CHEMICAL REVIEWS

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Hydrogels for Tissue Engineering

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I. Introduction

Every year, millions of patients suffer the loss or failure of an organ or tissue as a result of accidents or disease. Over 8 million surgical procedures are performed to treat these patients in the U.S. each year, and the overall cost of these problems to the U.S. economy is estimated to exceed $400 billion per year.[1] Tissue or organ transplantation is a generally accepted therapy to treat these patients. However, this approach is extremely limited by a donor shortage. For example, according to the American Heart Association, only 2300 people received a heart transplant in 1997, while approximately 40 000 patients in the U.S. alone could benefit from this therapy. Similarly, over 10 000 patients require skin grafts to treat severe burns or skin cancers in the U.S. each year.[2]

An exciting and revolutionary strategy to treat patients who need a new organ or tissue is the engineering of man-made organs or tissues (Figure 1). Tissues or organs can be potentially engineered with a number of different strategies, but a particularly appealing approach utilizes a combination of a patient's own cells combined with polymer scaffolds. In this approach, tissue-specific cells are isolated from a small tissue biopsy from the patient and harvested in vitro. The cells are subsequently incorporated into three-dimensional polymer scaffolds that act as analogues to the natural extracellular matrices found in tissues. These scaffolds deliver the cells to the desired site in the patient's body, provide a space for new tissue formation, and potentially control the structure and function of the engineered tissue.[3,4] A variety of tissues are being engineered using this approach including fabricated artery, bladder, skin, cartilage, bone, ligament, and tendon. Several of these tissues are now at or near clinical uses.[5−10] In addition, various approaches have been introduced to coax differentiated or undifferentiated cells (i.e., stem cells) into the desired cell phenotype.[11]

A critical element in virtually all tissue engineering approaches is the polymer scaffold. The polymer potentially mimics many roles of extracellular matrixes found in tissues. Extracellular matrices, comprised of various amino acids and sugar-based macromolecules, bring cells together and control the tissue structure, regulate the function of the cells, and allow the diffusion of nutrients, metabolites, and growth factors.[12] Various types of polymers have been studied and utilized to date in tissue engineering.[13] Aliphatic polyesters including poly(glycolic acid) (PGA),

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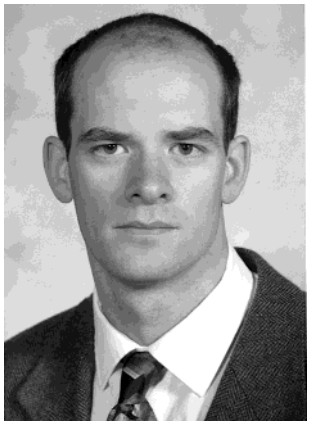
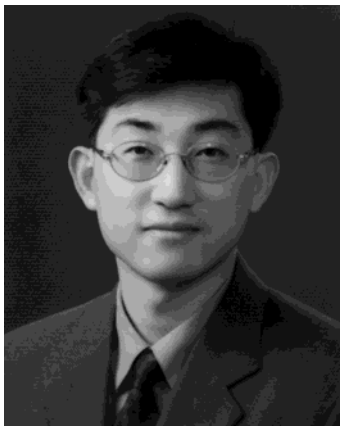


David Mooney was born in Madison, WI in 1964. He received a B.S. at the University of Wisconsin (1987) and a Ph.D. from MIT (1992), both in Chemical Engineering. He served as a postdoctoral fellow in the Surgery Department at Harvard Medical School, before moving to the University of Michigan. He is currently an Associate Professor in Chemical Engineering, Biomedical Engineering, and Biologic & Materials Sciences. Research in his laboratories is focused on elucidating the mechanisms by which cells receive information from materials, and utilizing this information to design new biomaterials that precisely regulate cellular gene expression. The resultant biomaterials are currently being tested in a variety of drug delivery and tissue engineering applications.



Kuen Yong Lee was born in Seoul, Korea in 1968. He received his B.S. degree in Fiber and Polymer Science from Seoul National University (1992), and his M.S. (1994) and Ph.D, (1998) degrees from Seoul National University in Polymer Chemistry. He held postdoctoral positions at the Korea Institute of Science and Technology (1998) and at the University of Michigan (1998–2001). He is currently an Assistant Research Scientist of Biologic & Materials Sciences at the University of Michigan. His research interest has included design, modification, and characterization of biomaterials for drug delivery and tissue engineering applications. His current research activities are focused on elucidating interactions between polymers and cells, degradable polymeric scaffolds, and delivery of growth factors for tissue engineering.

poly(lactic acid) (PLA), and copolymers (PLGA) of these materials are the most widely used synthetic polymers (Figure 2).[14,15] These polymers have a long history of use in medical applications and are considered safe in many situations by the FDA. However, the use of these types of polymer scaffolds requires the surgeon to make incisions (cuts) sufficiently large to enable placement of the polymer/cell constructs.

An exciting alternative approach to cell delivery for tissue engineering is the use of polymers (i.e., hydrogels) that can be injected into the body. This approach enables the clinician to transplant the cell and polymer combination in a minimally invasive

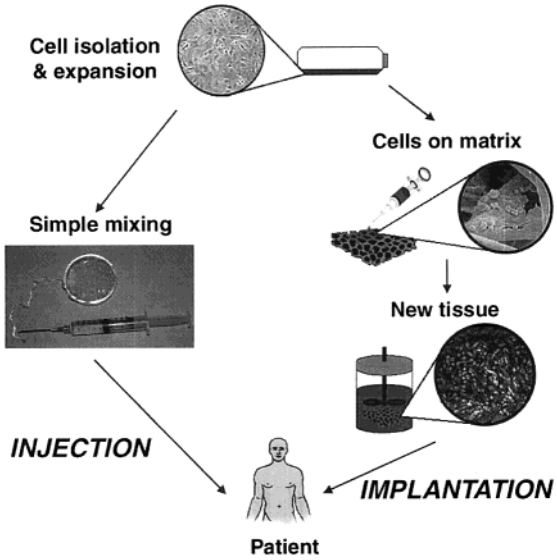


Figure 1. Schematic illustration of typical tissue engineering approaches. Cells are obtained from a small biopsy from a patient, expanded in vitro, and transplanted into the patient either by injection using a needle or other minimally invasive delivery approach, or by implantation at the site following an incision (cut) by the surgeon to allow placement.

