Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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J. Mat. Chem. B

RSCPublishing

ARTICLE

An overview of the suitability of hydrogel-forming polymers for extrusion-based 3D-printing

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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This review evaluates hydrogel-forming polymers that are suitable for soft tissue engineering with a focus on materials that can be fabricated using additive manufacturing (3D-printing). An overview of the specific material requirements for hydrogel-based tissue engineering constructs is presented. This is followed by an explanation of the various hydrogel-forming polymer classes that includes a detailed examination of material properties that are critical for extrusion printing. Specifically, mechanisms for hydrogel formation, degradation, and biological response, activity and compatibility are explored. A discussion of extrusion printing strategies for printable hydrogel-forming polymers is then presented in conjunction with a list of considerations to guide future tissue engineering developments.

Introduction

Tissue engineering is an interdisciplinary field that applies the principles of materials engineering and life sciences towards the development of technologies that can restore, maintain and improve tissue function[1]. These technologies have the potential to provide alternatives to human tissue and organ donations as well as to augment our existing anatomy[1]. The fundamental component of most tissue engineering strategies is the creation of a cellular scaffold. Scaffolds are 3D architectures comprised of various components that can include structural materials, biological materials like cells, proteins, or growth factors, and in some cases even functional materials like conductors[2]. Ideally the properties of these scaffolds should resemble the extracellular matrix and should be designed to initially hold cells in place and deliver bioactive molecules, whilst reserving a space for tissue to develop[3,4].

Hydrogels are an appealing class of materials for scaffold development because their composition and structure is somewhat similar to natural tissue, and can offer a synthetic surrogate for extracellular matrix (ECM). For example, the natural biopolymer chitosan is structurally similar to the ECM component glycoaminoglycans. The materials are comprised mainly of water (up to ~99%) with the remainder being a hydrophilic polymer network that confines the water within its boundaries[5]. Hydrogels also possess similar mechanical properties to soft tissues and can be processed using relatively mild conditions and aqueous chemistries[3].

Over the past 30 years, an extensive array of both naturally derived and synthetically produced hydrogel-forming polymers have been utilised for various soft tissue engineering objectives[6]. However, hydrogel-forming polymers need to be tailored for their specific application. For tissue engineering, the hydrogels must be prepared from biocompatible polymers using either non-toxic reagents, or in the case of toxic reagents, using those which can be completely removed after the scaffold has been fabricated[3,7]. Often, hydrogel-forming polymers must possess gel-forming mechanisms which allow the encapsulation of cells during processing as well; i.e. if cells must be integrated in the scaffold during fabrication, the gel formation process must not harm the cells[8,9]. Furthermore, control of the degradability of hydrogel-forming polymers is critical as the lifetime of the material has to suit the tissue engineering application; specifically temporary scaffolds need to degrade but permanent implants must not[10].

The hydrogel fabrication method employed influences the choice of polymers used, in particular the gel forming mechanism must be amenable to the fabrication technique used for constructing the scaffold as well as satisfy the basic criteria for tissue engineering[9]. Historically, researchers have used a variety of hydrogel fabrication techniques such as porogen leaching[11,12], casting[13], gas foaming[14–16], phase separation[17] and electrospinning[18–20] to provide a porous scaffold architecture. Each technique has shown some degree of organisation of pore size, distribution and inter-connectivity[21], however, recently, Additive Manufacturing technologies have been utilized to

produce novel complex tissue engineering contructs[22]. Known also as 3D-printing, Additive Manufacturing (AM) describes a range of technologies that use computer aided design and automation to build structures through a layer-by-layer process. Additive Manufacturing aligns particularly well with building patient specific parts as it blends the concepts of computer aided tissue engineering (CATE) which incorporates 3D medical imaging, computer aided design and modelling, and solid free-form fabrication of tissue and organs (Figure 1) [22,23]. An assortment of AM equipment has been developed that have the precise spatial control needed for complex hydrogel fabrication, including laser[24,25], stereolithography[26], inkjet printer and extrusion based prototyping systems[21,27,28]. These technologies have enabled novel 3D scaffold designs, which when coupled with established medical imaging techniques, can produce custom designed tissue implants suited to the individual patient's requirements[23,29].

In this review we take a detailed look at hydrogel forming polymers which are suitable for tissue engineering through extrusion printing. We initially investigate the various hydrogel-forming polymers with focus on i) the classes of polymers according to origin, monomer types and electrical nature, ii) the hydrogel-forming mechanisms, and iii) their degradability and biocompatibility. This is followed by a discussion on extrusion printing, particularly the relationships between choice of hydrogel material, applicability for tissue engineering and suitability for extrusion printing. This review includes a table that lists the major hydrogel forming polymers used in tissue engineering with the characteristics most pertinent to tissue engineering and extrusion printing which could serve as a valuable resource to guide future soft tissue scaffold development.

Hydrogel-forming polymers

Hydrogel-forming polymers can be classified according to their synthetic origins, composition, electrostatic nature and gel forming mechanism (Figure 2). These same traits are critical considerations when selecting a hydrogel-forming polymer for specific tissue engineering applications and the ability to utilise viable fabrication methods.

Hydrogels are formed from either naturally produced polymers (also referred to as biopolymers) or synthetic polymers. Biopolymers are derived from various organisms including human, animals, plants and bacteria and are generally more compatible and more likely to interact positively with cells[30]. Human derived biopolymers such as collagen and fibrin have the greatest biological compatibility and possess proteolytic pathways of degradation (proteolysis by specific enzymes), whilst those non-human derived biopolymers such as alginate or chitosan have less compatibility and degradability[31]. Notwithstanding, many human derived biopolymers are often preferentially derived from non-human sources because they are more available and cheaper to produce. For example hyaluronan, which is a glycosamnioglycan produced in humans, is much more efficiently produced from bacteria[32]. Historically, plant derived biopolymers (e.g. alginate, agarose, cellulose) have been used in cell culture and are inexpensive and easy to obtain, however, because they are completely foreign molecules to humans they have at best intermediate biocompatibility at best and no proteolytic degradation mechanism within the body[32]. There are major drawbacks with using biopolymers, including significant variations in molecular weight and structure from batch to batch and they presents a potential risk of pathogen transfer from the originating organism[33].

Synthetic polymers usually possess superior mechanical properties and can be produced in large quantities, consistently, cheaply, and above all, are easy to modify to produce hydrogels with desirable properties. There is no risk of pathogens being present in a synthetic polymer hydrogel; however care must be taken to ensure that there is no trace of toxic unpolymerised/uncross-linked reagents left in the hydrogel prior to use (e.g. residual acrylamide from poly(acrylamide))[34]. Most synthetic polymers are not biocompatible; have limited biodegradability; and have poor cellular adhesion, however, many of these shortcomings have been addressed to some extent with clever processing and modification strategies[30,35,36].

Polymers can also be classified based on their composition and more specifically the monomers/types of monomers from which they are made. Most biopolymers used for tissue engineering are either proteins/polypeptides, polysaccharides, or glycosaminoglycans while the most prevalent synthetic polymers are polyols, polyethers or polyesters. Proteins and polypeptides, are the most functional of all biopolymers because they contain peptide domains that interact directly with cells[37] and perform specific functions (e.g., signalling and cellular adhesion), and usually there are enzymes in the body that specialise in degradation of these polymers[38,39]. Conversely, proteins are also relatively expensive to mass produce and have limited lifetimes[38,39]. Polysaccharides are a diverse class of biopolymers obtainable from many plant and microbial lifeforms which makes them a very versatile and cost effective hydrogel materials[30]. Many polysaccharides are also polyelectrolytes that can form ionotropic hydrogels or complex coacervate hydrogels such as gellan gum[40] and gelatin[41]. Glycosaminoglycans are a class of polysaccharides that contain amine functionality and deserve special mention because they, in combination with various proteins, form the natural extracellular matrix of human cells and consequently have excellent biocompatibility and cellular affinity[4,42]. Polyols, polyethers and polyesters can be produced cheaply with consistency but are generally less degradable than biopolymers, with the exception of polyesters of derived from naturally occurring α-hydroxy acids which possess greater biocompatibility and are to some extent biodegradable or excretable[43].

Another important basis of classification for hydrogel-forming polymers is by their electrical nature as this directly relates to how a hydrogel can be formed and hence how processable they

are using AM technologies. The electrical nature of a polymer is derived from the inherent functionality of the monomers it is constructed from. Certain functional groups are ionisable in aqueous solutions; for example the amino groups in gelatin have the potential to be positively charged and the carboxylate groups of alginate are negatively charged[44,45]. Polysaccharides are often anionic in nature due to an abundance of carboxylate or sulfate containing moieties within their structure such as gellan gum which has one carboxylate containing saccharide unit in every four[46]. There are also polysaccharides that are cationic due to an abundance of amine containing monomer units such as chitosan[47]. Glycosaminoglycans, by definition, contain amino groups in their structure but also usually contain an excess of carboxylic acid functionality and hence are almost exclusively anionic in nature such as hyaluronan[48]. Proteinaceous biopolymers are comprised of a mixture of amino acids with many different negative and positively charged functional groups. Proteinaceous polymers are amphoteric polyelectrolytes as the distribution of positive and negative charges in these protein molecules is mediated both by solution pH and the isoelectric point (pI) of the protein. Not all biopolymers are polyelectrolytes, as there are a number of neutrally charged polysaccharides such as agarose, dextran and cellulose. Almost all polyols, polyethers and polyesters are neutral in nature[49–52].

