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Review

Hydrogels for tissue engineering: scaffold design variables and applications

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Abstract

Polymer scaffolds have many different functions in the field of tissue engineering. They are applied as space filling agents, as delivery vehicles for bioactive molecules, and as three-dimensional structures that organize cells and present stimuli to direct the formation of a desired tissue. Much of the success of scaffolds in these roles hinges on finding an appropriate material to address the critical physical, mass transport, and biological design variables inherent to each application. Hydrogels are an appealing scaffold material because they are structurally similar to the extracellular matrix of many tissues, can often be processed under relatively mild conditions, and may be delivered in a minimally invasive manner. Consequently, hydrogels have been utilized as scaffold materials for drug and growth factor delivery, engineering tissue replacements, and a variety of other applications.

Keywords: Alginate; Chitosan; Hyaluronic acid; PEG; Drug delivery

Contents

1. Introduction

The field of tissue engineering has developed to meet the tremendous need for organs and tissues [1–4]. In the most general sense, tissue engineering seeks to fabricate, living replacement parts for the body [5]. The necessity of tissue engineering is illustrated by the ever-widening supply and demand mismatch of organs and tissues for

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transplantation (Fig. 1) [6]. This trend persists, as demonstrated by the fact that only 23,407 people received transplants from July 2000 to July 2001, while 79,902 people awaited them [7].

Numerous strategies currently used to engineer tissues depend on employing a material scaffold. These scaffolds serve as a synthetic extracellular matrix (ECM) to organize cells into a three-dimensional architecture and to present stimuli, which direct the growth and formation of a desired tissue [8]. Depending on the tissue of interest and the specific application, the required scaffold material and its properties will be quite different. Common scaffold materials include poly(lactide-co-glycolide) (PLG). PLG are hydrolytically degradable polymers that are FDA approved for use in the body and mechanically strong [9,10]. However, they are hydrophobic and typically processed under relatively severe conditions, which makes factor incorporation and entrapment of viable cells potentially a challenge. As an alternative, a variety of hydrogels, a class of highly hydrated polymer materials (water content $\geqslant 30\%$ by weight) [11], are being employed as scaffold materials. They are composed of hydrophilic polymer chains, which are either synthetic or natural in origin. The structural integrity of hydrogels depends on crosslinks formed between polymer chains via various chemical bonds and physical interactions. Hydrogels used in these applications are typically degradable, can be processed under relatively mild conditions, have mechanical and structural properties similar to many tissues and the ECM, and can be delivered in a minimally invasive manner [12].

This review will focus on the use of hydrogels as scaffolds for tissues engineering. Adequate scaffold design and material selection for each specific application depend on several variables, including physical properties (e.g. mechanics, degradation, gel formation), mass transport properties (e.g. diffusion), and biological properties (e.g. cell adhesion and signaling). We have identified three categories of scaffolds applications in this review: space filling agents, bioactive molecule delivery, and cell/tissue delivery. The materials available for use in hydrogel formation are first discussed along with a description of the pertinent design variables. The current use of hydrogels for each of the major categories of applications will subsequently be reviewed.

2. Gel forming materials

A variety of synthetic and naturally derived materials may be used to form hydrogels for tissue engineering scaffolds. Synthetic materials include poly(ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(propylene furmarate-co-ethylene glycol) (P(PF-co-EG)), and polypeptides. Representative naturally derived polymers include agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid (HA). We have chosen to focus on a subset of these hydrogels (PEO, PVA, P(PF-co-EG), alginate, chitosan, collagen, and HA) because of their current prevalent use in tissue engineering applications. The chemistry, gelling conditions, and degradation modes of each are described in this section.

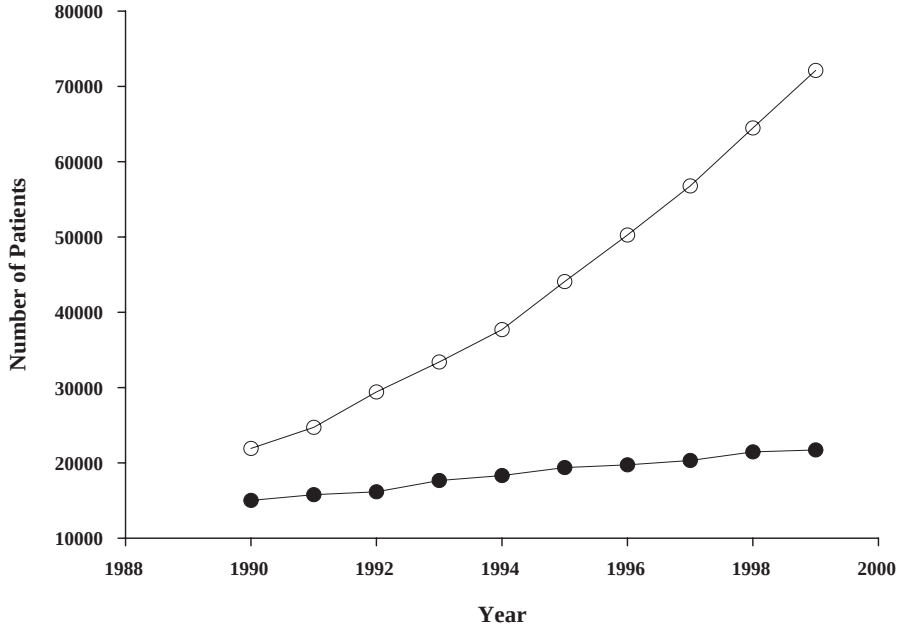

Fig. 1. UNOS organ transplant statistics for 1990 to 1999 [6] documenting the wait-listed patients (○) and transplants (●).

2.1. Synthetic materials

Synthetic hydrogels are appealing for tissue engineering because their chemistry and properties are controllable and reproducible. For example, synthetic polymers can be reproducibly produced with specific molecular weights, block structures, degradable linkages, and crosslinking modes. These properties in turn, determine gel formation dynamics, crosslinking density, and material mechanical and degradation properties. Examples of such synthetic materials discussed here are PEO, PVA, and P(PF-co-EG).