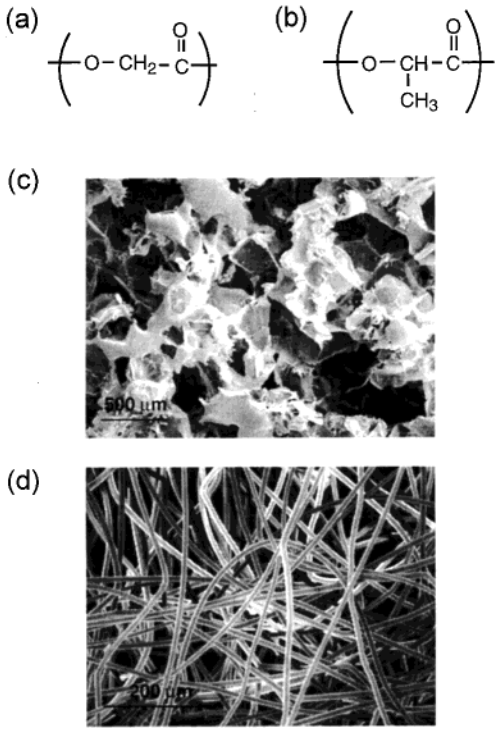


Figure 2. Chemical structure of (a) poly(glycolic acid), (b) poly(lactic acid), and typical structures of (c) porous scaffolds of poly(lactide-*co*-glycolide) and (d) nonwoven fabrics of poly(glycolic acid). These latter materials have been widely used for tissue engineering applications. (Reprinted from ref 14 with permission. Copyright 1998 John Wiley & Sons, Inc.)

manner. Hydrogels have structural similarity to the macromolecular-based components in the body and are considered biocompatible.[16] Hydrogels have found numerous applications in tissue engineering as well as in drug delivery. Tissue engineering is a most recent application of hydrogels, in which they are used as scaffolds to engineer new tissues (Figure 1).[17]

In this review, we discuss the critical design parameters of hydrogels to be used in tissue engineering. Hydrogels, currently used or with potential applications in tissue engineering, are divided into two categories, according to their natural or synthetic origin. Hydrogels from natural polymers have been widely used for tissue engineering approaches. However, limitations of gels from natural polymers have motivated approaches to modify these polymers as well as to use various synthetic polymers. A wide range of synthetic polymers may potentially have suitable chemical and physical properties for these applications. In addition, incorporation of growth factors and the role of mechanical signals to enhance tissue development will be discussed as future directions.

II. Design Parameters for Hydrogels in Tissue Engineering

Hydrogels in tissue engineering must meet a number of design criteria to function appropriately and promote new tissue formation. These criteria include both classical physical parameters (e.g., degradation and mechanics) as well as biological performance parameters (e.g., cell adhesion). An absolutely critical parameter is the biocompatibility of hydrogels. Biocompatibility relates to the material's ability to exist within the body without damaging adjacent cells or lead to significant scarring or otherwise elicit a response that detracts from its desired function. This may be especially problematic as the inflammatory response to a hydrogel can affect the immune response toward the transplanted cells and vice versa.[18,19] Naturally derived polymers frequently demonstrate adequate biocompatibility, while synthetic polymers may elicit significant negative responses from the body. Therefore, one may have some restrictions when preparing hydrogels from synthetic polymers for these applications.

The mechanism of gelling, which may include ionic or covalent cross-linking and inherent phase transition behavior, should be considered next. Ionic cross-linking with multivalent counterions is a simple way to form hydrogels. However, those ions could be exchanged with other ionic molecules in aqueous environments, resulting in an uncontrolled deterioration of the original properties of hydrogels.[20] Covalent cross-linking is a common method to precisely control the cross-linking density of hydrogels. However, the toxicity of cross-linking molecules must be considered, and nondegradable cross-link formation may be disadvantageous in most tissue engineering applications. One recent approach to form hydrogels is the utilization of the phase transition behavior of certain polymers.[21] For example, a very small change of temperature near the lower critical solution temperature (LCST) can trigger the phase transition of a polymer solution to a gel, and significant research has been performed to control the LCST (e.g., design it to be close to body temperature).[22] Hydrogels, cross-linked in situ with minimal temperature rise during polymerization, have also been reported and utilized for orthopedic tissue engineering.[23]

