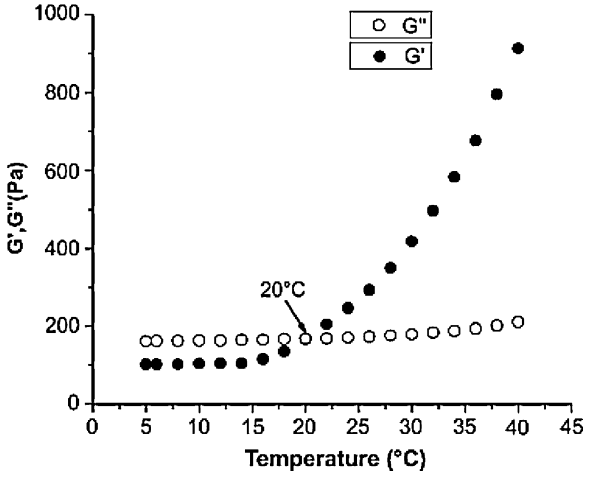

Fig. 3. Temperature dependence of storage and loss moduli (G' and G'') of a 1 wt% hydroxybutyl chitosan aqueous solution.
From Zhu et al. (2010).

they were associated to polymers featuring an intrinsic thermosensitivity. Many polysaccharides like chitosan, alginates, dextrans were mixed with thermo-sensitive polymers as recently reviewed by Prabaharan and Mano (2006). As mentioned above, pNIPAM has been the most widely used thermoresponsive polymer (Sun, Zhang, & Chu, 2007) but also other polymers like pluronics were associated with chitosan (for instance Gupta et al., 2007). An injectable composite hydrogel based on this strategy was developed by Wang et al., capable of releasing insulin-like growth factor (IGF-1) and delivering mesenchymal stroma cells. The hydrogel resulted from the association of two naturally occurring polymers, type I collagen and chondroitin sulfate with a pNIPAM derivative. This mixture gelled within 6 s at 37 °C, producing a gel whose moduli matched those of human myocardium and in which cells could proliferate and maintain their pluripotent differentiation potential. The pNIPAM derivative was designed so as to increase its biodegradation and biocompatibility (Wang, Li, et al., 2010), as pNIPAM itself suffers from toxicity (Vihola, Laukkanen, Valtola, Tenhu, & Hirvonen, 2005) and is not biodegradable (Pérez, Gallardo, Corrigan, & Román, 2008).

An innovative approach toward thermally sensitive polysaccharide was initiated by Chenite, Buschmann, Wang, Chaput, and Kandani (2001) and Chenite et al. (2000) who establish that chitosan/glycerophosphate solutions could undergo a sol–gel transformation on increasing temperature, around body temperature. The degree of acetylation of chitosan (DA), its molar mass and the reactant concentration impacted the appearance and structure of the hydrogel (Zhou et al., 2008). The suggested mechanism considers that, on adding the glycerophosphate, a partial neutralization of the chitosan solution took place, up to pH 6.8–7.2 without inducing any precipitation of the polymer, the two hydroxyl moieties of glycerophosphate maintaining a high level of hydration of the polymer chains which remained soluble. On heating, hydrogen bonds between water molecules, glycerophosphate and chitosan were broken and, because of this desolvation, polymer chains associated in a 3D hydrogel (Ruel-Gariépy, Chenite, Chaput, Guirguis, & Leroux, 2000). Lavertu, Filion, and Buschmann (2008) established that the increase in temperature induced a proton release from chitosan that could be trapped by glycerophosphate, whose pK_a is not affected by temperature, inducing the gelation process. In a subsequent investigation, Kim et al. (2010) actually evidenced by FTIR the protonation of phosphate groups in the chitosan gel forming solution with increasing temperature. This mechanism probably explains why, Rossi et al. (2010) found that trimethylchitosan gelled in the presence of GP only at low degree of substitution (namely 3%) and not at higher ones (78%), because not enough primary amines remained to allow the proton exchange. From this mechanism, it is quite clear that, to confer thermo-responsiveness to chitosan, amounts of glycerophosphate such as 45 wt% should be used, but severe inflammatory responses have been observed in rats (Molinaro, Leroux, Damas, & Adam, 2002). Nevertheless, this toxicity was not systemic as no hepatic, nor renal toxicities were noticed (Zhou, Zhang, Zhang, & Chen, 2011) and, moreover, its intensity depended on the route of administration with an acute edema by subcutaneous injections and a less pronounced one by transdermal injection of the same product in the same animal model (Molinaro et al., 2002). This biocompatibility issue was further investigated in 2010, in the context of the development of an injectable material for culturing stem cells. Hydrogels obtained with 80 wt% glycerophosphate induced severe cell growth inhibition in comparison with gels obtained with 40 wt% of GP. But theses latter gels could not be used for *in situ* gelation purposes as the gel temperature was around 46 °C. To drop this temperature down to 15 °C, the authors added, to the chitosan–GP mixture, hydroxyethyl cellulose at a concentration of 2 wt% (Yan et al., 2010). Another strategy proposed by Wu, Wei, Wang, Su, and Ma (2007)

