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Review

Bioresponsive Hydrogels: Chemical Strategies and Perspectives in Tissue Engineering

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Abstract: Disease, trauma, and aging account for a significant number of clinical disorders. Regenerative medicine is emerging as a very promising therapeutic option. The design and development of new cell-customized biomaterials able to mimic ECM functionalities represent one of the major strategy to control the cell fate and stimulate tissue regeneration. Recently, hydrogels have received a considerable interest for their use in the modulation and control of cell fate during regeneration processes. Several synthetic bioresponsive hydrogels are being developed in order to facilitate cell-matrix and cell-cell interactions. In this review new strategies and future perspectives of such synthetic cell microenvironment will be highlighted.

Keywords: hydrogels; tissue engineering; bioconjugation

1. Introduction

Bioresponsive hydrogels are dynamic systems that are capable of responding to or stimulating specific signals through the natural biological processes. The possibility to tailor hydrogel composition, mechanical properties and degradation rate makes this class of materials promising tools for tissue engineering and regenerative medicine applications. Hydrogels are cross-linked 3D networks containing hydrophilic polymer chains that can absorb a significant amount of water [1] with excellent mechanical and chemical versatility, which favors their biomedical applications. In addition, hydrogels can be easily implanted by non-invasive injection in order to avoid complex surgical intervention, even in not defined or irregular target site of injured tissues [1]. Among these, bioresponsive hydrogels are often referred to as "smart" systems and are attracting great interest because they exhibit specific and tunable physical and biochemical properties when exposed to environmental factors in our body such as pH, temperature, or enzyme and receptors [2]. These general features may result in hydrogel responses (i.e. in term of swelling, degradation, mechanical deformation) [3] and/or in cells and tissue responses. This ability can be obtained and controlled by the spatial functionalization of hydrogel constituents with specific biological entities (biocues) in order to induce the desidered stimuli. Biological entities used with this aims can be native or synthetic biomacromolecules, such as enzymes, antibodies, nucleic acids, [4] or small bioactive molecules such as carbohydrates [5] or peptides. [6] In this review, we will focus on a brief overview of the strategies employed to obtain bioresponsive hydrogel through functionalization with bioactive molecules.

2. Classification of the hydrogels

On the basis of their composition, it is possible to distinguish naturally, synthetic and composite or semisynthetic hydrogels (Figure 1). Natural hydrogels are composed of natural polymers including polynucleotides, proteins and polysaccharides. These natural polymers have different natural origins (animal or from plants and microorganisms). Several polysaccharide-based or protein-based hydrogels have been developed. A number of examples of polysaccharides based hydrogel with tuneable properties are cited in the literature for cartilage and bone tissue engineering applications. Most cited hydrogels for these applications were be prepared using hyaluronic acid, chitosan and alginate biopolymers. Hyaluronic acid (HA), alginate and chitosan are hydrophilic

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polysaccharides. It is known that HA is the simplest glycosaminolglycan (GAG) found in almost every mammalian tissue and fluid. It was found prevalently during wound healing and in synovial fluids of joints. It is a linear polysaccharide composed of a repeating disaccharide of (1–3) and (1–4)-linked β-D-glucuronic acid and *N*-acetyl-β-D-glucosamine units.

HA-based hydrogels have been produced by covalent crosslinking for example by hydrazide derivatives, by esterification, by carbodiimide chemistry and Huisgen-type cycloaddition (click chemistry) [7]. Additionally, HA has been combined with both collagen and other proteins to form composite hydrogels [8]. Chitosan has been studied and employed for many tissue engineering applications; in fact it has a similar structure compared to naturally occurring GAGs, and it is degradable by human enzymes. It is a linear polysaccharide made up of (1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine residues derived from chitin, which is found in arthropod exoskeletons. One of the main chitosan property is its solubility in dilute acids by protonation of the free amino groups, so, it can be gelled, for example, by increasing the pH. Chitosan hydrogels were obtained also *via* glutaraldehyde crosslinking, UV irradiation or thermal variations [9].

Alginates are composed of guluronic acid and mannuronic acid. Its abundance, and low prices, allows a widespread use in the food industry as thickener, emulsifier, and in tissue engineering applications. Alginate was used for different medical purposes, such as cell encapsulation and drug delivery, because it is able to gel under mild conditions, and has low toxicity. Hydrogel production takes place when divalent cations such as Ca^{2+}, Ba^{2+}, or Sr^{2+} cooperatively interact with monomers to form ionic-bridges between different polymer chains. Alginate-based hydrogel has also been obtained by covalently crosslinking with adipic hydrazide or PEG using common carbodiimide chemistry [10].

Thanks to their structural role in nature, also protein-based hydrogels gained great interest as polymers to design smart hydrogels for tissue engineering. Collagen-based hydrogels have found application due to its ubiquitous presence in different tissues of human body. However, collagen is naturally degraded by metalloproteases, in particular collagenases, and serine proteases, allowing cells to degrade it. Several crosslinking strategies were performed to control mechanical properties and 3D structures using for example BDGE linkers or genipin crosslinking [11,12]. Another protein, gelatin (obtained from collagen hydrolysis) has captured increasing attention as it has relatively low antigenicity although maintaining the properties of biocompatibility and biodegradability; moreover, gelatin is significantly less expensive than collagen [13]. Several examples of photocrosslinkable gelatin (i.e., gelatin methacrylamide (GelMA)) with tunable mechanical, degradation, and biological properties were investigated for tissue engineering applications. [14,15]. Other chemoselective crosslinking strategies involved thiol-ene photopolymerization between thiolated gelatin and pentenoyl gelatin [16] or thiolated gelatin and PEGdA [17]. These are just a few examples of natural biopolymers used for tissue engineering applications. Despite hydrogels derived from natural biomacromolecules such as protein or polysaccharides can actively support cell proliferation, migration and differentiation, these materials are poorly mechanically robust and hard to process, and it is also difficult to maintain product consistency. To overcome these limitations, synthetic materials represent promising starting materials in scaffold design. Synthetic hydrogels are interesting biomaterials for tissue engineering because their chemistry and properties can be controlled and reproducible. For example, synthetic polymers can be designed with specific molecular weights, degradable linkers, and crosslinking modes. Consequently, these features allow to fine tune gel formation dynamics, material mechanical properties, crosslinking density and degradation. Examples of synthetic materials are PEG ((poly(ethylene glycol)), PLA and PCL. PEG is currently FDA approved for a lot of medical applications and is one of the most frequently applied synthetic polymer for hydrogel preparation. PEG is a hydrophilic polymer usually photocrosslinked by modifying each end of the polymer either with acrylates or methacrylates [18]. Thermally reversible hydrogels have also been produced from block copolymers of the hydrophilic PEG and several polyesters as the hydrophobic block; poly(lactide) (PLA), poly(glycolic-co-lactic acid) (PLGA) and poly(ε-caprolactone) (PCL) and poly(l-lactic acid) (PLLA) are extensively used [19], because of

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their biocompatibility, biodegradability and facile synthesis by ring opening polymerization of lactide, glycolide or ε-caprolactone monomers. .