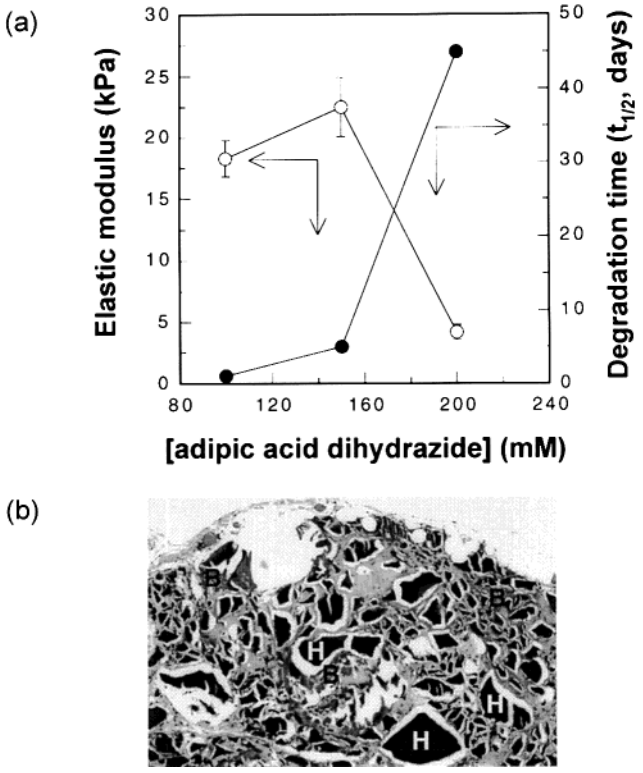


Figure 3. (a) Decoupled degradation behavior of poly(aldehyde guluronate) (PAG) hydrogels cross-linked with adipic acid dihydrazide. $t_{1/2}$ indicates the time when gels lose 50% of their initial modulus.[27] (b) Photomicrograph of representative tissue section following osteoblast/PAG gel constructs transplantation into mice. The tissue section was taken after 9 weeks and stained with hematoxylin and eosin. The original picture was taken at 100× magnification, and the photomicrograph has labels for remaining hydrogel (H) and newly formed bone tissue (B) (unpublished data).

The mechanical properties of hydrogels are important design parameters in tissue engineering, as the gel must create and maintain a space for tissue development. In addition, the adhesion and gene expression of cells are tightly related to the mechanical properties of the polymer scaffold.[24] The mechanical properties of hydrogels mainly depend on the original rigidity of polymer chains, types of cross-linking molecules and the cross-linking density, and swelling as a result of hydrophilic/hydrophobic balance.[25]

The controlled degradation of hydrogels is also critical in tissue engineering, whether the gels are originated from natural resources or are synthetically created. Typically, one desires to coordinate the degradation rate of a scaffold to tissue development, and this time will be dependent on the tissue type to be engineered.[26] Degradation of hydrogels can be due to hydrolysis, the action of enzymes, and/or dissolution. The degradation rate and mechanical properties of cross-linked gels are typically coupled to each other. However, sometimes those properties can be decoupled by intentionally introducing network defects, resulting in the formation of soft hydrogels with longer degradation times than stiffer, more cross-linked gels (Figure 3).[27]

The interactions of cells with hydrogels significantly affects their adhesion as well as migration and

differentiation. The adhesion may be cell-type specific and is dependent on the interaction of specific cell receptors with ligands that are a component or adsorbed onto the materials.[28] Inappropriate interactions could cause undesirable tissue formation.

III. Hydrogels from Natural Polymers

A. Collagen and Gelatin

Collagen is the most widely used tissue-derived natural polymer, and it is a main component of extracellular matrices of mammalian tissues including skin, bone, cartilage, tendon, and ligament. Physically formed collagen gels are thermally reversible and offer a limited range of mechanical properties. Chemical cross-linking of collagen using glutaraldehyde[29] or diphenylphosphoryl azide[30] can improve the physical properties. However, these gels are still short of physical strength, potentially immunogenic, and can be expensive.[31] Furthermore, there can be big variations between produced collagen batches. However, collagen meets many of the biological design parameters, as it is composed of specific combinations of amino acid sequences that are recognized by cells and degraded by enzymes secreted from the cells (i.e., collagenase). Collagen has been used as a tissue culture scaffold or artificial skin due to the ready attachment of many different cell types and its cell-based degradation. The attachment of cells to collagen can be altered by chemical modification, including the incorporation of fibronectin, chondroitin sulfate, or low levels of hyaluronic acid into the collagen matrix.[32] Collagen gels have been utilized for reconstruction of liver,[33] skin,[34] blood vessel,[35] and small intestine.[36]

Gelatin is a derivative of collagen, formed by breaking the natural triple-helix structure of collagen into single-strand molecules. There are two types of gelatin, gelatin A and gelatin B. Gelatin A is prepared by acidic treatment before thermal denaturation, while gelatin B is processed by alkaline treatment that leads to a high carboxylic content.[37] Gelatin easily forms gels by changing the temperature of its solution. It has been used in many tissue engineering applications due to its biocompatibility and ease of gelation. Gelatin gels have also been utilized for delivery of growth factors to promote vascularization of engineered new tissue.[38] However, the weakness of the gels has been a problem, and a number of chemical modification methods have been investigated to improve the mechanical properties of gelatin gels.[39,40]

B. Hyaluronate

Hyaluronate is one of the glycosaminoglycan components in natural extracellular matrices and plays a significant role in wound healing. Hyaluronate can be formed into hydrogels by covalent cross-linking with various kinds of hydrazide derivatives (Figure 4)[41,42] and radical polymerization of glycidyl methacrylate.[43] Hyaluronate is degraded by hyaluronidase, which exists in cells and serum.[44] Hyaluronate has shown excellent potential for tissue engineering applications such as artificial skin,[45] facial intradermal implants,[46] would healing,[47] and soft tissue augmentation.[48] However, hyaluronate requires thorough purification to remove impurities and endotoxins that may potentially transmit disease or act as an adjuvant in eliciting an immune response.[49] In addition, hyaluronate gels typically possess low mechanical properties. These issues have limited the applications of hyaluronate.

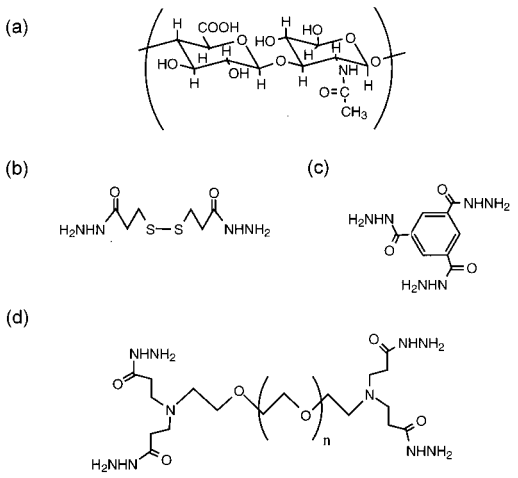


Figure 4. Chemical structure of (a) hyaluronic acid and various cross-linking molecules that can be used to form hydrogels, including (b) 3,3′-dithiobis(propanoic dihydrazide), (c) 1,3,5-benzene(tricarboxylic trihydrazide), and (d) poly(ethylene glycol)-diamine tetrapropanoic tetrahydrazide.