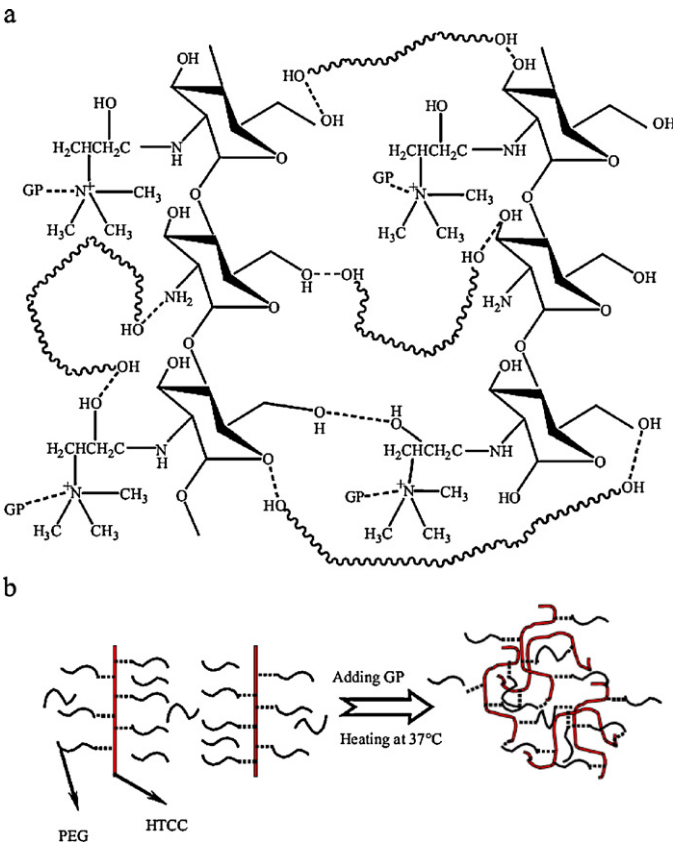

Fig. 4. Mechanism of the thermosensitivity of hydrogel from quaternized chitosan (HTCC)/PEG/GP using low GP concentration.
From Wu et al. (2007) with permission.

to reduce the GP content in the 3D-matrix consisted in adding a polyethylenoxide, PEG-4000, to a mixture comprising 3.6 wt% of quaternized chitosan and 3 wt% of GP. Both components, GP and PEG-4000, brought the thermosensitivity to the chitosan derivative (Fig. 4), hence the low viscosity solution could gel at 37 °C. Still with the objective to reduce the toxicity related to the use of high GP concentrations, Kim et al. modified the experimental protocol for the preparation of thermally sensitive GP-chitosan gels. The excess of acid used to solubilize chitosan in an aqueous solution was dialysed and, as a result, the amount of GP necessary to induce gelation could significantly be reduced to a few weight percents. Another means for reducing the amount of GP consisted in adding another partner to chitosan such as collagen, when rBMSCs were co-injected sub cut in rats, the stem cells survived at least 28 days at the injection site (Huang et al., 2011).

4. pH responsive hydrogels

Polysaccharides can bear ionogenic groups whose ionization may depend on the pH of the medium, like in the case of carboxylic acid moieties such as in alginates or amine groups as in chitosan. This ionization ensures the water solubility of the polymers, which have thus become polyelectrolytes, either positively charged, for chitosan, or negatively charged for alginates. For the sol–gel transition to occur, two conditions should be met (i) the polymers should be at a concentration higher than the critical chain entanglement C^* and (ii) the charge density should be reduced to favour chain interactions and create physical cross-links (Montembault, Viton, & Domard, 2005). This charge reduction can be achieved by altering the pH of the polysaccharide solution, in such a way that precipitation is prevented as it could hamper the gelation process.