Hydrogel formation mechanisms

The gelation behavior (mechanism by which a hydrogel forms) has a direct impact on the methods used to fabricate the hydrogel component for tissue engineering. In general, certain gel forming processes lend themselves to rapid prototyping fabrication methods while others require more time to develop into robust hydrogels and are suited to slower fabrication techniques such as porogen leaching[11,17]. All hydrogels possess some level of physical attraction between macromers as a result of hydrogen bonding and entanglements amongst one another[9]. Often these physical interactions are strong enough to form a weak gel but these are seldom strong enough for tissue engineering applications or layer upon layer fabrication. Usually a hydrogel intended for tissue engineering applications must be strengthened through additional electrostatic interactions or chemical cross-linking (Figure 3)[53].

Ionotropic hydrogels are those formed as a result of electrostatic interactions between polyanions and cations or polycations and anions. For example, alginate is a polyanionic polymer comprised of mannuronic and glucaronic acid residues which forms a firm ionotropic hydrogel upon addition of calcium ions[54]. Another example is chitosan, a polycationic polymer containing glucosamine residues, which are positively charged above its isoelectric point and will form a firm ionotropic hydrogel with phosphate ions[55]. Ionotropic hydrogels are usually able to form a firm hydrogel upon cooling and are therefore particularly useful for *in situ* tissue engineering or for use in rapid prototyping fabrication techniques[54,56]. Ionic cross-links are able to self-repair which can be beneficial for a number of bio-medical applications. Hydrogels intended for use as cartilage tissue scaffolds comprised of gellan gum and epoxy amine polymers have been demonstrated to recover after physical deformation[57].

Complex coacervate hydrogels, also sometimes referred to as polyion complexes or polyelectrolyte complexes, are formed upon mixing of a polyanion and a polycation with one another such as alginate and poly(L-lysine) or sometimes also with an amphoteric polymer such as chondroitin sulfate and gelatin[9,41].

Hydrogels can be either directly cross-linked with various chemical cross-linkers, or are able to be pre-functionalised so that they can be subsequently cross-linked[58]. The variety of cross-linking methods and reagents are large and many cross-linking reagents are toxic and must be fully removed from the hydrogel before they come into contact with cells or a body[55]. It is also possible to incorporate proteolytically degradable sequences using covalent cross-linking chemistries to improve the degradability of otherwise non-degradable polymers[59].

Degradation behaviour and biocompatability

It is often advantageous for the polymer to be degradable via a natural process whose degradation rate matches the rate of the production of new extracellular matrix[6], but in some cases a permanent implant is desirable[60]. Hydrogel polymers are generally degraded by either proteolysis or by hydrolysis[61]. Proteolysis occurs when an enzyme that is produced by the cells in or around the implant is able to recognise a degradable peptide sequence in the polymer which it can sever[39,59]. Collagen for example, is a proteinaceous biopolymer which can be degraded through the action of a variety of matrix metalloproteases called collagenases[62]. The main advantage of proteolytically degradable polymers are that they will be degraded at a rate that more closely matches that of cellular growth because the cells are programmed to produce these enzymes to make room for themselves to grow into[42]. Many hydrogel polymers are also able to be hydrolysed without the aid of an enzyme under physiological conditions but at a significantly slower rate[63] which can be advantageous if a longer lasting implant is desired. There are potentially negative effects of degradation by-products of materials, for example, the degradable products of ester-based polymers are acidic and can lead to auto-catalytic degradation.

Synthetic polyesters of α-hydroxy acids are the only synthetic polymers that can be degraded in a natural way into their naturally occurring monomers and subsequently consumed in the tricarboxylic acid cycle[64]. Poly(lactic acid) and poly(glycolic acid) are examples of α-hydroxy acid based polymers which have been used extensively in biomedical engineering as degradable stents, sutures and wound dressings[65–67]. Most other synthetic polymers used in tissue engineering are generally non-degradable and are often either selected for use in applications that require more persistent materials or limited to low molecular weight analogues (< 5,000 Da) which are able to be removed via the renal system[43,68,69]. Alternatively, non-degradable hydrogel-forming

polymers may have degradable regions built into their structure to impart finely controlled degradability[59]. Poly(ethylene glycol) is a prevalent example of a synthetic polymer with poor inherent degradability which can and has been modified to include enzyme-cleavable domains and improve its degradability[36,70,71].

Materials for use in tissue engineering must be compatible with the body of the intended patient. Further, the interaction between cells and biomaterials as well as biomaterials and the body needs to be carefully considered when selecting hydrogel-forming polymers for tissue engineering applications. The term biocompatibility is often used to describe this concept, which is an ambiguous concept that has evolved and changed meaning in line with the evolution of our understanding of the interaction between biomaterials and the body[72]. In this review, we define a biocompatible material as one which does not incite a foreign body reaction on its own, is non-inflammatory or otherwise immunogenic, and is non-cytotoxic. The foreign body response is a reaction to the inclusion of a foreign material such as a tissue engineered construct which can be detrimental to the function of the implant[73]. Often a hydrogel scaffold on its own can be responsible for a foreign body reaction, but cells and other inclusions in an implant may also contribute[73]. Certain hydrogel-forming polymers have been observed to stimulate particularly strong foreign body reactions such as carrageenan which is frequently used to test the efficacy of anti-inflammatory reagents in animal models by stimulating the initial foreign body response[74,75].

The ability of cells to adhere to the scaffold is also an important aspect of hydrogel-forming polymers to consider. The adhesion of cells to the hydrogel scaffold has been demonstrated to provide important stimulation to the cells and directs their differentiation and activity[76,77]. Lack of cellular adhesion can also result in anoikis – apoptosis induced by inadequate cell-matrix interactions[78]. Cells can adhere to a scaffold through specific "lock and key" type interactions such as the integrin and heparin binding domains in cells and extracelleular matrix proteins[79]. Many hydrogel-forming polymers do not inherently possess specific cellular adhesion regions but instead may be modified to do so by immobilising proteins onto the polymer[80]. By far the most prevalent strategy to improvement of cellular adhesion is the tethering of the integrin binding RGD domain to the polymer backbone (Figure 4)[33,81–83]. The non-adherent polysaccharide gellan gum has been demonstrated to have significantly improved cellular adhesion when the RGD peptide sequence has been tethered to it[84].

Extrusion printing of hydrogel-forming polymers

Extrusion printing is a technique based on building structures by driving material out of a nozzle and onto a stage. The extruded material is either directed by moving the nozzle above the stage or by moving the stage underneath the nozzle; irrespectively, 3D structures are created through continuously depositing material layer-upon-layer. In order to successfully build 3D structures in this manner, the first layer needs to have structural integrity before the second layer is deposited. Consequently, parameters such as polymer rheology and the gel forming mechanism are critically important; polymer solutions must be either viscous or viscoelastic initially, and then become self-supporting gels before additional layers are deposited. Temporal control of gelation is crucial to avoid premature gelation of the polymer solution while it is still in the printer. To this end several strategies for printing hydrogel-forming polymers are presented.

Polymers which form hydrogels mainly through physical associations tend to possess a gel transition temperature below which the solution gels, such as agarose, methylcellulose, gelatin and collagen. Hot solutions of these polymers can be printed onto a cooled stage whereupon the polymer traverses its gel transition temperature and solidifies. Agarose is an example of a polymer which has been printed in this manner where the polymer solution was held in the printer reservoir at 60°C-80°C and printed into a cool bath below the gel transition temperature (Figure 5A)[85,86]. A limitation of this approach is that physical hydrogels tend to be very weak and may need to be reinforced using other polymers or with a post-print cross-linking step. A compromise must also be made with respect to the magnitude of the temperature drop; if the temperature drop is small, the polymer solution will have a high viscosity and require high pressure to expel, but if the temperature drop is large it will take a long time to cool down and gel. In some instances the initial and final temperature of the polymer solution/gel may also preclude it from being able to include cells during printing as temperatures far outside normal body ranges could damage the living material.

Photo-curable hydrogel-forming polymers can be printed onto an illuminated stage where they will form firm hydrogels upon the incidence of light[87]. Some polymers can be directly photo-cured if the appropriate photoinitiator is incorporated. For example, it has been demonstrated that any proteinaceous biopolymer which contains tyrosine residues (such as collagen, fibrin and gelatin) can be cross-linked with white light in the presence of Ru(II)bpy32+ photoinitiator (Figure 5B)[88–90]. Even polymers which are not ordinarily photo-curable (such as gellan gum or dextran) may be modified to become photo-curable, often through a straightforward reaction with a acrylate or methacrylate based agent[91,92]. Photo-curable polymer printing has been reported using PEG-acrylate and PPO-acrylate functionalised polymers without cells, as well as with solutions of gelatin-methacrylate and hyaluronan-methacrylate functionalised polymers mixed with cells[26,87,93,94]. The main advantage of photo-curable polymer printing is that this type of polymerisation is usually very rapid (a few seconds to a few minutes) and is generally cell-friendly[91]. Also, because the reactive stimulus is light in this case, a bath is not needed and the time scale of gelation can be adjusted by changing the intensity of the light.