PEO is currently FDA approved for several medical applications and is one of the most commonly applied synthetic hydrogel polymers for tissue engineering. PEO and the chemically similar poly(ethylene glycol) (PEG) are hydrophilic polymers (Figs. 2a and b, respectively) that can be photocrosslinked by modifying each end of the polymer with either acrylates or methacrylates [13–15]. Hydrogels are then formed when the modified PEO or PEG is mixed with the appropriate photoinitiator and crosslinked via UV exposure [14,16]. Thermally reversible hydrogels have also been formed from block copolymers of PEO and poly(L-lactic acid) (PLLA) [17] and PEG and PLLA [18]. In addition to the thermally reversible hydrogels, degradable PEO and PEG hydrogels have been formed by synthesizing block copolymers containing hydrolytically degradable poly(lactic acid) (PLA) [19] and enzyme specific cleavage sequences of oligopeptides [14,15].

Another synthetic hydrophilic polymer widely explored for use in space filling and drug delivery applications is PVA (Fig. 2c). It can be physically crosslinked by repeated freeze-thawing cycles of aqueous polymer solutions [20] or chemically crosslinked with glutaraldehyde [21], succinyl chloride, adipoyl chloride, and sebacoyl chloride [22] to form hydrogels.

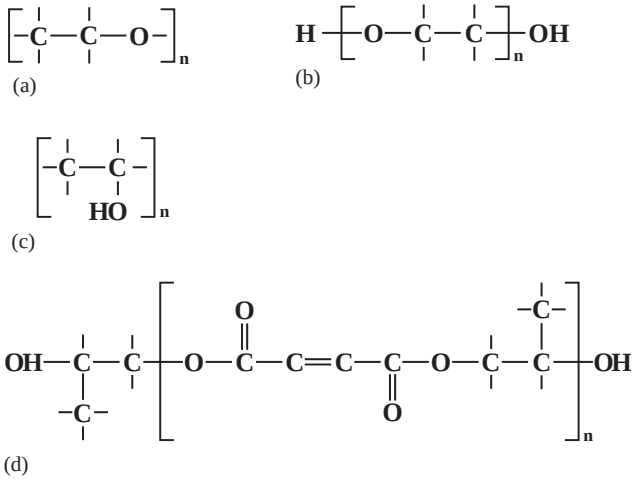

Fig. 2. Structure of synthetic hydrogel forming polymers: (a) PEO, (b) PEG, (c) PVA (100% hydrolyzed), and (d) PPF.

It can also be blended with other water-soluble polymers and again crosslinked either physically or chemically [23–25]. It these forms, it is not dissolvable in aqueous solutions.

A newer, synthetic hydrogel block copolymer, P(PF-co-EG) has been created for use as an injectable carrier for bone and blood vessel engineering [26]. The homopolymer poly(propylene fumarate) (PPF) is a hydrophobic, linear polyester (Fig. 2d), which undergoes degradation by hydrolysis of the ester linkage. It can form hydrogels when synthesized as a block copolymer with hydrophilic PEG and crosslinked either chemically [27] or via UV exposure [28].

2.2. Naturally derived materials

Naturally derived hydrogel forming polymers have frequently been used in tissue engineering applications because they are either components of or have macromolecular properties similar to the natural ECM. For example, collagens are the main protein of mammalian tissue ECM and comprise 25% of the total protein mass of most mammals [29,30]. Similarly, HA is found in varying amounts in all tissues of adult animals [29]. Like HA, both alginate and chitosan are hydrophilic, linear polysaccharides [31,32]. They have also been shown to interact in a favorable manner in vivo and thus have been utilized as hydrogel scaffold materials for tissue engineering [12,32].

Collagen is an attractive material for biomedical applications as it is the most abundant protein in mammalian tissues [30] and is the main component of natural ECM [29]. There are at least 19 different types of collagen, but the basic structure of all collagen is composed of three polypeptide chains, which wrap around one another to form a three-stranded rope structure [30]. The strands are held together by both hydrogen and covalent bonds. Collagen strands can self-aggregate to form stable fibers [30]. In addition, collagen fibers and scaffolds can be created and their mechanical properties enhanced by introducing various chemical crosslinkers (i.e. glutaraldehyde, formaldehyde, carbodiimide) [33,34], by crosslinking with physical treatments (i.e. UV irradiation, freeze-drying, heating) [33,35], and by blending it with other polymers (i.e. HA, PLA, poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA), chitosan, PEO) [33,34,36–38]. Collagen is naturally degraded by metalloproteases, specifically collagenase, and serine proteases [29], allowing for its degradation to be locally controlled by cells present in the engineered tissue.

HA is the simplest glycosaminolglycan (GAG) and is found in nearly every mammalian tissue and fluid [29]. It is especially prevalent during wound healing and in the synovial fluid of joints. It is a linear polysaccharide composed of a repeating disaccharide (Fig. 3a) of (1–3)

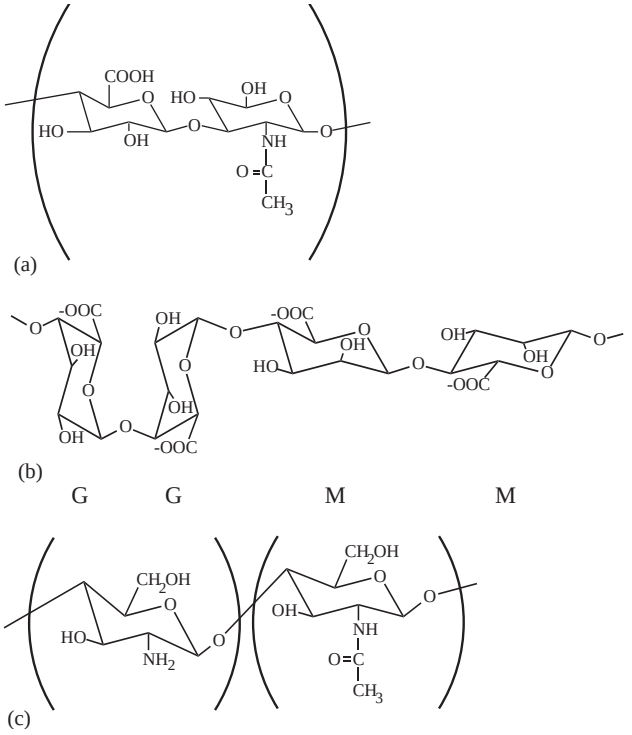

Fig. 3. Structure of naturally derived hydrogel forming polymers: (a) HA, (b) alginate, and (c) chitosan.

