Biopolymer-Based Hydrogels As Scaffolds for Tissue Engineering Applications: A Review

ARTICLE *in* BIOMACROMOLECULES · MARCH 2011

Impact Factor: 5.75 · DOI: 10.1021/bm200083n · Source: PubMed

3 AUTHORS, INCLUDING:

Peter Dubruel
Ghent University
276 PUBLICATIONS **3,259** CITATIONS
SEE PROFILE

Etienne Schacht
Ghent University
391 PUBLICATIONS **9,510** CITATIONS
SEE PROFILE

REVIEW

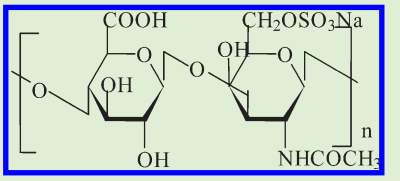
pubs.acs.org/Biomac

Biopolymer-Based Hydrogels As Scaffolds for Tissue Engineering Applications: A Review

S. Van Vlierberghe, P. Dubruel, and E. Schacht*

Polymer Chemistry & Biomaterials Research Group, Ghent University, Ghent, Belgium

ABSTRACT: Hydrogels are physically or chemically cross-linked polymer networks that are able to absorb large amounts of water. They can be classified into different categories depending on various parameters including the preparation method, the charge, and the mechanical and structural characteristics. The present review aims to give an overview of hydrogels based on natural polymers and their various applications in the field of tissue engineering. In a first part, relevant parameters describing different hydrogel properties and the strategies applied to finetune these characteristics will be described. In a second part, an important class of biopolymers that possess thermosensitive properties (UCST or LCST behavior) will be discussed. Another part of the review will be devoted to the application of cryogels. Finally, the most relevant biopolymer-based hydrogel systems, the different methods of preparation, as well as an in depth overview of the applications in the field of tissue engineering will be given.



1. INTRODUCTION

The application of hydrogels dates back to 1960, when Wichterle and Lim introduced the use of hydrophilic networks of cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA, Figure 1) as soft contact lens material.[1] During the last few decades, hydrogels have gained increasing interest, as indicated by the increasing number of papers on hydrogel-based materials published from 1995 up to now (Figure 2). A large variety of definitions exists for hydrogels. The most frequently referred definition is the one given by Peppas.[2] According to his definition, hydrogels are water-swollen, cross-linked polymeric structures containing (1) covalent bonds produced by the reaction of one or more comonomers, (2) physical cross-links due to chain entanglements, (3) association bonds including hydrogen bonds or strong van der Waals interactions between chains, or (4) crystallites bringing together two or more macromolecular chains.

Hydrogels can be classified into different categories depending on various parameters including the preparation method, the overall charge, and the mechanical and structural characteristics. On the basis of the preparation method, homopolymer and copolymer hydrogels can be distinguished. Alternatively, hydrogels can also be classified as neutral, anionic, or cationic depending on the charges of the building blocks. Finally, classification can be made according to the physical structure: amorphous, semicrystalline, hydrogen-bonded, supramolecular, or hydrocolloidal.

Hydrogels are extremely suitable for a variety of applications in the pharmaceutical and medical industry. Because they are capable of retaining large amounts of water and because of their soft and rubbery consistence, they closely resemble living tissues. Moreover, their high water content also contributes to their excellent biocompatibility, as already indicated by Ovsianikov et al.[3] They have already demonstrated the potential of porous gelatin-based hydrogels produced using two-photon polymerization to be applied as carriers for mesenchymal stem cells. In addition, upon applying osteogenic stimulation, the seeded cells differentiated into the anticipated lineage. Hydrogels also show minimal tendency to adsorb proteins from body fluids because of their low interfacial tension.[4] However, it should be noted that natural polymers including collagen, gelatin, glycosaminoglycans, and derivatives thereof often possess a high affinity for proteins present in serum. For example, Van Vlierberghe et al. have shown that (methacrylamide-modified) gelatin shows a high affinity for fibronectin.[5] The manuscript describes the results of complementary assays including radiolabeling, surface plasmon resonance, and quartz crystal microbalance to study the interaction between gelatin and fibronectin. Interestingly, this property offers potential for materials with specific protein affinity to be applied as scaffolds for tissue engineering applications. Research has already indicated that cell attachment preferentially occurs where (ECM) proteins have been surface-deposited. Furthermore, drugs can be incorporated into the matrices and can be released subsequently according to various release profiles depending on the hydrogel properties. Hydrogels can thus function as excellent drug delivery vehicles. Peppas et al. have already described the application of (chitosan-based) hydrogels for pharmaceutical applications in several excellent reviews.[6,7] The present review aims to give an overview of hydrogels based on natural polymers and their various applications in regenerative medicine, more specifically, in the field of tissue engineering. In a first part, relevant parameters describing hydrogel properties and the strategies applied to finetune these characteristics will be described. Next, the most relevant biopolymer-based hydrogel systems, the different methods of preparation, as well as an in-depth overview of the applications in the field of tissue engineering will be given.

Received: October 15, 2010
Revised: March 8, 2011

dx.doi.org/10.1021/bm200083n | *Biomacromolecules* XXXX, XXX, 000–000

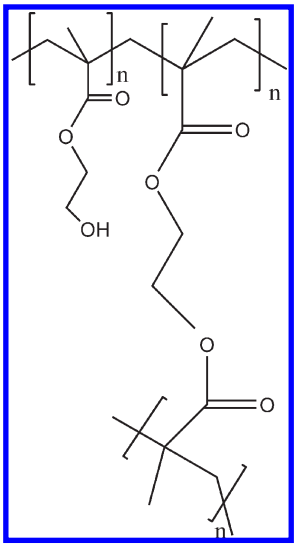


Figure 1. Structure of cross-linked poly(2-hydroxyethyl methacrylate).

A complete description of all existing natural hydrogels is beyond the scope of this Review. The focus of this Review is on the most recent developments during the past decade.

2. HYDROGEL NETWORK PROPERTIES

The suitability of hydrogels as biomedical materials and their performance in a particular application depend to a large extent on their bulk structure. The most important parameters used to characterize the network structure of hydrogels include: (1) the molecular weight of the polymer chains between two neighboring cross-links (M_c), (2) the corresponding mesh size (ξ), and (3) the effective network density (ν_e). These parameters are inter-related and can be determined by applying the equilibrium-swelling theory and the rubber-elasticity theory. For an in depth description of both theories, the authors refer to some excellent reviews.[8−12]

Recently, an interesting alternative to characterize cross-linked hydrogel networks has been presented.[13] High-resolution magic-angle spinning (HR-MAS) NMR spectroscopy enables to both characterize and quantify any unreacted cross-linkable moieties present in a chemically cross-linked, swollen hydrogel network. This technique has been applied for the first time to quantify unreacted methacrylamide moieties in a chemically cross-linked gelatin hydrogel. Because HR-MAS NMR spectroscopy is a fast, accurate, straightforward, and nondestructive technique, we anticipate that it will be applied more frequently in the future to study the hydrogel network properties.

3. TEMPERATURE-INDUCED HYDROGEL FORMATION

As previously mentioned, hydrogels are cross-linked 3-D networks containing covalent bonds, physical cross-links, hydrogen bonds, strong van der Waals interactions, and crystallite associations. Very often, combinations of the previously mentioned associations are involved in hydrogel formation. This will be further outlined in the description of the various biopolymers that have already been applied in tissue engineering applications. (See Section 5.) Interestingly, a large number of these biopolymers possess the property to self-structure upon temperature variation. Two different types of temperature-sensitive materials can be distinguished: upper critical solution temperature (UCST) and lower critical solution temperature (LCST) materials. Both types of systems possess an interesting potential for biomedical applications because materials that gel or dissolve in situ can be developed, depending on the exact UCST or LCST. Because of the impact of temperature-sensitive hydrogels, a description on the theoretical background of UCST and LCST- behavior will be given.

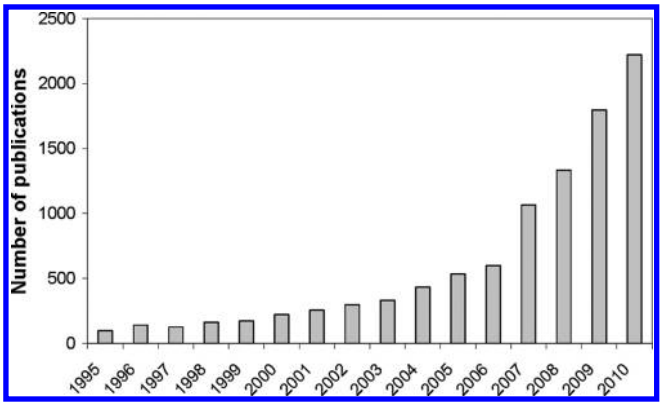


Figure 2. Overview of the number of publications concerning hydrogels during the last 16 years.

3.1. Upper Critical Solution Temperature. The gelation of many biopolymers is induced by the reversible temperature-sensitive formation of intermolecular hydrogen bonds. This thermoreversible process is characteristic for gelatin (i.e., partially hydrolyzed collagen) and certain polysaccharides including agarose, amylose, amylopectin, and carrageenan.[14] The nucleation and growth of the helical aggregates is driven by the formation of double (for polysaccharides) or triple helices (for gelatin).

A number of synthetic polymers are also capable of forming physical hydrogels via hydrogen bonding. Examples include polyethylene oxide and polyvinylalcohol.[15] An alternative system based on hydrogen-bond-mediated self-assembly was reported by Salem et al.[16] In that paper, microparticles of lactic acid/ethylene oxide copolymers were modified using biotin. The addition of an aqueous solution of avidin led to cross-linking by molecular interaction between the biotin-modified synthetic polymer and the multiple binding sites on avidin. This methodology was applied for the fabrication of cell carriers.

3.2. Lower Critical Solution Temperature. A second class of temperature-sensitive materials includes the LCST systems. At low temperatures, a homogeneous solution is obtained. Upon heating, aggregation of the hydrophobic groups occurs, inducing phase separation and hydrogel formation. The endothermal gelation is driven by an entropy change. In contrast with the increase in order during the aggregation of the hydrophobic segments, the entropy increases during the hydrogel formation. This is due to the large amount of water molecules released by the hydrophobic part of the polymer.[17] Gelation thus occurs spontaneously upon heating because the entropy ($T\Delta S$) compensates for the unfavorable enthalpy (ΔH).

Examples of such systems include graft copolymers composed of a polyacetal backbone with pendant poly(ethylene glycol) side chains, linear poly(*N*-isopropylacrylamide-*co*-butylmethacrylate-*co*-acrylic acid) terpolymers, and poly(*N*-isopropylacrylamide-*co*-methacrylic acid).[18−20]

Gehrke et al. selected chemically cross-linked hydrogels based on methylcellulose (MC), HPMC, hydroxypropylcellulose, and carboxymethylcellulose for drug release applications.[21] These

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

Table 1. Application Fields of Cryogels with Their Respective Building Blocks[a]

type of polymer	application	reference
gelatin, poly(acrylamide), agarose, poly(2-hydroxyethyl methacrylate), dextrane, chitosan	cell carrier	35, 38, 41
carbon particles	biosensor	42
poly(acrylamide)	chromatography	22
galactomannan, xanthan, starch	food	23, 43−45
poly(acrylamide), gelatin	drug release	46−49

[a] For more specific information regarding cryogels, the authors wish to refer to some excellent reviews.[22,27,50]

cellulose derivatives show LCST behavior, which makes them particularly interesting to be applied as drug delivery vehicles.

4. CRYO-INDUCED HYDROGEL FORMATION

In addition to hydrogel formation at ambient temperature, hydrogels can also be synthesized by applying a cryogenic treatment. Lozinsky et al. have referred to porous hydrogels produced using a cryogenic treatment using the term "cryogels".[22] Interestingly, the phenomenon of cryogelation decreases both the critical monomer/polymer concentration and the reaction time required for gelation. Cryotropic gelation (aka cryogelation) is a specific type of gelation taking place upon cryogenic treatment of gel-forming systems. A requirement for the processes resulting in the formation of cryogels is crystallization of the bulk of the low-molecular-weight liquid present in the initial system.[22] Because of the crystallization of the pure solvent, the total volume of the nonfrozen liquid microphase (NFLMP) is lower than the initial reaction volume. Consequently, the concentration of polymer or monomer in the NFLMP is significantly higher than the initial concentration. The polymer gel phase can be formed during one of the stages of cryogenic treatment: during freezing of the initial system, during storage of the samples in the frozen state, or during thawing of the frozen specimens.[22−26] The result of the above-mentioned process is a so-called "cryogel" (i.e., porous scaffold composed of the hydrogel starting material).[22] For a detailed description of the term "cryogel" and its specific applications, we refer to some excellent reviews from Lozinsky et al.[22,27]

Recently, the structuring of different polymers by cryogenic treatment has attracted much of attention. For a thiol-containing poly(acryl amide) derivative, the conditions to obtain an insoluble gel after freezing-thawing were optimized. The gel retained the shape of the cryo-mold, whereas the polymer solution at room temperature remained liquid.[28] Both the reaction rate and yield to transform macromolecular thiols into the corresponding disulfides were increased by freeze−thawing of aqueous solutions of thiol-containing polymers in the presence of oxidants.[29]

The processes of cryo-induced association and aggregation of β-glucans resulted in the formation of soft or hard matter depending on the amount of applied freeze−thaw cycles.[30]

Multiple freezing cycles influenced the structure of poly(vinyl alcohol) (PVA) hydrogels in such a way that secondary crystallites were superimposed on primary crystallites, which were formed after the first temperature cycle. This resulted in materials with improved mechanical properties.[31]

More recently, the process of cryogelation for the production of porous materials has been widely applied.[32−34] Gelatin cryogels were prepared as cell carriers for a panel of human cells.[35,36] A well-defined "curtain-like" pore architecture was induced by applying a cryogenic treatment on scaffolds containing both gelatin and chondroitin sulfate.[37] Agarose cryogel sponges were evaluated as scaffolds for culturing both isolated pancreatic islets and insulinoma cells.[38] Blends of PVA and different biological macromolecules, including hyaluronic acid, dextran, and gelatin were used to produce bioartificial hydrogels functioning as potential tissue engineering scaffolds.[39] Macroporous gels, based on agarose, poly(acrylamide), or polymethacrylates were patented as separation media for application within the field of chromatography.[40] An overview of the most recent applications of cryogels is given in Table 1.

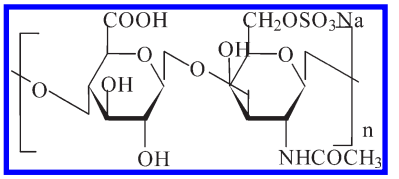


Figure 3. Chemical structure of chondroitin sulfate C.

5. BIOPOLYMER-BASED HYDROGEL SYSTEMS

From the above overview of materials showing temperature- and cryo-induced gelation, the relevance of biopolymers in the field of tissue engineering was already clearly demonstrated. (See Sections 3 and 4.) In what follows, an overview of the most frequently applied polysaccharides and proteins for regenerative medicine, the different hydrogel preparation methods applied, as well as their applications will be given.

5.1. Polysaccharide Hydrogels. *5.1.1. Chondroitin Sulfate.* Chondroitin sulfate (CS) is a glycosaminoglycan composed of alternating units of *N*-acetyl-D-galactosamine and D-glucuronic acid. (See Figure 3.) It possesses excellent biocharacteristics including the binding and modulation of certain growth factors.

Because natural CS is readily water-soluble, chemical cross-linking of CS is required for in vitro or in vivo hydrogel application. In literature, a variety of methods was already described for cross-linking CS. Kirker et al. have prepared biocompatible hydrogel films using the adipic dihydrazide derivative of chondroitin sulfate (CS-ADH), in which a pendant hydrazide functionality generated a gel using a small molecule or a macromolecular cross-linker (e.g., poly(ethylene glycol)-propiondialdehyde, PEG-dialdehyde, Figure 4).[51] The most frequently applied cross-linking reagents include a combination of 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS).[52] The cross-linking reaction has often been performed in the presence of collagen (Figure 5)[53] or other amine-containing reagents (e.g., 1,12-diaminododecane).[54] However, cross-linking using EDC often resulted in (partial) matrix collapse in aqueous media. This could be (partially) prevented by performing the

dx.doi.org/10.1021/bm200083n |Biomacromolecules XXXX, XXX, 000–000

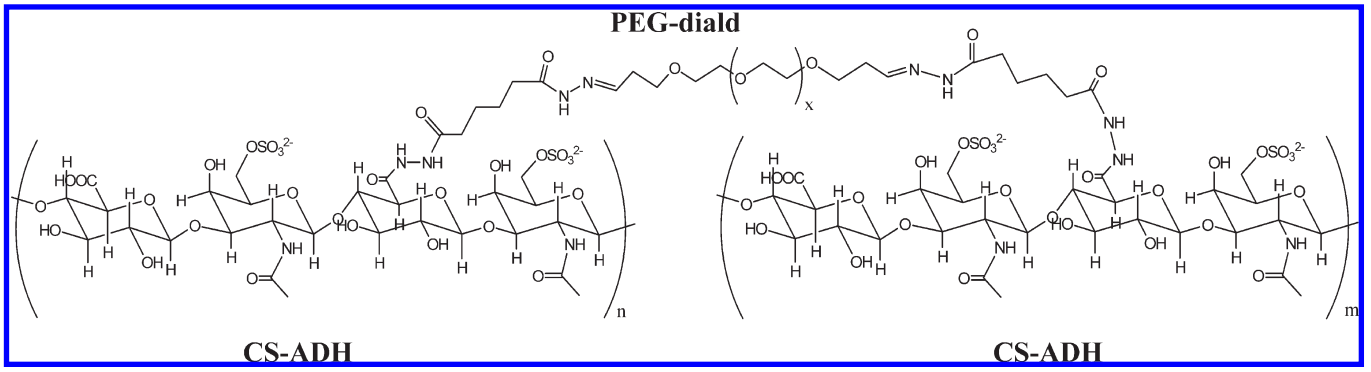


Figure 4. CS hydrogel cross-linking chemistry.

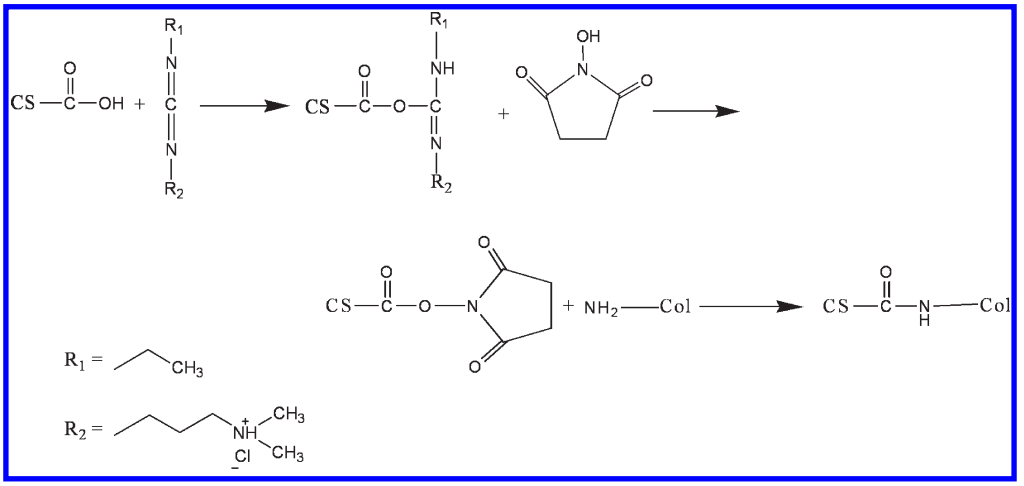


Figure 5. Cross-linking of CS and collagen using EDC and NHS.

cross-linking step in the presence of ethanol.[55] Alternatively, CS was functionalized with thiol groups by EDC-mediated condensation with a disulfide-containing hydrazide, followed by dithiothreitol reduction. In a subsequent step, thiol-modified CS was cross-linked using poly(ethylene glycol) diacrylate.[56] Li et al. have utilized glycidyl methacrylate (GMA) in a heterogeneous reaction in aqueous medium regardless of GMA's potential side-reaction with water. Two reactions, including a rapid transesterification and a slow irreversible epoxide ring-opening took place simultaneously. (See Figure 6.)[57]

CS-based hydrogels have previously found widespread application in the field of tissue engineering. Hydrogels composed of gelatin and CS were applied as controlled release systems for antibacterial proteins. Incorporation of CS in cross-linked gelatin gels significantly increased the protein loading capacity of the gels and extended the release time.[58] Alternatively, gelatin-CS-hyaluronan tricopolymer scaffolds were selected to mimic natural cartilage.[59,60] It was observed that the presence of CS promoted the secretion of proteoglycan and type II collagen.[60] Bilayer gelatin-CS-hyaluronan biomatrices have also been studied for wound treatment. The results showed that in addition to a permanent coverage with histologically normal and adequately differentiated epithelial tissue, a well-defined dermal-epidermal junction and a collagen network in the dermis were present. As a result, the skin substitute had a positive effect on the promotion of the wound healing process and could be used to assist the regeneration of full-thickness skin defects.[61,62] Another application of the tricopolymer scaffold included the regeneration of the human nucleus pulposus.[63] Furthermore, both microcarriers and membranes, composed of CS and gelatin, were prepared in view of different therapeutic strategies.[64−70]

A nonexhaustive overview ranked alphabetically by application, is given in Table 2.