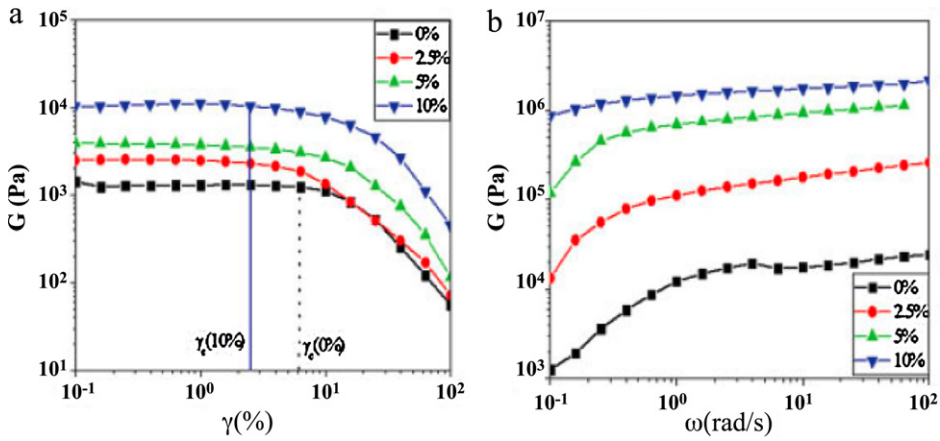

Fig. 5. Plots of dynamic storage modulus G' versus (a) strain γ and (b) frequency at 37 °C for PLA microspheres and 0.6% KGM/1% CML hydrogel composites with different content of microspheres.
From Hong et al. (2008) with permission.

Montembault et al. (2005) carried out this charge reduction uniformly, on at least 2 dimensions of the considered volume, by letting gaseous ammonia diffuse through the whole volume of a chitosan solution. But these experimental requirements are hard to achieve *in vivo*, during injection of the gel precursor in a defect or subcutaneously. As observed by Chiu et al., a chitosan solution injected in phosphate buffer saline (PBS, pH 7.4, 150 mM NaCl) led to a local precipitation of the polysaccharide. To circumvent this, the authors used a hydrophobic derivative of chitosan with an optimum degree of substitution by palmitoyl chains of ca 15% and obtained a homogenous gel (Chiu, Chen, Chen, et al., 2009). The pre-organized solution, comprising micellar aggregates linked to one another, yielded a homogenous gel organized as a network of nanodomains. On the biocompatibility standpoint, these implanted gels were found biodegradable, with an initial macrophage response, suggesting that these gels could be used as injectable cell delivery systems (Chiu, Chen, Su, et al., 2009).

Silylated compounds, in particular silanols, can ionize via proton abstraction or can condense onto one another just like for silicon synthesis. In 2005, Trojani et al. took advantage of this property to form 3D structures from silylated hydroxymethylpropyl cellulose. The polymer was a trimethoxy silyl derivative of HMPC that could only be solubilized in alkaline pH (around 12). Mixing this solution with an appropriate cell culture medium decreased the pH of the Si-HMPC solution, as a result, silanol groups formed and their condensation led to the macrogel. Human osteoblastic cells were successfully cultured in these gels for more than 3 weeks, with maintenance of their osteogenic potential.