and (1–4)-linked β-D-glucuronic acid and *N*-acetyl-β-D-glucosamine units [29]. Hydrogels of HA are formed by covalent crosslinking with hydrazide derivatives [39–41], by esterification [42–44], and by annealing [45]. Additionally, HA has been combined with both collagen and alginate to form composite hydrogels [34,41,46,47]. HA is naturally degraded by hyaluronidase [29], again allowing cells in the body to regulate the clearance of the material in a localized manner.

Alginate has been used in a variety of medical applications including cell encapsulation and drug stabilization and delivery, because it gels under gentle conditions, has low toxicity, and is readily available. It is a linear polysaccharide copolymer of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers (Fig. 3b), and is derived primarily from brown seaweed and bacteria [31,48]. Within the alginate polymer, the M and G monomers are sequentially distributed in either repeating or alternating blocks [31,48,49]. The amount and distribution of each monomer depends on the species, location, and age of seaweed from which the alginate is isolated [31,48]. Gels are formed when divalent cations such as Ca^{2+}, Ba^{2+}, or Sr^{2+} cooperatively interact with blocks of G monomers to form ionic bridges between different polymer chains [31]. The crosslinking density and thus mechanical properties and pore size of the ionically crosslinked gels can be readily manipulated by varying the M to G ratio and molecular weight of the polymer chain. Gels can also be formed by covalently crosslinking alginate with adipic hydrazide and PEG using standard carbodiimide chemistry [50,51]. Ionically crosslinked alginate hydrogels do not specifically degrade but undergo slow, uncontrolled dissolution. Mass is lost through ion exchange of calcium followed by dissociation of individual chains, which results in loss of mechanical stiffness over time [52]. Hydrolytically degradable forms of alginate have been synthesized by partial oxidation of alginate and an alginate derivative, polyguluronate [53], to form oxidized alginate [54] and poly(aldehyde guluronate) (PAG) [55], respectively.

Chitosan has been investigated for a variety of tissue engineering applications because it is structurally similar to naturally occurring GAGs and is degradable by enzymes in humans. It is a linear polysaccharide of (1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine residues derived from chitin (Fig. 3c), which is found in arthropod exoskeletons [32,56,57]. The degree of *N*-deacetylation usually varies from 50% to 90% [12,32] and determines the crystallinity, which is greatest for 0% and 100% *N*-deacetylation [32]. Chitosan is soluble in dilute acids which protonate the free amino groups [12,32,58]. Once dissolved, chitosan can be gelled by increasing the pH [12,32,58] or extruding the solution into a nonsolvent [32]. Chitosan derivatives and blends have also been gelled via glutaraldehyde crosslinking [59,60], UV irradiation [61], and thermal variations [23,56,58]. Chitosan is degraded by lysozyme; the kinetics of degradation are inversely related to the degree of crystallinity [62–64].

3. Scaffold design variables

Selection or synthesis of the appropriate hydrogel scaffold materials is governed by the physical property, the mass transport property, and the biological interaction requirements of each specific application. These properties or design variables are specified by the intended scaffold application and environment into which the scaffold will be placed. For example, scaffolds designed to encapsulate cells must be capable of being gelled without damaging the cells, must be nontoxic to the cells and the surrounding tissue after gelling, must allow appropriate diffusion of nutrients and metabolites to and from the encapsulated cells and surrounding tissue, and require sufficient mechanical integrity and strength to withstand manipulations associated with implantation and in vivo existence [65]. Here, the defined physical properties include mechanical strength and gel formation dynamics, while diffusion requirements specify the mass transport properties. In addition, the biological properties are designated by the required nontoxicity. What follows are definitions and discussions

of the physical, mass transport, and biological design variables relevant to the design of hydrogel scaffolds for tissue engineering.

3.1. Physical properties

Many scaffolds for tissue engineering initially fill a space otherwise occupied by natural tissue, and then provide a framework by which that tissue may be regenerated. In this capacity, the physical properties of the material are inherent to the success of the scaffold. Specific physical properties include gel formation mechanisms and dynamics, mechanical characteristics, and degradation behavior. In hydrogels, these properties are prescribed by the intrinsic properties of the main chain polymer and the crosslinking characteristics (i.e. amount, type, and size of crosslinking molecules), as well as environmental conditions.

Gel formation mechanisms and dynamics dictate how molecules and cells are incorporated into a scaffold and how that scaffold is then delivered. Common fabrication processes and reagents such as temperature increases, pH changes, and various solvents can denature proteins [66] and cause cell damage or death [29]. One approach to bypass this issue is to process the material and create a scaffold prior to incorporating bioactive molecules and cells. However, an exciting feature of many hydrogels is their ability to be mixed with cells and molecules prior to injection and in vivo gel formation. Injectable, in vivo gelling forms of alginate [67–70], PEO [17,71], chitosan [58], and P(PF-co-EG) [26,28] have all been successfully combined with cells and/or bioactive molecules and delivered in a minimally invasive manner. The success of this approach depends on the ability to control both pre- and post-gel properties including gel formation rates and liquid flow properties.

Once the scaffold is produced and placed, formation of tissues with desirable properties relies on scaffold material mechanical properties on both the macroscopic and the microscopic level. Macroscopically, the scaffold must bear loads to provide stability to the tissues as it forms and to fulfill its volume maintenance function. On the microscopic level, evidence suggests that cell growth and differentiation and ultimate tissue formation are dependent on mechanical input to the cells [72–75]. As a consequence, the scaffold must be able to both withstand specific loads and transmit them in an appropriate manner to the surrounding cells and tissues.