Reactive printing of ionotropic polymers is a very successful method used to date for extrusion printing of scaffolds. It involves printing a polymer solution into a bath of reactive substance that induces gelation. Usually, this is performed with

ionotropic hydrogels and a bath containing a solution of the appropriate counter-ion[85,95]. It has been reported in several instances that alginate has been printed into a calcium solution in this manner to produce microspheres as well as more complex structures (Figure 5C)[96–98]. The main advantage of reactive printing of ionotropic polymers is the very rapid gelation (approximately 1 second). The polymer solution and the bath can be held at cell culture temperature (37°C) and the gel forming method itself is cell-friendly. In fact, it is possible to print gellan gum solutions with cell culture media whereupon a gel is formed instantly (Figure 5D)[99].

Recommendations and Conclusions

For those considering potential hydrogels for tissue engineering applications, it is fundamental to not only examine the desired characteristics of the material for a scaffold but also the ability of the material to be fabricated in the desired scaffold design. For extrusion printing in particular, the gel formation mechanism and the printing technique are intrinsically tied.
Table 1 presents the most prevalent hydrogel-forming polymers used in tissue engineering with a summation of their most important characteristics (polymer class, functionality, degradability, biological response, activity and compatibility, and gel formation mechanism). These polymers include familiar polymers which have been used in biomedical devices, formulations and in cell culture protocols for decades such as gelatin and collagen, but also include more recently investigated materials like carrageenans and gellan gum. The information presented in Table 1 is also directly relevant to extrusion printing and it is our hope that the CATE community finds this table a useful resource.

Finally, we would like to remark on some opportunities (and caveats) that we have identified for future endeavors into extrusion 3D-printing of soft tissue engineering scaffolds: (1) Photo-curability. Almost all polymers can be chemically modified to become photo-curable hydrogel-forming polymers. This avoids the need of a bath during printing. Other advantages include the ability to tune ink rheology (e.g. gelation behaviour) using the intensity of the light and the concentration of photocatalyst and/or initiator; (2) Bathless reactive printing. Using smart printer nozzle designs (e.g. core/sheath integrated nozzle) would allow for reactive printing to be carried without the need of a bath. An as yet untested but hypothetically promising reactive printing option could be to combine a proteinaceous polymer with cross-linking enzymes. For example, fibrinogen is rapidly polymerised (several seconds) in the presence of thrombin to produce fibrin[103–105]; and (3) Integrated bioprinting. Extrusion-based printing methods can allow the use of a bio-ink containing growth factors and/or cells, thereby facilitating the placement of cells in the construct. However, the inclusion of cells in the ink adds further complication to the fabrication process as maintaining cell viability during and after printing is essential. To overcome these challenges, other cell printing methods such as inkjet printing99, could be integrated with extrusion printing systems. In one potential setup, an extrusion printed scaffold that could not include viable cells could be selectively seeded with inkjet printed cells in a secondary step.

Thus, it is expected that a great number of hydrogel-forming polymers could be used (or adapted) for additive manufacturing (3D-printing) with a view to fabricating soft scaffolds for tissue engineering applications.

Acknowledgements

The authors wish to acknowledge the financial support provided by the University of Wollongong (UOW) and the Australian Research Council (ARC) through its Centre of Excellence and Future Fellowship programs.

Notes and references

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Electronic Supplementary Information (ESI) available: See DOI: 10.1039/b000000x/

(1) Langer, R.; Vacanti, J. P. Tissue engineering. *Science (80-.).* **1993**, *260*, 920–926.
(2) Karp, J. M.; Dalton, P. D.; Shoichet, M. S. Scaffolds for tissue engineering. *MRS Bull.* **2003**, *28*, 301–306.
(3) Drury, J. L.; Mooney, D. J. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* **2003**, *24*, 4337–4351.
(4) Akhyari, P.; Kamiya, H.; Haverich, A.; Karck, M.; Lichtenberg, A. Myocardial tissue engineering: The extracellular matrix. *Eur. J. Cardio-thoracic Surg.* **2008**, *34*, 229–241.
(5) Calvert, P. Hydrogels for soft machines. *Adv. Mater.* **2009**, *21*, 743–756.
(6) Ma, P. X. Biomimetic materials for tissue engineering. *Adv. Drug Deliv. Rev.* **2008**, *60*, 184 – 198.
(7) Billiet, T.; Vandenhaute, M.; Schelfhout, J.; Van Vlierberghe, S.; Dubruel, P. A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. *Biomaterials* **2012**, *33*, 6020–6041.
(8) Nicodemus, G. D.; Bryant, S. J. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng. Part B Rev.* **2008**, *14*, 149–165.
(9) Hoffman, A. S. Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **2012**, *43*, 3–12.
(10) Gilbert, T. W.; Stewart-Akers, A. M.; Badylak, S. F. A quantitative method for evaluating the degradation of biologic scaffold materials. *Biomaterials* **2007**, *28*, 147–150.
(11) Ma, Z.; Gao, C.; Gong, Y.; Shen, J. Paraffin spheres as porogen to fabricate poly(L-lactic acid) scaffolds with improved

cytocompatibility for cartilage tissue engineering. *J. Biomed. Mater. Res. B. Appl. Biomater.* **2003**, *67*, 610–617.

(12) Nazhat, S. N.; Neel, E. A. A.; Kidane, A.; Ahmed, I.; Hope, C.; Kershaw, M.; Lee, P. D.; Stride, E.; Saffari, N.; Knowles, J. C.; Brown, R. A. Controlled microchannelling in dense collagen scaffolds by soluble phosphate glass fibers. *Biomacromolecules* **2007**, *8*, 543–551.

(13) Chen, C. W.; Betz, M. W.; Fisher, J. P.; Paek, A.; Chen, Y. Macroporous hydrogel scaffolds and their characterization. *Tissue Eng. Part C Methods* **2011**, *17*, 101–112.

(14) Barbetta, A.; Rizzitelli, G.; Bedini, R.; Pecci, R.; Dentini, M. Porous gelatin hydrogels by gas-in-liquid foam templating. *Soft Matter* **2010**, *6*, 1785.

(15) Harris, L. D.; Kim, B.-S.; Mooney, D. J. Open pore biodegradable matrices formed with gas foaming. *J. Biomed. Mater. Res.* **1998**, *42*, 396–402.

(16) Chung, E. J.; Sugimoto, M.; Koh, J. L.; Ameer, G. A. Low-pressure foaming: A novel method for the fabrication of porous scaffolds for tissue engineering. *Tissue Eng. Part A* **2012**, 1–9.

(17) Liu, X.; Ma, P. X. Phase separation, pore structure, and properties of nanofibrous gelatin scaffolds. *Biomaterials* **2009**, *30*, 4094–103.

(18) Han, J.; Lazarovici, P.; Pomerantz, C.; Chen, X.; Wei, Y.; Lelkes, P. I. Co-electrospun blends of PLGA, gelatin, and elastin as potential nonthrombogenic scaffolds for vascular tissue engineering. *Biomacromolecules* **2011**, *12*, 399–408.

(19) Nam, J.; Huang, Y.; Agarwal, S.; Lannutti, J. Improved cellular infiltration in electrospun fiber via engineered porosity. *Tissue Eng.* **2007**, *13*, 2249–2257.

(20) Martins, A.; Reis, R. L.; Neves, N. M. Electrospinning: processing technique for tissue engineering scaffolding. *Int. Mater. Rev.* **2008**, *53*, 257–274.

(21) Mironov, V.; Reis, N.; Derby, B. Bioprinting: A beginning. *Tissue Eng.* **2006**, *12*, 631–634.

(22) Kang, H.-W.; Park, J. H.; Kang, T.-Y.; Seol, Y.-J.; Cho, D.-W. Unit cell-based computer-aided manufacturing system for tissue engineering. *Biofabrication* **2012**, *4*, 015005.

(23) Sun, W.; Darling, A.; Starly, B.; Nam, J. Computer-aided tissue engineering: Overview, scope and challenges. *Biotechnol. Appl. Biochem.* **2004**, *39*, 29–47.

(24) Bryant, S. J.; Cuy, J. L.; Hauch, K. D.; Ratner, B. D. Photo-patterning of porous hydrogels for tissue engineering. *Biomaterials* **2007**, *28*, 2978–86.

(25) Leslie-Barbick, J. E.; Shen, C.; Chen, C.; West, J. L. Micron-scale spatially patterned, covalently immobilized vascular endothelial growth factor on hydrogels accelerates endothelial tubulogenesis and increases cellular angiogenic resposnses. *Tissue Eng. Part A* **2011**, *17*, 221–229.

(26) Dhariwala, B.; Hunt, E.; Boland, T. Rapid prototyping of tissue-engineering constructs , using photopolymerizable hydrogels and sterolithography. *Tissue Eng.* **2004**, *10*, 1316–1322.

(27) Mironov, V.; Trusk, T.; Kasyanov, V.; Little, S.; Swaja, R.; Markwald, R. Biofabrication: A 21st century manufacturing paradigm. *Biofabrication* **2009**, *1*, 1–16.