5.1.2. Hyaluronic Acid. Hyaluronic acid (i.e., hyaluronan, HA) is a nonsulphated glycosaminoglycan, composed of alternating units of D-glucuronic acid and D-*N*-acetylglucosamine, linked together via alternating β-1,4 and β-1,3 glycosidic bonds (Figure 7).[71,72] HA is one of the major components of the extracellular matrix of skin, cartilage, and the vitreous humor.[56,73] The first hyaluronan-based biomedical product (Healon) was developed in the 1970s and is FDA approved for the use in eye surgery (e.g., corneal transplantation).[74] At present, the most often used commercially available HA-based product is HYAFF (i.e., benzyl ester of HA).[75,76] The product exists with varying esterification degrees, and various research groups have already reported on their differences in mechanical properties and biological response.[75] However, at research level, a widely applied strategy to cross-link chemically HA was and still is the polymerization of methacrylate-functionalized HA.[77−82] In combination with collagen to form semi-interpenetrating networks (semi-IPNs), endothelial cell attachment was already realized within microfluidic channels aiming at blood vessel formation.[77] In addition, the semi-IPNs were suitable to enable fibroblast[78,83] and chrondrocyte encapsulation[84] and subsequent proliferation.

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

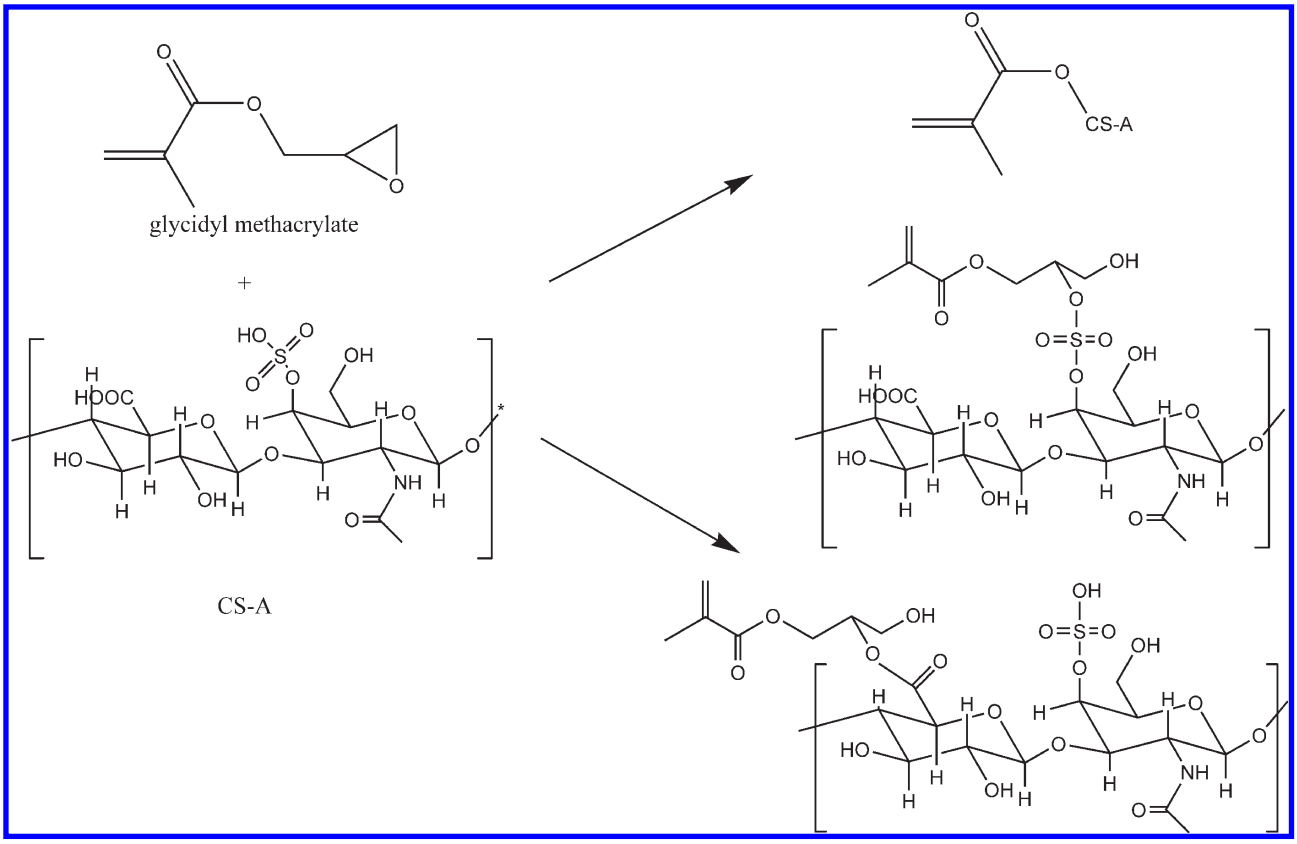


Figure 6. Reaction mechanism of CS and glycidyl methacrylate.

Table 2. Overview of Biomedical Applications of Chondroitin Sulfate

type of chondroitin sulfate	application
gelatin/chondoitin-6-sulfate/hyaluronan, methacrylate- and aldehyde-modified chondroitin sulfate, chondroitin sulfate/chitosan/dermatan sulfate, poly(L-lactide)-*g*-chondroitin sulfate, poly(ethylene glycol)/chondroitin sulfate	cartilage
EDC cross-linked chondroitin sulfate/collagen/elastin, EDC cross-linked chondroitin sulfate/collagen, thiolated chondroitin sulfate/hyaluronan/gelatin	general tissue engineering application
chondroitin sulfate/collagen	heart
gelatin/chondoitin-6-sulfate/hyaluronan, glutaraldehyde cross-linked gelatin/chondroitin-6-sulfate	intervertebral disk
chondroitin sulfate/heparin/collagen	liver
chondroitin sulfate/collagen	lung
EDC cross-linked chondoitin-6-sulfate/gelatin/hyaluronan	skin
chitosan/chondroitin sulfate, chondroitin sulfate/Pluronic F127 nanogel, chondroitin sulfate spheres	drug release

HA has also been combined with alginate[85] and poly-L-lysine[86−88] to develop scaffolds for a variety of tissue engineering applications including nerve regeneration.[89] More recently, composite scaffolds were also prepared starting from complementary chemical functionalities. Tan et al. reported on the development of a new class of biocompatible and biodegradable composite hydrogels derived from water-soluble chitosan and oxidized hyaluronic acid without the addition of a chemical cross-linking agent. The gelation was attributed to the Schiff base reaction between amino and aldehyde groups of polysaccharide derivatives including *N*-succinyl-chitosan and aldehyde-modified hyaluronic acid.[90] In addition, that research group has also elaborated a novel strategy to synthesize aminated hyaluronic acid-*g*-poly

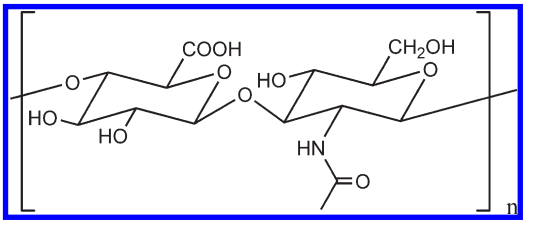


Figure 7. Chemical structure of hyaluronic acid.

(*N*-isopropylacrylamide) (AHA-*g*-PNIPAAm).[91] AHA prepared by grafting adipic dihydrazide to the HA backbone was coupled

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

Table 3. Overview of Biomedical Applications of Hyaluronic Acid

type of hyaluronic acid	application
ester-containing hyaluronic acid	adipose tissue
amine/aldehyde-containing hyaluronic acid, hyaluronic acid/poly(vinylalcohol), MMP-sensitive hyaluronic acid	bone
hyaluronic acid/collagen I, hyaluronan/gelatin/chondoitin-6-sulfate, adipic dihydrazide-modified	cartilage
collagen/hyaluronic acid, fibrin/hyaluronic acid, chitosan/hyaluronic acid, chitosan/hyaluronic acid, carrageenan/fibrin/hyaluronic acid	
thiolated hyaluronan/poly(ethyleneglycol) diacrylate, hyaluronic acid/gelatin gradient, poly(*N*-isopropylacrylamide)/hyaluronic acid, hyaluronic acid/pendant L-benzoyl-cysteine, methacrylated hyaluronic acid, collagen/hyaluronan/chitosan, collagen/hyaluronic acid, silk fibroin/hyaluronan	general
acryl-modified hyaluronic acid/poly(ethylene glycol) acryl	gene therapy
ester-containing hyaluronan/butyric and retinoic acid, methacrylated hyaluronan, divinylsulfone cross-linked hyaluronan	heart
benzyl esters of hyaluronic acid, hyaluronan/gelatin/chondoitin-6-sulfate	intervertebral disk
benzyl esters of hyaluronic acid	liver
hyaluronic acid	muscle
photo-cross-linked hyaluronic acid, collagen/hyaluronic acid, fibroin/hyaluronic acid, antibody-modified hyaluronic acid, hyaluronic acid/polylysine	nerve
hyaluronic acid derivatives, carbodiimide-cross-linked hyaluronic acid	ophthalmology
benzyl esters of hyaluronic acid, hyaluronan-gelatin, EDC cross-linked hyaluronan/chondoitin-6-sulfate/gelatin, adipic dihydrazide derivatives of hyaluronic acid/PEG-propiondialdehyde, hyaluronic acid/chitosan/gelatin	skin
thiol-modified hyaluronic acid	spinal cord
methacrylated hyaluronic acid	vascular tissue

to carboxylic end-capped PNIPAAm (PNIPAAm-COOH) produced via radical polymerization using 4,4′-azobis(4-cyanovaleric acid) as an initiator.[91] Horn et al. combined thiol-modified HA with acrylate-functionalized PEG to create hydrogels suitable for spinal cord repair using Michael's addition.[92] When targeting hard tissue engineering including cartilage repair, the mechanical properties of the above-mentioned material were insufficient. Therefore, various research groups studied the possibility to develop HA-based composites possessing synthetic polymers including poly lactic-glycolic acid (PLGA)[93] and poly(propylene fumarate).[84] Because proteins (e.g., gelatin,[94,95] fibrin,[96] fibroin,[97] collagen[98,99]) are often part of these composites, EDC is in most studies applied to realize chemical cross-linking.[61,62,94,95,99] HA has also been modified with moieties including RGD peptide or galactose targeting, respectively increased or specific cell attachment for hepatocytes.[100] More recently, MMP-sensitive HA-based scaffolds have been developed to finetune the material degradation to the time needed for new tissue formation. In general, cross-linkers are selected possessing MMP-cleavable peptides to mimic the remodeling characteristics of natural extracellular matrices by cell-derived MMPs.[101] In addition to porous HA-based scaffolds, nanofibers and microbeads have also already been developed starting from glycosaminoglycans using electrospinning[102] and phase separation,[103] respectively. Finally, HA has also been combined with stem cells to function as injectable material for tissue augmentation purposes.[104]

In Table 3, an overview is given of various hyaluronic acid derivatives and their respective biomedical applications.

5.1.3. Chitosan. Chitosan is the partial deacetylated derivative of chitin, which is obtained from the shells of crabs and shrimp (Figure 8). This biocompatible, cationic polymer dissolves in water up to a pH of 6.2. An increased basicity results in a gel-like precipitation of the hydrated polymer by neutralization of the amine groups. The pH-responsiveness can be extended to a pH-dependent, thermoresponsive system (i.e., LCST-characterized

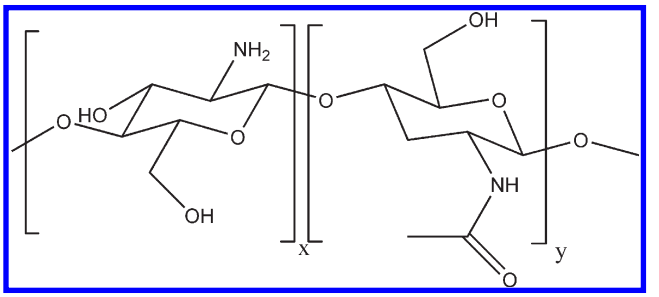


Figure 8. Structure of chitosan.

system) by adding polyol salts including β-glycerophosphate (GP). These formulations dissolve at neutral pH and ambient temperature. Upon heating to body temperature, gelation occurs. It was observed that both the stability at room temperature and the gelation time increase with decreasing deacetylation degree.[105]

The solubility at ambient temperature and pH 7 is induced by the hydration of the chitosan chain, promoted by the GP. Upon heating to body temperature, the bound water is partially released inducing chain interactions and subsequent gelation. Various interactions are involved in the gelation mechanism:

1 electrostatic attractions between chitosan ammonium groups and the GP phosphate group,
2 hydrogen bonds between chitosan chains, due to the decreased electrostatic repulsion after neutralization of the ammonium groups by GP,
3 chitosan−chitosan hydrophobic interactions.

Chitosan microspheres have already been prepared by adding a chitosan solution drop-by-drop in a sodium tripolyphosphate solution.[106] Next, the microspheres were transferred in a mold, followed by sintering to develop chitosan matrices suitable for bone tissue engineering applications. To develop scaffolds with a channel-like pore morphology, Bagnaninchi et al. have already reported on the potential of freeze-drying in the presence of a

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

Table 4. Overview of Biomedical Applications of Chitosan

type of chitosan	application
glutaraldehyde-cross-linked collagen/chitosan	adipose tissue
freeze-dried chitosan/gelatin, electrospun collagen/chitosan nanofiber	blood vessel
sintered chitosan microspheres, poly(ε-caprolactone)/poly(vinylalcohol)/chitosan, chitosan/fibroin/hydroxyapatite, β-TCP/chitosan, β-FGF-loaded hydroxyapatite/chitosan, polycaprolactone/chitosan, chitosan/alginate multilayer scaffold, chitosan/gelatin, titania/chitosan composite, photo-cross-linkable chitosan, chitosan/collagen, ceramic nanoparticles/chitosan, chitosan/polyethylene glycol dimethacrylate/*N*,*N*-dimethylacrylamide, silk/chitosan, nanohydroxyapatite/chitosan/carboxymethyl cellulose	bone
chitosan beads, EDC-cross-linked collagen/chitosan/GAG, chitosan/poly(butylene succinate), CS/dermatan sulfate/chitosan, chitosan/hyaluronic acid, chitosan/hyaluronic acid, chitosan/polyester-based, insulin-loaded chitosan, chitosan/gelatin, alginate/chitosan, chitosan/gelatin/hyaluronan, chitosan/Pluronic, polyethylene oxide/chitosan, glutaraldehyde/oxidized dextran/chitosan	cartilage
hydroxypropyl chitosan/gelatin	corneal stroma
chitosan/starch, hydroxyapatite/chitosan, chitosan/soy protein/TEOS, collagen/hyaluronan/chitosan, genipin-cross-linked chitosan, thiolated chitosan, electrosprayed chitosan microbeads, chitosan/poly(vinyl alcohol), poly(caprolactone)/chitosan, chitosan/collagen, nanofibrous PLLA/chitosan fibers, disulfide cross-linked chitosan, chitosan/poly-L-lysine, chitosan/gelatin, chitosan-*graft*-β-cyclodextrin, calcium phosphate/chitosan, carboxymethyl chitosan-*graft*-D-glucuronic acid, chitosan/PEG/gelatin, chitosan-*g*-lactic acid, chitosan/phopholipid	general tissue engineering applications
chitosan/glycerophosphate, chitosan/glycerophosphate/hydroxyethylcellulose	intervertebral disk
collagen/chitosan, silk fibroin/chitosan/heparin, chitosan/gelatin	liver
alginate dialdehyde cross-linked chitosan/calcium polyphosphate	meniscus
poly(lysine)-functionalized chitosan, polypyrrole/chitosan, PLGA/chitosan/HA, chitosan/polyglycolic acid	nerve
chitosan/gelatin/glycerol phosphate	nucleus pulposus
DTBP-cross-linked chitosan, gold colloid/chitosan, collagen/chitosan, bFGF/chitosan, β-glycerol phosphate/collagen/chitosan	skin
chitosan-based hyaluronan, chitosan microchannel	tendon

series of pore-forming needles possessing a diameter of 50 μm.[107] The potential of the scaffolds developed was already demonstrated for tendon tissue engineering.[107] However, matrices possessing elongated channels could also find some potential in the field of nerve regeneration. Crompton et al. have modified chitosan with poly-D-lysine via azidoaniline photocoupling.[108] The results indicated that cortical cell survival was improved for poly-D-lysine modifications up to 0.1%. When exceeding this number, neurite outgrowth was hindered significantly.[108] Jiao et al. have reported on the development of chitosan/polyglycolic acid nerve grafts for axon regeneration.[109]

Alternative techniques to develop porous scaffolds are supercritical fluid technology[110,111] and stereolithography.[112] Choi et al. have developed chitosan inverse opal scaffolds due to their unique uniform pore size and regular 3-D interconnectivity.[113] PCL microspheres were selected as template, followed by fabrication of a cubic close-packed lattice. Finally, the PCL template was dissolved selectively.[113] The scaffolds showed potential to be applied for bone tissue engineering. He et al. have developed composite scaffolds of chitosan and gelatin for liver tissue engineering by combining stereolithography and freeze-drying.[114]

Chitosan-based scaffolds and nanofibers have also already been applied for bone regeneration, either as such[115] or as part of composites with synthetic polymers including poly(L-lactic acid), poly(butylene-succinate),[116] or ceramics including hydroxyapatite.[117−130] When targeting skin tissue, engineering ECM constituents such as collagen are often included in chitosan-based scaffolds.[131−133] In addition to collagen[134,135] (and derivatives thereof[41,121,136,137]), synthetic polymers including poly(ethylene glycol)[138,139] and Pluronics[140,141] have also already been combined with chitosan when aiming at tissue regeneration.

In the presence of glycosaminoglycans[142] (i.e., dermatan sulfate, CS,[143] or hyaluronic acid [90,103,134,144]), EDC is often applied as a cross-linking agent. The matrices developed enable the adhesion and subsequent proliferation of chondrocytes[145] and hepatocytes.[100] In the latter case, galactose moieties were first coupled to hyaluronic acid using ethylene diamine to target specifically hepatocytes.[100] She et al. also evaluated composite scaffolds of chitosan and silk fibroin for liver tissue engineering.[146−148] In addition, growth factors[149−152] including bFGF, BMP-2, BMP-6, and peptides (e.g., RGD)[140] have already been combined with chitosan to upregulate the cell-interactive properties.

A commonly applied approach to cross-link chitosan-based scaffolds is the use of glutaraldehyde.[127,153−155] In addition, oxidized dextran has also been already selected as cross-linker.[153]

In contrast with photocurable chitosans, which are cross-linked irreversibly, thiolated chitosans have also been developed. In that case, the cross-linking occurs via air oxidation of the thiols forming disulfide linkages.[156]

Recently, injectable chitosan-based hydrogels have also gained increasing interest because of their potential applications in the field of tissue engineering.[157] De Souza et al. have reported on the in vivo biocompatibility of blends consisting of chitosan, phospholipids, and lauric aldehyde or lauric chloride.[157] Alternatively, enzymatic cross-linking using horseradish peroxidase and H_2O_2 has also been applied to obtain injectable chitosan hydrogels.[158]

In Table 4, an overview of the various biomedical applications of chitosan is given.