Adequate mechanical performance of a scaffold depends on specifying, characterizing, and controlling the material mechanical properties including elasticity, compressibility, viscoelastic behavior, tensile strength, and failure strain. For hydrogels, these properties are affected by polymer and crosslinker characteristics, gelling conditions (e.g. temperature and pH), swelling, and degradation [76]. For example, the mechanical strength and compression modulus of alginate hydrogels increase with increasing ratios of G to M subunits [31] as well as increasing lengths of G blocks [31]. In addition, increasing the volume fraction of alginate from 1% to 3% results in an increase in both the compression modulus and equilibrium shear modulus [52]. Similar increases in compression modulus were observed for PEG gels when the weight fraction of PEG was increased from 10% to 40% [77] and for PVA hydrogels [78]. Hydrogel mechanical properties are also affected by the crosslinker type and density. The mechanical strength of ionically crosslinked alginate hydrogels increases when the ion concentration is increased and when divalent ions that have a higher affinity for alginate are used for crosslinking [31]. Similarly, the mechanical shear modulus of covalently crosslinked alginate is dependent on the crosslinker density [51]. In addition to the polymer and crosslinker characteristics, gel swelling usually results in a decrease in the mechanical strength of hydrogels [28,76,77]. However, the mechanical properties and swelling have been independently controlled in covalently crosslinked alginate hydrogels by varying both the crosslinker type and density [51]. Hydrogel degradation and dissolution usually lead to a weakening of the gels [52,77] unless tissue ingrowth acts to strengthen them [77] or these properties are decoupled [55].

The desired kinetics for scaffold degradation depends on the tissue engineering application. Degradation is essential in many small and large molecule release applications and in functional tissue regeneration applications. However, it may not be warranted if the application is related to cell encapsulation for immunoisolation. Ideally, the rate of scaffold degradation should mirror the rate of new tissue formation or be adequate for the controlled release of bioactive molecules. Thus, it is important to understand and control both the mechanism and the rate by which each material is degraded. For hydrogels, there are three basic degradation mechanisms: hydrolysis, enzymatic cleavage, and dissolution. Most of the synthetic hydrogels are degraded through hydrolysis of ester linkages [19,26,79]. As hydrolysis occurs at a constant rate in vivo and in vitro, the degradation rate of hydrolytically labile gels (e.g. PEG-PLA copolymer) can be manipulated by the composition of the material but not the environment [79]. As discussed in the materials section above, collagen, HA, and chitosan are all degraded by enzymatic action [29,62,63,64]. Synthetic linkages have also been introduced into PEO to render it susceptible to enzymatic degradation [14,15]. The rate of enzymatic degradation will depend both on the number of cleavage sites in the polymer and the amount of available enzymes in the scaffold environment [14,15]. Ionically crosslinked alginate normally undergoes dissolution [52], but can also undergo controlled hydrolysis

after partial oxidization [54]. The rate of dissolution of ionically crosslinked alginate depends on the ionic environment in which the scaffold is placed [52].

3.2. Mass transport properties

The success of scaffolds for tissue engineering are typically coupled to the appropriate transport of gases, nutrients, proteins, cells, and waste products into, out of, and/or within the scaffold. Here, the primary mass transport property of interest, at least initially, is diffusion. In a scaffold, the rate and distance a molecule diffuses depend on both the material and molecule characteristics and interactions. Gel properties such as polymer fraction, polymer size, and crosslinker concentration determine the gels nanoporous structure [31,80]. As a consequence, diffusion rates will be affected by the molecular weight and size of the diffusion species (defined by Stokes radii) compared to these pores. For example, molecules such as glucose, oxygen, and vitamin B_{12}, with molecular weights less than 1300 Da and Stokes radii less then 1 nm, are able to freely diffuse into and from ionically crosslinked alginate microspheres [81,82]. However, higher molecular weight molecules, including myoglobin, albumin, and fibrinogen are not able to freely diffuse, and their rate of diffusion is further decreased by increases in alginate concentration, in Ca^{2+} concentration, and/or in extent of gellation. The diffusion rates of molecules through glutaraldehyde crosslinked chitosan gels are also decreased when the crosslinker concentration is increased [59]. For PEO hydrogels the size and molecular weight of molecules that are able to diffuse and the rate at which they diffuse both increase as functions of increasing polymer molecular weight and hydrolyzable linkages [80]. Interestingly, for alginate and likely other charged polymers, the diffusion rates of charged molecules are not solely size-dependent [31,83]. Rather, they are also affected by charge interactions with the negatively charged alginate chains.

Ultimately, diffusion requirements and subsequent material choice depend on the scaffold application. In the case of small and large molecule delivery, limiting free diffusion out of the scaffold may be a priority [80]. In contrast, enhancing the supply of oxygen and nutrients and the removal of waste products is essential to the survival of implanted cells. In vivo, most cells exist within 100 μm of a capillary [84], and diffusion is usually adequate for cell and tissue survival over this distance. However, for larger distances, other means of transport (e.g. simultaneous angiogenesis) must be incorporated.

3.3. Biological properties

Materials used to form gels engineered to exist in the body must simultaneously promote desirable cellular functions for a specific application (i.e. adherence, proliferation, differentiation) and tissue development, while not eliciting a severe and chronic inflammatory response. Hydrogel forming polymers are generally designed to be nontoxic to the cells they are delivering and to the surrounding tissue. Both collagen and HA are major components of the native ECM and tissues [29,30]. Both should theoretically interact favorably with the body, provided that they have not been contaminated during processing and that there are no cross-species immunological issues (both are typically derived from bovine sources). PEO is currently FDA approved for many medical applications, while P(PF-co-EG) has been shown to be slightly toxic to cultured cells in vitro and to not induce a significant inflammatory response in vivo [27]. Chitosan has also been shown to be nontoxic despite its chemotaxic effect on neutrophils [62,85]. For alginate, it was thought that high M content induced an immune response, but recent data suggest that contaminants were the more likely cause of the response [86]. When purified, alginate is relatively immune quiescent; however, the purity of commercially available alginate continues to be a problem [87].