C. Fibrin

Fibrin has been used as a sealant and an adhesive in surgery as it plays an important role in natural wound healing. Fibrin gels can be produced from the patient's own blood and can be used as an autologous scaffold for tissue engineering. No toxic degradation or inflammatory reactions are expected from this natural component of the body. Fibrin forms gels by the enzymatic polymerization of fibrinogen at room temperature in the presence of thrombin.[50] An interesting feature of fibrin is the degradation and remodeling by cell-associated enzymatic activity during cell migration and wound healing, and its degradation rate can be controlled by apronitin, a proteinase inhibitor.[51]

Fibrin gels might promote cell migration, proliferation, and matrix synthesis through the incorporation of platelet-derived growth factors and transforming growth factor β.[52] Bidomain peptides with a factor XIIIa substrate in one domain and a bioactive peptide containing RGD sequence in another domain have been covalently incorporated into fibrin gels during coagulation through the action of the transglutaminase factor XIIIa, resulting in gels with potential neurological applications.[53] Fibrin gels have also been utilized to engineer tissues with skeletal muscle cells,[51] smooth muscle cells,[54] and chondrocytes.[55] However, fibrin gels are limited in mechanical strength, and this prevents their use in certain applications.

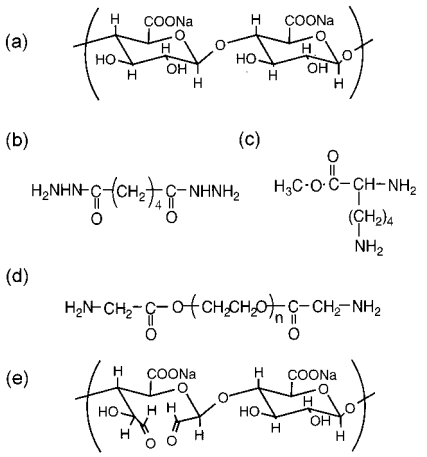


Figure 5. Chemical structure of (a) sodium alginate and various cross-linking molecules used in covalent cross-linking reactions, including (b) adipic acid dihydrazide, (c) L-lysine, and (d) poly(ethylene glycol)-diamine. Alginate can be oxidized with sodium periodate under mild reaction conditions to infer main chain lability to hydrolysis as well (e).

D. Alginate

Alginate is a well-known biomaterial obtained from brown algae and is widely used for drug delivery and in tissue engineering due to its biocompatibility, low toxicity, relatively low cost, and simple gelation with divalent cations such as Ca^{2+}, Mg^{2+}, Ba^{2+}, and Sr^{2+} (Figure 5a).[56] Alginate has found uses to date as an injectable cell delivery vehicle[57] as well as wound dressing, dental impression, and immobilization matrix.[58,59] Alginate gel beads have also been prepared and used for transplantation of chondrocytes,[60] hepatocytes,[61] and islets of Langerhans to treat diabetes.[62]

Despite its advantageous features, alginate itself may not be an ideal material because it degrades via a process involving loss of divalent ions into the surrounding medium, and subsequent dissolution. This process is generally uncontrollable and unpredictable. Therefore, covalent cross-linking with various types of molecules and different cross-linking densities has been attempted to precisely control the mechanical and/or swelling properties of alginate gels (Figure 5b−d).[25] In addition, the molecular weights of many alginates are typically above the renal clearance threshold of the kidney.[63] Recently, hydrolytically degradable and covalently cross-linked hydrogels derived from alginate were reported.[64] An attractive approach to control the degradation of alginate involves the isolation of polyguluronate blocks with molecular mass of 6000 Da from alginate, and the subsequent oxidation and covalent cross-linking of these derivatives with adipic acid dihydrazide. The gelling of these polymers could be readily controlled, and their mechanical and degradation properties were also regulated depending on the cross-linking density.[27] In addition, slightly oxidized alginate itself was found to be degradable in aqueous media depending on the pH and temperature of the solution (Figure 5e).[65]

Another potential limitation in using alginate gels in tissue engineering is the lack of cellular interaction. Alginate is known to discourage protein adsorption due to its hydrophilic character, and it is unable to specifically interact with mammalian cells.[66] Therefore, alginate has been modified with lectin, a carbohydrate specific binding protein, to enhance ligand-specific binding properties.[67] An RGD-containing cell adhesion ligand has also been covalently coupled to alginate gels to enhance cell adhesion. These modified alginate gels have been demonstrated to provide for the adhesion, proliferation, and expression of differentiated phenotype of skeletal muscle cells (Figure 6).[68]