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

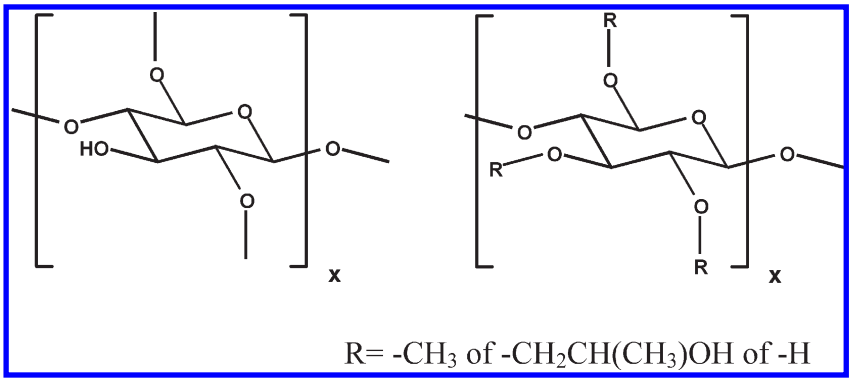


Figure 9. Structure of methylcellulose (left part) and hydroxypropylmethylcellulose (right part).

Table 5. Overview of Biomedical Applications of Cellulose Derivatives

type of cellulose	application
Ca^{2+}-activated cellulose, cellulose/lactide, bacterial cellulose, nanohydroxyapatite/bacterial cellulose	bone
cellulose/collagen, injectable cellulose	cartilage
bacterial cellulose	cornea
various cellulose-based hydrogels	general
cellulose acetate and regenerated cellulose	heart
bacterial cellulose	muscle
cellulosic hydrogels	nerve
carboxymethylcellulose	nucleus pulposus
bacterial cellulose	vascular

5.1.4. Cellulose Derivatives. In contrast with most other biopolymers, gelation of various cellulose derivatives including MC and hydroxypropylmethylcellulose (HPMC) (Figure 9) occurs upon heating.[159] The LCST values of MC and HPMC are, respectively, situated between 40 and 50 °C and 75−90 °C. Because the transition temperature of both polymers is above 37 °C, both physical and chemical methods have been applied to decrease the LCST values: addition of NaCl or decrease in the hydroxypropyl degree.[159]

The gelation of both MC and HPMC is mainly induced by intermolecular, hydrophobic interactions of the methoxy side groups. Consequently, the macromolecules are fully hydrated at low temperatures. Upon heating, gradual dehydratation occurs, resulting in a viscosity increase. Near the transition temperature, polymer−polymer interactions are dominant and result in the formation of a polymer network.[160] These polymers were evaluated for tissue engineering applications by Tate et al.[161] From the results, it was demonstrated that MC is a promising candidate material to be applied as brain cell support. The potential advantages of MC scaffolding in the brain were numerous, including the treatment of multiple-site injuries and irregular defects. Bacterial cellulose has also been reported on for tissue engineering applications by various research groups.[162−165] However, bacterial cellulose is not enzymatically degradable in vivo. Therefore, Li et al. have developed 2,3-dialdehyde-modified bacterial cellulose via periodate oxidation.[166] Verma et al. further modified this cellulose derivative with hydrazine yielding 2,3-dihydrazone.[167]

In combination with hydroxyapatite, cellulose shows potential to be applied for bone tissue engineering.[123,168−171] Cellulose is often combined with proteins (e.g., gelatin),[172] polysaccharides (e.g., chitosan), or both.[123,173,174]

An overview of the most recent biomedical applications of a selected number of cellulose derivatives is given in Table 5.

5.1.5. Alginate. Alginate is a brown-algae-derived polysaccharide composed of β-D-mannuronic acid and α-L-guluronic acid units.[175] The molecular weight can vary between 10 and 1000 kDa depending on the source and production process. Upon adding multivalent cations, an alginate solution rapidly forms an ionotropical gel what makes it extremely interesting to be applied in the biomedical field.[176,177] To enable in vivo injection of a Ca^{2+}-cross-linked alginate hydrogel, the cross-linking rate should be reduced. Interestingly, polyols have already been applied to slow down the hydrogel formation. It has been anticipated that the polyols hinder the immediate complexation of Ca^{2+} by alginate. In addition, this formulation containing polyols to reduce the hydrogel formation rate has even been filed as a patent. Alginate has been proven to be mucoadhesive, biocompatible, and nonimmunogenic.[178] An overview of the most recent biomedical applications of alginates is given in Table 6. Alginate is often processed into microcarriers for cell encapsulation.[179] Although alginate does not possess cell-interactive properties, as such, several authors have overcome this issue by coupling cell-interactive peptides (e.g., RGD) or growth factors (e.g., VEGF)[180] to the alginate backbone.[176] In addition, alginate-based semi-interpenetrating polymer networks have been prepared possessing stimuli-responsive behavior. Wang et al. have developed a pH-sensitive superabsorbent hydrogel composed of sodium alginate-*g*-poly(sodium acrylate) and polyvinylpyrrolidone by free-radical solution polymerization using ammonium persulfate as initiator and *N*,*N*-methylene-bis-acrylamide as cross-linker.[181] Thermosensitive hydrogels were prepared by Zhao et al. via in situ copolymerization of *N*-isopropylacrylamide with poly(ethylene glycol)-*co*-poly(ε-caprolactone) in the presence of sodium alginate by UV irradiation technology.[182] Electroresponsive behavior has been induced by grafting poly(acrylic acid) to sodium alginate using ammonium persulfate as initiator and *N*,*N*′-methylene-bis-acrylamide as cross-linker.[183]

When aiming at bone tissue engineering, alginate is often combined with calcium phosphates.[184−186] In addition, proteins including gelatin are also often included in alginate-based scaffolds to improve the cell-interactive properties.[184,187−190]

In addition to stereolithography[191] and freeze-drying,[192] porous alginate-based scaffolds have also been developed by stacking successively alginate gel layers for cell encapsulation

Table 6. Overview of Biomedical Applications of Alginate

type of polymer	application
alginate/elastin/PEG, angiogenic factors/alginate	blood vessel
alginate microbeads, alginate/gelatin/hydroxyapatite, oxidized alginate/gelatin/tricalcium phosphate, chitosan/alginate, alginate/poly (lactic-*co*-glycolic acid)/calcium phosphate, collagen/alginate/nanohydroxyapatite	bone
sodium alginate, chitosan/alginate, gelatin/alginate	bone marrow
alginate/fibrin, agarose/alginate/gelatin, chitosan/alginate/hyaluronate, PLGA/alginate, transforming growth factor-β(1) loaded alginate	cartilage
alginate, alginate-*cis*-aconityl-daunomycin, calcium alginate/silk fibroin, hyaluronic acid/alginate, PLGA/Ca-alginate	drug delivery
alginate, alginate/polyvinyl alcohol, laminated alginate, carbon nanotube/alginate, iron-cross-linked alginate, alginate/poly(L-lysine)-hyaluronic acid, alginate/chitosan, copper-capillary alginate	general
injectable alginate, gelatin/alginate	heart
alginate/chitosan	ligament
macroporous alginate, alginate/galactosylated chitosan, sodium alginate	liver
chitosan/calcium polyphosphate	meniscus
photo-cross-linked alginate	nucleus pulposus
gelatin/alginate	skin
alginate	spinal cord
alginate/chitosan	tendon

and poly-L-lysine-hyaluronic acid multilayer films functioning as reservoirs for bioactive molecules.[89]

5.2. Protein Hydrogels. *5.2.1. Collagen.* Collagen is the major protein of the ECM. As summarized in Table 7, at least 12 types of collagen exist in various tissues.[193−201] Types I, II, and III are the most abundant and form fibrils of similar structure. Type IV collagen forms a 2D reticulum and is a major component of the basal lamina.

In general, porous collagen-based scaffolds are produced using freeze-drying techniques or stereolithography methods. In addition, Kim et al. have developed a cryogenic direct-plotting system to fabricate 3-D collagen matrices.[202]

When aiming at bone tissue regeneration, porous collagen scaffolds are often combined with calcium phosphates.[203−209] Many researchers have also already reported on composite scaffolds both with synthetic polymers[210,211] including poly(lactic acid),[212−214] poly(glycolic acid),[213−215] or poly(caprolactone) as well as (modified) glycosaminoglycans[207,216−220] such as (photo-cross-linkable) hyaluronic acid[98] forming semi-IPNs.[78,83]

Specific scaffold geometries including cylindrical tubes have also been developed for blood vessel regeneration. Boccafoschi et al. assembled both collagen and vascular cells onto a rotating cylinder.[221] In addition to microporous scaffolds, collagen-based nanofibers have already been developed using electrospinning.[222−229] Moreover, collagen microbeads were also already applied for adipogenic differentiation of stem cells.[230] In most reports, carbodiimide is applied as cross-linking agent.[222,231−233] In addition, aldehydes,[155] a dehydrothermal treatment, or natural cross-linkers (e.g., genipin) have also been applied to cross-link collagen as such or in the presence of glycosaminoglycans.[218]

To improve further the cell-interactive properties of collagen-based matrices, specific peptides, growth factors[151,234] (e.g., BMP-2),[235] or both have already been incorporated. Duan et al. have applied the 2-polypropyleneimine octaamine dendrimer in the presence of EDC as cross-linker for collagen scaffolds.[236] In addition, these dendrimers have been modified with YIGSR peptides.[236] Lee et al. incorporated VEGF in bioprinted composite scaffolds possessing fibrin for neural tissue engineering.[237] More recently, recombinant human-like collagen

Table 7. Overview of the Different Collagen Types, Localized in Various Tissues

collagen type	localization
I	skin, tendon, bone
II	cartilage, vitreous humor
III	skin, muscle, frequently associated with type I
IV	all basal lamina
V	most interstitial tissue, associated with type I
VI	most interstitial tissue, associated with type I
VII	epithelia
VIII	some endothelial cells
IX	cartilage, associated with type II
X	hypertrophic and mineralizing cartilage
XI	cartilage
XII	cartilage, associated with type I and III

was also developed and applied for safety issues (e.g., risk related to BSE infection).[135,238,239]

In Table 8, an overview of collagen-based materials and their applications is shown.

5.2.2. Gelatin. Gelatin is a biopolymer derived from collagen by hydrolytic degradation. Because of its unique functionality, gelatin is used in a wide variety of applications, ranging from food-related over pharmaceutical and photographic to technical products. However, gelatin has also been frequently applied as a material for biomedical applications. Because gelatin has a sol−gel transition temperature around 30 °C, gelatin should be cross-linked chemically to avoid dissolution at body temperature. Because gelatin is composed of a large variety of side chains, a wide variety of chemical modification methods, introducing cross-linkable groups, have been proposed.[240,241] The choice of potential reagents is limited to water-stable ones because gelatin only dissolves in water and in a number of alcohols. In most cases, bifunctional reagents including glutaraldehyde,[242] diisocyanates,[243,244] carbodiimides,[245] genipin,[246−248] polyepoxy-compounds,[249] and acyl azides[250] have been applied. When gelatin

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

Table 8. Overview of Biomedical Applications of Collagen-Based Materials

type of collagen	application
glutaraldehyde-cross-linked collagen/chitosan, bFGF/collagen, collagen microbeads	adipose tissue
compressed collagen	bladder
collagen/cell assembly, p(DLLA-*co*-TMC)/collagen, collagen-chitosan nanofiber, PLGA microsphere/collagen, fibroin/collagen, TMC/DNA-containing collagen, collagen/citric acid derivative, polylactide/silk fibroin/gelatin	blood vessel
collagen/nanohydroxyapatite, dense collagen, polyvinyl alcohol/collagen/hydroxyapatite, collagen microspheres, collagen/nanotube, collagen I/PLGA-β-TCP, collagen fiber/PLA, collagen/glycosaminoglycan, nano-HA/collagen/PLLA, collagen/OP-1, PCL/collagen, RhBMP-2 microspheres/chitosan/collagen, adenovirus vectors/collagen/chitosan	bone
collagen/chitosan/GAG, adipic dihydrazide-modified collagen/hyaluronic acid, PLGA/collagen, micronized collagen sponges, type II collagen, collagen propeptides, type II collagen/chondroitin sulfate/hyaluronan, collagen/HA/chondroitin sulfate	cartilage
dendrimer-cross-linked collagen, hydroxypropyl chitosan/gelatin	cornea
CO(3)Ap-collagen	dental
photo-cross-linked collagen, EDC-cross-linked electrospun collagen, poly(lactic-*co*-glycolic acid)/collagen, PHBV/collagen, collagen/hyaluronan/chitosan, collagen/hyaluronic acid, TPU/collagen, collagen/glycosaminoglycan, poly(lactic acid-*co*-caprolactone)/collagen, stromal cell-derived factor 1α-loaded heparinized collagen, collagen/hyaluronan/chitosan, gelatin/alginate	general
type I collagen, collagen/GAG	heart
type I and II collagen/GAG	intervertebral disk
collagen/silk	ligament
poly(lactic-*co*-glycolic acid)/collagen, collagen/chitosan/heparin	liver
cross-linked atelocollagen	muscle
collagen/microchannels, collagen/hyaluronic acid, collagen/heparan sulfate	nerve
collagen II/hyaluronan/chondroitin-6-sulfate, collagen	nucleus pulposus
UV-cross-linked collagen	ophtalmology
PLGA/collagen	pancreas
compressed collagen, cross-linked collagen/chondroitin sulfate/hyaluronic acid, β-glycerol phosphate/collagen/chitosan, collagen/elastin, electrospun collagen/PCL, poly[(D,L-lactide)-*co*-glycolide]/collagen	skin
collagen	urological

is combined with sugars (e.g., agarose), 1,1-carbonyldiimidazole can be applied as cross-linker.[251] Gelatin derivatization occurs mostly via the amine groups of lysine and hydroxylysine.[252] The guanidinium group of arginine is protonated under mild basic conditions, which excludes this group from nucleophilic reaction. The imidazole group of histidine can react but leads to the formation of unstable products.[253] Methacrylamide-modified gelatin can be cross-linked in the presence of a photoinitiator upon applying UV irradiation.[254−260] Van Den Bulcke at al modified the primary amines of gelatin with methacrylamide moieties using methacrylic anhydride. The subsequent chemical cross-linking occurred upon UV irradiation in the presence of a UV-active photoinitiator Irgacure 2959.[261] The obtained hydrogels appeared to be very promising to be applied for wound treatment. Van Vlierberghe et al. selected methacrylamide-modified gelatin as starting material to produce porous scaffolds for tissue regeneration purposes. The gelatin-based cell carriers were prepared by applying a cryogenic treatment, followed by lyophilization.[5,254,262] The cryogels developed supported the attachment and growth of a large variety of human cells including fibroblasts, endothelial cells, glial cells, osteoblasts, and epithelial cells.[35] In addition, porous scaffolds were developed based on combinations of methacrylamide-modified gelatin and methacrylate-modified CS.[37] Alternatively, redox initiators also enable the polymerization of gelatins possessing methacrylamide moieties. Another possibility to obtain chemically cross-linked hydrogels includes high-energy irradiation, such as ebeam and gamma-rays. The major advantages of high-energy irradiation include a reagent- and solvent-free reaction and the simultaneous cross-linking and sterilization. The latter is particularly interesting in view of future applications because the production process is drastically shortened by performing cross-linking and sterilization simultaneously.

Hu et al. reported on the enzymatic cross-linking of gelatin-hydroxypropionic acid.[263] Moreover, the obtained material was processed into hollow fibers for general tissue engineering applications using a novel fiber spinning method.[263] Sakai et al. utilized peroxidase-mediated cross-linking of incorporated phenolic hydroxyl groups.[264] Gelatin can also be processed using other techniques including electrospinning[265−272] and stereolithography.[112,273−275] Because gelatin is derived from collagen, it is often combined with GAGs,[61,62,144,276,277] calcium phosphates,[278−282] or both when targeting the regeneration of specific tissues. Moreover, gelatin has also been part of composites with synthetic polymers including poly(L-lactic acid),[283] polyurethanes,[284] and PCL.[270] Boudet et al. achieved chemical cross-linking using a thermosensitive reactive copolymer based on *N*-isopropylacrylamide.[285] The copolymer consisted of acrylic acid units that formed amide bonds with the amino groups of gelatin in the presence of a water-soluble carbodiimide. By setting the temperature above or below the LCST, it was possible to finetune the reactivity of the system and control the gelation process.

For the production of porous gelatin-based scaffolds for tissue engineering purposes, freeze-drying and phase separation techniques are often applied.[255,286,287]

A few examples, illustrating the potential of gelatin-based materials, are summarized below. Co-release of basic fibroblast growth factor, insulin, and insulin-like growth factor I from styrenated gelatin-based microspheres promoted de novo

Table 9. Overview of Biomedical Applications of Gelatin-Based Materials

type of gelatin	application
gelatin sponge	adipose tissue
gelatin/poly(ε-caprolactone) nanofibers, VEGF immobilized gelatin, polyethylene-glycol diacrylate/gelatin, chitosan/gelatin, gelatin/PET nanofibers, gelatin/PES fibers, gelatin/PTFE	blood vessel
hydroxyapatite chitosan/gelatin, gelatin/poly(α-hydroxy acids), glutaraldehyde cross-linked gelatin, hydroxyapatite/gelatin, β-tricalcium phosphate/gelatin, gelatin/poly(ε-caprolactone) nanofibers, gelatin microcarriers/polyester, micro- and nanohydroxyapatite/chitosan/gelatin, rhBMP-2-loaded gelatin/nanohydroxyapatite/fibrin, poly[(L-lactide)-*co*-(epsilon-caprolactone)]/gelatin, gelatin-based photopolymers	bone
gelatin/chondoitin-6-sulfate/hyaluronan, plasmid DNA/chitosan/gelatin, gelatin microparticle/OPF, gelatin microparticle/poly(D,L-lactide-ε-caprolactone), TGF-β1-loaded gelatin, ceramic/gelatin, esterified hyaluronan/gelatin, gelatin/chitosan/hyaluronan	cartilage
transglutaminase cross-linked gelatin, proanthocyanidin cross-linked chitosan/gelatin, gelatin/poly(D,L-lactide), gelatin fibers, PHBHHx/gelatin, PVA/gelatin, PNIPAM/gelatin, gelatin- and fibronectin-coated PE multilayer nanofilms, gelatin/montmorillonite/cellulose, chitosan/PEG/gelatin, gelatin/hydroxyphenylpropionic acid, gelatin microparticles, gelatin/chitosan cryogels, genipin-cross-linked PCL/gelatin nanofibers, silk sericin/gelatin, α-chitin/gelatin, agarose/gelatin cryogel, hyaluronan/gelatin	general
gelatin/polyurethane, photo-cross-linked gelatin, alginate/gelatin	heart
gelatin/chondoitin-6-sulfate/hyaluronan, gelatin, glutaraldehyde cross-linked gelatin/chondroitin-6-sulfate	intervertebral disk
gelatin/silk fibroin	ligament
cross-linked sodium alginate/gelatin, chitosan/gelatin	liver
gelatin/PCL nanofibers	muscle
photo cross-linkable gelatin, gelatin/hydroxyphenylpropionic acid	nerve
chitosan/gelatin/glycerol phosphate	nucleus pulposus
gelatin/agarose	pancreas
glutaraldehyde cross-linked gelatin	skin

formation of adipose tissue.[288] Poly(*N*-isopropylacrylamide)-grafted gelatin has been used in cardiac tissue engineering applications.[289] A variety of other applications in the field of tissue engineering are summarized in Table 9.[290−293]

5.2.3. Elastin. Elastin forms the greater part of elastic, thus mechanically active tissues including tendon, blood vessels, and elastic cartilage.[294] A commercially available dermal substitute (i.e., Matriderm) composed of elastin and collagen has already been evaluated and described frequently in literature.[295,296] Because of its extensive covalent cross-linking, only a few research groups have applied native elastin as cell carriers for tissue engineering applications.[297] Therefore, Rodriguez-Cabello et al. have developed a promising alternative being recombinant elastin.[298−303] These repetitive polypeptides are composed of VPGXG pentapeptide sequences, where X can be every natural amino acid except for proline.[299] Interestingly, recombinant elastin shows thermoresponsive LCST behavior. Below the transition temperature, the polymer remains soluble, whereas above this critical temperature, the hydrophobic chains self-assemble into a more ordered structure. Poly(VPAVG) is the recombinant elastin resembling native elastin to the highest extent. Above its transition temperature, poly(VPAVG) forms micelles. Therefore, the materials are very promising to be applied for drug delivery purposes.[304,305] Annabi et al. have developed porous scaffolds starting from hexamethylene diisocyanate cross-linked α-elastin using high pressure CO_2. The obtained pore size was influenced by varying the pressure applied.[306] Elastin has also been processed into nanofibers using electrospinning, either as such or in the presence of collagen or gelatin.[228,271,307−309] To obtain homogeneous and continuous fibers, small PEO quantities had to be added. The produced fibers were cross-linked using EDC/NHS.[309] In addition, elastin-like polymers show excellent biocompatibility because they resemble natural elastin and their degradation products are native amino acids.[299] For enhancement of its cell-interactive properties, peptide sequences including RGD[300] and growth factors such as bFGF[310] have already been combined with elastin. Table 10 shows an overview of elastin-based constructs applied for tissue engineering applications.