(28) Peltola, S. M.; Melchels, F. P. W.; Grijpma, D. W.; Kellomäki, M. A review of rapid prototyping techniques for tissue engineering purposes. *Ann. Med.* **2008**, *40*, 268–280.

(29) Hollister, S. J. Porous scaffold design for tissue engineering. *Nat. Mater.* **2005**, *4*, 518–524.

(30) Baldwin, A. D.; Kiick, K. L. Polysaccharide-modified synthetic polymeric biomaterials. *Biopolymers* **2010**, *94*, 128–140.

(31) Pariente, J.-L.; Kim, B.-S.; Atala, A. In vitro biocompatibility assessment of naturally derived and synthetic biomaterials using normal human urothelial cells. *J. Biomed. Mater. Res.* **2001**, *55*, 33–39.

(32) Rinaudo, M. Main properties and current applications of some polysaccharides as biomaterials. *Polym. Int.* **2008**, *430*, 397–430.

(33) Collier, J. H.; Segura, T. Evolving the use of peptides as components of biomaterials. *Biomaterials* **2011**, *32*, 4198–4204.

(34) Sung, H.-W.; Huang, R.-N.; Huang, L. L. H.; Tsai, C.-C. In vitro evaluation of cytotoxicity of a naturally occurring cross-linking reagent for biological tissue fixation. *J. Biomater. Sci. Polym. Ed.* **1999**, *10*, 63–78.

(35) Saik, J. E.; Gould, D. J.; Watkins, E. M.; Dickinson, M. E.; West, J. L. Covalently immobilized platelet derived growth factor-BB promotes angiogenesis in biomimetic poly(ethylene glycol) hydrogels. *Acta Biomater.* **2010**, *7*, 133–143.

(36) Tessmar, J. K.; Göpferich, A. M. Customized PEG-derived copolymers for tissue-engineering applications. *Macromol. Biosci.* **2007**, *7*, 23–39.

(37) Cen, L.; Liu, W. E. I.; Cui, L. E. I.; Zhang, W.; Cao, Y. Collagen tissue engineering: Development of novel biomaterials. *Pediatr. Res.* **2008**, *63*, 492–496.

(38) Nagase, H.; Woessner, J. F. Matrix metalloproteinases. *J. Biol. Chem.* **1999**, *274*, 21491–21494.

(39) Frederick Woessner, J. Matrix metalloproteinases and their inhibitors in connective tissue remodelling. *J. Fed. Am. Soc. Exp. Biol.* **1991**, *5*, 2145–2154.

(40) Smith, A. M.; Shelton, R. M.; Perrie, Y.; Harris, J. J. An initial evaluation of gellan gum as a material for tissue engineering applications. *J. Biomater. Appl.* **2007**, *22*, 241–254.

(41) Jin, K.-M.; Kim, Y.-H. Injectable, thermo-reversible and complex coacervate combination gels for protein drug delivery. *J. Control. Release* **2008**, *127*, 249–256.

(42) Barker, T. H. The role of ECM proteins and protein fragments in guiding cell behavior in regenerative medicine. *Biomaterials* **2011**, *32*, 4211–4214.

(43) Ramesh, R. C.; Zentner, G. M. Biodegradable low molecular weight triblock poly(lactide-co-glycolide) polyethylene glycol copolymers having reverse thermal gelation propreties. 6004573, 1999.

(44) Djabourov, M.; Leblond, J.; Papon, P. Gelation of aqueous gelatin solutions I: Structural investigation. *J. Phys.* **1988**, *49*, 319–332.

(45) Oliveira, J. T.; Reis, R. L. Polysaccharide-based materials for cartilage tissue engineering applications. *J. Tissue Eng. Regen. Med.* **2010**, *5*, 421–436.

(46) Ferris, C. J.; in het Panhuis, M. Conducting bio-materials based on gellan gum hydrogels. *Soft Matter* **2009**, *5*, 3430-3437.

(47) Sashiwa, H.; Aiba, S. Chemically modified chitin and chitosan as biomaterials. *Prog. Polym. Sci.* **2004**, *29*, 887–908.
(48) Allison, D. D.; Grande-Allen, K. J. Review. Hyaluronan: A powerful tissue engineering tool. *Tissue Eng. Part B Rev.* **2006**, *12*, 2131–2140.
(49) Sigma-Aldrich Product information: Agarose *1*.
(50) Naji, L.; Schiller, J.; Kaufmann, J.; Stallmach, F.; Kärger, J.; Arnold, K. The gel-forming behaviour of dextran in the presence of KCl: A quantitative 13C and pulsed field gradient (PFG) NMR study. *Biophys. Chem.* **2003**, *104*, 131–140.
(51) Lévesque, S. G.; Lim, R. M.; Shoichet, M. S. Macroporous interconnected dextran scaffolds of controlled porosity for tissue-engineering applications. *Biomaterials* **2005**, *26*, 7436–7446.
(52) Sannino, A.; Demitri, C.; Madaghiele, M. Biodegradable cellulose-based hydrogels: Design and applications. *Materials (Basel).* **2009**, *2*, 353–373.
(53) Lii, C.; Liaw, S. C.; Lai, V. M. F.; Tomasik, P. Xanthan gum–gelatin complexes. *Eur. Polym. J.* **2002**, *38*, 1377–1381.
(54) Hatefi, A.; Amsden, B. Biodegradable injectable in situ forming drug delivery systems. *J. Control. Release* **2002**, *80*, 9–28.
(55) Hennink, W. E.; van Nostrum, C. F. Novel crosslinking methods to design hydrogels. *Adv. Drug Deliv. Rev.* **2002**, *54*, 13–36.
(56) Park, S. A.; Lee, S. H.; Kim, W. Fabrication of hydrogel scaffolds using rapid prototyping for soft tissue engineering. *Macromol. Res.* **2011**, *19*, 694–698.
(57) Bakarich, S. E.; Pidcock, G. C.; Balding, P.; Stevens, L.; Calvert, P.; in het Panhuis, M. Recovery from applied strain in interpenetrating polymer network hydrogels with ionic and covalent cross-links. *Soft Matter* **2012**, *8*, 9985–9988.
(58) Rault, I.; Frei, V.; Herbage, D. Evaluation of different chemical methods for cross-linking collagen gel, films and sponges. *J. Mater. Sci. Mater. Med.* **1996**, *7*, 215–221.
(59) Patterson, J.; Hubbell, J. A. Enhanced proteolytic degradation of molecularly engineered PEG hydrogels in response to MMP-1 and MMP-2. *Biomaterials* **2010**, *31*, 7836–7845.
(60) Griffith, L. G. Emerging design principles in biomaterials and scaffolds for tissue engineering. *Ann. N. Y. Acad. Sci.* **2002**, *961*, 83–95.
(61) Meyvis, T. K. L.; De Smedt, S. C.; Demeester, J.; Hennink, W. E. Influence of the degradation mechanism of hydrogels on their elastic and swelling properties during degradation. *Macromolecules* **2000**, *33*, 4717–4725.
(62) Metzmacher, I.; Radu, F.; Bause, M.; Knabner, P.; Friess, W. A model describing the effect of enzymatic degradation on drug release from collagen minirods. *Eur. J. Pharm. Biopharm.* **2007**, *67*, 349–360.
(63) Pomerantseva, I.; Krebs, N.; Hart, A.; Neville, C. M.; Huang, A. Y.; Sundback, C. A. Degradation behavior of poly(glycerol sebacate). *J. Biomed. Mater. Res.* **2009**, *91*, 1038–1047.
(64) Athanasiou, K. A.; Niederauer, G. G.; Agrawal, C. M. Biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* **1996**, *17*, 93–102.
(65) Ravi, S.; Chaikof, E. L. Biomaterials for vascular tissue engineering. *Regen. Med.* **2010**, *5*, 107–120.
(66) Queen, D.; Evans, J. H.; Gaylor, J. D. S.; Courtney, J. M.; Reid, W. H. Burn wound dressings - a review. *Burns* **1987**, *13*, 218–228.
(67) Cutright, D. E.; Hunsuck, E. E. Tissue reaction to the biodegradable polylactic acid suture. *Oral Surg. Oral Med. Oral Pathol.* **1971**, *31*, 134–139.
(68) Arturson, G.; Wallenics, G. The renal clearance of dextran of different molecular sizes in normal humans. *Scand. J. Clin. Lab. Invest.* **1964**, *1*, 81–86.
(69) Place, E. S.; George, J. H.; Williams, C. K.; Stevens, M. M. Synthetic polymer scaffolds for tissue engineering. *Chem. Soc. Rev.* **2009**, *38*, 1139–1151.
(70) Rosso, F.; Marino, G.; Giordano, A.; Barbarisi, M.; Parmeggiani, D.; Barbarisi, A. Smart materials as scaffolds for tissue engineering. *J. Cell. Physiol.* **2005**, *203*, 465–470.
(71) Banta, S.; Wheeldon, I. R.; Blenner, M. Protein engineering in the development of functional hydrogels. *Annu. Rev. Biomed. Eng.* **2010**, *12*, 167–86.
(72) Jons Hilborn; Bjursten, L. M. A new and evolving paradigm for biocompatibility. *J. Tissue Eng. Regen. Med.* **2007**, *1*, 110–119.
(73) Anderson, J. M.; Rodriguez, A.; Chang, D. T. Foreign body reaction to biomaterials. *Semin. Immunol.* **2009**, *20*, 86–100.
(74) Thomson, A. W.; Fowler, E. F. Carrageenan: A review of its effects on the immune system. *Agents Actions* **1981**, *11*, 265–273.
(75) Winter, C. A.; Risley, E. A.; Nuss, G. W. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Exp. Biol. Med.* **1962**, *111*, 544–547.
(76) Bettinger, C. J. Biodegradable elastomers for tissue engineering and cell-biomaterial interactions. *Macromol. Biosci.* **2011**, *11*, 467–482.
(77) Annabi, N.; Mithieux, S. M.; Boughton, E. A.; Ruys, A. J.; Weiss, A. S.; Dehghani, F. Synthesis of highly porous crosslinked elastin hydrogels and their interaction with fibroblasts in vitro. *Biomaterials* **2009**, *30*, 4550–4557.
(78) Frisch, S. M.; Screaton, R. A. Anoikis mechanisms. *Curr. Opin. Cell Biol.* **2001**, *13*, 555–562.
(79) Joung, Y.-K.; Bae, J.-W.; Park, K.-D. Controlled release of heparin-binding growth factors using heparin-containing particulate systems for tissue regeneration. *Expert Opin. Drug Deliv.* **2008**, *5*, 1173–1184.
(80) Liu, X.; Won, Y.; Ma, P. X. Surface modification of interconnected porous scaffolds. *J. Biomed. Mater. Res.* **2005**, *74*, 84–91.
(81) Bellis, S. L. Advantages of RGD peptides for directing cell association with biomaterials. *Biomaterials* **2011**, *32*, 4205–4210.
(82) Hersel, U.; Dahmen, C.; Kessler, H. RGD modified polymers: Biomaterials for stimulated cell adhesion and beyond. *Biomaterials* **2003**, *24*, 4385–4415.
(83) Klein, F.; Richter, B.; Striebel, T.; Franz, C. M.; von Freymann, G.; Wegener, M.; Bastmeyer, M. Two-component polymer scaffolds for controlled three-dimensional cell culture. *Adv. Mater.* **2011**, *23*, 1341–1345.
(84) Ferris, C. J.; Gilmore, K. J.; Wallace, G. G.; in het Panhuis, M. Modified gellan gum hydrogels for tissue engineering applications. *Soft Matter* **2013**, *9*, 3705–3711.