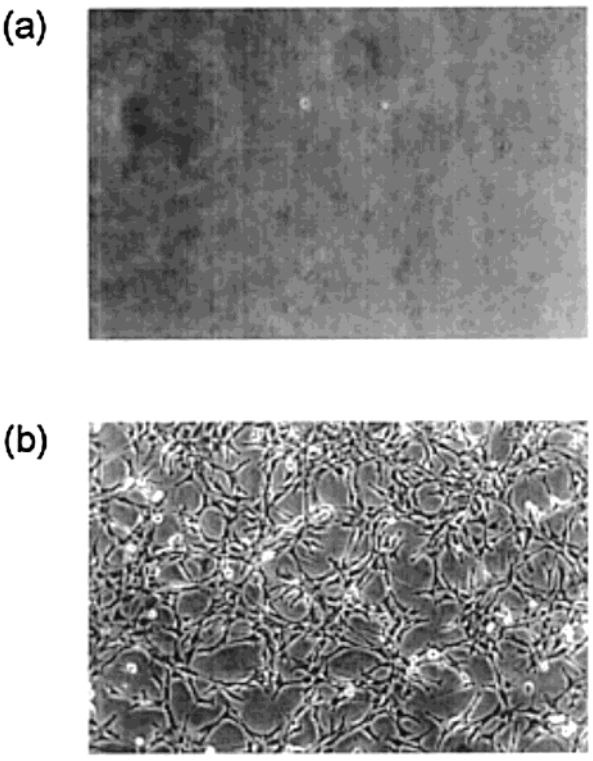


Figure 6. Myoblast adhesion onto (a) unmodified and (b) GRGDY-modified alginate hydrogels. Very few cells adhere to unmodified alginate gels, while cells readily adhere, spread, and function on the modified gels. (Reprinted from ref 68 with permission. Copyright 1999 Elsevier Science.)

E. Agarose

Agarose is another type of marine algal polysaccharide, but unlike alginate it forms thermally reversible gels.[69] The proposed gel structure is bundles of associated double helices, and the junction zones consist of multiple chain aggregation.[70] The physical structure of the gels can be mainly controlled by using a range of agarose concentrations, which results in various pore sizes. The large pores and low mechanical stiffness of the gels at low concentrations of agarose may enable the migration and proliferation of cells, and these factors have been found to affect neurite growth in vitro.[71] Chitosan has been covalently bound to agarose gels to incorporate charge into the gels, and this significantly contributed to neurite growth as well.[3] Cell adhesion peptides (CDPGYIGSR) have also been covalently coupled to enhance the interaction with cells.[72]

F. Chitosan

Chitosan is prepared by *N*-deacetylation of chitin and usually contains less than 40% of *N*-acetyl-D-

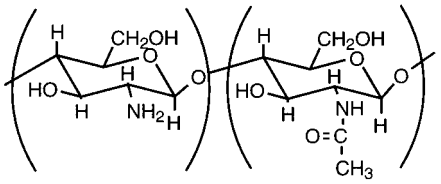


Figure 7. Chemical structure of chitosan.

glucosamine residues (Figure 7). Chitosan has found many biomedical applications, including tissue engineering approaches, due to its biocompatibility, low toxicity, structural similarity to natural glycosaminoglycans, and degradation by enzymes such as chitosanase and lysozyme.[73] However, chitosan is easily soluble in the presence of acid, and generally insoluble in neutral conditions as well as in most organic solvents due to the existence of amino groups and the high crystallinity. Therefore, many derivatives have been reported to enhance the solubility and processibility of this polymer.[74,75] Chitosan forms hydrogels by ionic[76] or chemical cross-linking with glutaraldehyde.[77] Azide-derivatized chitosan was also reported to form gels by UV irradiation.[78]

Numerous derivatives have been developed to alter the biological functions of chitosan, including enhancement of cellular interactions for tissue engineering approaches. Chitosan has been modified with sugar residues such as fructose or galactose for culture of hepatocytes,[79,80] and with proteins such as collagen, gelatin, and albumin for neural tissue engineering.[81] In addition, methylpyrrolidinone-derivatized chitosan has been reported to promote bone formation.[82]

IV. Hydrogels from Synthetic Polymers

A. Poly(acrylic acid) and Its Derivatives

One of the most studied synthetic hydrogels is hydrolytically stable cross-linked poly(2-hydroxyethyl methacrylate) (HEMA). The permeability and hydrophilicity of these gels are dependent on the cross-linking agents.[83] Poly(HEMA) has been used for ophthalmic uses including contact lens,[84] as well as in many drug delivery applications.[85] Macroporous poly(HEMA) gels have been prepared by freeze/thaw, or particulate leaching techniques for cartilage replacement.[86] Many different types of molecules and cells have also been encapsulated into poly(HEMA) gels, and this approach has been reported to be successful for delivery of insulin or other proteins into the body.[87] Poly(HEMA) gels are not degradable in physiological conditions. Therefore, dextran-modified poly(HEMA) gels have been synthesized, and reported to be degradable by enzymes.[88] In addition, enantiomeric oligo(L-lactide) and oligo(D-lactide) were grafted to poly(HEMA) to induce stereocomplexation, resulting in the formation of poly(HEMA) gels without using any toxic chemical reagents.[89]

Poly(*N*-isopropylacrylamide) (PNIPAAm) is potentially very attractive for tissue engineering applications as it exhibits phase transition behavior above the lower critical solution temperature (LCST). The LCST of PNIPAAm in water is approximately 32 °C

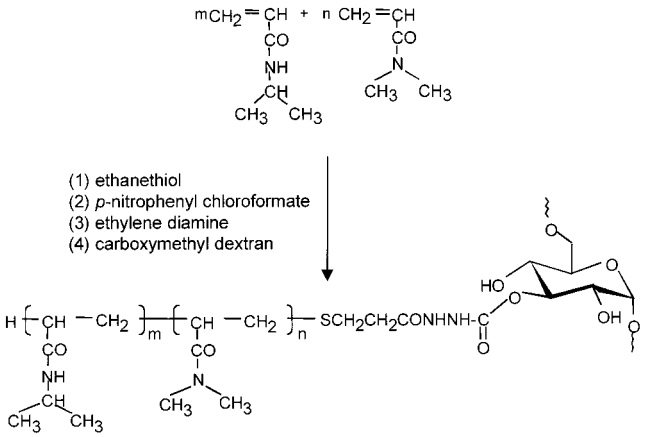


Figure 8. Synthesis of dextran-grafted poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (NIPAAm-*co*-DMAAm).[99]

and can be matched to body temperature by copolymerization.[90] Therefore, the use of PNIPAAm and its copolymers in tissue engineering would be very beneficial as one can easily prepare a mixed solution of cells and the polymer at room temperature or even at a lower temperature and inject it into the desired site. This will result in the formation of a solid cell/polymer construct as the gel warms to body temperature. NIPAAm has been copolymerized with acrylic acid, methacrylic acid, or butylmethacrylic acid, depending on the desired final applications.[91−93] Acrylamide derivatives have also been cross-linked with native proteins,[94] oligodeoxyribonucleotides,[95] or engineered coiled-coil proteins[96] to form temperature-responsive gels. In this situation, conventional polymers could potentially be modified to exhibit thermal transition behavior by utilizing a variety of cross-linking molecules that can induce phase separation in response to temperature changes.