5. Composite hydrogels

As we have seen above, *in situ* forming hydrogels have a great potential for regenerative medicine applications as they allow the simple delivery and encapsulation of viable cells, thanks to the high hydration level of their microenvironment, maintaining the exchanges of cellular nutrients and wastes. These conditions are favourable for cells whose fate does not depend on binding onto a substrate, but for anchorage-dependent cells (ADCs) like fibroblasts, osteoblasts, endothelial, epithelial and smooth muscle cells, it is essential to provide them with adhesion zones within the 3D culture device. To address this issue, the use of composite hydrogels comprising microparticles entrapped within a hydrogel has been fairly documented (Wang, Varshney, & Wang, 2010). The role of the dispersed particles is to provide the cells with anchorage sites of well-adapted dimensions to maintain ADCs' viability and this aspect was recently reviewed by Sun, Lin, Li, Harn, and Chiou (2011). The optimal microparticle size range is 150–400 μm and they can be composed of collagen, gelatin (Lau, Wang, Png, Su, & Wang, 2011) or with polysaccharides like dextran or gellan gum eventually coated with gelatin (Wang, Gong, Lin, Shen, & Wang, 2008). For instance, Wang et al. in 2009 encapsulated gelatin grafted gellan microspheres loaded with human mesenchymal cells into agarose gels and observed that the cells remained viable after 7 days in culture. In contrast, the same cells dispersed free in the same hydrogel without any possible focal adhesion, had regularly gone into apoptosis over the 7 day period (Wang et al., 2009). An *in situ* gelling system based on the radical initiated polymerization of a chitosan derivative in the presence of collagen coated poly(lactic acid) microparticles was used by Hong et al. as an injectable scaffold for cartilage regeneration. Not only the microparticles helped improve the viability of chondrocytes but also impacted the mechanical properties of the final system (Fig. 5). On increasing the amount of microparticles, the storage modulus G' increased (100 fold compared to the gel alone), but the critical strain at which G' started to decrease lowered, suggesting that their presence induced defects in the hydrogel matrix (Hong, Gong, Gao, & Shen, 2008). An other example of thermosensitive hybrid hydrogel was obtained from chitosan, GP, collagen and hydroxyapatite and could gel at body temperature. In this particular case, the purpose of adding hydroxyapatite to the collagen fibers embedded within the chitosan–GP hydrogel was to produce a microenvironment close to that of the bone. With such a composite system, the authors injected rat bone marrow stems cells into rats, keeping the cells alive for 28 days (Huang et al., 2011).

6. Shear responsive gels

In situ forming gels can be achieved without any need of chemical cross-linker, nor physical stimuli like pH change or heat, simply by stiffening thanks to electrostatic, van der Waals or steric interactions. These interactions will develop at rest and will be broken at shear, bringing reversibly the material back to solution. These materials are shear thinning or thixotropic hydrogels and, hence, have a great potential of use in cell delivery as, once again, these systems do not need potentially toxic chemical cross-linkers, nor physico-chemical processing.

Van Tomme, van Steenbergen, De Smedt, van Nostrum, and Hennink (2005), by using dextran microspheres derivatized with poly(sodium methacrylate) or dimethylaminoethyl methacrylate for respectively negatively and positively charged particles,

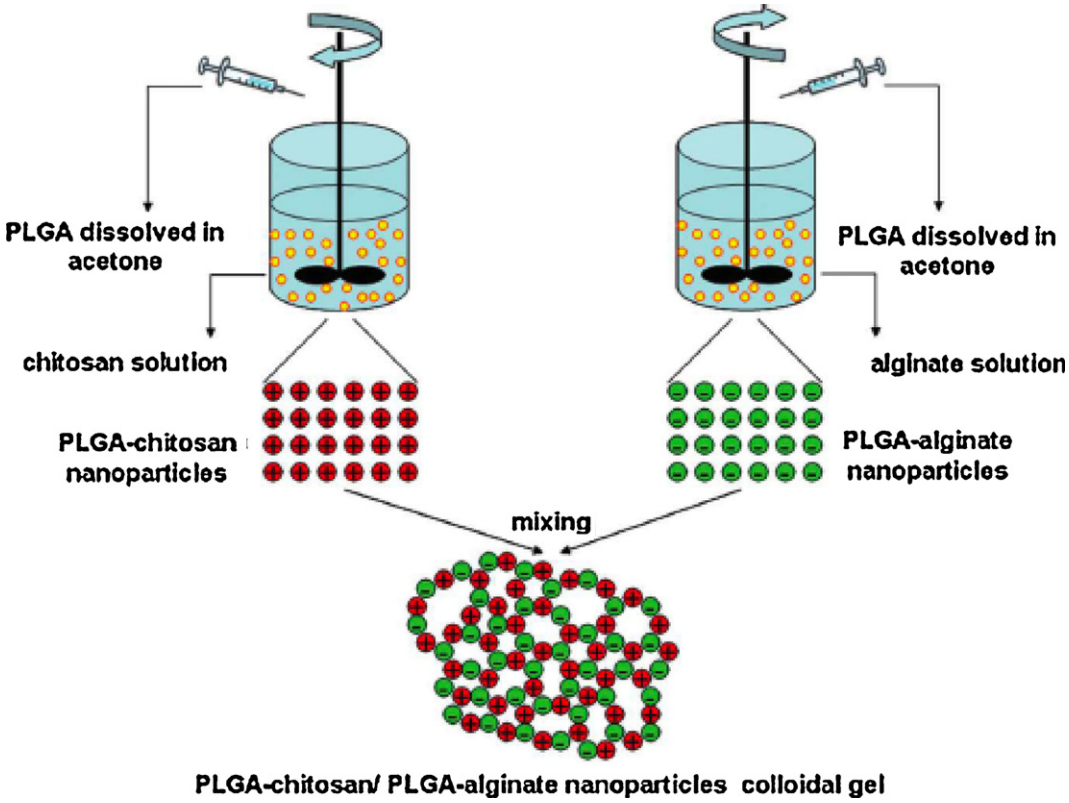

Fig. 6. Elaboration of colloidal gels for the culture of mesenchymal stem cells. From Wang et al. (2011) with permission.