(85) Landers, R.; Pfister, A.; Hubner, U.; John, H.; Schmelzeisen, R.; Mulhaupt, R. Fabrication of soft tissue engineering scaffolds by means of rapid prototyping techniques. *J. Mater. Sci.* **2002**, *37*, 3107–3116.

(86) Landers, R.; Hübner, U.; Schmelzeisen, R.; Mülhaupt, R. Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. *Biomaterials* **2002**, *23*, 4437–4447.

(87) Skardal, A.; Zhang, J.; Mccoard, L.; Xu, X.; Oottamasathien, S.; Prestwich, G. D. Photocrosslinkable hyaluronan-gelatin hydrogels for two-step bioprinting. *Tissue Eng. Part A* **2010**, *16*, 2675–2685.

(88) Fancy, D. A.; Kodadek, T. Chemistry for the analysis of protein-protein interactions: Rapid and efficient cross-linking triggered by long wavelength light. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 6020–6024.

(89) Elvin, C. M.; Danon, S. J.; Brownlee, G.; White, J. F.; Hickey, M.; Liyou, N. E.; Edwards, G.; Ramshaw, J. M.; Werkmeister, J. Evaluation of photo-crosslinked fibrinogen as a rapid and strong tissue adhesive. *J. Biomed. Mater. Res. A* **2010**, *93*, 687–695.

(90) Sando, L.; Danon, S.; Brownlee, A. G.; McCulloch, R. J.; Ramshaw, J. A.; Elvin, C. M.; Werkmeister, J. A. Photochemically crosslinked matrices of gelatin and fibrinogen promote rapid cell proliferation. *J. Tissue Eng. Regen. Med.* **2011**, *5*, 337–343.

(91) Nguyen, K. T.; West, J. L. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials* **2002**, *23*, 4307–4314.

(92) Ifkovits, J. L.; Burdick, J. Review: photopolymerizable and degradable biomaterials for tissue engineering applications. *Tissue Eng.* **2007**, *13*, 2369–2385.

(93) Di Biase, M.; Saunders, R. E.; Tirelli, N.; Derby, B. Inkjet printing and cell seeding thermoreversible photocurable gel structures. *Soft Matter* **2011**, *7*, 2639–2646.

(94) Billiet, T.; Gevaert, E.; De Schryver, T.; Cornelissen, M.; Dubruel, P. The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability. *Biomaterials* **2014**, *35*, 49–62.

(95) Khalil, S.; Nam, J.; Sun, W. Multi-nozzle deposition for construction of 3D biopolymer tissue scaffolds. *Rapid Prototyp. J.* **2005**, *11*, 9–17.

(96) Delaney, J. T.; Liberski, A. R.; Perelaer, J.; Schubert, U. S. Reactive inkjet printing of calcium alginate hydrogel porogens - a new strategy to open-pore structured matrices with controlled geometry. *Soft Matter* **2010**, *6*, 866–869.

(97) Boland, T.; Xu, T.; Damon, B.; Cui, X. Application of inkjet printing to tissue engineering. *Biotechnol. J.* **2006**, *1*, 910–917.

(98) Duan, B.; Hockaday, L. A.; Kang, K. H.; Butcher, J. T. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *J. Biomed. Mater. Res. Part A* **2013**, *101*, 1255–1264.

(99) Ferris, C. J.; Gilmore, K. J.; Beirne, S.; McCallum, D.; Wallace, G. G.; in het Panhuis, M. Bio-ink for on-demand printing of living cells. *Biomater. Sci.* **2013**, *1*, 101–232.

(100) Hockaday, L. A.; Kang, K. H.; Colangelo, N. W.; Cheung, P. Y. C.; Duan, B.; Malone, E.; Wu, J.; Girardi, L. N.; Bonassar, L. J.; Lipson, H.; Chu, C. C.; Butcher, J. T. Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. *Biofabrication* **2012**, *4*, 035005.

(101) Lee, K. Y.; Mooney, D. J. Hydrogels for tissue engineering. *Surgery* **2001**, *101*.

(102) Vasheghani-farahani, E.; Ganji, F. Hydrogels in controlled drug delivery. *Polym. J.* **2009**, *18*, 63–88.

(103) Weisel, J. W. Fibrinogen and fibrin. *Adv. Protein Chem.* **2005**, *70*, 248–285.

(104) Ahmed, T. A. E.; Dare, E. V; Hincke, M. Fibrin: A versatile scaffold for tissue engineering applications. *Tissue Eng. Part B Rev.* **2008**, *14*, 199–215.

(105) Schense, J. C.; Hubbell, J. A. Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. *Bioconjug. Chem.* **1999**, *10*, 75–81.

(106) Kao, J. M.; Rose, R.; Yousef, M.; Hunter, S. K.; Rodgers, V. G. In vivo biocompatibility evaluation of Cibacron blue-agarose. *J. Biomed. Mater. Res.* **1999**, *47*, 537–42.

(107) Hunt, N. C.; Grover, L. M. Cell encapsulation using biopolymer gels for regenerative medicine. *Biotechnol. Lett.* **2010**, *32*, 733–742.

(108) Rochas, C.; Heyraud, A. Acid and enzymic hydrolysis of kappa carrageenan. *Polym. Bull.* **1981**, *5*, 81–86.

(109) Rochas, C.; Rinaudo, M. Mechanism of gel formation in κ-carrageenan. *Biopolymers* **2004**, *23*, 735–745.

(110) Mi, F.-L.; Tan, Y.-C.; Liang, H.-F.; Sung, H.-W. In vivo biocompatibility and degradability of a novel injectable-chitosan-based implant. *Biomaterials* **2002**, *23*, 181–191.

(111) Chiono, V.; Pulieri, E.; Vozzi, G.; Ciardelli, G.; Ahluwalia, A.; Giusti, P. Genipin-crosslinked chitosan/gelatin blends for biomedical applications. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 889–898.

(112) Suzuki, S.; Saito, H.; Anno, T. Y. K.; Seno, N.; Kawai, Y.; Furuhashi, T. Formation of three types of disulfated disaccharides from chondroitin sulfates by chondroitinase digestion. *J. Biol. Chem.* **1968**, *243*, 1543–1550.

(113) Lynn, A. K.; Yannas, I. V; Bonfield, W. Antigenicity and immunogenicity of collagen. *J. Biomed. Mater. Res. B. Appl. Biomater.* **2004**, *71*, 343–354.

(114) A, R.; CC, C. Glutaraldehyde in collagen gel formation. *J. Biomater. Sci. Polym. Ed.* **1990**, *1*, 167–172.