However, all these synthetic materials lack biologically active sites. In order to overcome this drawback, extensive conjugation chemistry is needed to introduce bioactive molecules, such as the well-known RGD peptide (arginine–glycine–aspartic acid) to allow cell adhesion [20] or bioactive molecules able to induce and guide specific biological phenomena (i.e growth factors, carbohydrates able to guide cell differentiation) [21]. Different complementary bioconjugation methods have also been used to introduce biological functions to synthetic materials [22].

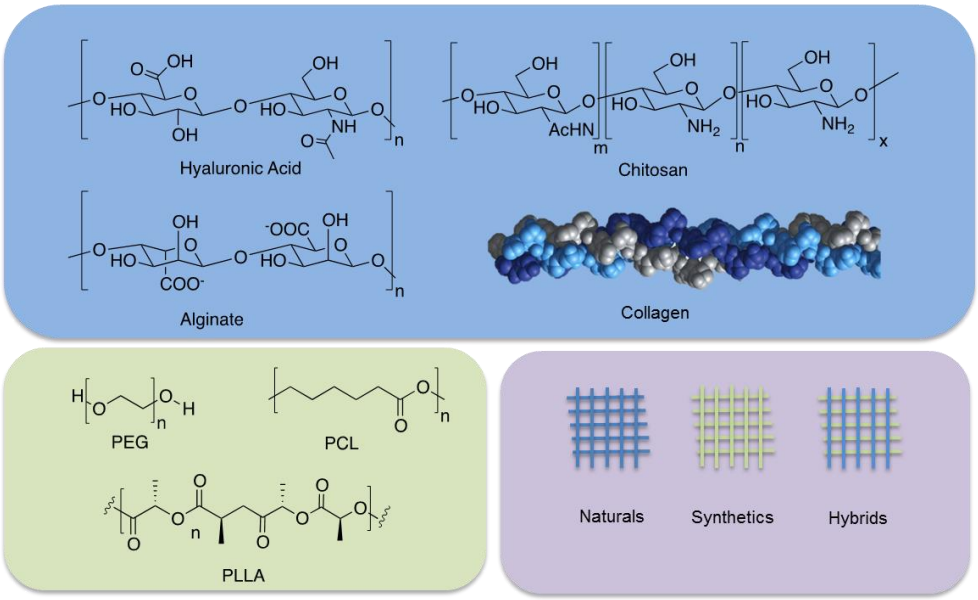

Figure 1. Naturally, synthetic and composite or semisynthetic hydrogels

3. From tissue complexity to hydrogel design: the simplification game

In the field of tissue engineering, hydrogels have the necessity to satisfy different design parameters for their integration in damaged site, and therefore to operate in an appropriate manner and support new tissue formation. Hydrogel scaffolds, for example, should provide a 3D architecture able to support cell growth and tissue integration and differentiation. This 3D structure better mimics the natural tissues and allows for morphology and gene expression. In order to develop hydrogels for tissue engineering applications, there is the necessity to take into consideration different parameters among which we must consider: mechanical and physicochemical criteria (i.e. biodegradation) and biological performance parameters (i.e. biocompatibility, cell adhesion and proliferation). Moreover, it is important to consider the availability and commercial feasibility when producing hydrogel scaffolds for tissue engineering applications. The native ECM supplies a plethora of signals to neighbouring cells that modulate functional outputs, in combination with cell-cell and cell-ECM signalling [23]. Moreover, the extracellular matrix should be imagined as a dynamic *milieu*, where the local environment can be reshaped through cell-mediated secretion and deposition of biomolecules or degraded through cell secreted enzymes called matrix metalloproteinases (MMPs). Tissue development and remodelling *in vivo* is coordinated by several regulatory factors interacting at multiple levels, in time and space. In the fields of tissue engineering and regenerative medicine, driving cells to differentiate in the right manner, at the right time, in the right place, and into the right phenotype requires an environment able to supply the same factors that drive cellular processes *in vivo* [24]. Hydrogels composed of biologically derived building blocks, such as extracellular matrix (ECM) components (for example collagen or GAGs), are being studied for a number of applications in the field of regenerative medicine, since proteins, glycoproteins and proteoglycans are essential models for biomaterials design and production, providing chemical, physical and biochemical properties able to mimic the native cellular microenvironment. The extracellular matrix mechanically

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supports interactions between cells for healthy tissue generation and preservation, like a scaffold that provides the framework for a high-rise building. Mimicking the natural tissue and microenvironment is not an easy job, but with the growth in the knowledge about physicochemical and biochemical cell signalling processes, this goal is now feasible for the design and production of tissue engineering scaffolds. To promote understanding of the cell-niche interactions, hydrogels have been extensively employed as artificial cell niche, given their tissue-like water content as well as tunable physico-chemical properties. However, only few hydrogels produced to date allow the control of cell microenvironment properties such as biochemical signals and mechanical stiffness [25].

4. Bioactivation strategies

Bio(macro)molecule conjugation (bioconjugation) is a useful approach that allows the improvement of the performance of hydrogels through cell-responsive molecules, such as proteins, peptides, [26,27] or even carbohydrate epitopes [28,29,30], in order to modulate and drive cellular fate. The conjugated biomolecules can be chosen in order to mediate different cellular events fundamental in regenerative medicine approaches, such as adhesion, migration, proliferation, and differentiation. Probably, the idea of bioresponsive hydrogel came from the pivotal study by Tirrell and co-workers [31] on self-assembly and gelation of a triblock artificial protein that was conjugated to a PEG moiety in order to control the gelling properties as a function of pH and temperature. Generally speaking, bioconjugated hydrogels may be classified into different groups depending on the bioactive molecule used for bioresponse activation: hydrogel-peptide conjugates, hydrogel-protein conjugates, hydrogel-glycan conjugates and hydrogel-small molecule conjugates.