The unique temperature-responsive nature of these polymers is leading to a variety of biological applications. In standard cell culture, cells are recovered from the dishes by treatment with proteases (e.g., trypsin). However, the culture of cells on PNIPAAm enables one to easily recover intact cell sheets without damage by simply decreasing the temperature and modulating the hydrophilicity of the gel.[97] These polymers are also being investigated as an injectable delivery vehicle for cartilage and pancreas engineering.[91,98] This mechanism of phase transition may be ideal for delivery of cells, as unlike chemically controlled cross-linking (e.g., alginate), the timing of phase transition is not set, but simply depends on the temperature change upon introduction to the body. However, limitations of these gels are the nondegradable cross-links, and the vinyl monomers and cross-linking molecules are toxic, carcinogenic, or teratogenic.[21] In an effort to obviate these issues, dextran-grafted PNIPAAm copolymers have been synthesized, and these may modulate degradation in synchronization with temperature (Figure 8).[99]

B. Poly(ethylene oxide) and Its Copolymers

Poly(ethylene oxide) (PEO) has been approved by the FDA for several medical applications due to its

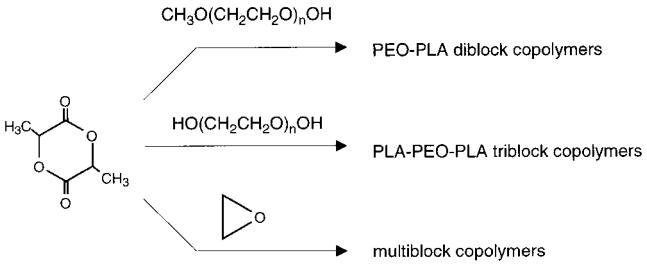


Figure 9. Synthetic scheme of poly(ethylene oxide)-poly(lactic acid) (PEO−PLA) block copolymers.

biocompatibility and low toxicity. It has been extensively studied for uses including preparation of biologically relevant conjugates,[100] surface modification of biomaterials,[101] and induction of cell membrane fusion.[102] PEO itself is very hydrophilic and can be synthesized by anionic or cationic polymerization of ethylene oxide. PEO gels can be prepared by UV photopolymerization of the precursor that consists of PEO with acrylate termini at each end in the presence of α-hydroxy acid.[103] The peptide sequence of Ala−Pro−Gly−Leu has also been introduced into these gels, to make the gels susceptible to enzymes existing in the body, and these gels may be useful for tissue engineering applications.[104] Branched PEO having a cinnamylidene acetyl moiety as a pendant group has been synthesized and photopolymerized to form gels, and these gels demonstrated antithrombogenic properties.[105] Star-shaped PEO was cross-linked by irradiation to form hydrogels and also modified with galactose moieties to enhance the interaction with liver cells.[106]

Various PEO-based copolymers have been reported and utilized, especially in drug delivery applications.[107−109] One interesting copolymer is a triblock copolymer of PEO and poly(propylene oxide) (PEO-*b*-PPO-*b*-PEO), which is known by the trade name Pluronics or Poloxamers, and is commercially available in various lengths and compositions. These polymers form thermally reversible gels without any permanent cross-links, unlike PNIPAAm and its copolymer gels. In addition, it was reported that PEO−PPO−PEO triblock copolymers could be designed to form gels at body temperature by forming a liquid crystalline phase.[110] PEO−PPO triblock copolymers have been mainly used for drug delivery applications, as they are known to enhance drug penetration, and also enhance the activity of antineoplastic agents against tumors.[111] There have been few reports on the utilization of these gels in tissue engineering to date,[112] but they may find multiple applications in this field.

Although PEO−PPO−PEO triblock copolymers form hydrogels in aqueous solutions in response to temperature changes, they have limitations for biomedical uses. These limitations include the lack of biodegradation. Therefore, a variety of biodegradable di- or triblock copolymers of PEO and poly(lactic acid) (PLA) have been synthesized, as PLA is degradable and has been already proved to be safe in many medical applications (Figure 9).[113] Alternating multiblock copolymers of PEO and PLA were also synthesized by the condensation reaction of L-lactic acid in the presence of succinic acid. These gels exhibited

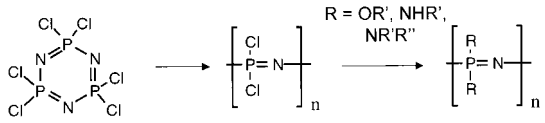


Figure 10. Synthetic scheme of polyphosphazene using poly(dichlorophosphazene) as an intermediate.

temperature-dependent reversible gel−sol transitions near body temperature.[114] These gels may be useful in tissue engineering as they can be easily formulated with protein drugs or cells at room temperature or lower, and subsequently delivered to the desired site in a minimally invasive manner.

C. Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is generally obtained from poly(vinyl acetate) by alcoholysis, hydrolysis, or aminolysis.[115] The hydrophilicity and solubility of PVA can be readily controlled by the extent of hydrolysis and molecular weight. PVA forms hydrogels by chemical cross-linking with glutaraldehyde[116] or epichlorohydrin.[117] To avoid the toxicity and leaching problems of chemical cross-linking agents, a repeated freezing/thawing method,[118] or electron beam[119] has been applied to form PVA hydrogels. The gels formed by the repeated freezing/thawing method were reported to be stable at room temperature, and highly elastic.[118] However, these gelling methods are not appropriate inside the body in situ, and PVA is not degradable in most physiological situations. Therefore, these gels are mostly likely to be useful as a long-term or permanent scaffold. PVA hydrogels have been utilized in tissue engineering for regeneration of artificial articular cartilage,[120] hybrid-type artificial pancreas,[121] and bone-like apatite formation.[122]

Oligopeptide sequences have been introduced onto the surface of PVA gels to enhance cellular interaction. For example, a Gly−His−Lys sequence responsible for hepatocyte attachment,[123] and an RGDS sequence for the adhesion of corneal epithelial cells[124] have been investigated.

D. Polyphosphazene

Polyphosphazenes have been attractive in many biomedical applications, as they are degradable in physiological situations. The degradation kinetics can be controlled by changes in the side-chain structure rather than the polymer backbone, unlike aliphatic polyesters, polyanhydrides, or poly(ortho esters).[125] Polyphosphazene, an organometallic polymer, contains alternating phosphorus and nitrogen atoms with two side groups attached to each phosphorus atom. Poly(dichlorophophazene) has been used as an intermediate to synthesize stable polyphosphazenes (Figure 10). Poly(dichlorophosphazene) is usually synthesized by thermal ring-opening polymerization of hexachlorocyclotriphosphazene,[126] solution condensation of *p*-trichloro-*N*-(dichlorophosphoryl)monophosphazene,[127] or living cationic polymerization of phosphoamines to control molecular weight distribution.[128] A hydrophilic backbone, as well as the structural versatility as a result of various substitution reactions, offers possibilities in designing new classes

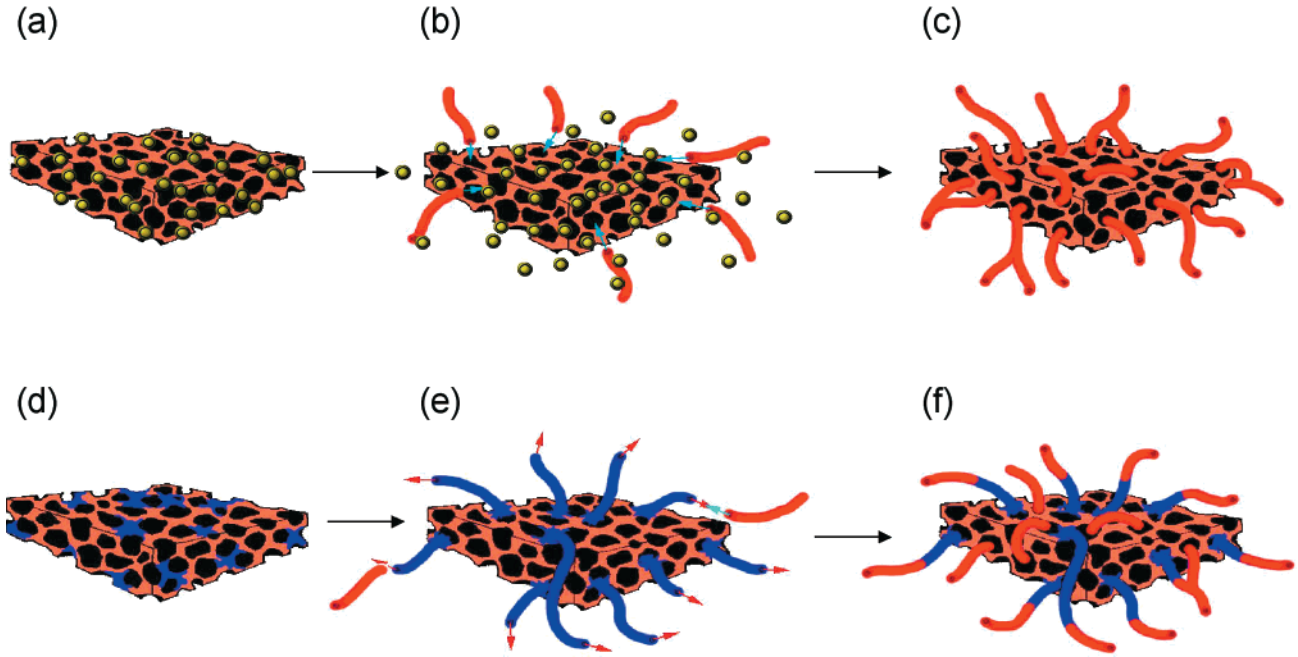


Figure 11. Schematic illustration of blood vessel formation promoted by including growth factors (a) or by seeding endothelial cells (d) into the polymer scaffold. Growth factors encourage existing blood vessels in the surrounding host tissue to grow into the scaffold (b), and the transplanted endothelial cells will form new blood vessels within the scaffold and grow outward toward the host tissue (e). Ultimately, new vessels combine with existing blood vessels to create functional blood vessels capable of blood flow (c,f).

of polyphosphazene gels. Various modifications of polyphosphazenes have been reported including poly(aryl/alkyl) phosphazenes,[129] poly[(amino acid-ester) phosphazenes],[130] and methoxy-poly(ethylene glycol)-substituted polyphosphazenes with temperature-responsive features.[131] Two types of hydrogels, nonionic and ionic, can be prepared from polyphosphazenes. Nonionic polyphosphazene gels are based on water-soluble polyphosphazenes containing glucosyl or glyceryl side groups.[132] Ionic polyphosphazene hydrogels, formed with divalent ions or ^{60}Co gamma irradiation, have been extensively studied in controlled delivery of protein drug due to their ability to respond to environmental changes such as pH or ionic strength.[133,134] These polymers might be useful for skeletal tissue regeneration[135] or encapsulation of hybridoma cells.[136]