5.2.4. Fibroin. Silk fibroin is a natural protein synthesized by the silkworm *Bombyx mori*.[311] Its primary structure mainly consists of glycine, alanine, and serine.[178] The protein can be processed into films,[312−314] nanofibers,[267,268,315−317] scaffolds,[147] membranes,[318] gels,[319,320] and powders,[321,322] which renders it extremely suitable to be applied in a large variety of applications in the field of biomaterials and drug delivery.[178] Fan et al. have developed porous gelatin-based hybrid scaffolds[323] for ligament tissue engineering.[287] In addition to composite scaffolds with other proteins (e.g., collagen),[225,238,324,325] fibroin has also been combined with GAGs including hyaluronan.[97,326] Aqueous solutions of fibroin and hyaluronan were freeze-dried to induce porosity and incubated subsequently in methanol to induce water insolubility of silk fibroin. The scaffolds developed were suitable to support mesenchymal stem cell adhesion.[326] In addition to freeze-drying,[327−329] leaching out of porogens can also be applied to render fibroin scaffolds porous. Makaya et al. evaluated the effect of porogens including salt and sucrose on the final scaffold properties.[330] For the production of scaffolds with a homogeneous pore size and geometry, stereolithography was already applied starting from silk fibroin.[331] In addition, fibroin-based microtubes have already been developed by incubating stainless steel tubes in fibroin solutions containing low amounts of PEO.[332] The latter enabled us to control the microporosity of

dx.doi.org/10.1021/bm200083n |*Biomacromolecules* XXXX, XXX, 000–000

Table 10. Overview of Biomedical Applications of Elastin-Based Materials

type of polymer	application
collagen/elastin, alginate/elastin/PEG, collagen/elastin/PCL, copper nonparasitic/elastin, bFGF/elastin, polydioxanone/elastin/collagen, poliglecaprone/PCL/elastin/gelatin, polyglyconate/elastin	blood vessel
BMP-containing elastin	bone
hexamethylene diisocyanate-cross-linked α-elastin, recombinant elastin, tropo-elastin, collagen/elastin, collagen/elastin/chitosan/poly(lactic acid), poly(lactide-*co*-glycolide)/gelatin/elastin	general
elastin-like proteins	nerve
recombinant elastin	ocular
collagen/elastin	skin

Table 11. Overview of Biomedical Applications of Fibroin-Based Materials

type of polymer	application
nonmulberry and mulberry silk gland fibroin	adipose tissue
fibroin, collagen/fibroin, polylactide/silk fibroin-gelatin, fibroin modified-polyhydroxyalkanoate	blood vessel
silk fibroin/chitosan/PLLA, chitosan/fibroin-hydroxyapatite, nonmulberry silk gland fibroin, nonmulberry and mulberry silk gland fibroin	bone
silk fibroin modified porous poly(e-caprolactone), plasma-treated fibroin	cartilage
alginate/fibroin, silk fibroin/gelatin	drug delivery
gelatin/silk fibroin, hyaluronan/silk fibroin, chitosan/silk fibroin, fibroin/recombinant human-like collagen, antheraea assama silk fibroin, nanohydroxyapatite/fibroin, silk fibroin-modified PHBHHx, polylactide/silk fibroin-gelatin	general
gelatin/silk fibroin	ligament
fibroin/recombinant human-like collagen, PLLA/fibroin, chitosan/silk fibroin, chitosan/silk fibroin/heparin	liver
antheraea pernyi silk fibroin	tendon

the processed tubes. An overview of the most recent tissue-engineered constructs using fibroin-based scaffolds is highlighted in Table 11. Different surface modifications including plasma treatment have been applied to fibroin-based materials to implement specific properties.[333] Vepari et al. have grafted poly(ethylene glycol) onto silk fibroin films using cyanuric chloride poly(ethylene glycol) to induce antiadhesive and antithrombotic properties. Wenk et al. applied sulfonated silk fibroin to control binding, delivery, and potency of FGF-2.[334] Several research groups finetuned the cell adhesion behavior of silk-based materials using lactose surface modification.[335−337] When aiming at bone tissue engineering, fibroin scaffolds were modified with hydroxyapatite.[338,339]

6. CONCLUSIONS AND FUTURE PROSPECTS

The current Review clearly shows that a variety of biopolymers, including polysaccharides and proteins, are a very versatile class of materials that have found widespread application in the field of regenerative medicine. Interestingly, a large number of these biopolymers possess thermoresponsive solubility behavior. This opens perspectives to develop systems that gel or dissolve at body temperature. Polymers that do not possess this property can still be applied to develop hydrogel materials by functionalizing them with cross-linkable groups.

The high number of research groups working in the field of hydrogel development and characterization clearly illustrates that hydrogels are ideal candidate materials to be applied in the field of tissue engineering. In the future, materials that mimic better the natural extracellular matrix in terms of composition, structural characteristics, and mechanical properties will be developed. We anticipate that the ideal tissue engineering scaffold will combine the mechanical tailoring possibilities of synthetic polymers with the biomimetic properties of natural materials. To mimic the extracellular matrix to a great extent, various biopolymers including glycosaminoglycans and proteins including collagen and elastin will need to be combined in one ideal matrix. However, to ensure reproducibility and avoid certain risks related to the use of natural materials, material scientists will be obliged to apply selectively recombinant proteins and so on.

In addition, the optimal scaffold architecture will undoubtedly combine the microstructure to be obtained with stereolithography techniques with the nanoroughness, which can be realized using electrospinning. At present, a large number of devices already exists to realize only one of the above-mentioned prerequisites. However, merging different processing techniques into one multifunctional device to finetune all morphological parameters both on the macroscale as well as on a micro- and nanolevel will become a major, even essential, challenge. Moreover, in view of the interdisciplinary character of tissue engineering, a close collaboration between various research disciplines including, among others, materials science, polymer chemistry, cell biology, medicin, and pharmacy will be essential to develop finally the ideal tissue engineering scaffold. In addition, this interdisciplinary approach will undoubtedly offer new possibilities in this direction.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Etienne.Schacht@UGent.be. Tel: 003292644497. Fax: 003292644972.

■ ACKNOWLEDGEMENT

S.V.V. would like to acknowledge the Research Foundation Flanders (FWO, Belgium) for the financial support under the form of a postdoctoral fellowship. P.D. would like to acknowledge Ghent University and the IWT for financial support in the frameworks of the UGent-BOF project 2009-2013 (Production of porous polymer structures via Bioplotting for cardiovascular applications), the UGent-GOA project 2010-2015 (BOF10/GOA/005, Biomedical Engineering for Improved Diagnosis and Patient-Tailored Treatment of Aortic Aneurysms and Dissection), the UGent Multidisciplinary Research Partnership Nano- and biophotonics (2010-2014) and the SBO HEPSTEM project IWT990066 respectively. The authors would also like to thank the PolExGene consortium. PolExGene is a STREP project (contract number 019114) funded under the EU 6th framework programme.

■ REFERENCES

(1) Wichterle, O.; Lim, D. Hydrophilic gels for biological use. *Nature* **1960**, *185*, 117–118.

(2) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* **2000**, *50* (1), 27–46.

(3) Ovsianikov, A.; Deiwick, A.; Van Vlierberghe, S.; Dubruel, P.; Moller, L.; Drager, G.; Chichkov, B., Laser fabrication of three-dimensional cad scaffolds from photosensitive gelatin for applications in tissue engineering. *Biomacromolecules*, published online March 2, http://dx.doi.org/10.1021/bm1015305.

(4) Gupta, P.; Vermani, K.; Garg, S. Hydrogels: from controlled release to pH-responsive drug delivery. *Drug Discovery Today* **2002**, *7*, 569–579.

(5) Van Vlierberghe, S.; Vanderleyden, E.; Dubruel, P.; De Vos, F.; Schacht, E. Affinity study of novel gelatin cell carriers for fibronectin. *Macromol. Biosci.* **2009**, *9*, 1105–1115.

(6) Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Peppas, N. A.; Gurny, R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 19–34.

(7) Peppas, N. A. Intelligent therapeutics: biomimetic systems and nanotechnology in drug delivery. *Adv. Drug Delivery Rev.* **2004**, *56*, 1529–1531.

(8) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27–46.

(9) Peppas, N. A.; Merrill, E. W. Crosslinked poly(vinyl alcohol) hydrogels as swollen elastic networks. *J. Appl. Polym. Sci.* **1977**, *21*, 1763–1770.

(10) Kloczkowski, A. Application of statistical mechanics to the analysis of various physical properties of elastomeric networks -- a review. *Polymer* **2002**, *43*, 1503–1525.

(11) Boyce, M. C.; Arruda, E. M. Constitutive models of rubber elasticity: a review. *Rubber Chem. Technol.* **2000**, *73*, 504–523.

(12) Gent, A. N. Rubber and rubber elasticity - review. *J. Polym. Sci., Part C: Polym. Symp.* **1974**, 1–17.

(13) Van Vlierberghe, S.; Fritzinger, B.; Martins, J. C.; Dubruel, P. Hydrogel network formation revised: high-resolution magic angle spinning nuclear magnetic resonance as a powerful tool for measuring absolute hydrogel cross-link efficiencies. *Appl. Spectrosc.* **2010**, *64*, 1176–1180.

(14) Ross-Murphy, S. B. Thermoreversible and Irreversible Physical Gels from Biopolymers. In *Polymer Gels: Fundamentals and Applications*; Bohidar, H. B., Dubin, P., Osada, Y., Eds.; ACS Symposium Series 833; American Chemical Society: Washington, D.C.; pp 51−69.

(15) Inoue, T.; Osaki, K. Rheological properties of poly(vinyl alcohol)/sodium borate aqueous-solutions. *Rheol. Acta* **1993**, *32*, 550–555.

(16) Salem, A. K.; Rose, F.; Oreffo, R. O. C.; Yang, X. B.; Davies, M. C.; Mitchell, J. R.; Roberts, C. J.; Stolnik-Trenkic, S.; Tendler, S. J. B.; Williams, P. M.; Shakesheff, K. M. Porous polymer and cell composites that self-assemble in situ. *Adv. Mater.* **2003**, *15*, 210–+.

(17) Bromberg, L. E.; Ron, E. S. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. *Adv. Drug Delivery Rev.* **1998**, *31*, 197–221.

(18) Schacht, E.; Toncheva, V.; Vandertaelen, K.; Heller, J. Polyacetal and poly(ortho ester)-poly(ethylene glycol) graft copolymer thermogels: preparation, hydrolysis and FITC-BSA release studies. *J. Controlled Release* **2006**, *116*, 219–225.

(19) Serres, A.; Baudyš, M.; Kim, S. W. Temperature and pH-sensitive polymers for human calcitonin delivery. *Pharm. Res.* **1996**, *13*, 196–201.

(20) Brazel, C. S.; Peppas, N. A. Pulsatile local delivery of thrombolytic and antithrombotic agents using poly(*N*-isopropylacrylamide-*co*-methacrylic acid) hydrogels. *J. Controlled Release* **1996**, *39*, 57–64.

(21) Gehrke, S. H. Synthesis, equilibrium swelling, kinetics, permeability and applications of environmentally responsive gels. *Adv. Polym. Sci.* **1993**, *110*, 81–144.

(22) Lozinsky, V. I. Cryogels on the basis of natural and synthetic polymers: preparation, properties and application. *Usp. Khim.* **2002**, *71*, 559–585.

(23) Giannouli, P.; Morris, E. R. Cryogelation of xanthan. *Food Hydrocolloids* **2003**, *17*, 495–501.

(24) Podorozhko, E. A.; Kurskaya, E. A.; Kulakova, V. K.; Lozinsky, V. I. Cryotropic structuring of aqueous dispersions of fibrous collagen: influence of the initial pH values. *Food Hydrocolloids* **2000**, *14*, 111–120.

(25) Lozinsky, V. I.; Damshkaln, L. G.; Brown, R.; Norton, I. T. Study of cryostructuration of polymer systems. XVIII. Freeze-thaw influence on water-solubilized artificial mixtures of amylopectin and amylose. *J. Appl. Polym. Sci.* **2000**, *78*, 371–381.

(26) Lozinsky, V. I.; Damshkaln, L. G. Study of cryostructuration of polymer systems. XVII. Poly(vinyl alcohol) cryogels: dynamics of the cryotropic gel formation. *J. Appl. Polym. Sci.* **2000**, *77*, 2017–2023.

(27) Lozinsky, V. I.; Galaev, I. Y.; Plieva, F. M.; Savinal, I. N.; Jungvid, H.; Mattiasson, B. Polymeric cryogels as promising materials of biotechnological interest. *Trends Biotechnol.* **2003**, *21*, 445–451.

(28) Vainerman, E. S.; Lozinsky, V. I.; Rogozhin, S. V. Study of cryostructurization of polymer systems. I. Structure formation in solutions of thiol-containing polymers under freezing-thawing. *Colloid Polym. Sci.* **1981**, *259*, 1198–1201.

(29) Lozinsky, V. I.; Golovina, T. O.; Gusev, D. G. Study of cryostructuration of polymer systems: XIII. Some characteristic features of the behaviour of macromolecular thiols in frozen aqueous solutions. *Polymer* **2000**, *41*, 35–47.

(30) Lazaridou, A.; Biliaderis, C. G. Cryogelation of cereal β-glucans: structure and molecular size effects. *Food Hydrocolloids* **2004**, *18*, 933–947.

(31) Willcox, P. J.; Howie, D. W.; Schmidt-Rohr, K.; Hoagland, D. A.; Gido, S. P.; Pudjijanto, S.; Kleiner, L. W.; Venkatraman, S. Microstructure of poly(vinyl alcohol) hydrogels produced by freeze/thaw cycling. *J. Polym. Sci., Part B: Polym. Phys.* **1999**, *37*, 3438–3454.

(32) Bajpai, A. K.; Saini, R. Designing of macroporous biocompatible cryogels of PVA-haemoglobin and their water sorption study. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 2063–2074.

(33) Bolgen, N.; Vargel, I.; Korkusuz, P.; Guzel, E.; Plieva, F.; Galaev, I.; Matiasson, B.; Piskin, E. Tissue responses to novel tissue engineering biodegradable cryogel scaffolds: an animal model. *J. Biomed. Mater. Res., Part A* **2009**, *91A*, 60–68.

(34) Vrana, N. E.; O'Grady, A.; Kay, E.; Cahill, P. A.; McGuinness, G. B. Cell encapsulation within PVA-based hydrogels via freeze-thawing: a one-step scaffold formation and cell storage technique. *J. Tissue Eng. Regener. Med.* **2009**, *3*, 567–572.

(35) Dubruel, P.; Unger, R.; VanVlierberghe, S.; Cnudde, V.; Jacobs, P. J. S.; Schacht, E.; Kirkpatrick, C. J. Porous gelatin hydrogels: 2. in vitro cell interaction study. *Biomacromolecules* **2007**, *8*, 338–344.

(36) VanVlierberghe, S.; Cnudde, V.; Dubruel, P.; Masschaele, B.; Cosijns, A.; DePaepe, I.; Jacobs, P. J. S.; VanHoorebeke, L.; Remon, J. P.;

Schacht, E. Porous gelatin hydrogels: 1. cryogenic formation and structure analysis. *Biomacromolecules* **2007**, *8*, 331–337.

(37) Van Vlierberghe, S.; Dubruel, P.; Lippens, E.; Masschaele, B.; Van Hoorebeke, L.; Cornelissen, M.; Unger, R.; Kirkpatrick, C. J.; Schacht, E. Toward modulating the architecture of hydrogel scaffolds: curtains versus channels. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 1459–1466.

(38) Bloch, K.; Lozinsky, V. I.; Galaev, I. Y.; Yavriyanz, K.; Vorobeychik, M.; Azarov, D.; Damshkaln, L. G.; Mattiasson, B.; Vardi, P. Functional activity of insulinoma cells (INS-1E) and pancreatic islets cultured in agarose cryogel sponges. *J. Biomed. Mater. Res., Part A* **2005**, *75A*, 802–809.

(39) Cascone, M. G.; Lazzeri, L.; Sparvoli, E.; Scatena, M.; Serino, L. P.; Danti, S. Morphological evaluation of bioartificial hydrogels as potential tissue engineering scaffolds. *J. Mater. Sci.: Mater. Med.* **2004**, *15*, 1309–1313.

(40) Mattiasson, B.; Galaev, I.; Lozinsky, V.; Plieva, F. Separation Medium, Its Preparation and Its Use. U.S. Patent 2008/0090918 A1, April 17, 2008.

(41) Kathuria, N.; Tripathi, A.; Kar, K. K.; Kumar, A. Synthesis and characterization of elastic and macroporous chitosan-gelatin cryogels for tissue engineering. *Acta Biomater.* **2009**, *5*, 406–418.

(42) Park, Y. M.; Kim, Y. H.; Yamamoto, T. Determination of dye concentration in water using mesoporous particle coated QCR sensor. *Sens. Actuators, B* **2007**, *125*, 468–473.

(43) Busto, M. D.; Meza, V.; Ortega, N.; Perez-Mateos, M. Immobilization of naringinase from *Aspergillus niger* CECT 2088 in poly-(vinyl alcohol) cryogels for the debittering of juices. *Food Chem.* **2007**, *104*, 1177–1182.

(44) Doyle, J. P.; Giannouli, P.; Martin, E. J.; Brooks, M.; Morris, E. R. Effect of sugars, galactose content, and chainlength on freeze-thaw gelation of galactomannans. *Carbohydr. Polym.* **2006**, *64*, 391–401.

(45) Filatova, A. G.; Volkov, I. O.; Krikunova, N. I.; Misharina, T. A.; Golovnya, R. V. Microstructure and surface composition of corn starch cryogels with sorbed organic flavoring agents. *Russ. Chem. Bull.* **2000**, *49*, 314–316.

(46) Bajpai, A. K.; Saini, R. Preparation and characterization of novel biocompatible cryogels of poly (vinyl alcohol) and egg-albumin and their water sorption study. *J. Mater. Sci.: Mater. Med.* **2006**, *17*, 49–61.

(47) Bajpai, A. K.; Saini, R. Preparation and characterization of biocompatible spongy cryogels of poly(vinyl alcohol)-gelatin and study of water sorption behaviour. *Polym. Int.* **2005**, *54*, 1233–1242.

(48) Xue, W.; Champ, S.; Huglin, M. B.; Jones, T. G. J. Rapid swelling and deswelling in cryogels of crosslinked poly(*N*-isopropylacrylamide-*co*-acrylic). *Eur. Polym. J.* **2004**, *40*, 703–712.

(49) Zhang, X. Z.; Chu, C. C. Thermosensitive PNIPAAm cryogel with superfast and stable oscillatory properties. *Chem. Commun.* **2003**, *12*, 1446–1447.

(50) Lozinsky, V. I.; Plieva, F. M.; Galaev, I. Y.; Mattiasson, B. The potential of polymeric cryogels in bioseparation. *Bioseparation* **2001**, *10*, 163–188.

(51) Kirker, K. R.; Luo, Y.; Nielson, J. H.; Shelby, J.; Prestwich, G. D. Glycosaminoglycan hydrogel films as bio-interactive dressings for wound healing. *Biomaterials* **2002**, *23*, 3661–3671.

(52) Daamen, W. F.; van Moerkerk, H. T. B.; Hafmans, T.; Buttafoco, L.; Poot, A. A.; Veerkamp, J. H.; van Kuppevelt, T. H. Preparation and evaluation of molecularly-defined collagen-elastin-glycosaminoglycan scaffolds for tissue engineering. *Biomaterials* **2003**, *24*, 4001–4009.

