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Polymeric Scaffolds for Cartilage Tissue Engineering

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Summary: Polymeric scaffolds are three-dimensional, porous structures that may be used as a vehicle to deliver cells or therapeutic factors to repair tissue defects. Both biodegradable and non-biodegradable polymers have been developed for this purpose. In this review, we survey the polymers that have been investigated for cartilage tissue engineering and discuss the critical parameters for successful applications in the future.

Keywords: biodegradable; biomaterials; cartilage tissue engineering; matrix

Introduction

Tissue engineering has been recognized for some time as a promising alternative to the use of autografts or allografts for tissue reconstruction and regeneration. The approach utilizes cells, biomaterial scaffolds, and signaling molecules for the repair of disease or trauma-related tissue defects. The biomaterial scaffold serves as a three-dimensional graft to deliver either cells or bioactive factors to the defect site, or to function as a space filler to recruit surrounding host cells to conduct the repair process. Articular cartilage defects that result from traumatic injury or from degenerative joint diseases, such as osteoarthritis, present a major challenge in terms of tissue repair. For cartilage regeneration, a large quantity of chondrocytes or chondroprogenitor cells are required to fill the structural defect site. Furthermore, these cells need to be exposed to a chondrogenically favorable environment. In particular, it is important that the cells are seeded at a high density, to simulate the cell condensation process that takes place during embryonic cartilage development. The cell condensation process increases cell-cell contact, a prerequisite for chondrogenic differentiation. Subsequently, a cartilage-specific extracellular matrix (ECM) is produced by the chondrocytes, that acts as the mechanical support for cartilage tissue, as well as regulating cellular activities.[1] A principal objective of scaffold design for cartilage tissue engineering is a structure that can simulate and replace the natural

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ECM, until cells seeded within the scaffold and/or derived from the host tissue can synthesize a new, natural matrix. Some of the critical parameters for tissue engineering scaffold include biodegradability, non-toxicity, promotion of cell attachment, optimal mechanical strength, and ability to regulate appropriate cellular activities.

A large number of polymeric biomaterials have been used for cartilage engineering applications, including non-biodegradable and biodegradable polymers, the latter consisting of both natural and synthetic polymers. Since non-biodegradable polymers would interfere with tissue turnover and remodeling, the current trend in cartilage tissue engineering is to use biodegradable polymers, although the non-biodegradable polymers have the advantage that their chemical and mechanical properties are not affected by degradation. On the other hand, polymer biodegradation, by the combined effect of enzymatic and hydrolytic activities, generates space within the scaffold to accumulate cell proliferation and the deposition of newly synthesized ECM. Ideally, optimal tissue regeneration occurs upon complete biodegradation of the polymeric matrix, and biological functions are thus restored. For cartilage tissue engineering, polymer scaffolds consisting of hydrogels as well as structures of defined shape and porosity have been used. This review surveys the current state of polymer applications in cartilage tissue engineering.

Non-biodegradable polymers

Poly(vinyl alcohol) (PVA) – PVA is a biocompatible hydrogel and has a long history of being used for cartilage regeneration applications.[2] PVA can be engineered to have similar water content as native cartilage, and molded into a scaffold with desired shape, or incorporated with another material to form an osteochondral construct.[3] However, like other gel materials, PVA hydrogels suffer from intrinsic weak mechanical strength, which may be improved by alteration of the synthetic process.[4] A PVA hydrogel scaffold, modified to yield improved mechanical properties, showed promise in the repair of cartilage defects *in vivo*.[5]

Polyethylene (PE) – PE has been widely accepted for biomaterial applications, e.g., as liner material for joint prostheses. Although PE of different molecular weights have been fabricated into three-dimensional, porous structures for the repair of osteochondral defects *in vivo*,[6] the extent of cartilage formation in the scaffold was not satisfactory.