4.1 Hydrogel-protein conjugates

Proteins are fundamental and ubiquitous macromolecules playing a crucial role in the body mediating a plethora of cell processes and for this reason relevant for the design of hydrogel for tissue regeneration [32,33]. The incorporation of proteins and especially growth factors in hydrogels allows to mimic the native cellular microenvironment, and may be a valuable tool to induce cell–substrate and cell–cell interactions for tissue engineering and regenerative medicine applications. However, their complex structure, and in some cases the limited availability through biotechnology and molecular biology techniques may hamper the development of protein-conjugated hydrogels. Moreover, protein may have poor chemical stability and, together with their tendency to aggregate, their bioactivity may be limited. Despite these issues, various approaches have been developed to incorporate and deliver proteins into hydrogels. In general, different strategies can be used to include proteins into hydrogels, such as physical entrapment or covalent linkage to the hydrogel macromolecules. The bioconjugation through covalent bonds may offer some advantages in terms of stability against *in vivo* degradation forces and of the maintenance of the therapeutic concentration in the hydrogel. A few examples of smart strategies for covalent protein bioconjugated hydrogels will be given in this section.

Anseth and co-workers fabricated PEG hydrogels *via* a thiol-acrylate photopolymerization reaction; in particular, PEG-diacrylate precursors were conjugated to thiolated EphA5-Fc receptor and ephrinA5-Fc ligand for enhancing pancreatic β-cell survival (Figure 2) [34]. EphA receptor and ephrinA ligand are cell surface-bound proteins involved, among others, in insulin secretion from pancreatic β-cells, in promoting cell adhesion and motility/morphology changes through the integrin signalling pathway. These bioresponsive hydrogels were shown to provide crucial cell-cell communication cues for cell survival and proliferation.

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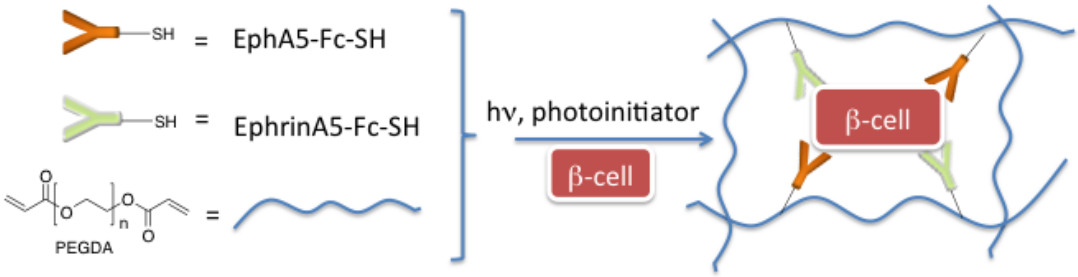

Figure 2. Schematic representation of PEG-based hydrogels conjugated to EphA5-Fc and EphrinA5-Fc.

In other examples, transforming growth factor-β1 (TGF-β1) was linked to photopolymerizable methacrylated chitosan (MeGC) hydrogels [35], and to acrylated HA hydrogels [36]. The first hydrogel was successfully used to induce chondrogenesis in hMSCs, while the second one showed enhanced cell survival and engraftment of encapsulated murine cardiac progenitors to the host tissue after transplantation, accompanied by vascularization.

Toward cardiac tissue regeneration, several hydrogels functionalised with different or multiple growth factors have been proposed. For example, stromal derived factor-1α (SDF-1α), conjugated to a PEG hydrogel [37] *in vivo* was demonstrated to sustain colonization of progenitor cells in the ischemic tissue and to promote the angiogenetic process. Similarly, the recruitment of progenitor cells inducing re-epithelization and revascularization was observed with alginate-based hydrogels bioconjugated to SDF-1α [38]. Another hot issue in regenerative medicine is neural tissue regeneration [39,40]. In this respect, an increase of axon outgrowth of dopaminergic neurons from rat embryos or differentiated from stem cells in culture was obtained through an injectable PEG-silica composite hydrogel conjugated to semaphorin 3A. Semaphorins are a class of secreted and membrane proteins particularly relevant in neural system development, involved in axonal growth cone guidance and in the deflection of axons from inappropriate regions. However, the presence of non-degradable silica particles embedded in the hydrogel resulted in an increase in macrophages and glial cells in long term implantation studies. The chemical bioconjugation of complex signalling proteins is a hard task, since the activity and stability of proteins can be compromised by the conjugation reaction that might not be sufficiently mild and/or chemoselective. In order to address this issue, an extremely interesting approach was proposed by Lutolf and co-workers, who obtained the spatiotemporally controlled enzyme-mediated bioconjugation of vascular endothelial growth factor 121 ($VEGF_{121}$) and the recombinant fibronectin type III repeat 9-10 fragment (FN_{9-10}) [41]. Briefly, the authors synthesised a PEG-based hydrogel, functionalised with a "masked" enzyme substrate, that could be rendered accessible to the related enzyme by a photocalysed reaction (Figure 3). Once the substrate is accesible, the enzyme, that is FXIIIa, a key enzyme involved in the blood coagulation cascade, catalyses the reaction between the ε-amino group of lysine (K) and the carboxamide side chain of glutamine (Q), to the corresponding ε-(γ-glutamyl)lysine isopeptide. Using recombinant $VEGF_{121}$ engineered with an exogenous peptide domain at the *N*-terminus containing the required glutamine to enable enzymatic cross-linking (Q-peptide in Figure 3), the enzymatic reaction affords the PEG hydrogel conjugated to the growth factor. The patterned PEG hydrogels resulted biocompatible for MSC cells.