While many hydrogels are nontoxic and do not activate a chronic immune response, they also do not readily promote cellular adhesion and function. With the exception of collagen, which is a natural ECM protein, most cells do not have receptors to hydrogel forming polymers and thus cannot adhere. Furthermore, because of the hydrophilic nature of hydrogels, ECM proteins such as laminin, fibronectin, and vitronectin typically do not readily absorb to the gel surface [88]. This fact has been exploited in the application of post-operative adhesion barriers [89] and in the design of specific adhesion surfaces [88,90]. A common approach to design a highly specific adhesive surface is to covalently couple an entire ECM protein [21,88] or peptide sequences capable of binding to cellular receptors [15,88,91] to the polymer. The most common peptide used in this approach is the amino acid sequence arginine–glycine–aspartic acid (abbreviated Arg–Gly–Asp or more commonly, RGD), derived from numerous ECM proteins including fibronectin, laminin, vitronectin, and collagen [88]. Other common peptides include arginine–glutamic acid–aspartic acid–valine (REDV) (from fibronectin), tyrosine–isoleucine–glycine–serine–arginine (YIGSR) (from laminin), and isoleucine–lysine–valine–alanine–valine (IKVAV) (from laminin) [88]. Most cell types are able to bind to RGD, thus both alginate [69,91] and PEG [15,92,93] have been modified with this peptide to promote cellular adhesion (Fig. 4). In an alternative approach, PVA was modified with the complete fibronectin protein to promote cell adhesion [21].

Growth factor tethering and incorporation are other avenues available by which hydrogels can be modified to

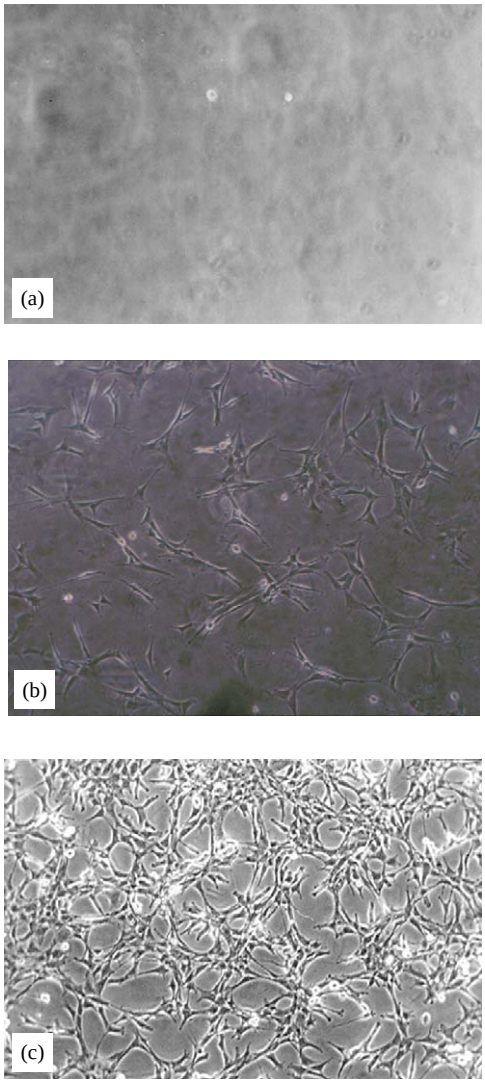

Fig. 4. Effect of coupling RGD to alginate on cell adhesion: (a) unmodified alginate seeded with C2C12 myoblasts (reprinted from [91] with permission, copyright Elsevier Sciences Ltd), (b) rat-derived calvarial osteoblasts on G_4RGDY-modified alginate (reprinted from [69] with permission, copyright IARD), and (c) C2C12 myoblasts on G_4RGDY-modified alginate (reprinted from [91] with permission, copyright Elsevier Sciences Ltd). Note: Image of rat-derived calvarial osteoblasts seeded on unmodified alginate not shown because no adhesion occurred [91], as in (a) with C2C12 myoblasts.

regulate the functions of interacting cells. As with adhesion proteins and peptides, growth factors and growth factor derived peptides have been covalently attached to hydrogel polymers. For example, transforming growth factor-β (TGF-β) was tethered to PEG to enhance smooth muscle cell ECM production [93]. Alternatively, an oligopeptide derived from bone morphogenetic protein-2 (BMP-2) was covalently attached to alginate to promote osteoblast migration into gels and subsequent calcification of the scaffolds [94]. Multiple factors may also be incorporated into hydrogels to manipulate tissue formation [95].

4. Hydrogel applications

Hydrogels have many different functions in the field of tissue engineering. They are applied as space filling agents, as delivery vehicles for bioactive molecules, and as three-dimensional structures that organize cells and present stimuli to direct the formation of a desired tissue. Space filling agents are the simplest group of scaffolds and are used in a variety of applications, including bulking, adhesion prevention, and as a biological "glue". In addition, bioactive molecules are delivered from hydrogel scaffolds in a variety of applications including promotion of angiogenesis and encapsulation of secretory cells. Finally, hydrogel scaffolds are being applied to transplant cells and to engineer nearly every tissue in the body, including cartilage, bone, and smooth muscle.

4.1. Space filling scaffolds

Space-filling agents encompass scaffolds that provide bulking, prevent adhesions, or function as bioadhesives. In this capacity, the most basic design requirements for a hydrogel are the abilities to maintain a desired volume and structural integrity for the required time. As a bulking material, these implants are used to treat conditions such as urinary incontinence [96–99] and vesicoureteral reflux [100,101], and are needed for both plastic and reconstructive surgery [102]. They have also found great utility in preventing post-operative adhesions [89,103–107] and can function as biological adhesives for soft tissues [61,108].