demonstrated that gelation occurred when equal amounts of oppositely charged microspheres were mixed and the shear modulus varied from 30 to 6500 Pa when the water content decreased. In a latter work from the same team, the authors used enantiomeric pure L or D oligolactates to functionalize the interface of the dextran microspheres. These oligomers of opposite chirality formed stereocomplexes that led to the reversible formation of elastic gels. To proceed further to a cell delivery system, Wang et al. mixed PLGA particles coated with chitosan, as the positively charged counterpart, or with alginates for the negative particles (Fig. 6). A 3D porous network was formed, via attractive electrostatic interactions, and could be extruded or molded to the desired shape. As for the dextran microparticle system, the obtained colloidal gels were shear thinning thanks to the disruption of the particle–particle interaction; at rest, these interaction were strong enough to allow the recovery of the cohesive properties of the assembly (Wang, Jamal, Detamore, & Berkland, 2011). In this investigation the particle diameter was in the submicron range, whereas Van Tomme et al. used particles in the micron-range. To obtain such a 3D structure a solid content of at least 10 wt% in particles was necessary, nevertheless, the structures observed by SEM depicted a high porosity favourable for the culture of human umbilical cord mesenchymal cells (UCMSCs) for a period of two weeks.

7. Conclusion and prospects

Tissue engineering and regenerative medicine is a fast expanding field of science based on a transdisciplinary approach, as many different parameters have to be taken into account in a sequential manner, which must be controlled spatiotemporally. Hence, cellular biology, medicine, materials sciences, pharmaceutical sciences, biophysics are needed to work together in a synergetic way. To allow for the regeneration of a new tissue/organ, starting from cells, one must create a local environment as close as the Extra Cellular Matrix as possible, both in terms of structure and of dynamics. The structural parameters can be viewed as the chemical composition of the new environment, its shape, its organization and mechanical properties; the dynamics concern (i) the evolution of the properties, organization and composition of environment with the cell development and neo-tissue formation, (ii) the capacities of the new environment to deliver and control specific cues during tissue/organ development, and (iii) the capacities of the new environment to integrate specific cues from the neighbouring existing tissues. Finally, the development of a therapeutic protocol should be the simplest possible, for economic reasons and for the comfort of both the patients and the practitioners.

Polysaccharide-based hydrogels are quite promising as, like the ECM they have a high water content and polysaccharides have many potentials in terms of biocompatibility, biodegradability and cellular interactions (including cell fate, cell motility and also cell degradability) since some polysaccharides enter in the composition of the ECM. We have seen that various approaches are under investigation to create an appropriate microenvironment that permits cell expansion and further development into a tissue or an organ, but also which fits to the tissue/organ to be replaced and be easy to implant in patients. The objective of the various investigations reviewed here was to confer to polysaccharides extra abilities (stimulus responsiveness, mechanical properties, etc.) but maintaining (and even improving) their exceptional capability at culturing viable and functional cells.

Future work will probably go into two major directions. First, is the translation of many academic results into (pre)clinical investigations in order to select the most promising/viable approaches in terms of medical development toward new commercially available products, addressing in particular the issues of large scale

production, batch to batch reproducibility and safety (biocompatibility, biodegradation and sterilization). On a more academic standpoint, it will be necessary to address the following issues: (i) delivery of some biological and physical cues, by encapsulating growth factors or adding mechanosensitive entities, and spatiotemporally controlling their delivery (by nanoengineering hydrogels or bringing more capabilities to positively response to biological stress), (ii) dealing with the concurrent/concomitant development of various cell types (or inducing from the same cell delivery hydrogel a controlled differentiation of stem cells), and (iii) adding development patterns to complex tissues/organs (here again via nanoengineering techniques). In this future, it will be essential to take into account what nature does, in other words to design systems capable of integrating the natural information system, i.e. the environmental cues originating from the local tissues and also eventually from the whole organism and capable of adapting its properties along with tissue/organ development.

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