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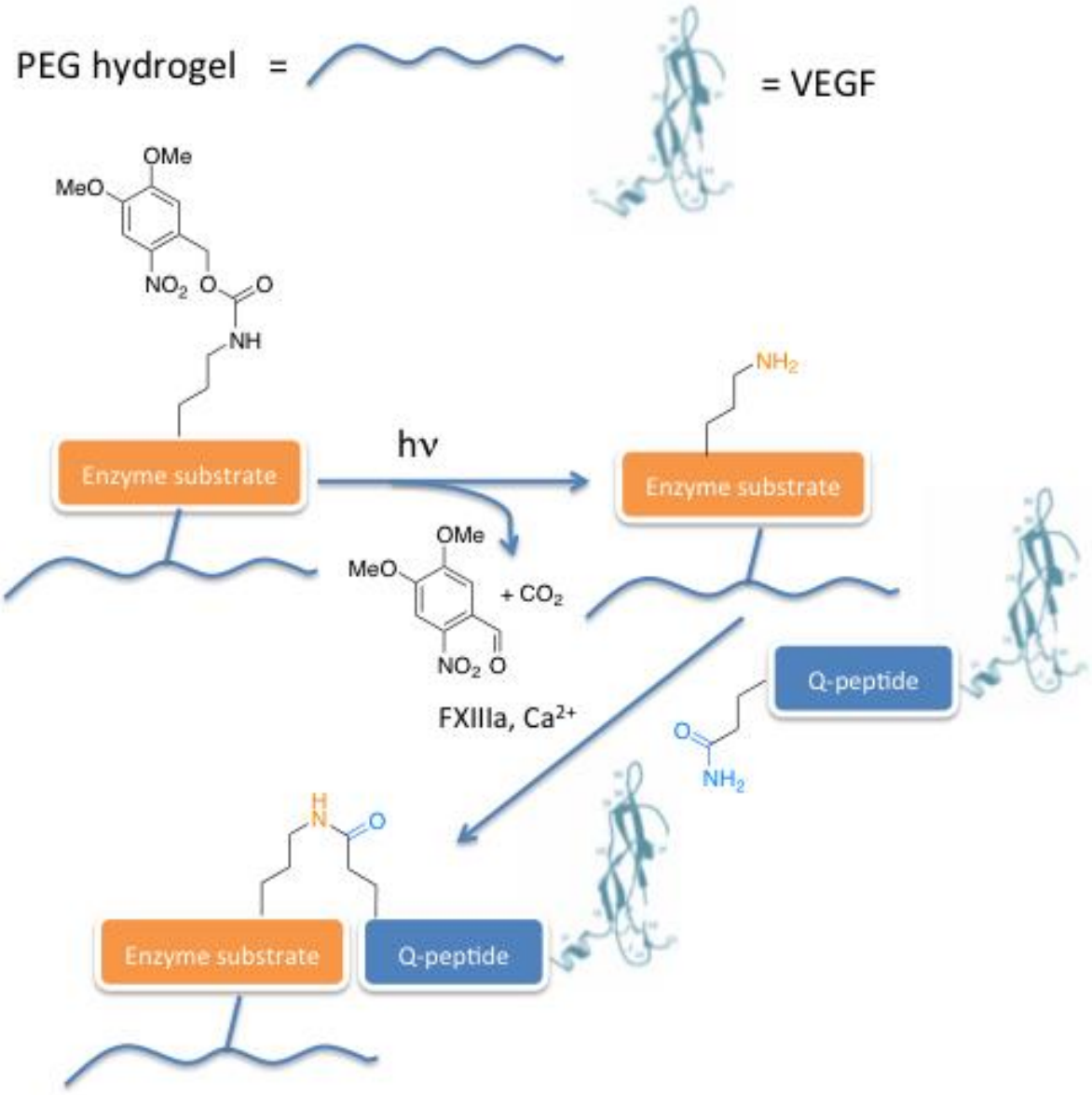

Figure 3. Strategy for the synthesis of PEG hydrogels conjugated to VEGF, through an enzyme-catalysed bioconjugation step.

4.2. Hydrogel-peptide conjugates

The bioconjugation of peptides in place of full-length proteins greatly simplifies the chemistry and may improve the efficacy of the bioconjugation. Since many mammalian cells are anchorage-dependent, cell adhesive properties of the hydrogel can be achieved by the introduction of small adhesive peptidic sequences into the hydrogel matrix [42]; for example they can be derived from laminin, such as RGD (Arg-Gly-Asp) [43], YIGSR, LGTIPG, IKVAV, PDGSR, LRE, LRGDN and IKLLI [44], or from type I collagen and fibronectin, i.e. DGEA, KQAGDV, REDV and PHSRN. PEG-based hydrogels are useful for tissue engineering applications due to their favourable porosity, mechanical properties, and biocompatibility. However, due to their chemical nature, PEG does not possess cell attachment motifs. The conjugation of adhesive peptides such as the RGD sequence improves PEG hydrogel biofunctionality, rendering PEG-based hydrogels more suitable mimics of ECM. In this respect, Anseth and co-workers synthesised a dynamically controlled PEG-based hydrogel containing the adhesive sequence RGD, through a double-click reaction (Figure 4) [45].

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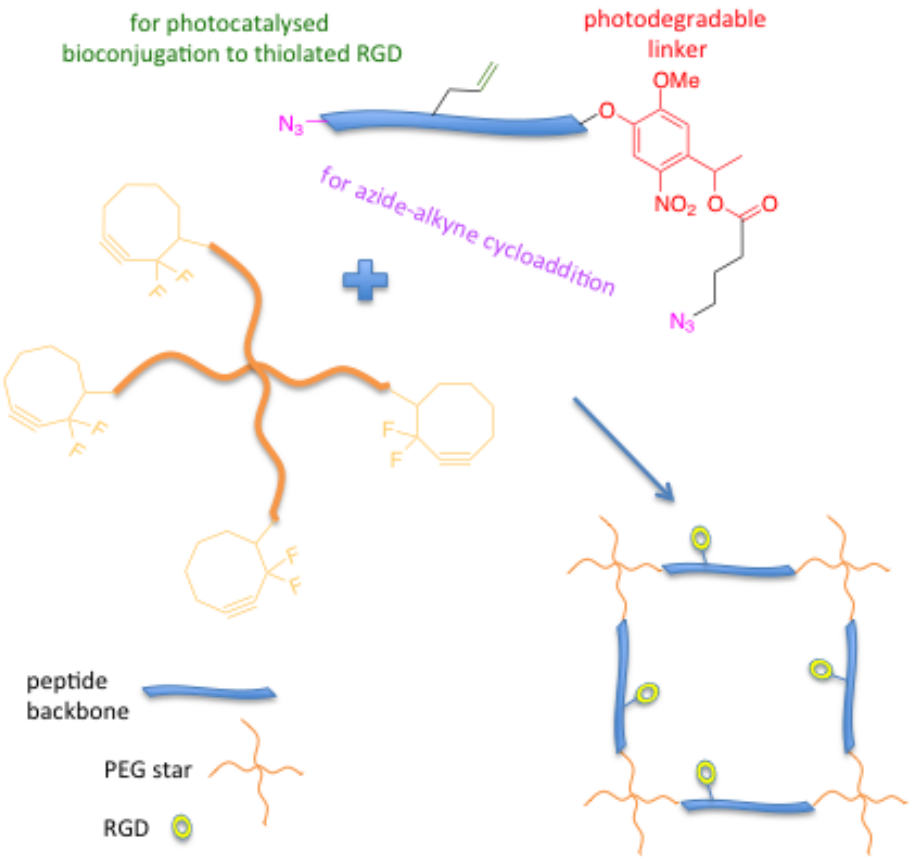

Figure 4. Strategy for the synthesis of photodegradable adhesive hydrogels.