Scaffolds composed of alginate, chitosan, and collagen show potential for use as general bulking agents. Porous scaffolds of RGD modified alginate have been successfully implanted into rats with minimal immune response, little encapsulation, and good tissue ingrowth [102]. Similar studies were completed for porous chitosan scaffolds implanted in mice [57]. Again, a minimal immune response and little implant encapsulation were noted. However, tissue ingrowth was limited to the edges of the scaffold. Glutaraldehyde crosslinked collagen scaffolds have been applied with mixed success as bulking agents for urinary incontinence [30,96,97,98,109]. Most problematic is the need for successive injections to maintain functionality. Assuming that an adequate volume of the bulking agent was properly placed, the need for additional injections is likely related to partial degradation of the collagen by collagenase. Crosslinking by glutaraldehyde partially protects against this degradation, but does not completely inhibit it. In addition, while there is a small amount of vascular invasion, these scaffolds remain relatively isolated from the surrounding tissue. Recently, a dextranomer/HA copolymer was employed as a bulking agent for vesicoureteral reflux [101]. Here, only a single injection

was necessary despite the fact that dextranomer/HA copolymer is biodegradable. Apparently, deposition of collagen due to the inflammatory response to the material acted to stabilize the implant volume.

Synthetic hydrogels are often appropriate materials for use as anti-adhesives because cells lack adhesion receptors to them and proteins often do not readily absorb to them if designed appropriately. PEG has been used to prevent post-operative adhesions [89,103–107] and to protect arteries from intimal thickening after damage [110,111]. PVA has also been used as an adhesion shield in tendon regeneration [112]. In both rat and rabbit models, photocrosslinked PEG reduced the extent of adhesions [89,103,104]. In these cases, the hydrogel was resorbed within 5 days of application. In a second approach, PEG was grafted to poly L-lysine (PLL) to form a copolymer (PLL-g-PEG) that would theoretically adhere to the tissue on one side and provide a nonadhesive brush-like surface on the other [107]. Here, the polymer liquid was dripped on the site of interest, but not gelled.

In contrast to anti-adhesives, biological adhesives are used in surgical procedures to seal small wounds out of which air and body fluids could leak, and to improve the effectiveness of wound dressings. Fibrin glue has commonly been used in this role, but hydrogels composed of chitosan [61] and chitin derivatives [108] are now being proposed for use as biological adhesives. Photocrosslinked chitosan gels appear to be nontoxic in vitro and have been effectively used to seal pinholes in the small intestines, aorta, and trachea of mice [61].

4.2. Scaffolds for bioactive molecule delivery

In a quite different application from space-filling agents, hydrogel scaffolds are also often utilized to stabilize and deliver bioactive molecules and encapsulate secretory cells. Currently, the majority of small and large molecule drugs are delivered into patients systemically (e.g. oral or intravenous delivery) without the use of a scaffold. Consequently, large doses are usually required for a desired local effect because of enzymatic degradation of the drug and nonspecific uptake by other tissues. This is not only costly, but can result in serious side effects. For example, systemic delivery of large amounts of vascular endothelial growth factor (VEGF), which is intended to locally promote angiogenesis, may lead to serious side-effects including neovascularization of nontarget tissues, expansion of atherogenic plaques, and growth of tumors [113,114]. In addition, many factors, which are necessary or beneficial to one tissue may be toxic for other tissues. Thus, a vehicle or scaffold allowing for local and specific delivery to the desired tissue site is highly desirable in many situations.

Several hydrogel systems currently exist in which proteins are successfully incorporated into a scaffold and then released. One of the most extensively studied proteins is the angiogenesis promoting growth factor, VEGF. VEGF has been incorporated into ionically crosslinked alginate hydrogels [115–118] as well as glutaraldehyde crosslinked collagen sponges [119]. It is released from alginate scaffolds both by diffusion [115,117] and by mechanical stimulation [116,118], and from collagen gels by hydrogel degradation [119]. Interestingly, the bioactivity of VEGF delivered from alginate microspheres has been shown to be greater than VEGF delivery alone [115], likely due to stabilization of the factor via the alginate interaction. The efficacy of this system has been demonstrated both in vitro [115] and in vivo (Fig. 5) to drive angiogenesis around the implant site [116–119]. Another angiogenesis promoting protein, basic fibroblast growth factor (bFGF), has also been released in a sustained manner from heparin-alginate gels [120–123] to enhance angiogenesis.

Osteogenesis promoting bone morphogenic proteins (BMPs) have also been delivered from various hydrogel scaffolds. For example BMP-2 has been photocrosslinked into PLA-PEG and PLA-DX-PEG systems and released as the polymer degraded [79]. Here, in vivo

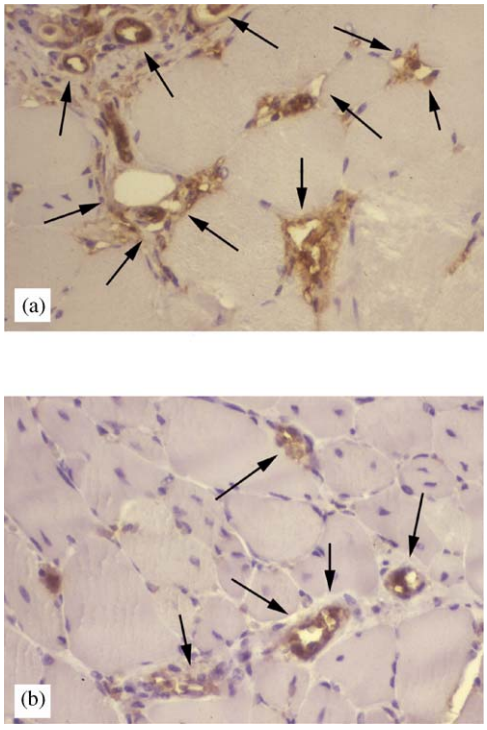

Fig. 5. VEGF release from alginate scaffolds resulting in angiogenesis: (a) with mechanical stimulation of alginate gels and (b) without mechanical stimulation of alginate gels. Arrows indicate blood vessel formation in the muscle tissue surrounding the implanted gels (reprinted from [116] with permission, copyright Macmillan Magazines Ltd).

results showed ectopic bone formation in PLA-DX-PEG scaffolds containing at least 0.5 μg of BMP-2 compared to no bone formation in scaffolds with 0.1 μg of BMP-2 and complete resorption of blank scaffolds. Ectopic bone formation has also been noted in PLA-PEG scaffolds containing at least 1 μg of BMP-2. Porous hyaluronic scaffolds can also be utilized for BMP-2 delivery [124], and in vitro results suggested that the BMP-2 was continuously released for controlled times in an active form.