(115) Ziv, O.; Avtalion, R. R.; Margel, S. Immunogenicity of bioactive magnetic nanoparticles: Natural and acquired antibodies. *J. Biomed. Mater. Res. A* **2008**, *85*, 1011–1021.

(116) Stenekes, R. J.; Loebis, A. E.; Fernandes, C. M.; Crommelin, D. J.; Hennink, W. E. Degradable dextran microspheres for the controlled release of liposomes. *Int. J. Pharm.* **2001**, *214*, 17–20.

(117) Daamen, W. F.; Veerkamp, J. H.; van Hest, J. C. M.; van Kuppevelt, T. H. Elastin as a biomaterial for tissue engineering. *Biomaterials* **2007**, *28*, 4378–4398.

(118) Taddese, S.; Weiss, A. S.; Neubert, R. H. H.; Schmelzer, C. E. H. Mapping of macrophage elastase cleavage sites in insoluble human skin elastin. *Matrix Biol.* **2008**, *27*, 420–8.

(119) Shin, H.; Olsen, B. D.; Khademhosseini, A. The mechanical properties and cytotoxicity of cell-laden double-network hydrogels based on photocrosslinkable gelatin and gellan gum biomacromolecules. *Biomaterials* **2012**, *33*, 3143–3152.

(120) Ozeki, M.; Tabata, Y. In vivo degradability of hydrogels prepared from different gelatins by various cross-linking methods. *J. Biomater. Sci. Polym. Ed.* **2005**, *16*, 549–561.

(121) Hellio, D.; Djabourov, M. Physically and chemically crosslinked gelatin gels. *Macromol. Symp.* **2006**, *241*, 23–27.

(122) Kirchmajer, D. M.; Watson, C. A.; Ranson, M.; in het Panhuis, M. Gelapin, a degradable genipin cross-linked gelatin hydrogel. *RSC Adv.* **2013**, *3*, 1073.

(123) Silva-Correia, J.; Miranda-Goncalves, V.; Salgado, A. J.; Sousa, N.; Oliveira, J. M.; Reis, R. M.; Reis, R. L. Angiogenic potential of gellan-gum-based hydrogels for application in nucleus pulposus regeneration: In vivo study. *Tissue Eng. Part A* **2012**, *18*, 1203–1212.

(124) Oliveira, J. T.; Martins, L.; Picciochi, R.; Malafaya, P. B.; Sousa, R. A.; Neves, N. M.; Mano, J. F.; Reis, R. L. Gellan gum: A new biomaterial for cartilage tissue engineering applications. *J. Biomed. Mater. Res.* **2010**, *93*, 852–863.

(125) Morris, E. R.; Nishinari, K.; Rinaudo, M. Gelation of gellan – A review. *Food Hydrocoll.* **2012**, *28*, 373–411.

(126) Zheng Shu, X.; Liu, Y.; Palumbo, F. S.; Luo, Y.; Prestwich, G. D. In situ crosslinkable hyaluronan hydrogels for tissue engineering. *Biomaterials* **2004**, *25*, 1339–1348.

(127) Krupa, J. C.; Marie Butler, A.; Mort, J. S. Quantitative bead assay for hyaluronidase and heparinase I. *Anal. Biochem.* **2003**, *319*, 280–286.

(128) Schmut, O.; Hofmann, H. Preparation of gels from hyaluronate solutions. *Graefe's Arch. Clin. Exp. Ophthalmol.* **1982**, *218*, 311–314.

(129) Caulfield, M. J.; Hao, X.; Qiao, G. G.; Solomon, D. H. Degradation on polyacrylamides. Part I. Linear polyacrylamide. *Polymer (Guildf).* **2003**, *44*, 1331–1337.

(130) Naghash, H. J.; Okay, O. Formation and structure of polyacrylamide gels. *1Journal Appl. Polym. Sci.* **1996**, *60*, 971–979.

(131) Serrano, M. C.; Pagani, R.; Vallet-Regí, M.; Peña, J.; Rámila, A.; Izquierdo, I.; Portolés, M. T. In vitro biocompatibility assessment of poly(epsilon-caprolactone) films using L929 mouse fibroblasts. *Biomaterials* **2004**, *25*, 5603–5611.

(132) Kweon, H.; Yoo, M. K.; Park, I. K.; Kim, T. H.; Lee, H. C.; Lee, H.-S.; Oh, J.-S.; Akaike, T.; Cho, C. S. A novel degradable polycaprolactone networks for tissue engineering. *Biomaterials* **2003**, *24*, 801–808.

(133) Santavirta, S.; Konttinen, Y. T.; Saito, T.; Gronblad, M.; Partio, E.; Kemppinen, P.; Rokkanen, P. Immune response to polyglycolic acid implants. *J. Jt. Bone Surg.* **1990**, *72*, 597–600.

(134) Shawe, S.; Buchanan, F.; Harkin-Jones, E.; Farrar, D. A study on the rate of degradation of the bioabsorbable polymer polyglycolic acid (PGA). *J. Mater. Sci.* **2006**, *41*, 4832–4838.

(135) Rai, R.; Tallawi, M.; Grigore, A.; Boccaccini, A. R. Synthesis, properties and biomedical applications of poly(glycerol sebacate) (PGS): A review. *Prog. Polym. Sci.* **2012**, *37*, 1051–1078.

(136) Sundback, C. a; Shyu, J. Y.; Wang, Y.; Faquin, W. C.; Langer, R. S.; Vacanti, J. P.; Hadlock, T. A. Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material. *Biomaterials* **2005**, *26*, 5454–5464.

(137) Park, B. H.; Han, Y. A.; Choi, J. H.; Lim, J. O. Biocompatibility of PHEMA and P(HEAM-co-SMA) hydrogels. *Key Eng. Mater.* **2005**, *277-279*, 51–55.

(138) Cutright, D. E.; Hunsuck, E. E. Tissue reaction to the biodegradable polylactic acid suture. *Oral Surg. Oral Med. Oral Pathol.* **1971**, *31*, 134–139.

(139) Chu, C. R.; Coutts, R. D.; Yoshioka, M.; Harwood, F. L.; Monosov, A. Z.; Amiel, D. Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): A tissue-engineering study. *J. Biomed. Mater. Res.* **1995**, *29*, 1147–1154.

(140) Suggs, L. J.; Krishnan, R. S.; Garcia, C. A.; Peter, S. J.; Anderson, J. M.; Mikos, A. G. In vitro and in vivo degradation of poly(propylene fumarate-co-ethylene glycol) hydrogels. *J. Biomed. Mater. Res.* **1998**, *42*, 312–320.

(141) Suggs, L. J.; Kao, E. Y.; Palombo, L. L.; Krishnan, R. S.; Widmer, M. S.; Mikos, A. G. Preparation and characterization of poly (propylene fumarate-co- ethylene glycol) hydrogels. *J. Biomater. Sci. Polym. Ed.* **2012**, *9*, 653–666.

(142) Schmedlen, R. H.; Masters, K. S.; West, J. L. Photocrosslinkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. *Biomaterials* **2002**, *23*, 4325–4332.

Table 1. Description of polymers used in tissue engineering categorised by class with information on hydrogel formation mechanism, degradability and their biological response, activity and biocompatibility.

Polymer	Class	Description and functionality	Biological response, activity and compatibility	Degradability	Hydrogel formation mechanism
Agarose	Neutral, polysaccharide biopolymer	Polymer comprised of D-galactose and 3,6-anhydro-L-galactose with ether functionality	Not biocompatible[45,106]	Non degradable[107]	Physical gel formation below 36˚C[49]
Alginate	Anionic, polysaccharide biopolymer	Polyelectrolyte comprised of D-mannuronic acid and L-guluronic acid with carboxylate and hydroxyl functionality	Varying biocompatibility, high L-guluronic acid content alginates are more immunogenic[32]	Hydrolysis[45] Ion exchange/chelation[45]	Ionotropic gel formation with divalent cations[45]
κ-Carrageenan	Anionic polysaccharide biopolymer	Polyelectrolyte comprised of D-galactose and 3,6-anhydro-D-galactose with hydroxyl and sulfate functionality	Inflammation inducing[75], but also demonstrated anti-tumoral[32]	Hydrolysis[108]	Ionotropic gel formation with monovalent cations[32,109]
Chitosan	Cationic, polysaccharide biopolymer	Polyelectrolyte comprised of D-glucosamine and N-acetyl-D-glucosamine with amine and hydroxyl functionality	Biocompatible, non-cytotoxic, anti-bacterial, anti-fungal, anti-tumoral[47,110]	Hydrolysis[110] Proteolysis (lysozyme)[110]	Chemical gel formation via cross-linking of amino groups[45,111]
Chondroitin Sulfate	Anionic glycosaminoglycan biopolymer	Polyelectrolyte comprised of N-acetyl-D-galactosamine and D-glucuronic acid with amide, carboxylate, hydroxyl and sulfate functionality	Biocompatible[45]	Proteolysis (chondroitinase)[112]	Complex coacervate gel formation with cationic polyelectrolytes[30]
Collagen	Amphoteric, proteinaceous biopolymer	Polyelectrolyte comprised of various amino acids with amine, carboxylate and hydroxyl functionality	Biocompatible, non-toxic, with good cellular adhesion but potentially immunogenic[113]	Proteolysis (collagenase)[62]	Physical self-assembling gel formation and chemical gel formation via cross-linking of amino or carboxylate groups[114]
Dextran	Neutral, polysaccharide biopolymer	Polymer comprised of D-Glucopyranose with hydroxyl functionality	Biocompatible but has poor protein and cellular adhesion[51], and is potentially immunogenic[115]	Hydrolysis[116]	Ionotropic gel formation in the presence of K^+ [50,51]
Elastin	Amphoteric, proteinaceous biopolymer	Polyelectrolyte comprised of various amino acids with amine and carboxylate functionality	Biocompatible, but is hydrophobic and insoluble[77,117]	Proteolysis (elastase)[118]	Covalent (self-assembling)[117]
Fibrin	Amphoteric, proteinaceous biopolymer	Polyelectrolyte comprised of various amino acids with amine, carboxylate and hydroxyl functionality	Biocompatible with excellent protein and cellular adhesion[65], thrombogenic[104]	Proteolysis[107]	Covalent (self-assembling)[103]