(53) Pieper, J. S.; van Wachem, P. B.; van Luyn, M. J. A.; Brouwer, L. A.; Hafmans, T.; Veerkamp, J. H.; van Kuppevelt, T. H. Attachment of glycosaminoglycans to collagenous matrices modulates the tissue response in rats. *Biomaterials* **2000**, *21*, 1689–1699.

(54) Sintov, A.; Dicapua, N.; Rubinstein, A. Cross-Linked Chondroitin Sulfate - Characterization for Drug-Delivery Purposes. *Biomaterials* **1995**, *16*, 473–478.

(55) Pieper, J. S.; Oosterhof, A.; Dijkstra, P. J.; Veerkamp, J. H.; van Kuppevelt, T. H. Preparation and characterization of porous crosslinked collagenous matrices containing bioavailable chondroitin sulphate. *Biomaterials* **1999**, *20*, 847–858.

(56) Cai, S. S.; Liu, Y. C.; Shu, X. Z.; Prestwich, G. D. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials* **2005**, *26*, 6054–6067.

(57) Li, Q.; Wang, D. A.; Elisseeff, J. H. Heterogeneous-phase reaction of glycidyl methacrylate and chondroitin sulfate: Mechanism of ring-opening-transesterification competition. *Macromolecules* **2003**, *36*, 2556–2562.

(58) Kuijpers, A. J.; Engbers, G. H. M.; Meyvis, T. K. L.; de Smedt, S. S. C.; Demeester, J.; Krijgsveld, J.; Zaat, S. A. J.; Dankert, J.; Feijen, J. Combined gelatin-chondroitin sulfate hydrogels for controlled release of cationic antibacterial proteins. *Macromolecules* **2000**, *33*, 3705–3713.

(59) Chang, C. H.; Kuo, T. F.; Lin, C. C.; Chou, C. H.; Chen, K. H.; Lin, F. H.; Liu, H. C. Tissue engineering-based cartilage repair with allogenous chondrocytes and gelatin-chondroitin-hyaluronan tri-copolymer scaffold: a porcine model assessed at 18, 24, and 36 weeks. *Biomaterials* **2006**, *27*, 1876–1888.

(60) Chang, C. H.; Liu, H. C.; Lin, C. C.; Chou, C. H.; Lin, F. H. Gelatin-chondroitin-hyaluronan tri-copolymer scaffold for cartilage tissue engineering. *Biomaterials* **2003**, *24*, 4853–4858.

(61) Wang, T. W.; Wu, H. C.; Huang, Y. C.; Sun, J. S.; Lin, F. H. Biomimetic bilayered gelatin-chondroitin 6 sulfate-hyaluronic acid biopolymer as a scaffold for skin equivalent tissue engineering. *Artif. Organs* **2006**, *30*, 141–149.

(62) Wang, T. W.; Sun, J. S.; Wu, H. C.; Tsuang, Y. H.; Wang, W. H.; Lin, F. H. The effect of gelatin-chondroitin sulfate-hyaluronic acid skin substitute on wound healing in SCID mice. *Biomaterials* **2006**, *27*, 5689–5697.

(63) Yang, S. H.; Chen, P. Q.; Chen, Y. F.; Lin, F. H. An in-vitro study on regeneration of human nucleus pulposus by using gelatin/chondroitin-6-sulfate/hyaluronan tri-copolymer scaffold. *Artif. Organs* **2005**, *29*, 806–814.

(64) Brown, K. E.; Leong, K.; Huang, C. H.; Dalal, R.; Green, G. D.; Haimes, H. B.; Jimenez, P. A.; Bathon, J. Gelatin/chondroitin 6-sulfate microspheres for the delivery of therapeutic proteins to the joint. *Arthritis Rheum.* **1998**, *41*, 2185–2195.

(65) Azhari, R.; Hirosue, S.; Leong, K. W. Chondroitin sulfate/gelatin microspheres - preparation, characterization and release kinetics. *Abstr. Pap. Am. Chem. Soc.* **1992**, *203*, 18-CELL.

(66) Yonese, M.; Nakagaki, M. Physicochemical studies of mixed membrane composed of gelatin and chondroitin sulfate. 0.5. Dependence of electrolyte permeabilities on membrane charge-density and water-content. *Yakugaku Zasshi* **1981**, *101*, 493–500.

(67) Yonese, M.; Nakagaki, M. Physicochemical studies of mixed membrane composed of gelatin and chondroitin sulfate. 0.4. Membrane charge-density and its pH dependency. *Yakugaku Zasshi* **1976**, *96*, 299–306.

(68) Yonese, M.; Nakagaki, M. Physicochemical studies of mixed membrane composed of gelatin and chondroitin sulfate. 0.2. Effect of chondroitin sulfate concentration and of complex-formation on swelling. *Yakugaku Zasshi* **1975**, *95*, 641–647.

(69) Yonese, M.; Nakagaki, M. Physicochemical studies of mixed membrane composed of gelatin and chondroitin sulfate. 0.3. Permeability of electrolytes and its pH-dependence. *Yakugaku Zasshi* **1975**, *95*, 665–671.

(70) Yonese, M.; Nakagaki, M. Physicochemical studies of mixed membrane composed of gelatin and chondroitin sulfate. 0.1. pH-dependence and anisotropy of swelling. *Yakugaku Zasshi* **1975**, *95*, 75–81.

(71) George, E. Intra-articular hyaluronan treatment for osteoarthritis. *Ann Rheum Dis* **1998**, *57*, 637–640.

(72) Mao, J. S.; Liu, H. F.; Yin, Y. J.; Yao, K. D. The properties of chitosan-gelatin membranes and scaffolds modified with hyaluronic acid by different methods. *Biomaterials* **2003**, *24*, 1621–1629.

(73) Ghosh, P.; Guidolin, D. Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent?. *Semin. Arthritis Rheum.* **2002**, *32*, 10–37.

(74) Cavallini, G. M.; Campi, L.; Delvecchio, G.; Lazzerini, A.; Longanesi, L. Comparison of the clinical performance of Healon 5 and Healon in phacoemulsification. *Eur. J. Ophthalmol.* **2002**, *12*, 205–211.

(75) Price, R. D.; Das-Gupta, V.; Leigh, I. M.; Navsaria, H. A. A comparison of tissue-engineered hyaluronic acid dermal matrices in a human wound model. *Tissue Eng.* **2006**, *12*, 2985–2995.

(76) Borzacchiello, A.; Mayol, L.; Ramires, P. A.; Pastorello, A.; Bartolo, C. D.; Ambrosio, L.; Milella, E. Structural and rheological characterization of hyaluronic acid-based scaffolds for adipose tissue engineering. *Biomaterials* **2007**, *28*, 4399–4408.

(77) Bick, A.; Gomez, E.; Shin, H.; Brigham, M.; Vu, M.; Khademhosseini, A. Fabrication of Microchannels in Methacrylated Hyaluronic Acid Hydrogels. In *2009 IEEE 35th Annual Northeast Bioengineering Conference*; Institute of Electrical and Electronics Engineers: Piscataway, NJ, 2009; pp 28−29.

(78) Brigham, M. D.; Bick, A.; Lo, E.; Bendali, A.; Burdick, J. A.; Khademhosseini, A. Mechanically robust and bioadhesive collagen and photocrosslinkable hyaluronic acid semi-interpenetrating networks. *Tissue Eng., Part A* **2009**, *15*, 1645–1653.

(79) Erickson, I. E.; Huang, A. H.; Sengupta, S.; Kestle, S.; Burdick, J. A.; Mauck, R. L. Macromer density influences mesenchymal stem cell chondrogenesis and maturation in photocrosslinked hyaluronic acid hydrogels. *Osteoarthritis Cartilage* **2009**, *17*, 1639–1648.

(80) Khademhosseini, A.; Eng, G.; Yeh, J.; Fukuda, J.; Blumling, J.; Langer, R.; Burdick, J. A. Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment. *J. Biomed. Mater. Res., Part A* **2006**, *79A*, 522–532.

(81) Patterson, J.; Stayton, P. S.; Li, X. D. In situ characterization of the degradation of PLGA microspheres in hyaluronic acid hydrogels by optical coherence tomography. *IEE Trans. Med. Imaging* **2009**, *28*, 74–81.

(82) Zawko, S. A.; Suri, S.; Truong, Q.; Schmidt, C. E. Photopatterned anisotropic swelling of dual-crosslinked hyaluronic acid hydrogels. *Acta Biomater.* **2009**, *5*, 14–22.

(83) Suri, S.; Schmidt, C. E. Photopatterned collagen-hyaluronic acid interpenetrating polymer network hydrogels. *Acta Biomater.* **2009**, *5*, 2385–2397.

(84) Liao, E.; Yaszemski, M.; Krebsbach, P.; Hollister, S. Tissue-engineered cartilage constructs using composite hyaluronic acid/collagen I hydrogels and designed poly(propylene fumarate) scaffolds. *Tissue Eng.* **2007**, *13*, 537–550.

(85) Nam, H. S.; An, J.; Chung, D. J.; Kim, J. H.; Chung, C. P. Controlled release behavior of bioactive molecules from photo-reactive hyaluronic acid-alginate scaffolds. *Macromol. Res.* **2006**, *14*, 530–538.

(86) Pan, L. J.; Ren, Y. J.; Cui, F. Z.; Xu, Q. Y. Viability and differentiation of neural precursors on hyaluronic acid hydrogel scaffold. *J. Neurosci. Res.* **2009**, *87*, 3207–3220.

(87) Ren, Y. J.; Zhou, Z. Y.; Cui, F. Z. Hyaluronic acid/polylysine hydrogel as a transfer system for transplantation of neural stem cells. *J. Bioact. Compat. Polym.* **2009**, *24*, 56–62.

(88) Wei, Y. T.; Sun, X. D.; Xia, X.; Cui, F. Z.; He, Y.; Liu, B. F.; Xu, Q. Y. Hyaluronic acid hydrogel modified with Nogo-66 receptor antibody and poly(L-lysine) enhancement of adherence and survival of primary hippocampal neurons. *J. Bioact. Compat. Polym.* **2009**, *24*, 205–219.

(89) Mjahed, H.; Porcel, C.; Senger, B.; Chassepot, A.; Netter, P.; Gillet, P.; Decher, G.; Voegel, J. C.; Schaaf, P.; Benkirane-Jessel, N.; Boulmedais, F. Micro-stratified architectures based on successive stacking of alginate gel layers and poly(L-lysine)-hyaluronic acid multilayer films aimed at tissue engineering. *Soft Matter* **2008**, *4*, 1422–1429.

(90) Tan, H. P.; Chu, C. R.; Payne, K. A.; Marra, K. G. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. *Biomaterials* **2009**, *30*, 2499–2506.

(91) Tan, H. P.; Ramirez, C. M.; Miljkovic, N.; Li, H.; Rubin, J. P.; Marra, K. G. Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. *Biomaterials* **2009**, *30*, 6844–6853.

(92) Horn, E. M.; Beaumont, M.; Shu, X. Z.; Harvey, A.; Prestwich, G. D.; Horn, K. M.; Gibson, A. R.; Preul, M. C.; Panitch, A. Influence of cross-linked hyaluronic acid hydrogels on neurite outgrowth and recovery from spinal cord injury. *J. Neurosurg.* **2007**, *6*, 133–140.

(93) Chang, N. J.; Yeh, M. L.; Jhung, Y. R. Fabricating PLGA Sponge Scaffold Integrated with Gelatin/Hyaluronic Acid for Engineering Cartilage. In *2009 IEEE 35th Annual Northeast Bioengineering Conference*; Institute of Electrical and Electronics Engineers: Piscataway, NJ, 2009; pp 147−148.

(94) Wang, T.-W.; Sun, J.-S.; Wu, H.-C.; Tsuang, Y.-H.; Wang, W.-H.; Lin, F.-H. The effect of gelatin-chondroitin sulfate-hyaluronic acid skin substitute on wound healing in SCID mice. *Biomaterials* **2006**, *27*, 5689–5697.

(95) Wang, T.-W.; Wu, H.-C.; Huang, Y.-C.; Sun, J.-S.; Lin, F.-H. Biomimetic bilayered gelatin-chondroitin 6 sulfate-hyaluronic acid biopolymer as a scaffold for skin equivalent tissue engineering. *Artif. Organs* **2006**, *30*, 141–149.

(96) Park, S. H.; Cui, J. H.; Park, S. R.; Min, B. H. Potential of fortified fibrin/hyaluronic acid composite gel as a cell delivery vehicle for chondrocytes. *Artif. Organs* **2009**, *33*, 439–447.

(97) Ren, Y. J.; Zhou, Z. Y.; Liu, B. F.; Xu, Q. Y.; Cui, F. Z. Preparation and characterization of fibroin/hyaluronic acid composite scaffold. *Int. J. Biol. Macromol.* **2009**, *44*, 372–378.

(98) Tang, S.; Vickers, S. M.; Hsu, H. P.; Spector, M. Fabrication and characterization of porous hyaluronic acid-collagen composite scaffolds. *J. Biomed. Mater. Res., Part A* **2007**, *82A*, 323–335.

(99) Wang, T. W.; Spector, M. Development of hyaluronic acid-based scaffolds for brain tissue engineering. *Acta Biomater.* **2009**, *5*, 2371–2384.

(100) Fan, J. Y.; Shang, Y.; Yuan, Y. J.; Yang, J. Preparation and characterization of chitosan/galactosylated hyaluronic acid scaffolds for primary hepatocytes culture. *J. Mater. Sci.: Mater. Med.* **2010**, *21*, 319–327.

(101) Kim, J.; Kim, I. S.; Hwang, S. J.; Kim, H. C.; Park, Y.; Sun, K. Bone Regeneration Using MMP Sensitive-Hyaluronic Acid Based Hydrogels. In *2009 IEEE 35th Annual Northeast Bioengineering Conference*; Institute of Electrical and Electronics Engineers: Piscataway, NJ, 2009; pp 42−43.

(102) Liu, Y.; Clark, R. A. F.; Huang, L.; Rafailovich, M. H. Hyaluronic acid-gelatin fibrous scaffold produced by electrospinning of their aqueous solution for tissue engineering applications. In *Advances in Material Design for Regenerative Medicine, Drug Delivery and Targeting/Imaging*, Shastri, V. P.; Lendlein, A.; Liu, L.; Mikos, A.; Mitragotri, S., Eds. **2009**; Vol. *1140*, pp 131-136.

(103) Park, S. J.; Yu, S. M.; Chun, M. H.; Chun, H. J.; Kim, C. H. Effect of hyaluronic acid on attachment and proliferation of chondrocyte on chitosan/hyaluronic acid bead scaffolds. *J. Tissue Eng. Regener. Med.* **2009**, *6*, 438–444.

(104) Okabe, K.; Yamada, Y.; Ito, K.; Kohgo, T.; Yoshimi, R.; Ueda, M. Injectable soft-tissue augmentation by tissue engineering and regenerative medicine with human mesenchymal stromal cells, platelet-rich plasma and hyaluronic acid scaffolds. *Cytotherapy* **2009**, *11*, 307–316.

(105) Ruel-Gariepy, E.; Chenite, A.; Chaput, C.; Guirguis, S.; Leroux, J. C. Characterization of thermosensitive chitosan gels for the sustained delivery of drugs. *Int. J. Pharm.* **2000**, *203*, 89–98.

(106) Abdel-Fattah, W. I.; Jiang, T.; El-Bassyouni, G. E. T.; Laurencin, C. T. Synthesis, characterization of chitosans and fabrication of sintered chitosan microsphere matrices for bone tissue engineering. *Acta Biomater.* **2007**, *3*, 503–514.

(107) Bagnaninchi, P. O.; Yang, Y.; Zghoul, N.; Maffulli, N.; Wang, R. K.; El Haj, A. J. Chitosan microchannel scaffolds for tendon tissue engineering characterized using optical coherence tomography. *Tissue Eng.* **2007**, *13*, 323–331.

(108) Crompton, K. E.; Goud, J. D.; Bellamkonda, R. V.; Gengenbach, T. R.; Finkelstein, D. I.; Horne, M. K.; Forsythe, J. S. Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. *Biomaterials* **2007**, *28*, 441–449.

(109) Jiao, H. S.; Yao, J.; Yang, Y. M.; Chen, X.; Lin, W. W.; Li, Y.; Gu, X. S.; Wang, X. D. Chitosan/polyglycolic acid nerve grafts for axon regeneration from prolonged axotomized neurons to chronically denervated segments. *Biomaterials* **2009**, *30*, 5004–5018.

(110) Temtem, M.; Silva, L. M. C.; Andrade, P. Z.; dos Santos, F.; da Silva, C. L.; Cabral, J. M. S.; Abecasis, M. M.; Aguiar-Ricardo, A. Supercritical CO_2 generating chitosan devices with controlled morphology. Potential application for drug delivery and mesenchymal stem cell culture. *J. Supercrit. Fluids* **2009**, *48*, 269–277.

(111) Duarte, A. R. C.; Mano, J. F.; Reis, R. L. Preparation of chitosan scaffolds loaded with dexamethasone for tissue engineering applications using supercritical fluid technology. *Eur. Polym. J.* **2009**, *45*, 141–148.

(112) He, J. K.; Li, D. C.; Liu, Y. X.; Yao, B.; Lu, B. H.; Lian, Q. Fabrication and characterization of chitosan/gelatin porous scaffolds with predefined internal microstructures. *Polymer* **2007**, *48*, 4578–4588.

(113) Choi, S. W.; Xie, J. W.; Xia, Y. N. Chitosan-based inverse opals: three-dimensional scaffolds with uniform pore structures for cell culture. *Adv. Mater.* **2009**, *21*, 2997–+.

(114) He, J. K.; Li, D. C.; Liu, Y. X.; Yao, B.; Zhan, H. X.; Lian, Q.; Lu, B. H.; Lv, Y. Preparation of chitosan-gelatin hybrid scaffolds with well-organized microstructures for hepatic tissue engineering. *Acta Biomater.* **2009**, *5*, 453–461.

(115) Heinemann, C.; Heinemann, S.; Lode, A.; Bernhardt, A.; Worch, H.; Hanke, T. In vitro evaluation of textile chitosan scaffolds for tissue engineering using human bone marrow stromal cells. *Biomacromolecules* **2009**, *10*, 1305–1310.

(116) Costa-Pinto, A. R.; Correlo, V. M.; Sol, P. C.; Bhattacharya, M.; Charbord, P.; Delorme, B.; Reis, R. L.; Neves, N. M. Osteogenic differentiation of human bone marrow mesenchymal stem cells seeded on melt based chitosan scaffolds for bone tissue engineering applications. *Biomacromolecules* **2009**, *10*, 2067–2073.

(117) Thein-Han, W. W.; Misra, R. D. K. Biomimetic chitosan-nanohydroxyapatite composite scaffolds for bone tissue engineering. *Acta Biomater.* **2009**, *5*, 1182–1197.

(118) Moreau, J. L.; Xu, H. H. K. Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate - chitosan composite scaffold. *Biomaterials* **2009**, *30*, 2675–2682.

(119) Malafaya, P. B.; Reis, R. L. Bilayered chitosan-based scaffolds for osteochondral tissue engineering: Influence of hydroxyapatite on in vitro cytotoxicity and dynamic bioactivity studies in a specific double-chamber bioreactor. *Acta Biomater.* **2009**, *5*, 644–660.

(120) Lian, Q.; Li, D. C.; Jin, Z. M.; Wang, J.; Li, A. M.; Wang, Z. Fabrication and in vitro evaluation of calcium phosphate combined with chitosan fibers for scaffold structures. *J. Bioact. Compat. Polym.* **2009**, *24*, 113–124.

(121) Li, J. J.; Dou, Y.; Yang, J.; Yin, Y. J.; Zhang, H.; Yao, F. L.; Wang, H. B.; Yao, K. D. Surface characterization and biocompatibility of micro- and nano-hydroxyapatite/chitosan-gelatin network films. *Mater. Sci. Eng., C* **2009**, *29*, 1207–1215.

(122) Kim, H. S.; Kim, J. T.; Jung, Y. J.; Ryu, S. C.; Son, H. J.; Kim, Y. G. Preparation of a porous chitosan/fibroin-hydroxyapatite composite matrix for tissue engineering. *Macromol. Res.* **2007**, *15*, 65–73.