A variety of other hydrogel systems have been previously used or are in development for drug delivery. These include thermal setting, degradable PEO-PLLA gels [17], thermally reversible PEG-PLLA gels [18], glutaraldehyde-crosslinked chitosan microspheres [59], and PEG bilayers [125].

Cell encapsulation for the express purpose of utilizing the cells to secrete a molecule of interest is an alternative and appealing method for long-term controlled, sustained drug delivery. Currently, most enzyme and hormonal deficiencies are treated by either oral administration or injection of the missing substance. Cell encapsulation offers a method by which a substance can be released over long periods of time, in a manner responsive to the needs of the body. To date, a variety of primary cells including pancreatic islets [65,87,126–128], hepatocytes [65], and adrenal cortical cells [65] as well as cells transfected to express specific enzymes and growth factors [129,130] have all been encapsulated using hydrogels.

Ionically crosslinked alginate has been the hydrogel material of choice for cell encapsulation because it is relatively nondegradable, cells can be easily mixed with it prior to gelling, and it is gelled under mild conditions [65]. Various Ca^{2+} or Ba^{2+} crosslinked alginate microspheres [87,128,129,130] and PLL coated alginate microcapsules have been utilized [65,126,129]. In addition, PEG has been applied in some instances to coat alginate microcapsules [131,132] and in other cases to directly encapsulate cells [127,133]. Islets microencapsulated in ionically crosslinked alginate have been successfully immunoisolated and have released sustained levels of glucose-sensitive insulin, allowing for xenotransplantation of islets [65,87]. Similar results were obtained for islets encapsulated with photocrosslinked PEG [127]. However, diffusion limitations, especially of oxygen, decrease cell viability in the center of the capsule and restrict the capsule size. In addition, fibrotic encapsulation of the alginate microcapsules, over time, has rendered them impermeable, resulting in loss of insulin release and cell death [87,132,134]. One approach pursued to limit fibrotic encapsulation was to coat alginate-PLL microspheres with an additional layer of photocrosslinked PEG [132]. Another study has suggested that alginate impurities are the primary cause of the overgrowth, and purification has led to prolonged functioning of the encapsulated islets [87] without additional coatings. Finally, an attempt to increase oxygen diffusion was made by immobilizing hemoglobin in the alginate matrix of the microcapsule [128]. In general, this approach to drug delivery has great potential as a method to treat diabetes as well as a range of diseases involving other hormonal and enzyme deficiencies.

4.3. Scaffolds for cell delivery

Hydrogel scaffolds are appealing for cell delivery and tissue development because they are highly hydrated three-dimensional networks of polymers that provide a place for cells to adhere, proliferate, and differentiate. They can also provide chemical signals to the cells through the incorporation of growth factors and mechanical signals by manipulation of the mechanical properties of the material. Currently, hydrogel scaffolds are being used in an attempt to engineer a wide range of tissues, including cartilage, bone, muscle, fat, liver, and neurons.

Hydrogels have a similar macromolecular structure to cartilage, which is a highly hydrated tissue composed of chondrocytes embedded in type II collagen and GAGs. Thus, cartilage is an obvious tissue to engineer using hydrogel scaffolds. To date, numerous hydrogel scaffolds embedded with chondrocytes have been synthesized and tested both in vitro and in vivo. In vitro studies have assessed the effects of scaffold mechanical strength, growth factor delivery, and scaffold materials on the phenotypic state of the embedded chondrocytes. Photocrosslinked PEO [77] and freeze-dried chitosan scaffolds [32] possess high moduli ($\sim$500 kPa), and studies suggest that stiffer matrices keep chondrocytes in a proliferative rather than differentiated phenotype, resulting in decreased collagen II deposition [33,77]. Dual release of insulin-like growth factor (IGF-I) and TGF-β also maintained chondrocytes in a proliferative state [95]. Conversely, relatively weak gels consisting of HA containing alginate and HA alone, promoted a differentiated chondrocyte phenotype and expression of type II collagen [47,135]. Similar studies also suggested that the presence of HA is sufficient to increase both chondrocyte proliferation and protein secretion [47,136]. In general, initial proliferation is important to ensure an adequate cell population, but ultimate cartilage formation depends on synthesis of GAGs and type II collagen. Overall, each of these studies address important features which may be incorporated into scaffolds to promote and control the formation of cartilage.

Alginate has been used more widely than other hydrogels to assess the in vivo potential of hydrogel scaffolds for cartilage engineering. In several studies, it has been mixed with chondrocytes and either injected into the site of interest [67,99,137] or molded and then

implanted [138]. Results of animal studies indicated that the chondrocytes were viable and producing ECM proteins consistent with cartilage as early as four weeks after implantation [67,137–139]. Mechanically, the implanted constructs were weak, achieving a compression modulus that was only about 15% that of native cartilage [138]. They also had a hydraulic permeability that was nearly 20 times that of the natural tissue [138]. In human studies, an alginate/autologous chondrocyte mixture was injected as a treatment for urinary incontinence and the majority of patients ($\sim$80%) showed improvement after a single injection [99]. This contrasts to results using collagen alone in this application, as it requires repeat injections to achieve long-term effect [98]. While this study did not directly demonstrate new tissue formation, animal studies have demonstrated tissue formation [137].