E. Polypeptides

Proteins are a major component of the natural matrixes of tissues, and there is wide interest in synthesizing polypeptides to mimic natural proteins. Polypeptides are usually prepared by using *N*-carboxyanhydride as a starting monomer, and a large number of polypeptides and copolypeptides can be synthesized from various combination of amino acids. However, it is very difficult to precisely control the sequence of amino acids as desired and thus very expensive. In addition, most polypeptides are insoluble in common organic solvents.[31] Recently, a polymerization strategy to synthesize polypeptides with well-defined amino acid sequences and a wide range of molecular weights was reported by using organonickel initiators that suppress chain-transfer and termination side reactions.[137]

One striking technique to bypass the above-mentioned problems is the synthesis of genetically engineered polypeptides. In brief, one may insert DNA templates of predetermined sequences into the genome of bacteria and produce polypeptides with predetermined structure and controlled properties.[138,139] This method enables one to design and engineer various sequences of polypeptides with known functions, including elasticity, stiffness, degradation, and cellular interactions. Silk-like polypeptides have been prepared by this technique,[140] and a Gly−Ala-rich sequence has been introduced into these artificial proteins to form reversible hydrogels in response to environmental changes of pH or temperature.[141] Elastin-mimetic polypeptides, comprised of a Gly−Val−Pro−Gly−any amino acid sequence, have also been studied and considered to have potential for artificial extracellular matrices in tissue engineering.[142−144] However, this technique is not appropriate to economically produce biomaterials in large scale at the current time, and one is also unable to easily modify the polymer product as any change requires re-engineering of the entire system.

V. Future Perspectives

We have summarized a wide range of hydrogels that are frequently used to date, or will potentially be useful in tissue engineering. Hydrogels should meet certain design parameters to be useful for this area, regardless of whether they originate from natural resources or are synthetically created. Hydrogels comprised of naturally derived macromolecules have potential advantages of biocompatibility, cell-controlled degradability, and intrinsic cellular interaction. However, they may exhibit batch

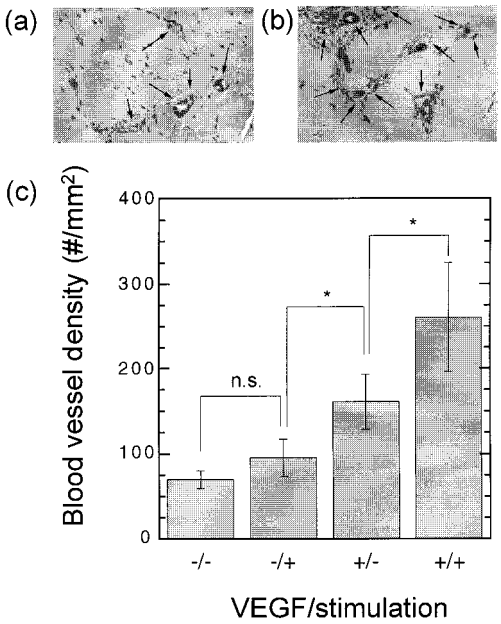


Figure 12. In vivo response to mechanical stimulation of VEGF-loaded alginate hydrogels implanted into the femoral artery ligation site of mice. Photomicrographs of representative tissue section (a) VEGF incorporated hydrogels without mechanical stimulation (+/−) and (b) VEGF incorporated hydrogels with mechanical stimulation (+/+). (c) Effect of mechanical stimulation on the blood vessel density. Control gels without VEGF under static conditions (−/−) or under mechanical stimulation (−/+) were also implanted. Original pictures were taken at 400× magnification and arrows indicate CD31-stained blood vessels. n.s. indicates no statistical difference. *statistical significance at a level of $p < 0.05$. (Reprinted from *Nature* (http://www.nature.com), ref 146 with permission. Copyright 2000 Macmillan Magazines Ltd.)

variations and generally exhibit a narrow and limited range of mechanical properties. In contrast, synthetic polymers can be prepared with precisely controlled structures and functions. However, many synthetic polymers do not degrade in physiological conditions, and the use of toxic chemicals in their synthesis or processing may require extensive purification steps. We believe no one material will satisfy all design parameters in all applications, but a wide range of materials will find uses in various tissue engineering applications.

A critical future challenge facing this field is how polymers may be used to promote blood vessel network formation in the tissue. This is critical to provide nutrient transport to the engineered tissue and integrate it with the rest of the body.[145] One important approach to actively modulate the vascularization process is the local delivery of either angiogenic factors or blood vessel forming cells (endothelial cells) to the engineered site using hydrogels (Figure 11).[2] Controlled and sustained release of angiogenic factors from hydrogels may optimize localized vessel formation. Various growth factors including vascular endothelial growth factor (VEGF),[146] basic fibroblast growth factor (bFGF),[147] epidermal growth factor (EGF),[148] and bone morphogenetic protein (BMP)[149] could be incorporated into hydrogels depending on the desired tissue type. Alternatively, delivery of plasmid DNA containing genes encoding the angiogenic proteins may be another approach to enhance vascular network formation in engineered tissues.[150] Co-transplantation of endothelial cells, which comprise blood vessels, along with the primary cell type of interest may allow one to rapidly form blood vessels in an engineered tissue. This approach is based on the observation that endothelial cells spontaneously form capillary-like structures in vitro if cultured in an appropriate environment.[151]

Another critical issue in the design of hydrogels for tissue engineering is that many tissues (e.g., bone, muscle, and blood vessels) exist in a mechanically dynamic environment. Many current hydrogels do not possess appropriate mechanical properties for these mechanically dynamic environments. In addition, it has been previously demonstrated that mechanical signals result in alterations of cellular structure, metabolism, and transcription and/or translation of various genes.[152−154] So the gels must appropriately convey the mechanical signals to these incorporated cells. We have recently reported that mechanical signals may be exploited to control growth factor release from hydrogels, and this could provide a novel approach to guide tissue formation in mechanically stressed environments (Figure 12).[146]

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VII. References

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