Polyacrylates – Currently, poly(2-hydroxyethyl methacrylate) (pHEMA), poly(ethyl methacrylate) (pEMA), poly(tetrahydrofuofurfuryl methacrylate) (pTHFMA), and poly(methyl methacrylate) (pMMA) have been used as biomaterials. To improve their mechanical properties for cartilage tissue engineering, different polyacrylates have been crosslinked.[7] Using pHEMA/MMA hydrogel, cartilage was formed at the repair site, but the rebuilt tissue was more compliant than the surrounding tissue.[8] Sawtell et al.[9] reported the cartilage inductive property of pEMA/THFMA and found higher GAG accumulation in pEMA/THFMA constructs compared to controls *in vitro*. Reissis et al.[10] reported hyaline cartilage regeneration at the defect site by using pEMA/THFMA hydrogel to repair subchondral defects in rabbits.

Poly(N-isopropylacrylamide) (pNIPAAm) – pNIPAAm is a thermo-sensitive hydrogel which exists in a liquid form below 32°C, the lower critical solution temperature (LCST), but undergoes solidification at 37°C, a useful feature that has been exploited for tissue engineering. Unlike other hydrogels needing either initiator or light for gelation, pNIPAAm polymerization can be achieved *in vivo* at body temperature. pNIPAAm is usually crosslinked with other polymers, such as acrylic acid, to minimize the expulsion of a large fraction of pore water resulting from structural collapse during phase transition. Several studies[11-12] reported that chondrocytes cultured in the pNIPAAm-based scaffold maintained their phenotype, with the production of a cartilage ECM, and showed regenerative activity for cartilage defects *in vivo*.

Natural biodegradable polymers

Two groups of biocompatible natural polymers have been used for cartilage tissue engineering: protein-based and carbohydrate-based polymers. Protein-based polymers include collagen and fibrin, and carbohydrate-based polymers include alginate, agarose, hyaluronan, and chitosan.

Collagen – Scaffolds based on collagen, the most abundant protein family in the body, have been extensively used to load chondrocytes[13] or mesenchymal stem cells (MSCs)[14] for *in vitro* and *in vivo* cartilage tissue engineering. Among the at least 19 different collagen types, collagen type I is the major collagen in connective tissues and collagen type II is exclusively abundant in hyaline cartilage. Both collagen types I and II have been used to repair cartilage defects[15-16] *in vivo* and collagen type II has been shown to be a more suitable scaffold material for neocartilage formation than collagen type I, because of better maintenance of chondrocytic phenotype.[17]

Fibrin – Produced by the enzymatic cleavage of fibrinogen, fibrin has been used as a clinical fixative due to its natural role in wound healing. Fibrin has recently attracted scientific attention for use as a scaffold to deliver chondrocytes,[18] MSCs,[19] or growth factors[20] for cartilage tissue engineering, including repair of articular cartilage defects.[21] Despite these promising results, the inherent poor mechanical properties and the tendency to trigger immune response in the host may limit the use of fibrin gel for scaffold applications.

Alginate – A brown algae-derived carbohydrate polymer consisting of repeating L-guluronic acid and D-mannuronic acid, alginate is polymerized in the presence of calcium or other divalent cations, and is reversibly depolymerized upon removal of the cations. Such a property makes alginate suitable for encapsulating chondrocytes and growth factors for delivery. Encapsulation in alginate forces cells to round up, and favors maintenance of chondrocytic phenotype[22] or induction of chondrogenic differentiation of MSCs.[23] While there have been reports of successful cartilage regeneration using alginate-chondrocytes,[24] a potential complication of using alginate for tissue engineering is that its incomplete degradation may induce immune response.[25]

Agarose – Another carbohydrate polymer, agarose, is purified from brown seaweed, and contains a repeated disaccharide sequence of L- and D-galactose. Like other gel-formatted scaffolds, agarose is injectable and has been used to redifferentiate chondrocytes.[26] While there are recent *in vitro* studies utilizing MSCs embedded in agarose for chondrogenesis,[27-28] the *in vivo* application of agarose is limited because of its foreign body giant cell reaction[29] and poor degradation.[30]