Hydrogel scaffolds are also being widely used in the area of nonload bearing bone tissue engineering. In general, hydrogels do not possess the mechanical strength to be used in load bearing applications, but can be placed into critical defects in a minimally invasive manner to promote regeneration, which would not otherwise occur. Both alginate and HA have been used in this application. For example, the degradable form of high G monomer alginate, PAG, was mixed with primary rat calvarial osteoblasts, gelled, and injected into the backs of immunodeficient mice [70]. In this system, mineralized bone was observed to form over a period of 9 weeks. However, due to transport limitations, this mineralization was limited to the outer 1/3 of the implant. Similarly, a Hyaff®11 (commercially available HA) scaffold was seeded with bone marrow stromal cells (BMSCs) and implanted into a rat model critical radial defect, leading to defect mineralization and healing [140]. This response was enhanced via preincubation of BMSCs with bFGF. Little to no healing or mineralization was observed when the defect was left alone and when a blank scaffold was implanted.

In both of the aforementioned examples, little effort was made to specifically control cell fate with the scaffold. Rather, these scaffolds were used as cell delivery vehicles. Alginate scaffolds have also been designed that contain covalently bound adhesion and signaling peptides to specifically influence cell fate and bone formation. The presence of the adhesion peptide RGD significantly increased the amount of bone formation in an alginate scaffold (Fig. 6) compared to gels without covalently bound peptide [69]. Another study indicated that a BMP-2 derived oligopeptide promoted ectopic bone formation when immobilized in alginate gels [94]. These studies clearly indicate that cell interaction properties of hydrogels can be designed to enhance and possibly direct in vivo bone formation.

Significant effort has also been directed to engineer vascular smooth muscle with PEG hydrogels. Photocrosslinked PEG scaffolds incorporating adhesion proteins [15,93], enzymatic degradation sites [15], and/or growth factors [93] have been utilized to increase collagen production by smooth muscle cells, as compared to unmodified PEG hydrogels. Additionally, incorporation of enzymatic degradation sites, allowed cells to migrate through the gel and produce more collagen than cells in nondegrading gels. Theoretically, there exists great potential to create functional vascular smooth muscle using these scaffolds in conjunction with external mechanical signals, which have been shown to enhance the mechanical and functional properties of engineered smooth muscle [72].

Beyond scaffolds for engineering bone, cartilage, and smooth muscle, hydrogels are being investigated to engineer nearly every other tissue in the body. For

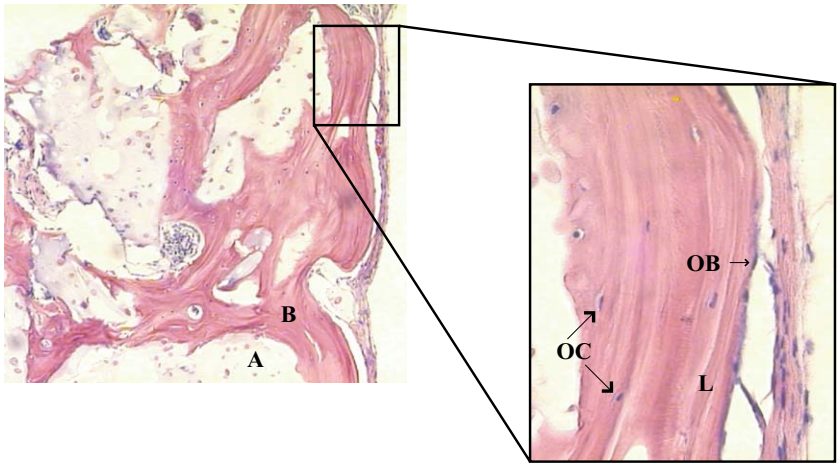

Fig. 6. Hematoxylin and eosin stained sections demonstrating bone formation at 24 weeks: G_4RGDY-modified alginate (A), newly formed bone (B), osteoblasts (OB), osteocytes (OC), and lamellae (L) (reprinted from [69] with permission, copyright IARD).

example, both collagen [141–143] and peptide modified alginate [144] have been employed as potential scaffolds for skeletal muscle engineering. In addition, preadipocyte seeded, freeze-dried collagen sponges have been explored as a means to engineer adipose tissue [145]. Collagen has been widely used for engineering large blood vessels [146]. Alginate hydrogels also show potential as Schwann cell matrices in the area of nerve grafting [147] and as scaffolds to promote hepatocyte function and synthesis of liver specific proteins [148,149]. As knowledge about these materials and the factors surrounding tissue development increases, so do the possible approaches to designing scaffolds to better engineer a tissue of interest.

5. Summary and future directions

The success of many space-filling agents, bioactive molecule delivery vehicles, and tissue constructs is highly dependent on the design of the scaffold. That design, in turn, depends on both the tissue as well as the environment in which the tissue resides. For example, when one desires to engineer bone or cartilage, a key issue is the magnitude of load bearing required from the new tissue. Similarly, the desired target of a bioactive molecule dictates the delivery mode and thus, the appropriate hydrogel. Accordingly, the numerous design requirements and variables, as well as the interactions between these variables, create an enormous potential design space. Consequently, many materials utilized to date have been used because they meet one requirement (e.g. cells can easily be mixed with the gel) without regard for other design parameters (e.g. degradation or mechanical properties).

Further material development will likely have a great impact on tissue engineering. As our understanding of the biological process of tissue development and healing expand, this information must be incorporated into the scaffolds such that specific signals are delivered in the appropriate spatial and temporal manner. In addition, more consideration needs to be made regarding the influence and requirements of external mechanical and electrical signals on development of engineered tissues. As discussed, some of this type of new material development is already underway. For example, novel methods to control material degradation have been engineered into many hydrogels. In addition, cell adhesion ligands have been attached to these materials, and growth factors have been incorporated into them to specifically regulate cell fate. Various methods of crosslinking have also been implemented, both to enhance the material biocompatibility as well as control the mechanical properties. Similarly, pre and post processing methods have been developed to control porosity, improve diffusion, and gently incorporate cells into the scaffold. Each of these advances has the potential to improve scaffolds and thus, support the development of more natural and functional tissues.

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