Spacer length between adhesive peptides and the material scaffold may be a critical parameter for regulating cell phenotype in tissue engineering. An interesting example of this issue was given with alginate hydrogels functionalised with RGD peptides linked through varying spacer arm lengths and assessed with primary human fibroblasts either on 2-D or 3-D scaffolds [46]. Alginate hydrogels were functionalised with G_nRGDSP moieties (n = number of glycine units) through EDC/sulfo-NHS coupling. A minimum number of four glycine units in the spacer arm resulted essential for enhanced adhesion and growth of fibroblasts. An optimal spacer arm length was also needed for minimizing cellular stress, as determined by the expression of heat shock proteins and Bcl-2.

Despite the extensive use of adhesive sequences, such as RGD and close peptides, other amino acid sequences have been conjugated to hydrogels in order to stimulate different cellular responses. For example, in order to control and reduce the oxidative stress experienced by cardiomiocytes (CMs) in myocardial infarction, the antioxidant tripeptide glutathione was bioconjugated to a chitosan-based hydrogel (Figure 5) [47]. The glutathione-conjugated chitosan resulted effective *in vitro* as antioxidant, biocompatible in the presence of cardiac myocytes, and was able to suppress the oxidative damage and apoptosis in CMs, removing the excessive intracellular ROS content.

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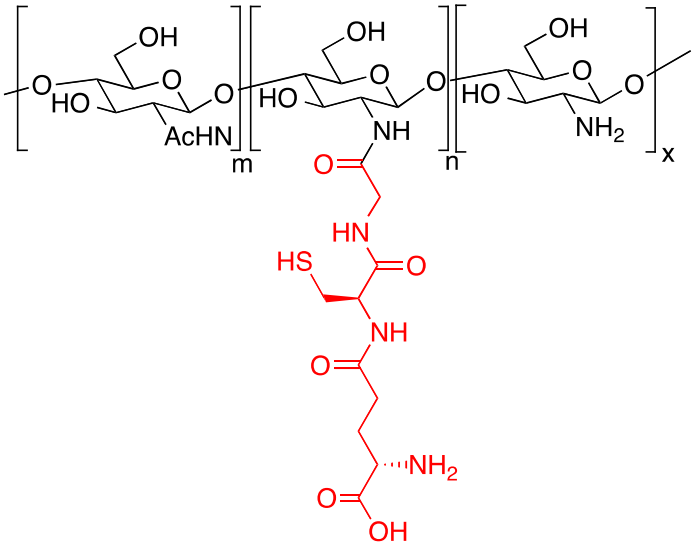

Figure 5. The chitosan-glutathione conjugated hydrogel.

Still in cardiac tissue engineering, the peptide sequence QHREDGS, derived from the fibrinogen-like domain of angiopoietin-1, was conjugated to a collagen-chitosan hydrogel. The peptide-modified hydrogels stimulated tube-like structure formation of encapsulated endothelial cells [48]. In order to promote osteogenesis, alginate hydrogels bioconjugated to peptide mimics of BMP-2 were studied with encapsulated osteoblasts and mesenchymal stem cells. Two different peptide sequences derived from bone morphogenetic protein 2 (BMP-2) were incorporated into the alginate backbone by two different chemoselective strategies, that could guarantee the spatial orientation of the peptides in their active form. The peptide DWIVA was covalently bound to alginate by carbodiimide chemistry, through a four glycine spacer (Figure 6a) [49], while the so-called "knuckle epitope" of BMP-2 (KIPKASSVPTELSAISTLYL), was modified with a cysteine residue at the *N*-terminal end; the thiol group was conjugated *via* a Michael addition to the alginate functionalized with maleimide groups (Figure 6b).

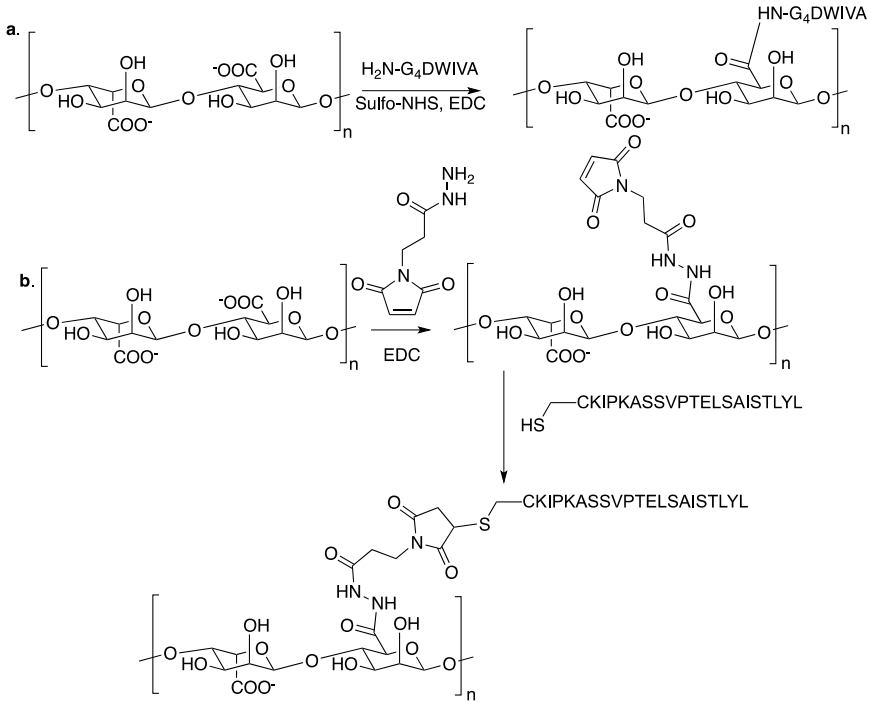

Figure 6. Alginate-based hydrogel designed to promote osteogenesis.

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Alginate functionalised with the knuckle epitope was shown to increase alkaline phosphatase activity in clonally derived murine osteoblasts, while with clonally derived murine mesenchymal stem cells it initiated Smad signalling, up-regulated osteopontin production, and increased mineral deposition.

Alginate-based hydrogel were also bioconjugated to the YIGSR peptide through amide bonds to the carboxylic acid groups of the alginate. The peptide-modified alginate hydrogels allowed adhesion of NB2a neuroblastoma cells and promoted neurite outgrowth [50]. The adhesion of NB2a neuroblastoma cells and neurite outgrowth was found to be a function of the peptide density.

4.3 Hydrogel-glycan conjugates

Heparin possesses binding domains to many growth factors, hence, when included into hydrogel design may be a useful tool to allow the retention and subsequent deliver of growth factors. This approach was used to promote angiogenesis. In more details, tyramine was first introduced into gelatin backbone as the cross-linking points, then heparin was covalently linked to gelatin-tyramine. Vascular endothelial growth factor (VEGF) was then incorporated into the gelatin derivative by non-bonding interactions with heparin binding motifs and finally enzymatic reaction with hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP) allowed the formation of the gel by oxidative cross-linking [51]. *In vivo* implantation experiments showed deeper and denser cell infiltration and angiogenesis in the heparin-modified gelatin/VEGF gels than in the controls.