Hyaluronan – The most commonly used carbohydrate-based natural polymer is hyaluronan (hyaluronic acid), a polysaccharide composed of repeating glucuronic acid and N-acetylglucosamine. However, as a physiological component of the cartilage ECM, natural, unmodified hyaluronan is hydrophilic. A hyaluronan-based scaffold is unstable and has inherently limited application in cartilage tissue engineering. Thus, approaches have been developed to increase the resistance of hyaluronan to degradation, including esterification,[31] and cross-linking.[32] A number of *in vitro* and *in vivo* studies have reported chondrocyte proliferation and synthesis of cartilaginous ECM using these modified hyaluronan scaffolds.[33-34]

Chitosan – Chitosan is derived from chitin, a polysaccharide formed in shellfish exoskeleton, and is composed of repeating glucosamine and N-acetylglucosamine disaccharides. Chitosan carries a high cationic charge density and can interact with a variety of anionic polymers such as chondroitin sulfate to form a hydrogel.[35] Because of its favorable biocompatibility and ease of processing in three-dimensional structure, chitosan is considered a candidate biomaterial for tissue engineering scaffolds. Chondrocytes cultured on chondroitin sulfate-augmented chitosan gel *in vitro* maintained their chondrocytic morphology and produced collagen type II,[36-37] and chitosan injection appeared to enhance the repair of rat knee cartilage.[38]

Chondroitin Sulfate – Chondroitin sulfate, a glycosaminoglycan and a major component of cartilage proteoglycan, is a desirable material for cartilage tissue engineering because of its biocompatible chemistry and integral role in cartilage macrostructure.[39-40] Chondroitin sulfate monomers are usually modified with methacrylate to produce a reactive side group for photopolymerization reaction to generate a crosslinked chondroitin sulfate gel. *In vitro* studies have demonstrated the viability of encapsulated chondrocytes in this crosslinked gel, suggesting potential applications for delivery or culture of cells in cartilage regeneration. Recent studies have shown an improvement in the stability of the chondroitin sulfate hydrogel in tissue culture by incorporating non-biodegradable poly(ethylene glycol) (PEG)[39] and PVA[40] to produce a co-gel, in which the negatively charged chondroitin sulfate regulates the water content and mechanical properties of this co-gel, a property that mimics the cartilage matrix *in vivo*.

Synthetic biodegradable polymers

Poly(α-hydroxy esters) – These are the most commonly used synthetic polymers in cartilage tissue engineering, because of their biodegradability and FDA approval for clinical use. The most commonly used poly(α-hydroxy esters), poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymer poly(lactic-co-glycolic acid) (PLGA), have been investigated for use in cartilage tissue engineering. PGA exhibits high crystallinity, is hydrophilic and sensitive to its environment, and tends to degrade in aqueous solution. Complete degradation of PGA results in glycolic acid, a natural metabolite. Two hypothetical metabolic fates for PGA *in vivo* are: (1) PGA is converted into CO_2 and water, subsequently removed by the respiratory and urinary system, respectively; and (2) PGA is first converted into glyoxylate and then glycine. These metabolic pathways result in non-toxic end products. PLA, with the addition of a methyl group, is more hydrophobic and more soluble in organic solvents, and degrades more slowly than PGA.[41] The three stereoisomers of PLA [D(-), L(+), and D, L] differ in the position of a methyl group in the lactic acid monomer, and exhibit distinct properties. For example, PLLA has a higher melting temperature than PDLLA because of its higher order crystal structure. On the other hand, PDLLA, composed of L and D stereoisomers, degrades faster than PLLA. Lactic acid is the end product of PLA after degradation and can be metabolized via the tricarboxylic acid cycle into CO_2 and water.[42] Generally speaking, PGA is hydrophilic and rigid, whereas PLA is flexible and slowly degrades; these two polymers can be copolymerized in different ratios to from a new polymer, PLGA, to optimize mechanical properties and degradation. The PLGA copolymer has an amorphous structure because PGA and PLA are not packed tightly, and exhibits a faster degradation rate compared to either PGA or PLA alone, although the degradation mechanism is identical. Another family member in the poly(α-hydroxy ester) group is poly (ε-caprolactone) (PCL), a semicrystalline biodegradable polymer. Compared to other polyester family members such as PLA, PGA, and PLGA, PCL has been used less frequently as a material for fabricating biomaterial scaffolds, mainly because of concern over its slower degradation kinetics. However, PCL may be suitable for applications such as long-term drug delivery[43]; in addition, its mechanical properties and degradation profile can be modified by blending or copolymerizing PCL with other polyesters.[41]