(123) Jiang, L. Y.; Li, Y. B.; Xiong, C. D. Preparation and biological properties of a novel composite scaffold of nano-hydroxyapatite/chitosan/carboxymethyl cellulose for bone tissue engineering. *J. Biomed. Sci.* **2009**, *16*, 65.

(124) Bao, X.; Teromoto, A.; Abe, K. Zstu/Su, poly (L-lactic acid)/chitosan nano/micro fiber-sheet promotes osteoblast adhesion and differentiation. *Proc. Int. Conf. Adv. Text. Mater. Manuf. Technol.* **2008**, 1–5.

(125) Chesnutt, B. M.; Viano, A. M.; Yuan, Y. L.; Yang, Y. Z.; Guda, T.; Appleford, M. R.; Ong, J. L.; Haggard, W. O.; Burngardner, J. D. Design and characterization of a novel chitosan/nanocrystalline calcium phosphate composite scaffold for bone regeneration. *J. Biomed. Mater. Res., Part A* **2009**, *88A*, 491–502.

(126) Chesnutt, B. M.; Yuan, Y. L.; Buddington, K.; Haggard, W. O.; Bumgardner, J. D. Composite chitosan/nano-hydroxyapatite scaffolds induce osteocalcin production by osteoblasts in vitro and support bone formation in vivo. *Tissue Eng., Part A* **2009**, *15*, 2571–2579.

(127) Cai, X.; Tong, H.; Shen, X. Y.; Chen, W. X.; Yan, J.; Hu, J. M. Preparation and characterization of homogeneous chitosan-polylactic acid/hydroxyapatite nanocomposite for bone tissue engineering and evaluation of its mechanical properties. *Acta Biomater.* **2009**, *5*, 2693–2703.

(128) Chen, F.; Su, Y.; Mo, X. M.; He, C. L.; Wang, H. S.; Ikada, Y. Biocompatibility, alignment degree and mechanical properties of an electrospun chitosan-P(LLA-CL) fibrous scaffold. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 2117–2128.

(129) Chen, J. D.; Wang, Y. J.; Chen, X. F. In situ fabrication of nano-hydroxyapatite in a macroporous chitosan scaffold for tissue engineering. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1555–1565.

(130) Fedotov, A. Y.; Komlev, V. S.; Smirnov, V. V.; Fomin, A. S.; Fadeeva, I. V.; Sergeeva, N. S.; Sviridova, I. K.; Kirsanova, V. A.; Barinov, S. M. Porous composite materials chitosan - bioactive calcium compound particulate for bone tissue engineering. *Tissue Eng., Part A* **2009**, *15*, 727–727.

(131) Chen, K. Y.; Liao, W. J.; Kuo, S. M.; Tsai, F. J.; Chen, Y. S.; Huang, C. Y.; Yao, C. H. Asymmetric chitosan membrane containing collagen I nanospheres for skin tissue engineering. *Biomacromolecules* **2009**, *10*, 1642–1649.

(132) Faikrua, A.; Jeenapongsa, R.; Sila-asna, M.; Viyoch, J. Properties of β-glycerol phosphate/collagen/chitosan blend scaffolds for application in skin tissue engineering. *ScienceAsia* **2009**, *35*, 247–254.

(133) Sun, L. P.; Wang, S.; Zhang, Z. W.; Wang, X. Y.; Zhang, Q. Q. Biological evaluation of collagen-chitosan scaffolds for dermis tissue engineering. *Biomed. Mater.* **2009**, *4*, 055008.

(134) Lin, Y. C.; Tan, F. J.; Marra, K. G.; Jan, S. S.; Liu, D. C. Synthesis and characterization of collagen/hyaluronan/chitosan composite sponges for potential biomedical applications. *Acta Biomater.* **2009**, *5*, 2591–2600.

(135) Zhu, C. H.; Fan, D. D.; Duan, Z. Z.; Xue, W. J.; Shang, L. A.; Chen, F. L.; Luo, Y. E. Initial investigation of novel human-like collagen/chitosan scaffold for vascular tissue engineering. *J. Biomed. Mater. Res., Part A* **2009**, *89A*, 829–840.

(136) Thein-Han, W. W.; Saikhun, J.; Pholpramoo, C.; Misra, R. D. K.; Kitiyanant, Y. Chitosan-gelatin scaffolds for tissue engineering: Physico-chemical properties and biological response of buffalo embryonic stem cells and transfectant of GFP-buffalo embryonic stem cells. *Acta Biomater.* **2009**, *5*, 3453–3466.

(137) Nagahama, H.; Maeda, H.; Kashiki, T.; Jayakumar, R.; Furuike, T.; Tamura, H. Preparation and characterization of novel chitosan/gelatin membranes using chitosan hydrogel. *Carbohydr. Polym.* **2009**, *76*, 255–260.

(138) Hong, H.; Liu, C. S.; Wu, W. J. Preparation and Characterization of Chitosan/PEG/Gelatin Composites for Tissue Engineering. *J. Appl. Polym. Sci.* **2009**, *114*, 1220–1225.

(139) Kuo, Y. C.; Hsu, Y. R. Tissue-engineered polyethylene oxide/chitosan scaffolds as potential substitutes for articular cartilage. *J. Biomed. Mater. Res., Part A* **2009**, *91A*, 277–287.

(140) Park, K. M.; Joung, Y. K.; Park, K. D.; Lee, S. Y.; Lee, M. C. RGD-conjugated chitosan-pluronic hydrogels as a cell supported scaffold for articular cartilage regeneration. *Macromol. Res.* **2008**, *16*, 517–523.

(141) Park, K. M.; Lee, S. Y.; Joung, Y. K.; Na, J. S.; Lee, M. C.; Park, K. D. Thermosensitive chitosan-pluronic hydrogel as an injectable cell delivery carrier for cartilage regeneration. *Acta Biomater.* **2009**, *5*, 1956–1965.

(142) Yan, J. H.; Qi, N. M.; Zhang, Q. Q. Rabbit articular chondrocytes seeded on collagen-chitosan-GAG scaffold for cartilage tissue engineering in vivo. *Artif. Cells, Blood Substitutes, Biotechnol.* **2007**, *35*, 333–344.

(143) Piai, J. F.; Rubira, A. F.; Muniz, E. C. Self-assembly of a swollen chitosan/chondroitin sulfate hydrogel by outward diffusion of the chondroitin sulfate chains. *Acta Biomater.* **2009**, *5*, 2601–2609.

(144) Tan, H. P.; Wu, J. D.; Lao, L. H.; Gao, C. Y. Gelatin/chitosan/hyaluronan scaffold integrated with PLGA microspheres for cartilage tissue engineering. *Acta Biomater.* **2009**, *5*, 328–337.

(145) Chen, Y.-L.; Lee, H.-P.; Chan, H.-Y.; Sung, L.-Y.; Chen, H.-C.; Hu, Y.-C. Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds for cartilage tissue engineering. *Biomaterials* **2007**, *28*, 2294–2305.

(146) She, Z. D.; Jin, C. R.; Huang, Z.; Zhang, B. F.; Feng, Q. L.; Xu, Y. X. Silk fibroin/chitosan scaffold: preparation, characterization, and culture with HepG2 cell. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 3545–3553.

(147) She, Z. D.; Liu, W. Q.; Feng, Q. L. Self-assembly model, hepatocytes attachment and inflammatory response for silk fibroin/chitosan scaffolds. *Biomed. Mater.* **2009**, *4*, 045013.

(148) She, Z. D.; Zhang, B. F.; Jin, C. R.; Feng, Q. L.; Xu, Y. X. Preparation and in vitro degradation of porous three-dimensional silk fibroin/chitosan scaffold. *Polym. Degrad. Stab.* **2008**, *93*, 1316–1322.

(149) Tigli, R. S.; Gumusderelioglu, M. Chondrogenesis on BMP-6 loaded chitosan scaffolds in stationary and dynamic cultures. *Biotechnol. Bioeng.* **2009**, *104*, 601–610.

(150) Lefler, A.; Ghanem, A. Development of bFGF-chitosan matrices and their interactions with human dermal fibroblast cells. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1335–1351.

(151) Shi, S. S.; Cheng, X. R.; Wang, J. W.; Zhang, W.; Peng, L.; Zhang, Y. F. RhBMP-2 microspheres-loaded chitosan/collagen scaffold enhanced osseointegration: an experiment in dog. *J. Biomater. Appl.* **2009**, *23*, 331–346.

(152) Yilgor, P.; Tuzlakoglu, K.; Reis, R. L.; Hasirci, N.; Hasirci, V. Incorporation of a sequential BMP-2/BMP-7 delivery system into chitosan-based scaffolds for bone tissue engineering. *Biomaterials* **2009**, *30*, 3551–3559.

(153) Hoffmann, B.; Seitz, D.; Mencke, A.; Kokott, A.; Ziegler, G. Glutaraldehyde and oxidised dextran as crosslinker reagents for chitosan-based scaffolds for cartilage tissue engineering. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 1495–1503.

(154) Mansur, H. S.; Costa, E. D.; Mansur, A. A. P.; Barbosa-Stancioli, E. F. Cytocompatibility evaluation in cell-culture systems of chemically crosslinked chitosan/PVA hydrogels. *Mater. Sci. Eng., C* **2009**, *29*, 1574–1583.

(155) Wu, X. M.; Black, L.; Santacana-Laffitte, G.; Patrick, C. W. Preparation and assessment of glutaraldehyde-crosslinked collagen-chitosan hydrogels for adipose tissue engineering. *J. Biomed. Mater. Res., Part A* **2007**, *81A*, 59–65.

(156) Wu, Z. M.; Zhang, X. G.; Zheng, C.; Li, C. X.; Zhang, S. M.; Dong, R. N.; Yu, D. M. Disulfide-crosslinked chitosan hydrogel for cell viability and controlled protein release. *Eur. J. Pharm. Sci.* **2009**, *37*, 198–206.

(157) De Souza, R.; Zahedi, P.; Allen, C. J.; Piquette-Miller, M. Biocompatibility of injectable chitosan-phospholipid implant systems. *Biomaterials* **2009**, *30*, 3818–3824.

(158) Jin, R.; Teixeira, L. S. M.; Dijkstra, P. J.; Karperien, M.; van Blitterswijk, C. A.; Zhong, Z. Y.; Feijen, J. Injectable chitosan-based hydrogels for cartilage tissue engineering. *Biomaterials* **2009**, *30*, 2544–2551.

(159) Sarkar, N. Thermal gelation properties of methyl and hydroxypropyl methylcellulose. *J. Appl. Polym. Sci.* **1979**, *24*, 1073–1087.

(160) Donhowe, I. G.; Fennema, O. The effects of solution composition and drying temperature on crystallinity, permeability and mechanical properties of methylcellulose films. *J. Food Process. Preserv.* **1993**, *17*, 231–246.

(161) Tate, M. C.; Shear, D. A.; Hoffman, S. W.; Stein, D. G.; LaPlaca, M. C. Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury. *Biomaterials* **2001**, *22*, 1113–1123.

(162) Schumann, D. A.; Wippermann, J.; Klemm, D. O.; Kramer, F.; Koth, D.; Kosmehl, H.; Wahlers, T.; Salehi-Gelani, S. Artificial vascular implants from bacterial cellulose: preliminary results of small arterial substitutes. *Cellulose* **2009**, *16*, 877–885.

(163) Backdahl, H.; Helenius, G.; Bodin, A.; Nannmark, U.; Johansson, B. R.; Risberg, B.; Gatenholm, P. Mechanical properties of bacterial cellulose and interactions with smooth muscle cells. *Biomaterials* **2006**, *27*, 2141–2149.

(164) Chen, Y. M.; Xi, T. F.; Zheng, Y. D.; Guo, T. T.; Hou, J. Q.; Wan, Y. Z.; Gao, C. In vitro cytotoxicity of bacterial cellulose scaffolds used for tissue-engineered bone. *J. Bioact. Compat. Polym.* **2009**, *24*, 137–145.

(165) Wippermann, J.; Schumann, D.; Klemm, D.; Kosmehl, H.; Satehi-Gelani, S.; Wahlers, T. Preliminary results of small arterial substitute performed with a new cylindrical biomaterial composed of bacterial cellulose. *Eur. J. Vasc. Endovasc. Surg.* **2009**, *37*, 592–596.

(166) Li, J.; Wan, Y. Z.; Li, L. F.; Liang, H.; Wang, J. H. Preparation and characterization of 2,3-dialdehyde bacterial cellulose for potential biodegradable tissue engineering scaffolds. *Mater. Sci. Eng., C* **2009**, *29*, 1635–1642.

(167) Verma, V.; Verma, P.; Ray, P.; Ray, A. R. 2,3-Dihydrazone cellulose: Prospective material for tissue engineering scaffolds. *Mater. Sci. Eng., C* **2008**, *28*, 1441–1447.

(168) Chen, Y. M.; Xi, T. F.; Zheng, Y. D.; Wan, Y. Z. In Vitro Cytotoxicity Study of the Nano-Hydroxyapatite/Bacterial Cellulose Nanocomposites. In *Materials Research, Parts 1 and 2*; Gu, Z. W., Han, Y. F., Pan, F. H., Wang, X. T., Weng, D., Zhou, S. X., Eds.; TRANT: Portland, 2009; Vol. 610−613, pp 1011−1016.

(169) Cromme, P.; Zollfrank, C.; Muller, L.; Muller, F. A.; Greil, P. Biomimetic mineralisation of apatites on Ca^{2+} activated cellulose templates. *Mater. Sci. Eng., C* **2007**, *27*, 1–7.

(170) Fang, B.; Wan, Y. Z.; Tang, T. T.; Gao, C.; Dai, K. R. Proliferation and osteoblastic differentiation of human bone marrow stromal cells on hydroxyapatite/bacterial cellulose nanocomposite scaffolds. *Tissue Eng., Part A* **2009**, *15*, 1091–1098.

(171) Muller, F. A.; Muller, L.; Hofmann, I.; Greil, P.; Wenzel, M. M.; Staudenmaier, R. Cellulose-based scaffold materials for cartilage tissue engineering. *Biomaterials* **2006**, *27*, 3955–3963.

(172) Haroun, A. A.; Gamal-Eldeen, A.; Harding, D. R. K. Preparation, characterization and in vitro biological study of biomimetic three-dimensional gelatin-montmorillonite/cellulose scaffold for tissue engineering. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 2527–2540.

(173) Kalson, N.; Richardson, S. M.; Hughes, N.; Freemont, A. J.; Hoyland, J. A. Evaluation of Chitosan/Glycerophosphate-Hydroxyethylcellulose (C/Gp-HEC) for use in tissue engineering of the intervertebral disc. *J. Pathol.* **2006**, *210*, 25–25.

(174) Zhao, L.; Mitomo, H.; Yoshii, F. Synthesis of pH-sensitive and biodegradable CM-cellulose/chitosan polyampholytic hydrogels with electron beam irradiation. *J. Bioact. Compat. Polym.* **2008**, *23*, 319–333.

(175) Augst, A. D.; Kong, H. J.; Mooney, D. J. Alginate hydrogels as biomaterials. *Macromol. Biosci.* **2006**, *6*, 623–633.

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

(177) Abbah, S. A.; Lu, W. W.; Chan, D.; Cheung, K. M. C.; Liu, W. G.; Zhao, F.; Li, Z. Y.; Leong, J. C. Y.; Luk, K. D. K. Osteogenic behavior of alginate encapsulated bone marrow stromal cells: an in vitro study. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 2113–2119.

(178) Sundar, S.; Kundu, J.; Kundu, S. C. Biopolymeric nanoparticles. *Sci. Technol. Adv. Mater.* **2010**, *11*, 014104.

(179) Grellier, M.; Granja, P. L.; Fricain, J. C.; Bidarra, S. J.; Renard, M.; Bareille, R.; Bourget, C.; Amedee, J.; Barbosa, M. A. The effect of the co-immobilization of human osteoprogenitors and endothelial cells within alginate microspheres on mineralization in a bone defect. *Biomaterials* **2009**, *30*, 3271–3278.

(180) Freeman, I.; Cohen, S. The influence of the sequential delivery of angiogenic factors from affinity-binding alginate scaffolds on vascularization. *Biomaterials* **2009**, *30*, 2122–2131.

(181) Wang, W. B.; Wang, A. Q. Synthesis and swelling properties of pH-sensitive semi-IPN superabsorbent hydrogels based on sodium alginate-*g*-poly(sodium acrylate) and polyvinylpyrrolidone. *Carbohydr. Polym.* **2010**, *80*, 1028–1036.

(182) Zhao, S. P.; Cao, M. J.; Li, H.; Li, L. Y.; Xu, W. L. Synthesis and characterization of thermo-sensitive semi-IPN hydrogels based on poly(ethylene glycol)-*co*-poly(epsilon-caprolactone) macromer, *N*-isopropylacrylamide, and sodium alginate. *Carbohydr. Res.* **2010**, *345*, 425–431.

(183) Yang, S. W.; Liu, G. Q.; Cheng, Y. Q.; Zheng, Y. H. Electro-responsive behavior of sodium alginate-*g*-poly (acrylic acid) hydrogel under DC electric field. *J. Macromol. Sci., Part A: Pure Appl.Chem.* **2009**, *46*, 1078–1082.

(184) Bernhardt, A.; Despang, F.; Lode, A.; Demmler, A.; Hanke, T.; Gelinsky, M. Proliferation and osteogenic differentiation of human bone marrow stromal cells on alginate-getatine-hydroxyapatite scaffolds with anisotropic pore structure. *J. Tissue Eng. Regener. Med.* **2009**, *3*, 54–62.

(185) Qi, X. P.; Ye, J. D.; Wang, Y. J. Alginate/poly(lactic-*co*-glycolic acid)/calcium phosphate cement scaffold with oriented pore structure for bone tissue engineering. *J. Biomed. Mater. Res., Part A* **2009**, *89A*, 980–987.

(186) Turco, G.; Marsich, E.; Bellomo, F.; Semeraro, S.; Donati, I.; Brun, F.; Grandolfo, M.; Accardo, A.; Paoletti, S. Alginate/hydroxyapatite biocomposite for bone ingrowth: a trabecular structure with high and isotropic connectivity. *Biomacromolecules* **2009**, *10*, 1575–1583.

(187) Cai, K.; Zhang, J.; Deng, L. H.; Yang, L.; Hu, Y.; Chen, C.; Xue, L.; Wang, L. Physical and biological properties of a novel hydrogel composite based on oxidized alginate, gelatin and tricalcium phosphate for bone tissue engineering. *Adv. Eng. Mater.* **2007**, *9*, 1082–1088.

(188) Rosellini, E.; Cristallini, C.; Barbani, N.; Vozzi, G.; Giusti, P. Preparation and characterization of alginate/gelatin blend films for cardiac tissue engineering. *J. Biomed. Mater. Res., Part A* **2009**, *91A*, 447–453.

(189) Xu, M.; Wang, X.; Yan, Y.; Yao, R.; Ge, Y. An cell-assembly derived physiological 3D model of the metabolic syndrome, based on adipose-derived stromal cells and a gelatin/alginate/fibrinogen matrix. *Biomaterials* **2010**, *31*, 3868–77.

(190) Yang, C. M.; Frei, H.; Rossi, F. A.; Burt, H. M. The differential in vitro and in vivo responses of bone marrow stromal cells on novel porous gelatin - alginate scaffolds. *J. Tissue Eng. Regener. Med.* **2009**, *3*, 601–614.

(191) Yildirim, E. D.; Yin, X.; Nair, K.; Sun, W. Fabrication, characterization, and biocompatibility of single-walled carbon nanotube-reinforced alginate composite scaffolds manufactured using freeform fabrication technique. *J. Biomed. Mater. Res., Part B* **2008**, *87B*, 406–414.

(192) Yuan, N. Y.; Lin, Y. A.; Ho, M. H.; Wang, D. M.; Lai, J. Y.; Hsieh, H. J. Effects of the cooling mode on the structure and strength of porous scaffolds made of chitosan, alginate, and carboxymethyl cellulose by the freeze-gelation method. *Carbohydr. Polym.* **2009**, *78*, 349–356.

(193) Lee, J. E.; Park, J. C.; Hwang, Y. S.; Kim, J. K.; Kim, J. G.; Suh, H. Characterization of UV-irradiated dense/porous collagen membranes: Morphology, enzymatic degradation, and mechanical properties. *Yonsei Med. J.* **2001**, *42*, 172–179.