Long-term *in vitro* maintenance of primary hepatocytes is a burden for hepatic tissue engineering, since these cells lose their phenotype in standard culture conditions. Foster et al. studied the use of heparin-containing hydrogels as scaffolds for cultivation and for the maintenance of functional primary hepatocytes. Thiolated heparin was conjugated to diacrylated PEG *via* a thiol-ene photocalysed reaction. Analysis of hepatic functionality of rat hepatocytes cultured on the hydrogel revealed that cells sustained albumin secretion for at least three weeks and increased cytochrome P450 activity. In addition, hepatocyte growth factor (HGF) was also entrapped into the gels, thanks to heparin interactions; in the presence of HGF, higher amounts of albumin could be observed.

4.4 Hydrogel-small molecule conjugates

Tyramine [52,53] and dopamine [54] conjugated hydrogels have also been prepared. For example, dopamine was conjugated to alginate (Figure 7), and subsequently gelled by enzymatic cross-linking mediated by horseradish peroxidase (HRP) and H_2O_2.

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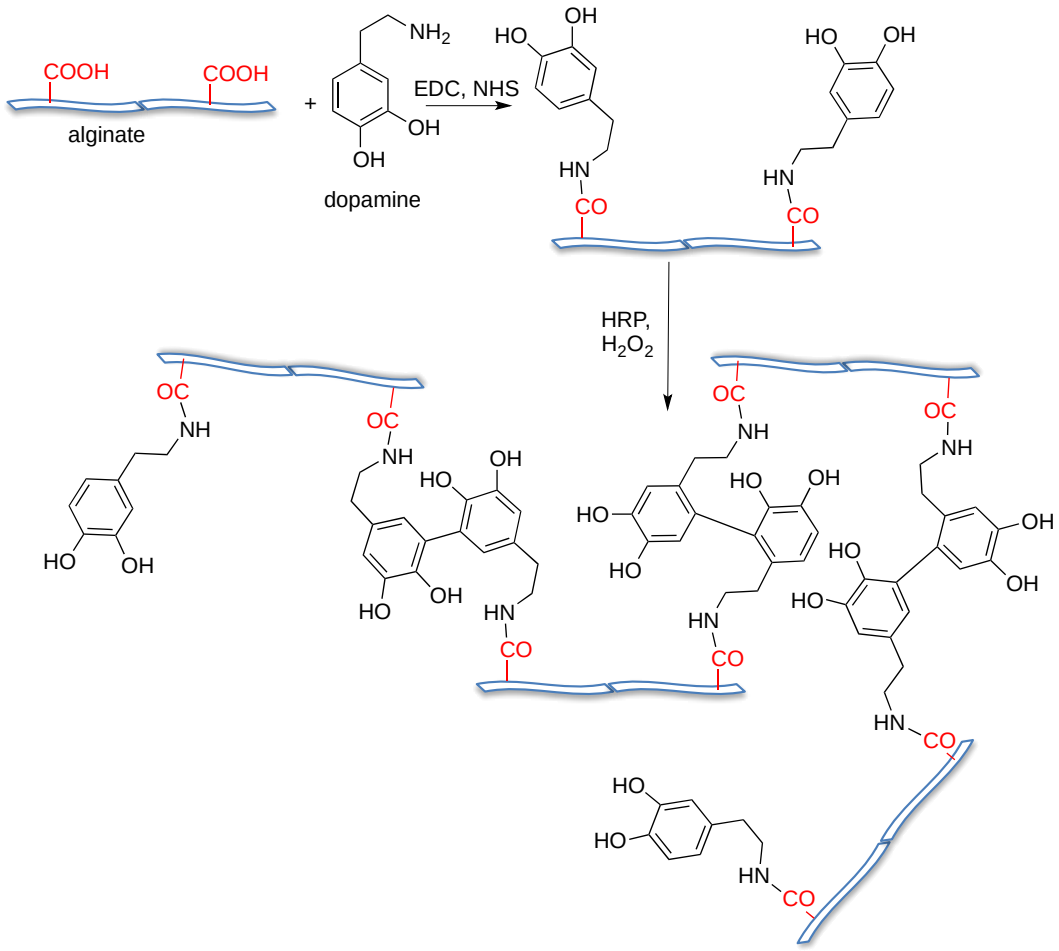

Figure 7. Alginate-based hydrogels cross-linked through dopamine oxidation by HRP.

The hydrogels resulted cytocompatible when assayed with NIH 3T3 cells. In addition, compared with unfunctionalised alginate hydrogels, the dopamine-conjugated hydrogel showed higher cell adhesion and elasticity properties.

5. Outlook and Perspectives

The field of hydrogels started in 1960s from the pioneering Wichterle and Lim in the 1960s. Since then, a remarkable development of hydrogels from simple chemically or physically crosslinked networks to complex bioresponsive systems was observed. Although not cited in this review, hydrogels are gaining a high level of sophistication, reflected, for example, in shape memory and self-healing hydrogels. The clinical needs for easy administration in regenerative medicine application fueled the research of injectable hydrogels and bioresponsive constructs able to drive cell response suitable for minimally invasive treatments. The selection of the cross-linking strategy is driven by the need of an immediate change from a low viscous solution before injection and quick formation of a strong network in situ. In addition, the possibility to modulate the degradation profiles after hydrogel administration and the bioactivation strategy can further improve the clinical translation of these scaffolds for tissue engineering applications. It is expected in the next years more sophisticated hydrogels suitably tuned to sustain and promote adhesion, migration and differentiation of specific cell lines, making cell therapies in tissue regeneration closer to the clinical application.

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Conflicts of Interest The authors declare no conflict of interest.