The use of poly(α-hydroxy esters) in cartilage tissue engineering applications has been reported since the early 1990's. Unlike most natural polymers fabricated into gel-based scaffolds, poly(α-hydroxy esters) are usually made into pre-fabricated forms, such as fibers or foam, before implantation into cartilage. Both *in vitro* and *in vivo* studies have shown the maintenance of chondrocyte phenotype in PLGA and PGA scaffolds.[44-45] It has been suggested that effective hyaline cartilage regeneration *in vivo* could be achieved by short term *in vitro* chondrocyte-PGA cultures, followed by long-term *in vivo* implantation.[46-47] The rationale is that, in conjunction with PGA degradation, a mechanically stable ECM structure is first secreted *in vitro* as a result of native ECM macromolecules deposition. PLA has also been used in cartilage tissue engineering and scaffolds made of this material degrade more slowly, thereby minimizing burst of acid release and maintaining structural mechanics.

Research done in our laboratory has recently involved the development of poly(α-hydroxy ester) based electrospun nanofibrous scaffolds as a three-dimensional structure for cell-based tissue engineering. Several types of poly(α-hydroxy esters) have been successfully fabricated into nanofibrous scaffolds (Figure 1). In *in vitro* studies,

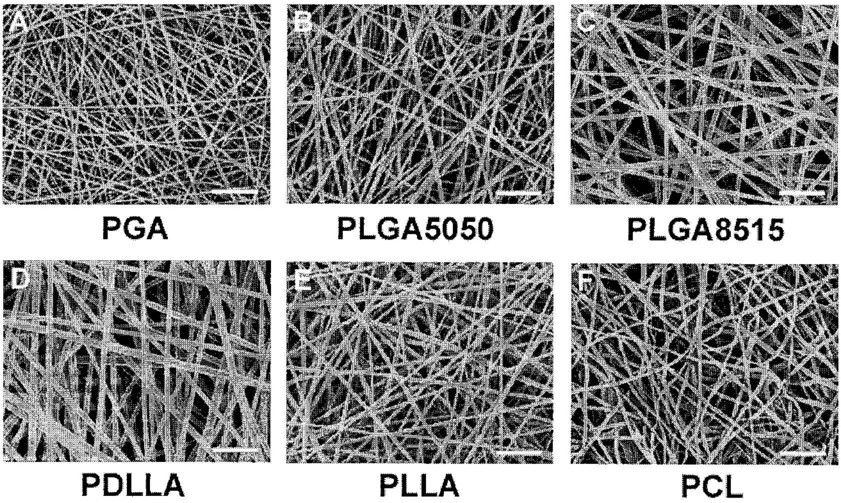

Figure 1. Scaning electron micrographs of electrospun poly(α-hydroxy ester) nanofibrous structures composed of randomly oriented ultra-fine fibers. (A) poly(glycolic acid) (PGA), (B) poly(D,L-lactic-co-glycolic acid 50:50) (PLGA5050), (C) poly(D,L-lactic-co-glycolic acid 85:15) (PLGA8515), (D) poly(D,L-lactic acid) (PDLLA), (E) poly(L-lactic acid) (PLLA), and (F) poly(ε-caprolactone) (PCL). Bar, 5 μm.