(194) Henson, F. M. D.; Davies, M. E.; Schofield, P. N.; Jeffcott, L. B. Expression of types II, VI and X collagen in equine growth cartilage during development. *Equine Vet. J.* **1996**, *28*, 189–198.

(195) Hurst, P. R.; Palmay, R. D.; Myers, D. B. Localization and synthesis of collagen types III and V during remodelling and decidualization in rat uterus. *Reprod., Fertil. Dev.* **1997**, *9*, 403–409.

(196) Smith, L. T. Patterns of type-VI collagen compared to type-I, type-III and type-V collagen in human embryonic and fetal skin and in fetal skin-derived cell-cultures. *Matrix Biol.* **1994**, *14*, 159–170.

(197) Berry, S. D. K.; Howard, R. D.; Akers, R. M. Mammary localization and abundance of laminin, fibronectin, and collagen IV proteins in prepubertal heifers. *J. Dairy Sci.* **2003**, *86*, 2864–2874.

(198) Wetzels, R. H. W.; Robben, H. C. M.; Leigh, I. M.; Schaafsma, H. E.; Vooijs, G. P.; Ramaekers, F. C. S. Distribution patterns of type-VII collagen in normal and malignant human tissues. *Am. J. Pathol.* **1991**, *139*, 451–459.

(199) Sawada, H.; Konomi, H. The alpha-1 chain of type-VIII collagen is associated with many but not all microfibrils of elastic fiber system. *Cell Struct. Funct.* **1991**, *16*, 455–466.

(200) Gregory, K. E.; Keene, D. R.; Tufa, S. F.; Lunstrum, G. P.; Morris, N. P. Developmental distribution of collagen type XII in cartilage: association with articular cartilage and the growth plate. *J. Bone Miner. Res.* **2001**, *16*, 2005–2016.

(201) Holmes, D. F.; Kadler, K. E. The 10 + 4 microfibril structure of thin cartilage fibrils. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17249–17254.

(202) Kim, G.; Ahn, S.; Yoon, H.; Kim, Y.; Chun, W. A cryogenic direct-plotting system for fabrication of 3D collagen scaffolds for tissue engineering. *J. Mater. Chem.* **2009**, *19*, 8817–8823.

(203) Ai-Munajjed, A. A.; O'Brien, F. J. Influence of a novel calcium-phosphate coating on the mechanical properties of highly porous collagen scaffolds for bone repair. *J. Mech. Behav. Biomed. Mater.* **2009**, *2*, 138–146.

(204) Al-Munajjed, A. A.; Plunkett, N. A.; Gleeson, J. P.; Weber, T.; Jungreuthmayer, C.; Levingstone, T.; Hammer, J.; O'Brien, F. J. Development of a biomimetic collagen-hydroxyapatite scaffold for bone tissue engineering using a SBF immersion technique. *J. Biomed. Mater. Res., Part B* **2009**, *90B*, 584–591.

(205) Cunniffe, G.; O'Brien, F. J.; Dickson, G. Investigation of a collagen NanoHA scaffold with potential for bone tissue engineering. *Tissue Eng.* **2007**, *13*, 1719–1719.

(206) Jungreuthmayer, C.; Donahue, S. W.; Jaasma, M. J.; Al-Munajjed, A. A.; Zanghellini, J.; Kelly, D. J.; O'Brien, F. J. A comparative study of shear stresses in collagen-glycosaminoglycan and calcium phosphate scaffolds in bone tissue-engineering bioreactors. *Tissue Eng., Part A* **2009**, *15*, 1141–1149.

(207) Ohyabu, Y.; Adegawa, T.; Yoshioka, T.; Ikoma, T.; Shinozaki, K.; Uemura, T.; Tanaka, J. A collagen sponge incorporating a hydroxyapatite/chondroitinsulfate composite as a scaffold for cartilage tissue engineering. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1861–1874.

(208) Sena, L. A.; Caraballo, M. M.; Rossi, A. M.; Soares, G. A. Synthesis and characterization of biocomposites with different hydroxyapatite-collagen ratios. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 2395–2400.

(209) Wahl, D. A.; Sachlos, E.; Liu, C. Z.; Czernuszka, J. T. Controlling the processing of collagen-hydroxyapatite scaffolds for bone tissue engineering. *J. Mater. Sci.: Mater. Med.* **2007**, *18*, 201–209.

(210) Ananta, M.; Aulin, C. E.; Hilborn, J.; Aibibu, D.; Houis, S.; Brown, R. A.; Mudera, V. A poly(lactic acid-*co*-caprolactone)-collagen hybrid for tissue engineering applications. *Tissue Eng., Part A* **2009**, *15*, 1667–1675.

(211) Chen, G. P.; Okamura, A.; Sugiyama, K.; Wozniak, M. J.; Kawazoe, N.; Sato, S.; Tateishi, T. Surface modification of porous scaffolds with nanothick collagen layer by centrifugation and freeze-drying. *J. Biomed. Mater. Res., Part B* **2009**, *90B*, 864–872.

(212) Gong, Y. H.; Zhu, Y. B.; Liu, Y. X.; Ma, Z. W.; Gao, C. Y.; Shen, J. C. Layer-by-layer assembly of chondroitin sulfate and collagen on aminolyzed poly(L-lactic acid) porous scaffolds to enhance their chondrogenesis. *Acta Biomater.* **2007**, *3*, 677–685.

(213) Wen, F.; Chang, S.; Toh, Y. C.; Teoh, S. H.; Yu, H. Development of poly(lactic-*co*-glycolic acid)-collagen scaffolds for tissue engineering. *Mater. Sci. Eng., C* **2007**, *27*, 285–292.

(214) Yang, Y.; Zhu, X. L.; Cui, W. G.; Li, X. H.; Jin, Y. Electrospun composite mats of poly[(D,L-lactide)-*co*-glycolide] and collagen with high porosity as potential scaffolds for skin tissue engineering. *Macromol. Mater. Eng.* **2009**, *294*, 611–619.

(215) Kawazoe, N.; Lin, X. T.; Tateishi, T.; Chen, G. P. Three-dimensional cultures of rat pancreatic RIN-5F cells in porous PLGA-collagen hybrid scaffolds. *J. Bioact. Compat. Polym.* **2009**, *24*, 25–42.

(216) Haugh, M. G.; Jaasma, M. J.; O'Brien, F. J. The effect of dehydrothermal treatment on the mechanical and structural properties of collagen-GAG scaffolds. *J. Biomed. Mater. Res., Part A* **2009**, *89A*, 363–369.

(217) Jungreuthmayer, C.; Jaasma, M. J.; Al-Munajjed, A. A.; Zanghellini, J.; Kelly, D. J.; O'Brien, F. J. Deformation simulation of cells seeded on a collagen-GAG scaffold in a flow perfusion bioreactor using a sequential 3D CFD-elastostatics model. *Med. Eng. Phys.* **2009**, *31*, 420–427.

(218) Ko, C. S.; Huang, J. P.; Huang, C. W.; Chu, I. M. Type II collagen-chondroitin sulfate-hyaluronan scaffold cross-linked by genipin for cartilage tissue engineering. *J. Biosci. Bioeng.* **2009**, *107*, 177–182.

(219) Tierney, C. M.; Haugh, M. G.; Liedl, J.; Mulcahy, F.; Hayes, B.; O'Brien, F. J. The effects of collagen concentration and crosslink density on the biological, structural and mechanical properties of collagen-GAG scaffolds for bone tissue engineering. *J. Mech. Behav. Biomed. Mater.* **2009**, *2*, 202–209.

(220) Tierney, C. M.; Jaasma, M. J.; O'Brien, F. J. Osteoblast activity on collagen-GAG scaffolds is affected by collagen and GAG concentrations. *J. Biomed. Mater. Res., Part A* **2009**, *91A*, 92–101.

(221) Boccafoschi, F.; Rajan, N.; Habermehl, J.; Mantovani, D. Preparation and characterization of a scaffold for vascular tissue engineering by direct-assembling of collagen and cells in a cylindrical geometry. *Macromol. Biosci.* **2007**, *7*, 719–726.

(222) Barnes, C. P.; Pemble, C. W.; Brand, D. D.; Simpson, D. G.; Bowlin, G. L. Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol. *Tissue Eng.* **2007**, *13*, 1593–1605.

(223) Chen, R.; Qiu, L. J.; Ke, Q. F.; He, C. L.; Mo, X. M. Electrospinning thermoplastic polyurethane-contained collagen nanofibers for tissue-engineering applications. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1513–1536.

(224) Heymer, A.; Bradica, G.; Eulert, J.; Noth, U. Multiphasic collagen fibre-PLA composites seeded with human mesenchymal stem cells for osteochondral defect repair: an in vitro study. *J. Tissue Eng. Regener. Med.* **2009**, *3*, 389–397.

(225) Yeo, I. S.; Oh, J. E.; Jeong, L.; Lee, T. S.; Lee, S. J.; Park, W. H.; Min, B. M. Collagen-based biomimetic nanofibrous scaffolds: preparation and characterization of collagen/silk fibroin bicomponent nanofibrous structures. *Biomacromolecules* **2008**, *9*, 1106–1116.

(226) Sell, S. A.; McClure, M. J.; Garg, K.; Wolfe, P. S.; Bowlin, G. L. Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering. *Adv. Drug Delivery Rev.* **2009**, *61*, 1007–1019.

(227) Powell, H. M.; Boyce, S. T. Engineered human skin fabricated using electrospun collagen-PCL blends: morphogenesis and mechanical properties. *Tissue Eng., Part A* **2009**, *15*, 2177–2187.

(228) McClure, M. J.; Sell, S. A.; Simpson, D. G.; Bowlin, G. L. Electrospun polydioxanone, elastin, and collagen vascular scaffolds: uniaxial cyclic distension. *J. Eng. Fibers Fabr.* **2009**, *4*, 18–25.

(229) Meng, W.; Kim, S. Y.; Yuan, J.; Kim, J. C.; Kwon, O. H.; Kawazoe, N.; Chen, G. P.; Ito, Y.; Kang, I. K. Electrospun PHBV/collagen composite nanofibrous scaffolds for tissue engineering. *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 81–94.

(230) Kim, I.; Park, H.; Shin, Y.; Kim, M. Adipogenic differentiation of adipose-derived stem cells on collagen microbeads. *J. Tissue Eng. Regener. Med.* **2009**, *6*, 924–930.

(231) Haaparanta, A. M.; Koivurinta, J.; Hawalainen, E. R.; Kellomaki, M. The effect of cross-linking time on a porous freeze-dried collagen scaffold using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as a cross-linker. *J. Appl. Biomater. Biomech.* **2008**, *6*, 89–94.

(232) Zeugolis, D. I.; Paul, G. R.; Attenburrow, G. Cross-linking of extruded collagen fibers-A biomimetic three-dimensional scaffold for tissue engineering applications. *J. Biomed. Mater. Res., Part A* **2009**, *89A*, 895–908.

(233) Madaghiele, M.; Piccinno, A.; Saponaro, M.; Maffezzoli, A.; Sannino, A. Collagen- and gelatine-based films sealing vascular prostheses: evaluation of the degree of crosslinking for optimal blood impermeability. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 1979–1989.

(234) Zhang, Y. F.; Shi, B.; Li, C. Z.; Wang, Y. N.; Chen, Y.; Zhang, W.; Luo, T.; Cheng, X. R. The synergetic bone-forming effects of combinations of growth factors expressed by adenovirus vectors on chitosan/collagen scaffolds. *J. Controlled Release* **2009**, *136*, 172–178.

(235) Niu, X. F.; Feng, Q. L.; Wang, M. B.; Guo, X. D.; Zheng, Q. X. Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2. *J. Controlled Release* **2009**, *134*, 111–117.

(236) Duan, X. D.; McLaughlin, C.; Griffith, M.; Sheardown, H. Biofunctionalization of collagen for improved biological response: scaffolds for corneal tissue engineering. *Biomaterials* **2007**, *28*, 78–88.

(237) Lee, Y. B.; Polio, S.; Lee, W.; Dai, G. H.; Menon, L.; Carroll, R. S.; Yoo, S. S. Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. *Exp. Neurol.* **2010**, *223*, 645–652.

(238) Hu, K.; Cui, F. Z.; Lv, Q.; Ma, J.; Feng, Q. L.; Xu, L.; Fan, D. D. Preparation of fibroin/recombinant human-like collagen scaffold to promote fibroblasts compatibility. *J. Biomed. Mater. Res., Part A* **2008**, *84A*, 483–490.

(239) Pulkkinen, H. J.; Tiitu, V.; Valonen, P.; Hamalainen, E. R.; Lammi, M. J.; Kiviranta, I. Recombinant human type II collagen as a material for cartilage tissue engineering. *Int. J. Artif. Organs* **2008**, *31*, 960–969.

(240) *The Science and Technology of Gelatin*; Ward, A. G., Courts, A., Eds.; Academic Press: New York, 1977.

(241) Kuijpers, A. J.; Engbers, G. H. M.; Krijgsveld, J.; Zaat, S. A. J.; Dankert, J.; Feijen, J. Cross-linking and characterisation of gelatin matrices for biomedical applications. *J. Biomater. Sci., Polym. Ed.* **2000**, *11*, 225–243.

(242) Jayakrishnan, A.; Jameela, S. R. Glutaraldehyde as a fixative in bioprotheses and drug delivery matrices. *Biomaterials* **1996**, *17*, 471–484.

(243) Olde Damink, L. H.; Dijkstra, P. J.; Van Luyn, M. J.; Van Wachem, P. B.; Nieuwenhuis, P.; Feijen, J. Cross-linking of dermal sheep collagen using hexamethylene diisocyanate. *J. Mater. Sci.: Mater. Med.* **1995**, *6*, 429–434.

(244) Bozzini, S.; Petrini, P.; Altomare, L.; Tanzi, M. C. Fabrication of chemically cross-linked porous gelatin matrices. *J. Appl. Biomater. Biomech.* **2009**, *7*, 194–199.

(245) Ofner, C. M.; Bubnis, W. A. Chemical and swelling evaluations of amino group cross-linking in gelatin and modified gelatin matrices. *Pharm. Res.* **1996**, *13*, 1821–1827.

(246) Lien, S. M.; Ko, L. Y.; Huang, T. J. Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering. *Acta Biomater.* **2009**, *5*, 670–679.

(247) Nickerson, M. T.; Patel, J.; Heyd, D. V.; Rousseau, D.; Paulson, A. T. Kinetic and mechanistic considerations in the gelation of genipin-crosslinked gelatin. *Int. J. Biol. Macromol.* **2006**, *39*, 298–302.

(248) Butler, M. F.; Ng, Y. F.; Pudney, P. D. A. Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 3941–3953.

(249) Sung, H.-W.; Hsu, H.-L.; Shih, C.-C.; Lin, D.-S. Cross-linking characteristics of biological tissues fixed with monofunctional or multifunctional epoxy compounds. *Biomaterials* **1996**, *17*, 1405–1410.

(250) Petite, H.; Rault, I.; Huc, A.; Mesnache, P.; Herbage, D. Use of the acyl azide method for cross-linking collagen-rich tissues such as pericardium. *J. Biomed. Mater. Res.* **1990**, *24*, 179–188.

(251) Sakai, S.; Hashimoto, I.; Kawakami, K. Synthesis of an agarose-gelatin conjugate for use as a tissue engineering scaffold. *J. Biosci. Bioeng.* **2007**, *103*, 22–26.

(252) Aoki, H.; Taguchi, T.; Saito, H.; Kobayashi, H.; Kataoka, K.; Tanaka, J. Rheological evaluation of gelatin gels prepared with a citric acid derivative as a novel cross-linker. *Mater. Sci. Eng., C* **2004**, *24*, 787–790.

(253) Van Den Bulcke, A. Synthese en evaluatie van hydrogelen op basis van gelatine, PhD dissertation Ghent University, Ghent, Belgium 2000.

(254) Van Vlierberghe, S.; Cnudde, V.; Dubruel, P.; Masschaele, B.; Cosijns, A.; De Paepe, I.; Jacobs, P. J. S.; Van Hoorebeke, L.; Remon, J. P.; Schacht, E. Porous gelatin hydrogels: 1. cryogenic formation and structure analysis. *Biomacromolecules* **2007**, *8*, 331–337.

(255) Van Vlierberghe, S.; Dubruel, P.; Lippens, E.; Cornelissen, M.; Schacht, E. Correlation between cryogenic parameters and physicochemical properties of porous gelatin cryogels. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1417–1438.

(256) Schuster, M.; Turecek, C.; Weigel, G.; Saf, R.; Stampfl, J.; Varga, F.; Liska, R. Gelatin-based photopolymers for bone replacement materials. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 7078–7089.

(257) 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**.

(258) Hu, X. H.; Ma, L.; Wang, C. C.; Gao, C. Y. Gelatin hydrogel prepared by photo-initiated polymerization and loaded with TGF-β 1 for cartilage tissue engineering. *Macromol. Biosci.* **2009**, *9*, 1194–1201.

(259) Benton, J. A.; DeForest, C. A.; Vivekanandan, V.; Anseth, K. S. Photocrosslinking of gelatin macromers to synthesize porous hydrogels

that promote valvular interstitial cell function. *Tissue Eng., Part A* **2009**, *15*, 3221–3230.

(260) Dubruel, P.; Unger, R.; Van Vlierberghe, S.; Cnudde, V.; Jacobs, P. J. S.; Schacht, E.; Kirkpatrick, C. J. Porous gelatin hydrogels: 2. in vitro cell interaction study. *Biomacromolecules* **2007**, *8*, 338–344.

(261) Van den Bulcke, A. I.; Bogdanov, B.; De Rooze, N.; Schacht, E. H.; Cornelissen, M.; Berghmans, H. Structural and rheological properties of methacrylamide modified gelatin hydrogels. *Biomacromolecules* **2000**, *1*, 31–38.

(262) Van Vlierberghe, S.; Dubruel, P.; Lippens, E.; Cornelissen, M.; Schacht, E. Correlation Between Cryogenic Parameters and Physico-Chemical Properties of Porous Gelatin Cryogels. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1417–1438.

(263) Hu, M.; Kurisawa, M.; Deng, R.; Teo, C. M.; Schumacher, A.; Thong, Y. X.; Wang, L.; Schumacher, K. M.; Ying, J. Y. Cell immobilization in gelatin-hydroxyphenylpropionic acid hydrogel fibers. *Biomaterials* **2009**, *30*, 3523–3531.

(264) Sakai, S.; Hirose, K.; Taguchi, K.; Ogushi, Y.; Kawakami, K. An injectable, in situ enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. *Biomaterials* **2009**, *30*, 3371–3377.

(265) Li, X. R.; Xie, J. W.; Yuan, X. Y.; Xia, Y. N. Coating electrospun poly(epsilon-caprolactone) fibers with gelatin and calcium phosphate and their use as biomimetic scaffolds for bone tissue engineering. *Langmuir* **2008**, *24*, 14145–14150.

(266) Zhang, S.; Huang, Y. Q.; Yang, X. P.; Mei, F.; Ma, Q.; Chen, G. Q.; Ryu, S.; Deng, X. L. Gelatin nanofibrous membrane fabricated by electrospinning of aqueous gelatin solution for guided tissue regeneration. *J. Biomed. Mater. Res., Part A* **2009**, *90A*, 671–679.

(267) Wang, S. D.; Zhang, Y. Z.; Yin, G. B.; Wang, H. W.; Dong, Z. H. Electrospun polylactide/silk fibroin-gelatin composite tubular scaffolds for small-diameter tissue engineering blood vessels. *J. Appl. Polym. Sci.* **2009**, *113*, 2675–2682.

(268) Wang, S. D.; Zhang, Y. Z.; Wang, H. W.; Yin, G. B.; Dong, Z. H. Fabrication and properties of the electrospun polylactide/silk fibroin-gelatin composite tubular scaffold. *Biomacromolecules* **2009**, *10*, 2240–2244.

(269) Qian, Y. F.; Li, X. Q.; Zhai, W.; Ke, Q. F.; King, M. W.; Mo, X. M. Characterization of Crosslinked Electrospun Nanofibers from Chitosan-Gelatin Complex. In *International Symposium on Fiber Based Scaffolds for Tissue Engineering, Proceedings*; Donghua University Press: Shanghai, Peoples Republic of China, 2008; pp 172−177.

(270) Rim, N. G.; Lee, J. H.; Jeong, S. I.; Lee, B. K.; Kim, C. H.; Shin, H. Modulation of osteogenic differentiation of human mesenchymal stem cells by poly[(L-lactide)-*co*-(epsilon-caprolactone)]/gelatin nanofibers. *Macromol. Biosci.* **2009**, *9*, 795–804.