Gelatin	Amphoteric, proteinaceous biopolymer	Polyelectrolyte comprised of various amino acids with amine, carboxylate and hydroxyl functionality	Biocompatible[115] with good cellular adhesion[119]	Proteolysis (collagenase)[120]	Physical gel formation below 27˚C and chemical gel formation via cross-linking of amino of carboxylate groups[121,122]
Gellan gum	Anionic, polysaccharide biopolymer	Polyelectrolyte comprised of D-glucose, D-glucuronic acid and L-rhamnose with carboxylate and hydroxyl functionality	Biocompatible[123], non-cytotoxic[124], but has poor cellular adhesion[84]	Hydrolysis[46] Ion-exchange/chelation[46]	Ionotropic gel formation with cations[125]
Hyaluronan	Anionic, glycosaminoglycan biopolymer	Polyelectrolyte comprised of glucuronic acid and N-acetyl-D-glucosamine with amide, carboxylate and hydroxyl functionality	Biocompatible[126], with good cellular adhesion[48]	Proteolysis (hyaluronidase)[127]	Ionotropic formation with cations[128]
Methylcellulose	Neutral, polysaccharide biopolymer	Polymer comprised of D-glucose with hydroxyl functionality	Biocompatible[52]	Non degradable[52]	Physical gel formation at a temperature dependant on the degree of methylation[52]
Poly(acrylamide)	Neutral, synthetic polymer	Polymer comprised of acrylamide with amide functionality	Non-toxic polymer, but monomer is neurotoxic[129]	Non degradable[129]	Covalent[130]
Poly(caprolactone)	Neutral, synthetic polyester	Polymer comprised of ε-caprolactone with ether functionality	Biocompatible[131]	Hydrolysis[132]	Covalent[132]
Poly(ethylene glycol)	Neutral, synthetic polyether	Polymer comprised of ethylene oxide with ether functionality	Biocompatible[36], but with poor protein and cellular adsorption[36]	Non degradable[36]	Covalent[36]
Poly(glycolic acid)	Neutral, synthetic polyester	Polymer comprised of glycolic acid with ester functionality	Intermediate biocompatibility, mildly immunogenic[133]	Hydrolysis[134]	Covalent[134]
Poly(glycerol sebacate)	Neutral, synthetic polyester	Polymer comprised of glycerol and sebacic acid with ester and hydroxyl functionality	Biocompatible, non-cytotoxic[135,136]	Hydrolysis[136]	Covalent[136]
Poly(2-hydroxyethyl methacrylate)	Neutral, synthetic polymer	Polymer comprise of 2-hydroxyethyl methacrylate with ester and hydroxyl functionality	Intermediate biocompatibility, mildly immunogenic[137]	Non degradable[24]	Covalent[69]
Poly(lactic acid)	Neutral, synthetic polyester	Polymer comprised of lactic acid with ester functionality	Intermediate biocompatibility, mildly immunogenic[138]	Hydrolysis[139]	Covalent[139]
Poly(propylene fumarate)	Neutral, synthetic polyester	Polymer comprised of propylene fumarate with ester functionality with ester and vinyl functionality	Inflammation causing material[140]	Hydrolysis[140]	Covalent[141]
Poly(vinyl alcohol)	Neutral, synthetic polyol	Polymer comprised of vinyl alcohol with hydroxyl functionality	Biocompatible, but with poor protein and cellular adhesion[142]	Non degradable[101]	Covalent[101,142]

Figures

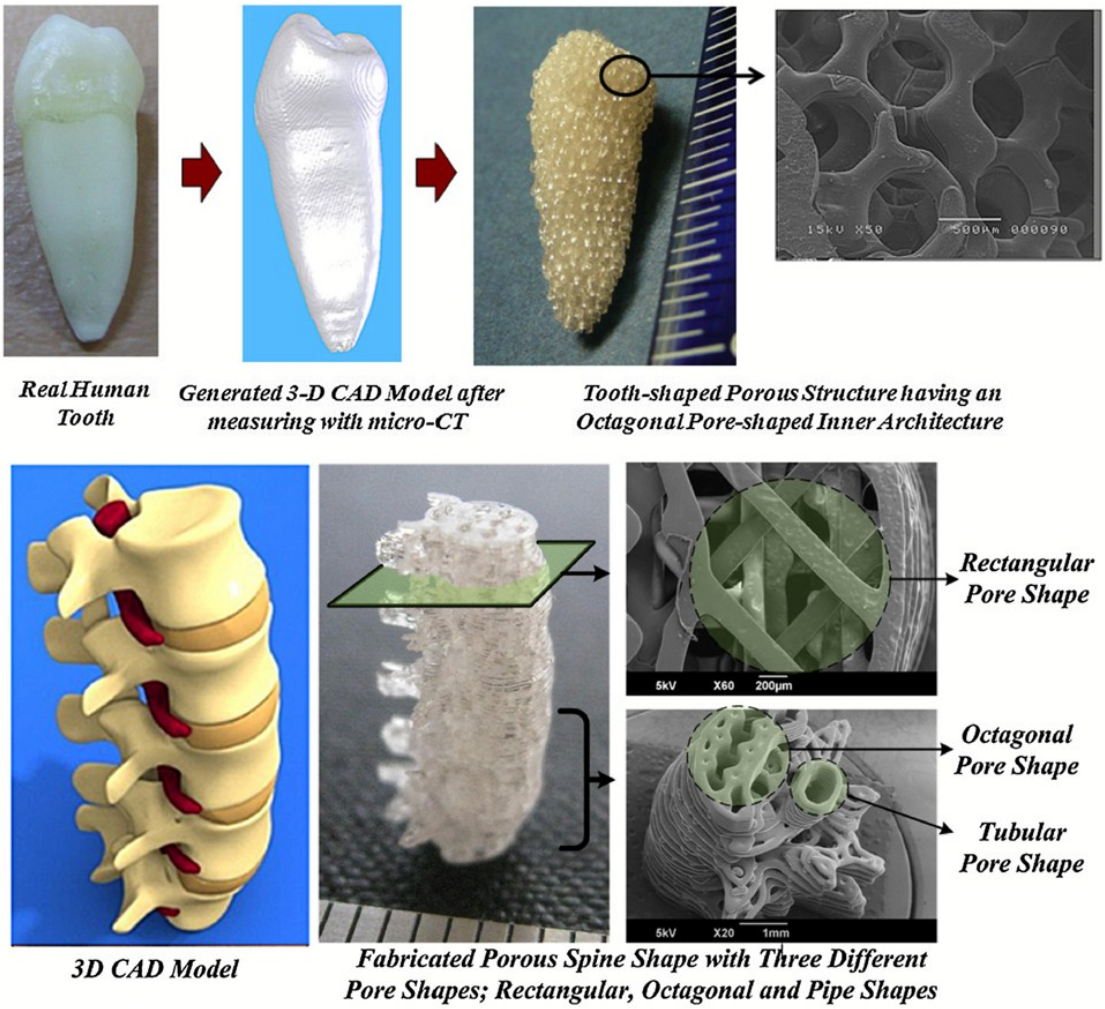

Figure 1. Computer aided tissue engineering of a tooth and section of spine[22] (© IOP Publishing. Reproduced by permission of IOP Publishing. All rights reserved.). The material used to create the structures is the photo-curable resin FA1260T (SK Cytec Inc, Korea).