chondrocytes cultured in PLLA (Figure 2) or PCL nanofibrous scaffolds maintained their chondrocytic phenotype[48], and MSCs in the same structure were successfully induced to differentiate into chondrocytes.[49] In an *in vivo* study, autologous chondrocyte-loaded

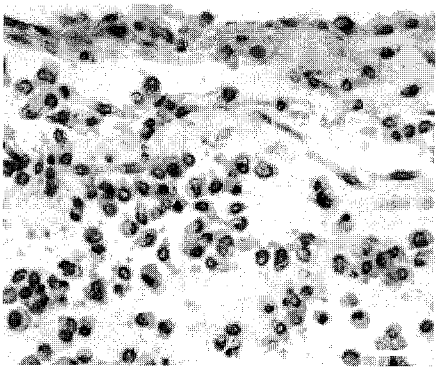

Figure 2. Histology of chondrocyte-PLLA nanofibrous scaffold cultures maintained in a chondrogenic medium supplemented with TGF-β1 for 28 days. Hematoxylin and Eosin staining showed round chondrocyte-like cells embedded in the scaffold. Bar, 25 μm.

PCL nanofibrous scaffolds successfully repaired full defects on pig femoral condyles (unpublished observation). Our results have also shown that, among the poly(α-hydroxy esters), PLLA and PCL based nanofibrous scaffolds exhibit the most optimal structural integrity in culture *in vitro*, suggesting their potential for cartilage tissue engineering application. Interestingly, the nanofibrous PLLA scaffold was a significantly more effective matrix compared to the microfibrous PLLA scaffold. Most recently, we have optimized the specific dimensions and mechanical properties of chondrocyte-loaded nanofibrous composites by using a dynamic horizontal axis rotation bioreactor culture system.[50]

Polyethylene oxide (PEO) – The capability of PEO to gel in situ is a useful property for cartilage tissue engineering, since a PEO gel, mixed with a desired cell type, can be directly injected into an irregular shaped defect site.[51] Recent efforts to improve the inherent, poor mechanical properties of the PEO hydrogel include UV crosslinking PEO with various acrylates. Elisseeff et al.[52] showed the *in vivo* formation of neocartilage in gels containing different concentrations of PEO dimethacrylate photopolymerized in situ. Currently, a major limitation is the depth of the injection site that permits sufficient light energy to photopolymerize the PEO gel. Different methodologies to polymerize PEO are being developed to overcome this problem.[53]

Poly(ethylene glycol) (PEG) – Because of their biocompatibility and non-immunogenicity, PEG hydrogels have been used in drug delivery devices and recently examined for cartilage tissue engineering. Unmodified PEG hydrogels, produced by γ-irradiation or electron beam irradiation, are not biodegradable, whereas chemically modified PEG hydrogels, produced by coupling with copolymers containing ester linkages, are biodegradable via hydrolytical activities. Various biomaterials, including natural and synthetic polymers, have been incorporated with PEG to form copolymer grafted PEG hydrogels.[54-55] In recent studies, ECM deposition by chondrocytes cultured in PEG hydrogels was controlled by hydrogel degradation,[56] and effective generation of cartilage tissue was observed.[57-58]

Conclusion

In tissue engineering, the three-dimensional, polymeric scaffold serves as a template to shape, guide, and protect the regenerated tissue. Traditionally, the properties of a tissue-engineered construct have been largely dictated by the polymeric scaffold. Current scaffold development aims to incorporate multiple polymers of different nature to design scaffolds that are able to respond to the biological activities of the seeded cells and the requirements of host tissue site. It is expected that modern polymer technologies will continue to enhance the performance of polymer scaffolds for cartilage tissue engineering.

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