(271) Li, M.; Mondrinos, M. J.; Chen, X.; Gandhi, M. R.; Ko, F. K.; Lelkes, P. I. Co-electrospun poly(lactide-*co*-glycolide), gelatin, and elastin blends for tissue engineering scaffolds. *J. Biomed. Mater. Res., Part A* **2006**, *79A*, 963–973.

(272) Cao, M. Y.; Chen, Z. J.; Tu, K. H.; Wang, L. Q.; Jiang, H. L. Studies on one-step electrospinning for preparing crosslinked gelatin fibers. *Acta Polym. Sin.* **2009**, *11*, 1157–1161.

(273) Wei, X.; Xiaohong, W.; Yongnian, Y.; Wei, Z.; Zhuo, X.; Feng, L.; Rendong, W.; Renji, Z. Rapid prototyping three-dimensional cell/gelatin/fibrinogen constructs for medical regeneration. *J. Bioact. Compat. Polym.* **2007**, *22*, 363–377.

(274) Xu, W.; Wang, X. H.; Yan, Y. N.; Zheng, W.; Xiong, Z.; Lin, F.; Wu, R. D.; Zhang, R. J. Rapid prototyping three-dimensional cell/gelatin/fibrinogen constructs for medical regeneration. *J. Bioact. Compat. Polym.* **2007**, *22*, 363–377.

(275) Wang, X. H.; Yan, Y. N.; Pan, Y. Q.; Xiong, Z.; Liu, H. X.; Cheng, B.; Liu, F.; Lin, F.; Wu, R. D.; Zhang, R. J.; Lu, Q. P. Generation of three-dimensional hepatocyte/gelatin structures with rapid prototyping system. *Tissue Eng.* **2006**, *12*, 83–90.

(276) Zhang, T.; Yan, Y.; Wang, X.; Xiong, Z.; Lin, F.; Wu, R.; Zhang, R. Three-dimensional gelatin and gelatin/hyaluronan hydrogel structures for traumatic brain injury. *J. Bioact. Compat. Polym.* **2007**, *22*, 19–29.

(277) Vanderhooft, J. L.; Alcoutlabi, M.; Magda, J. J.; Prestwich, G. D. Rheological properties of cross-linked hyaluronan-gelatin hydrogels for tissue engineering. *Macromol. Biosci.* **2009**, *9*, 20–28.

(278) Zhao, F.; Grayson, W. L.; Ma, T.; Bunnell, B.; Lu, W. W. Effects of hydroxyapatite in 3-D chitosan-gelatin polymer network on human mesenchymal stem cell construct development. *Biomaterials* **2006**, *27*, 1859–1867.

(279) Liu, Y.; Lu, Y.; Tian, X. Z.; Cui, G.; Zhao, Y. M.; Yang, Q.; Yu, S. L.; Xing, G. S.; Zhang, B. X. Segmental bone regeneration using an rhBMP-2-loaded gelatin/nanohydroxyapatite/fibrin scaffold in a rabbit model. *Biomaterials* **2009**, *30*, 6276–6285.

(280) Habraken, W.; Wolke, J. G. C.; Mikos, A. G.; Jansen, J. A. Porcine gelatin microsphere/calcium phosphate cement composites: an in vitro degradation study. *J. Biomed. Mater. Res., Part B* **2009**, *91B*, 555–561.

(281) Liu, X.; Smith, L. A.; Hu, J.; Ma, P. X. Biomimetic nanofibrous gelatin/apatite composite scaffolds for bone tissue engineering. *Biomaterials* **2009**, *30*, 2252–2258.

(282) Lien, S. M.; Chien, C. H.; Huang, T. J. A novel osteochondral scaffold of ceramic-gelatin assembly for articular cartilage repair. *Mater. Sci. Eng., C* **2009**, *29*, 315–321.

(283) Lazzeri, L.; Cascone, M. G.; Danti, S.; Serino, L. P.; Moscato, S.; Bernardini, N. Gelatine/PLLA sponge-like scaffolds: morphological and biological characterization. *J. Mater. Sci.: Mater. Med.* **2007**, *18*, 1399–1405.

(284) Wei, X.; Xiaohong, W.; Yongnian, Y.; Renji, Z. A polyurethane-gelatin hybrid construct for manufacturing implantable bioartificial livers. *J. Bioact. Compat. Polym.* **2008**, *23*, 409–422.

(285) Boudet, C.; Iliopoulos, I.; Poncelet, O.; Cloitre, M. Control of the chemical cross-linking of gelatin by a thermosensitive polymer: example of switchable reactivity. *Biomacromolecules* **2005**, *6*, 3073–3078.

(286) Fan, H. B.; Liu, H. F.; Toh, S. L.; Goh, J. C. H. Enhanced differentiation of mesenchymal stem cells co-cultured with ligament fibroblasts on gelatin/silk fibroin hybrid scaffold. *Biomaterials* **2008**, *29*, 1017–1027.

(287) Fan, H. B.; Liu, H. F.; Wang, Y.; Toh, S. L.; Goh, J. C. H. Development of a silk cable-reinforced gelatin/silk fibroin hybrid scaffold for ligament tissue engineering. *Cell Transplant.* **2008**, *17*, 1389–1401.

(288) Masuda, T.; Furue, M.; Matsuda, T. Photocured, styrenated gelatin-based microspheres for de novo adipogenesis through corelease of basic fibroblast growth factor, insulin, and insulin-like growth factor I. *Tissue Eng.* **2004**, *10*, 523–535.

(289) Naito, H; Takewa, Y; Mizuno, T; Ohya, S; Nakayama, Y; Tatsumi, E; Kitamura, S; Takano, H; Taniguchi, S; Y., T. Three-dimensional cardiac tissue engineering using a thermoresponsive artificial extracellular matrix. *ASAIO J.* **2004**, *50*, 344–348.

(290) Liu, H. F.; Mao, J. S.; Yao, K. D.; Yang, G. H.; Cui, L.; Cao, Y. L. A study on a chitosan-gelatin-hyaluronic acid scaffold as artificial skin in vitro and its tissue engineering applications. *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 25–40.

(291) Cronin, E. M.; Thurmond, F. A.; Bassel-Duby, R.; Williams, R. S.; Wright, W. E.; Nelson, K. D.; Garner, H. R. , Protein-coated poly(L-lactic acid) fibers provide a substrate for differentiation of human skeletal muscle cells. *J. Biomed. Mater. Res., Part A* **2004**, *69A*, 373–381.

(292) Gamez, E; Goto, Y; Nagata, K; Iwaki, T; Sasaki, T; T., M. Photofabricated gelatin-based nerve conduits: nerve tissue regeneration potentials. *Cell Transplant.* **2004**, *13*, 549–564.

(293) Kojima, K.; Ignotz, R. A.; Kushibiki, T.; Tinsley, K. W.; Tabata, Y.; Vacanti, C. A. Tissue-engineered trachea from sheep marrow stromal cells with transforming growth factor ?2 released from biodegradable microspheres in a nude rat recipient. *J. Thorac. Cardiovasc. Surg.* **2004**, *128*, 147–153.

(294) Krishna, O. D.; Kiick, K. L. Protein- and peptide-modified synthetic polymeric biomaterials. *Biopolymers* **2010**, *94*, 32–48.

(295) Keck, M.; Haluza, D.; Burjak, S.; Eisenbock, B.; Kamolz, L. P.; Frey, M. Cultivation of keratinocytes and preadipocytes on a collagen-elastin scaffold (Matriderm): first results of an in vitro study. *Eur. J. Surg.* **2009**, *41*, 189–193.

(296) Kolokythas, P.; Aust, M. C.; Vogt, P. M.; Paulsen, F. Dermal substitute with the collagen-elastin matrix Matriderm in burn injuries: a comprehensive review. *Handchirurgie Mikrochirurgie Plastische Chirurgie* **2008**, *40*, 367–371.

(297) Jia, X. Q.; Kiick, K. L. Hybrid multicomponent hydrogels for tissue engineering. *Macromol. Biosci.* **2009**, *9*, 140–156.

(298) Barbosa, J. S.; Costa, R. R.; Testera, A. M.; Alonso, M.; Rodriguez-Cabello, J. C.; Mano, J. F. Multi-layered films containing a biomimetic stimuli-responsive recombinant protein. *Nanoscale Res. Lett.* **2009**, *4*, 1247–1253.

(299) Bessa, P. C.; Machado, R.; Nurnberger, S.; Dopler, D.; Banerjee, A.; Cunha, A. M.; Rodriguez-Cabello, J. C.; Redl, H.; van Griensven, M.; Reis, R. L.; Casal, M. Thermoresponsive self-assembled elastin-based nanoparticles for delivery of BMPs. *J. Controlled Release* **2010**, *142*, 312–318.

(300) Costa, R. R.; Custodio, C. A.; Testero, A. M.; Arias, F. J.; Rodriguez-Cabello, J. C.; Alves, N. M.; Mano, J. F. Stimuli-responsive thin coatings using elastin-like polymers for biomedical applications. *Adv. Funct. Mater.* **2009**, *19*, 3210–3218.

(301) Martin, L.; Alonso, M.; Girotti, A.; Arias, F. J.; Rodriguez-Cabello, J. C. Synthesis and characterization of macroporous thermo-sensitive hydrogels from recombinant elastin-like polymers. *Biomacromolecules* **2009**, *10*, 3015–3022.

(302) Martinez-Osorio, H.; Juarez-Campo, M.; Diebold, Y.; Girotti, A.; Alonso, M.; Arias, F. J.; Rodriguez-Cabello, J. C.; Garcia-Vazquez, C.; Calonge, M. Genetically engineered elastin-like polymer as a substratum to culture cells from the ocular surface. *Curr. Eye Res.* **2009**, *34*, 48–56.

(303) Ozturk, N.; Girotti, A.; Kose, G. T.; Rodriguez-Cabello, J. C.; Hasirci, V. Dynamic cell culturing and its application to micropatterned, elastin-like protein-modified poly(*N*-isopropylacrylamide) scaffolds. *Biomaterials* **2009**, *30*, 5417–5426.

(304) Herrero-Vanrell, R.; Rincon, A. C.; Alonso, M.; Reboto, V.; Molina-Martinez, I. T.; Rodriguez-Cabello, J. C. Self-assembled particles of an elasin-like polymer as vehicles for controlled drug release. *J. Controlled Release* **2005**, *102*, 113–122.

(305) Rincon, A. C.; Molina-Martinez, I. T.; de Las Heras, B.; Alonso, M.; Bailez, C.; Rodriguez-Cabello, J. C.; Herrero-Vanrell, R. Biocompatibility of elastin-like polymer poly(VPAVG) microparticles: in vitro and in vivo studies. *J. Biomed. Mater. Res., Part A* **2006**, *78A*, 343–351.

(306) 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.

(307) Smith, M. J.; McClure, M. J.; Sell, S. A.; Barnes, C. P.; Walpoth, B. H.; Simpson, D. G.; Bowlin, G. L. Suture-reinforced electrospun polydioxanone-elastin small-diameter tubes for use in vascular tissue engineering: a feasibility study. *Acta Biomater.* **2008**, *4*, 58–66.

(308) Miyamoto, K.; Atarashi, M.; Kadozono, H.; Shibata, M.; Koyama, Y.; Okai, M.; Inakuma, A.; Kitazono, E.; Kaneko, H.; Takebayashi, T.; Horiuchi, T. Creation of cross-linked electrospun isotypic-elastin fibers controlled cell-differentiation with new cross-linker. *Int. J. Biol. Macromol.* **2009**, *45*, 33–41.

(309) Buttafoco, L.; Kolkman, N. G.; Engbers-Buijtenhuijs, P.; Poot, A. A.; Dijkstra, P. J.; Vermes, I.; Feijen, J. Electrospinning of collagen and elastin for tissue engineering applications. *Biomaterials* **2006**, *27*, 724–734.

(310) Kurane, A.; Simionescu, D. T.; Vyavahare, N. R. In vivo cellular repopulation of tubular elastin scaffolds mediated by basic fibroblast growth factor. *Biomaterials* **2007**, *28*, 2830–2838.

(311) Cao, Y.; Wang, B. C. Biodegradation of silk biomaterials. *Int. J. Mol. Sci.* **2009**, *10*, 1514–1524.

(312) Lawrence, B. D.; Wharram, S.; Kluge, J. A.; Leisk, G. G.; Omenetto, F. G.; Rosenblatt, M. I.; Kaplan, D. L. Effect of hydration on silk film material properties. *Macromol. Biosci.* **2010**, *10*, 393–403.

(313) Lu, Q.; Wang, X. Q.; Hu, X.; Cebe, P.; Omenetto, F.; Kaplan, D. L. Stabilization and release of enzymes from silk films. *Macromol. Biosci.* **2010**, *10*, 359–368.

(314) Nogueira, G. M.; Rodas, A. C. D.; Weska, R. F.; Aimoli, C. G.; Higa, O. Z.; Maizato, M.; Leiner, A. A.; Pitombo, R. N. M.; Polakiewicz, B.; Beppu, M. M. Bovine pericardium coated with biopolymeric films as an alternative to prevent calcification: In vitro calcification and cytotoxicity results. *Mater. Sci. Eng., C* **2010**, *30*, 575–582.

(315) Jin, H. J.; Chen, J. S.; Karageorgiou, V.; Altman, G. H.; Kaplan, D. L. Human bone marrow stromal cell responses on electrospun silk fibroin mats. *Biomaterials* **2004**, *25*, 1039–1047.

(316) Ki, C. S.; Kim, J. W.; Hyun, J. H.; Lee, K. H.; Hattori, M.; Rah, D. K.; Park, Y. H. Electrospun three-dimensional silk fibroin nanofibrous scaffold. *J. Appl. Polym. Sci.* **2007**, *106*, 3922–3928.

(317) Meinel, A. J.; Kubow, K. E.; Klotzsch, E.; Garcia-Fuentes, M.; Smith, M. L.; Vogel, V.; Merkle, H. P.; Meinel, L. Optimization strategies for electrospun silk fibroin tissue engineering scaffolds. *Biomaterials* **2009**, *30*, 3058–3067.

(318) Ghassemifar, R.; Redmond, S.; Zainuddin; Chirila, T. V. Advancing towards a tissue-engineered tympanic membrane: silk fibroin as a substratum for growing human eardrum keratinocytes. *J. Biomater. Appl.* **2010**, *24*, 591–606.

(319) Gong, Z. G.; Yang, Y. H.; Huang, L.; Chen, X.; Shao, Z. Z. Formation kinetics and fractal characteristics of regenerated silk fibroin alcogel developed from nanofibrillar network. *Soft Matter* **2010**, *6*, 1217–1223.

(320) Yucel, T.; Kojic, N.; Leisk, G. G.; Lo, T. J.; Kaplan, D. L. Non-equilibrium silk fibroin adhesives. *J. Struct. Biol.* **2010**, *170*, 406–412.

(321) Jang, E. S.; Park, J. W.; Kweon, H.; Lee, K. G.; Kang, S. W.; Baek, D. H.; Choi, J. Y.; Kim, S. G. Restoration of peri-implant defects in immediate implant installations by Choukroun platelet-rich fibrin and silk fibroin powder combination graft. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2010**, *109*, 831–836.

(322) Tao, Y. Z.; Yan, Y.; Xu, W. L. Physical characteristics and properties of waterborne polyurethane materials reinforced with silk fibroin powder. *J. Polym. Sci., Part B: Polym. Phys.* **2010**, *48*, 940–950.

(323) Mandal, B. B.; Mann, J. K.; Kundu, S. C. Silk fibroin/gelatin multilayered films as a model system for controlled drug release. *Eur. J. Pharm. Sci.* **2009**, *37*, 160–171.

(324) Lu, Q.; Hu, K.; Feng, Q. L.; Cui, F. Z. Growth of fibroblast and vascular smooth muscle cells in fibroin/collagen scaffold. *Mater. Sci. Eng., C* **2009**, *29*, 2239–2245.

(325) Hu, K.; Lv, Q.; Cui, F. Z.; Feng, Q. L.; Kong, X. D.; Wang, H. L.; Huang, L. Y.; Li, T. Biocompatible fibroin blended films with recombinant human-like collagen for hepatic tissue engineering. *J. Bioact. Compat. Polym.* **2006**, *21*, 23–37.

(326) Garcia-Fuentes, M.; Meinel, A. J.; Hilbe, M.; Meinel, L.; Merkle, H. P. Silk fibroin/hyaluronan scaffolds for human mesenchymal stem cell culture in tissue engineering. *Biomaterials* **2009**, *30*, 5068–5076.

(327) Mandal, B. B.; Kundu, S. C. Cell proliferation and migration in silk fibroin 3D scaffolds. *Biomaterials* **2009**, *30*, 2956–2965.

(328) Wang, Y.; Rudym, D. D.; Walsh, A.; Abrahamsen, L.; Kim, H. J.; Kim, H. S.; Kirker-Head, C.; Kaplan, D. L. In vivo degradation of three-dimensional silk fibroin scaffolds. *Biomaterials* **2008**, *29*, 3415–3428.

(329) Mauney, J. R.; Nguyen, T.; Gillen, K.; Kirker-Head, C.; Gimble, J. M.; Kaplan, D. L. Engineering adipose-like tissue in vitro and in vivo utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds. *Biomaterials* **2007**, *28*, 5280–5290.

(330) Makaya, K.; Terada, S.; Ohgo, K.; Asakura, T. Comparative study of silk fibroin porous scaffolds derived from salt/water and sucrose/hexafluoroisopropanol in cartilage formation. *J. Biosci. Bioeng.* **2009**, *108*, 68–75.

(331) Ghosh, S.; Parker, S. T.; Wang, X. Y.; Kaplan, D. L.; Lewis, J. A. Direct-write assembly of microperiodic silk fibroin scaffolds for tissue engineering applications. *Adv. Funct. Mater.* **2008**, *18*, 1883–1889.

(332) Lovett, M.; Cannizzaro, C.; Daheron, L.; Messmer, B.; Vunjak-Novakovic, G.; Kaplan, D. L. Silk fibroin microtubes for blood vessel engineering. *Biomaterials* **2007**, *28*, 5271–5279.

(333) Jin, S. C.; Baek, H. S.; Woo, Y. I.; Lee, M. H.; Kim, J. S.; Park, J. C.; Park, Y. H.; Rah, D. K.; Chung, K. H.; Lee, S. J.; Han, I. H. Beneficial effects of microwave-induced argon plasma treatment on cellular behaviors of articular chondrocytes onto nanofibrous silk fibroin mesh. *Macromol. Res.* **2009**, *17*, 703–708.

(334) Wenk, E.; Murphy, A. R.; Kaplan, D. L.; Meinel, L.; Merkle, H. P.; Uebersax, L. The use of sulfonated silk fibroin derivatives to control binding, delivery and potency of FGF-2 in tissue regeneration. *Biomaterials* **2010**, *31*, 1403–1413.

(335) Gotoh, Y.; Niimi, S. Effects of supplementation with insulin and dexamethasone on morphologies and maintenance of rat repatocytes onto lactose-silk fibroin conjugates during primary culture. *Kobunshi Ronbunshu* **2005**, *62*, 326–330.

(336) Gotoh, Y.; Niimi, S. Formation and maintenance of rat hepatocyte spheroids on lactose-silk fibroin conjugates in primary cultures. *Kobunshi Ronbunshu* **2008**, *65*, 312–316.

(337) Gotoh, Y.; Niimi, S.; Hayakawa, T.; Miyashita, T. Preparation of lactose-silk fibroin conjugates and their application as a scaffold for hepatocyte attachment. *Biomaterials* **2004**, *25*, 1131–1140.

(338) Liu, L.; Liu, J. Y.; Wang, M. Q.; Min, S. J.; Cai, Y. R.; Zhu, L. J.; Yao, J. M. Preparation and characterization of nano-hydroxyapatite/silk fibroin porous scaffolds. *J. Biomater. Sci., Polym. Ed.* **2008**, *19*, 325–338.

(339) Zhao, J.; Zhang, Z. Y.; Wang, S. Y.; Sun, X. J.; Zhang, X. L.; Chen, J.; Kaplan, D. L.; Jiang, X. Q. Apatite-coated silk fibroin scaffolds to healing mandibular border defects in canines. *Bone* **2009**, *45*, 517–527.