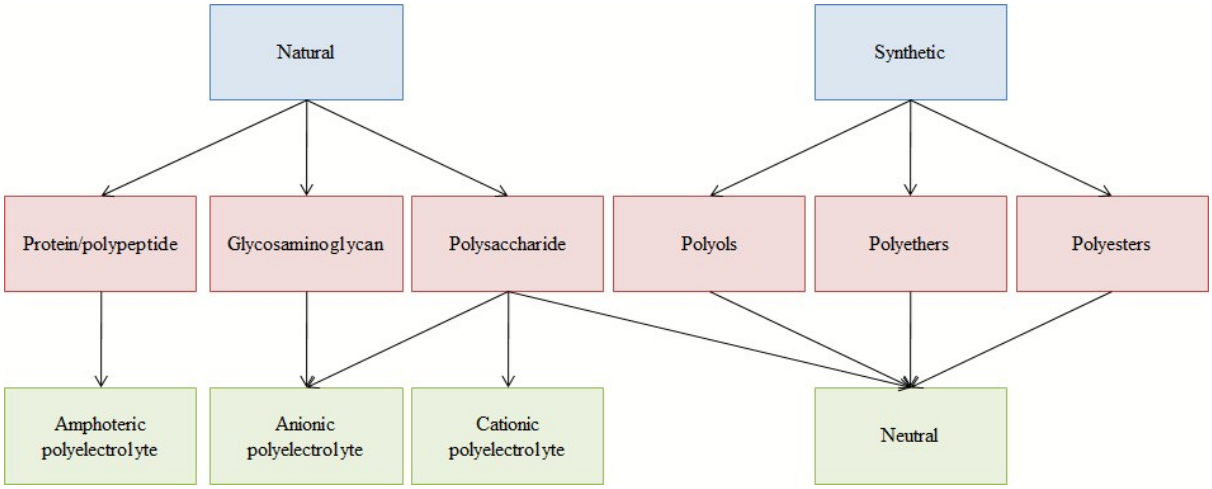

Figure 2. Hydrogel-forming polymers can be classified by origin (blue), composition (red) and electrical nature (green). To some extent, the origin, composition and electrical nature are related (arrows).

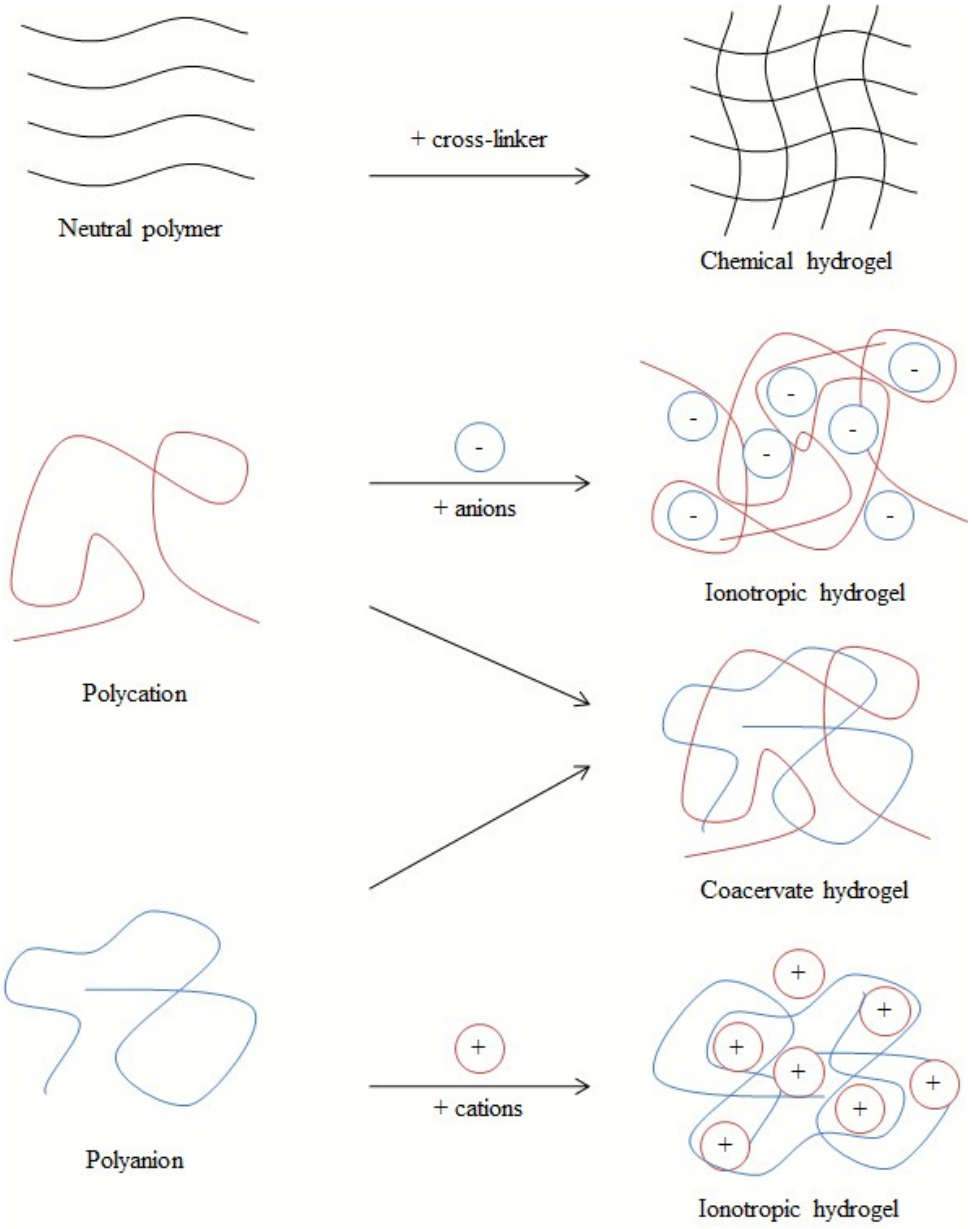

Figure 3. Schematic of different hydrogel-forming mechanisms: Chemical cross-linking, ionotropic cross-linking, and complex coacervate formation.

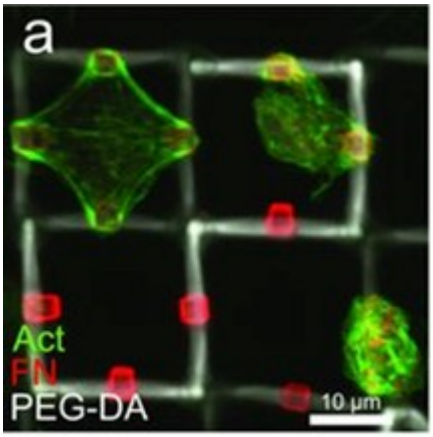

Figure 4. Primary chicken fibroblasts adhering to a 3D composite-polymer scaffold (PEG-DA) with fibronectin (FN) adhesion sites[83] (© 2011 John Wiley and Sons).

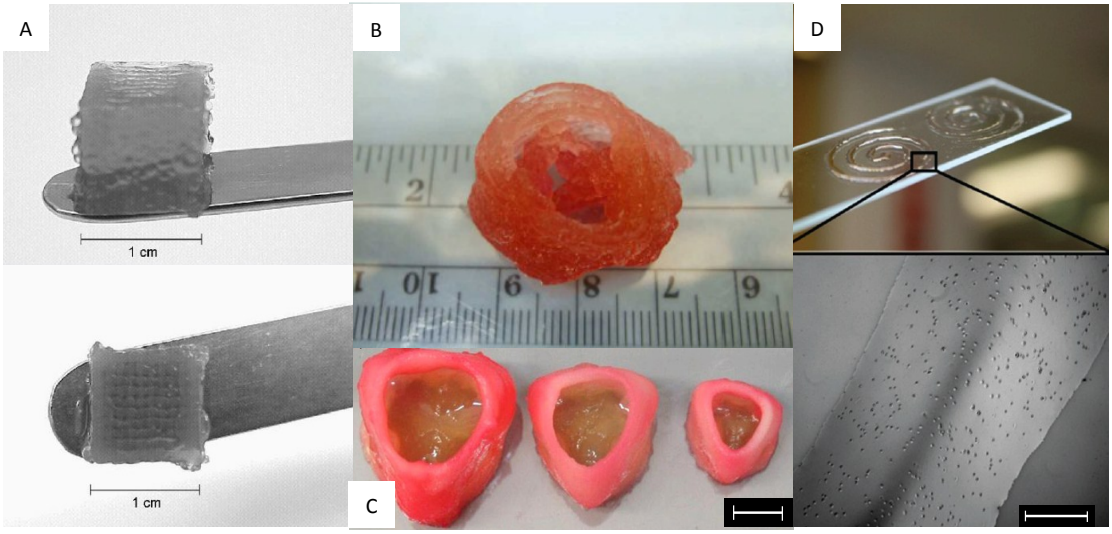

Figure 5. Examples of computer aided tissue engineering constructs made from hydrogels. A) a porous cube of agarose hydrogel printed using a thermal modulation approach[85] (with kind permission from Springer Science and Business Media); B) an aortic valve conduit printed with a blend of alginate/gelatin hydrogel[98] (© 2013 John Wiley and Sons) and C) differently scaled aortic valves printed from PEG-DA hydrogels (scale bar is 1 cm)[100] (© IOP Publishing. Reproduced by permission of IOP Publishing. All rights reserved.); D) a cellular ink of gellan gum and mouse myoblasts is printed in spirals on a glass microscopy slide (scale bar is 500 μm). Reproduced from reference [99].

Graphical abstract for Manuscript ID TB-REV-02-2015-000393 with the original title "*An overview of the suitability of hydrogel forming polymers for 3D-printing*" by D.M. Kirchmajer, R. Gorkin and M. in het Panhuis

Text highlighting novelty of our work:

In this review hydrogel-forming polymers that are suitable for extrusion-based 3D printing are evaluated.