References

1. Wu, Y.-L.; Chen, X.; Wang, W.; Loh, X.J. Engineering Bioresponsive Hydrogels toward Healthcare Applications. *Macromol. Chem. Physic.* **2016**, *217*, 175-188.
2. Ebara, M.; Kotsuchibashi, Y.; Narain, R.; Idota, N.; Kim, Y.-J.; Hoffman, J.M.; Uto, K.; Aoyagi, T.M. *Smart Biomaterials*, Publisher: Springer Japan, 2014.
3. Michael C. Koetting, M.C; d, Peters, J.T; Steichen, S.D; Peppas, N.A. Stimulus-responsive hydrogels: Theory, modern advances, and applications. *Mat. Sci. Eng. R.* **2015**, *93*, 1-49.
4. Carlini, A.S.; Adamiak, L.; Gianneschi, N.C. Biosynthetic Polymers as Functional Materials. *Macromolecules.* **2016**, *49*, 4379-4394.
5. Russo L.; Cipolla, L. Glycomics: New Challenges and Opportunities in Regenerative Medicine. *Chem. Eur. J.* **2016,** DOI: 10.1002/chem.201602156
6. Grim, J.C.; Marozas, I.A.; Anseth, K.S. Thiol-ene and photo-cleavage chemistry for controlled presentation of biomolecules in hydrogels. *J. Control. Release.* **2015**, *219*, 95-106.
7. Burdick, J.A; Prestwich, G.D. Hyaluronic Acid Hydrogels for Biomedical Applications. *Adv. Mater.* **2011**, *23*, H41-H56.
8. Zhu, J.; Marchant, R.E. Design properties of hydrogel tissue-engineering scaffolds. *Expert. Rev. Med. Devices.* **2011**, *8*, 607-626.
9. Ahmadi, F.; Oveisi, Z.; Samani, S.M.; Amoozgar, Z. Chitosan based hydrogels: characteristics and pharmaceutical applications. *Res. Pharm. Sci.* **2015**, *10*, 1–16.
10. Lee, K.Y.; Mooney, D.J. Alginate: properties and biomedical applications. *Prog. Polym. Sci.* **2012**, *37*, 106–126.
11. Rafat, M.; Koh. L.B.; Islam, M.M.; Liedberg, B.O.; Griffith, M. Highly elastic epoxy cross-linked collagen hydrogels for corneal tissue engineering. *Acta Ophtalmol.* **2012**, *90*, s249.
12. Mu, C.; Zhang, K.; Lin, W.; Li, D. Ring-opening polymerization of genipin and its long-range crosslinking effect on collagen hydrogel. *J. Biomed. Mater. Res. A.* **2013**, *101*, 385-393.
13. Xin Zhao, X.; Lang, Q.; Yildirimer, L.; Lin, Z.Y.; Cui, W.; Annabi, N.; Ng, K.W.; Dokmeci, M.R.; Ghaemmaghami, A.M.; Khademhosseini, A. Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering. *Adv. Healtcare Mater.* **2016**, *5*, 108-118.
14. Bajaj, P.; Schweller, R.M.; Khademhosseini, A.; West, J.L.; Bashi, R. 3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine. *Annu. Rev. Biomed. Eng.* **2014**, *16*, 247–276.
15. Greene, T.; Lin, C.-C. Modular Cross-Linking of Gelatin-Based Thiol−Norbornene Hydrogels for in Vitro 3D Culture of Hepatocellular Carcinoma Cells. *ACS Biomater. Sci. Eng.* **2015**, *1*, 1314–1323.
16. Russo, L; Sgambato, A.; Visone, R.; Occhetta, P.; Moretti, M.; Rasponi, M.; Nicotra, F.; Cipolla, L. Gelatin hydrogels via thiol-ene chemistry. *Monatsh. Chem*. **2016**, *147*, 587–592.
17. Xu, K.; Cantu, D.A.; Fu, Y.; Kim, J.; Zheng, X.; Hematti, P.; Kao, W.J. Thiol-ene Michael-type formation of gelatin/poly(ethylene glycol) biomatrices for three-dimensional mesenchymal stromal/stem cell administration to cutaneous wounds. *Acta Biomater.* **2013**, *9*, 8802–8814.
18. Hunt, J.A.; Chen, R.; van Veen, T.; Bryan, N. Hydrogels for tissue engineering and regenerative medicine. *J. Mater. Chem. B*, **2014**, *2*, 5319-5338.
19. Lee, J.; Bae, Y.H.; Sohn, Y.S.; Jeong, B. Thermogelling aqueous solutions of alternating multiblock copolymers of poly(l-lactic acid) and poly(ethylene glycol). *Biomacromolecules* **2006**, *7*, 1729–1734.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2016 doi:10.20944/preprints201608.0036.v1

Peer-reviewed version available at Gels 2016, 2, 28; doi:10.3390/gels2040028

20. Bellis, S.L. Advantages of RGD peptides for directing cell association with biomaterials. *Biomaterials* **2011**, *32*, 4205-4210.
21. Ventre, M.; Netti, P.A. Controlling Cell Functions and Fate with Surfaces and Hydrogels: The Role of Material Features in Cell Adhesion and Signal Transduction. *Gels* **2016**, *2*, 12.
22. Kyburz, K.A.; Anseth, K.S. Synthetic Mimics of the Extracellular Matrix: How Simple is Complex Enough? *Ann. Biomed. Eng.* **2015**, *43*, 489–500.
23. Zhang, H.; Dai, S.; Bi, J.; Liu, K.-K. Biomimetic three-dimensional microenvironment for controlling stem cell fate. *Interface Focus.* **2011**, *1*, 792–803.
24. Swinehart, I.T.; Badylak, S.F. Extracellular Matrix Bioscaffolds in Tissue Remodeling and Morphogenesis. *Dev. Dynam.* **2016**, *245*, 351-360.
25. Tong, X.; Yang, F. Engineering interpenetrating network hydrogels as biomimetic cell niche with independently tunable biochemical and mechanical properties. *Biomaterials* **2014**, *35*, 1807-1815.
26. Ahadian, S.; Sadeghian, R.B.; Salehi, S.; Ostrovidov, S.; Bae, H.; Ramalingam, M.; Khademhosseini, A. Bioconjugated Hydrogels for Tissue Engineering and Regenerative Medicine. *Bioconjugate Chem.* **2015**, *26*, 1984–2001.
27. Jabbari, E. Bioconjugation of hydrogels for tissue engineering. *Curr. Opin. Biotechnol.* **2011**, *22*, 655–660.
28. Russo, L.; Sgambato, A.; Lecchi, M.; Pastori, V.; Raspanti, M.; Natalello, A.; Doglia, S.M.; Nicotra, F.; Cipolla, L. Neoglucosylated collagen matrices drive neuronal cells to differentiate. *ACS Chem. Neurosci.* **2014**, *5*, 261-265.
29. Sgambato, A.; Russo, L.; Montesi, M.; Panseri, S.; Marcacci, M.; Caravà, E.; Raspanti, M.; Cipolla, L. Different Sialoside Epitopes on Collagen Film Surfaces Direct Mesenchymal Stem Cell Fate. *ACS Appl. Mater. Interfaces* **2016**, *8*, 14952−14957.
30. Russo, L.; Cipolla, L. Glycomics: new challenges and opportunities in regenerative medicine. *Chemistry, Eur. J.* **2016**, DOI: 10.1002/chem.201602156.
31. Petka, W.A.; Harden, J.L.; McGrath, K.P.; Wirtz, D., Tirrell, D.A. Reversible hydrogels from self-assembling artificial proteins. *Science* **1998**, *281*, 389–392
32. Vermonden, T.; Censi, R.; Hennink, W. E. Hydrogels for protein delivery. *Chem. Rev.* **2012**, *112*, 2853−2888.
33. Censi, R.; Di Martino, P.; Vermonden, T.; Hennink, D. W. Hydrogels for protein delivery in tissue engineering. *J. Control. Release.* **2012**, *161*, 680−692.
34. Lin, C.C.; Anseth, K.S. Cell–cell communication mimicry with poly(ethylene glycol) hydrogels for enhancing β-cell function. *Proc. Natl. Acad. Sci. U. S. A.*, **2011**, *108*, 6380–6385.
35. Kim, J.; Lin, B.; Kim, S.; Choi, B.; Evseenko, D.; Lee, M. TGF-β1 conjugated chitosan collagen hydrogels induce chondrogenic differentiation of human synovium-derived stem cells. *J. Biol. Eng.* **2015**, *9*, 1.
36. Jha, A.K.; Tharp, K.M.; Ye, J.; Santiago-Ortiz, J.L.; Jackson, W.M.; Stahl, A., Schaffer, D.V.; Yeghiazarians, Y.; Healy, K.E. Enhanced survival and engraftment of transplanted stem cells using growth factor sequestering hydrogels. *Biomaterials* **2015**, *47*, 1−12.
37. Prokoph, S.; Chavakis, E.; Levental, K.R.; Zieris, A.; Freudenberg, U.; Dimmeler, S.; Werner, C. Sustained delivery of SDF-1a from heparin-based hydrogels to attract circulating pro-angiogenic cells. *Biomaterials* **2012**, *33*, 4792−4800.
38. Rabbany, S.Y.; Pastore, J.; Yamamoto, M.; Miller, T.; Rafii, S.; Aras, R.; Penn, M. Continuous delivery of stromal cell-derived factor-1 from alginate scaffolds accelerates wound healing. *Cell Transplant.* **2010**, *19*, 399−408.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2016 doi:10.20944/preprints201608.0036.v1

Peer-reviewed version available at Gels 2016, 2, 28; doi:10.3390/gels2040028

39. Wang, T.Y.; Forsythe, J.S.; Parish, C.L.; Nisbet, D.R. Biofunctionalisation of polymeric scaffolds for neural tissue engineering. *J. Biomater. Appl.* **2012**, *27*, 369-390.
40. Tam, R.Y.; Fuehrmann, T.; Mitrousis, N.; Shoichet, M.S. Regenerative Therapies for Central Nervous System Diseases: a Biomaterials Approach. *Neuropsychopharmacol. Rev.* **2014**, *39*, 169–188.
41. Mosiewicz, K.A.; Kolb, L.; van der Vlies, A.J.; Martino, M.M.; Lienemann, P.S.; Hubbell, J.A.; Ehrbar, M.; Lutolf, M.P. In situ cell manipulation through enzymatic hydrogel photopatterning. *Nat. Mater.* **2013**, *12*, 1072−1078.
42. Wheeldon, I.; Farhadi, A.; Bick, A.G.; Jabbari, E.; Khademhosseini, A. Nanoscale tissue engineering: spatial control over cell-materials interactions. *Nanotechnology*, **2011,** *22*, 212001-212017.
43. Hersel, U.; Dahmen, C.; Kessler, H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* **2003**, *24*, 4385–4415.
44. Zhu, J. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials* **2010**, *31*, 4639-4656.
45. DeForest, C.A.; Anseth, K.S. Cytocompatible Click-based Hydrogels with Dynamically-Tunable Properties Through Orthogonal Photoconjugation and Photocleavage Reactions. *Nat. Chem*. **2011**, *3*, 925–931.
46. Lee, J.W.; Park, Y.J.; Lee, S.J.; Lee, S.K.; Lee, K.Y. The effect of spacer arm length of an adhesion ligand coupled to an alginate gel on the control of fibroblast phenotype. *Biomaterials* **2010**, *31*, 5545–5551.
47. Li, J.; Shu, Y.; Hao, T.; Wang, Y.; Qian, Y.; Duan, C.; Sun, H.; Lin, Q.; Wang, C. A chitosan-glutathione based injectable hydrogel for suppression of oxidative stress damage in cardiomyocytes. *Biomaterials* **2013**, *34*, 9071−9081.
48. Miklas, J. W.; Dallabrida, S.M.; Reis, L.A.; Ismail, N.; Rupnick, M.; Radisic, M. QHREDGS enhances tube formation, metabolism and survival of endothelial cells in collagen-chitosan hydrogels. *PLoS One* **2013**, *8*, e72956.
49. Madl, C.M.; Mehta, M.; Duda, G.N.; Heilshorn, S.C.; Mooney, D.J. Presentation of BMP-2 mimicking peptides in 3D hydrogels directs cell fate commitment in osteoblasts and mesenchymal stem cells. *Biomacromolecules* **2014**, *15*, 445−455.
50. Dhoot, N.O.; Tobias, C.A.; Fischer, I.; Wheatley, M.A. Peptide-modified alginate surfaces as a growth permissive substrate for neurite outgrowth. *J. Biomed. Mater. Res. A* **2004**, *71*, 191−200.
51. Li, Z.; Qu, T.; Ding, C.; Ma, C.; Sun, H.; Li, S.; Liu, X. Injectable gelatin derivative hydrogels with sustained vascular endothelial growth factor release for induced angiogenesis. *Acta Biomater.* **2015**, *13*, 88−100.
52. Park, K.M.; Shin, Y.M.; Joung, Y.K.; Shin, H.; Park, K.D. In Situ Forming Hydrogels Based on Tyramine Conjugated 4-Arm-PPO-PEO via Enzymatic Oxidative Reaction *Biomacromolecules* **2010**, *11*, 706–712.
53. Prodanovic, O.; Spasojevic, D.; Prokopijevic, M.; Radotic, K.; Markovic, N; Blazic, M.; Prodanovic, R. Tyramine modified alginates via periodate oxidation for peroxidase induced hydrogel formation and immobilization *React. Funct. Polym.* **2015**, *93*, 77–83.
54. Hou, J.; Li, C.; Guan, Y.; Zhang, Y.; Zhu, X.X. Enzymatically crosslinked alginate hydrogels with improved adhesion properties. *Polym. Chem*. **2015**, *6*, 2204-2213.

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