Chapter 4

Injectable Hydrogels for Cartilage Tissue Engineering

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Summary

Current clinical practices to treat damaged cartilage include the use of tissue transplants and prosthetic implants. While these surgical procedures restore joint function they can also present long-term complications. Tissue engineering is a discipline that focuses on the creation or regeneration of tissues that presents an alternative to cartilage replacements. Through the integration of engineering and the life sciences, tissues can be generated using scaffolds that support encapsulated cells and their production of tissue matrix. Hydrogels are a class of materials used for scaffold development and have been proven to sustain cellular and tissue function. Hydrogels have also been implanted in vivo through minimally invasive techniques, providing therapeutic treatments without invasive surgical procedures. This chapter presents an introduction to tissue engineering and hydrogels with a focus on cartilage repair, followed by reviews on selected polymers that may be used to synthesize hydrogels. A study of the biological response of stem cells to a synthetic-biological composite hydrogel is also presented. The composite was synthesized by photopolymerization of a liquid composition of poly(ethylene glycol)-diacrylate and Cartrigel, an extract of cartilage. Bone marrow-derived mesenchymal stem cells (MSCs) were encapsulated in the hydrogels and cultured with a chondrogenic stimulus, TGF-β3. Results indicated that the presence of Cartrigel altered MSC genetic expression and matrix production. Synthetic-biological composite hydrogels therefore demonstrated the ability to support MSC function and affect cell response to extracellular bioactive factors.

Keywords: Biomaterials, Cartilage, Extracellular Matrix, Hydrogels, Tissue Engineering

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Introduction

Cartilage loss from disease or trauma is a significant medical problem in the United States (1). Current clinical treatments for cartilage injury often involve surgical interventions to remove affected tissues and insert transplanted cartilage or prosthetic devices as replacements. While these procedures may provide relief from pain and restore joint mobility, they can present long-term complications (2-5). Tissue engineering, a discipline combining engineering and the life sciences, presents a potentially effective method of treating cartilage damage. The aims of tissue engineering are to restore tissue structure and function. Conceptually, there are three general components to tissue engineering systems: (1) isolated cells that form tissue matrix, (2) biomaterial scaffolds that function as carriers to promote cell activity and tissue production, and (3) bioactive factors that regulate and induce cellular behavior in a controlled manner (6, 7). There are numerous approaches to using the above mechanisms for various tissue applications, either as individual components or in a combined system.

Significant research efforts have been performed using biomaterials seeded with cells for tissue engineering. Hydrogels are an example of a scaffold with the ability to encapsulate cells and have demonstrated potential for cartilage repair (8-12). The benefits of hydrogels for tissue development and clinical usage are especially evident in injectable systems. This chapter presents a discussion on injectable hydrogels with a brief overview of hydrogels in tissue engineering and a study involving the use of a novel injectable synthetic-biological composite hydrogel for cartilage repair.

Overview

Hydrogels in Tissue Engineering

Tissue engineering has been defined as an "interdisciplinary field that applies the principles of engineering and the life sciences to the development of biological substitutes that restore, maintain, or improve tissue function" (13). A general approach to tissue engineering is to seed

cells on a three-dimensional polymer scaffold for incubation either *in vitro* or *in vivo* to stimulate matrix synthesis and, in the case of progenitor cells, produce cell differentiation.

The polymer scaffold is an important component of a tissue engineering system and merits discussion on its contribution to tissue engineered constructs. Tissue development is dependent on the structural environment, cell-biomaterial interaction, and biological signals incorporated in the scaffold (14, 15). The physical properties and biocompatibility of the polymer must also be able to ensure cellular function *in vivo*. Both synthetic and natural materials have been used to form scaffolds. While naturally-derived materials often have desirable biological properties, they also possess limited mechanical strength or fast degradation profiles (15, 16) that may not be suitable for clinical applications. Synthetic polymers have generated appropriate three dimensional environments to promote tissue development and are beneficial as cell carriers for musculoskeletal tissue engineering (17). Although not as bioactive as natural scaffolds, synthetic polymers provide the necessary properties to produce scaffolds with desired controllable physical and chemical characteristics (16, 18). A wide variety of synthetic polymers are available from which researchers can create scaffolds and incorporate bioactive factors for cell regulation. Commonly used scaffolds have been made from aliphatic polyesters such as poly(glycolic acid), poly(lactic acid), and poly(lactic-*co*-glycolic acid) (19, 20). These polymers have been applied to repair a number of tissues including vascular, hepatic, and orthopedic systems (21-23). Poly(anhydrides) and poly(phosphazenes) are two additional types of synthetic polymers used for orthopedic tissue engineering (18, 20, 24).

Hydrogels are another option for tissue engineering. Hydrogels have numerous desirable traits including high, tissue-like water content and moldable characteristics that are beneficial for clinical use (18). Hydrogels are insoluble, hydrophilic polymer networks formed by crosslinking water-soluble monomers through covalent or hydrogen bonding, or van der Waals interactions between the monomer chains (25). A defining physical characteristic of hydrogels is their ability to swell in liquid solutions (26). The swelling behavior of hydrogels provides an aqueous environment comparable to soft tissue for encapsulated cells. The presence of water and a porous structure also allows for the influx of low molecular weight solutes and nutrients crucial to cellular viability, as well as the transport of cellular waste out of the hydrogel (18, 27). The degree of transport through hydrogels is determined by the pore size of the network, or the average molecular

weight between crosslinks. Along with water content, the pore size also influences the mechanical properties of the hydrogel (27) and can affect the degree of cellular activity in tissue engineering applications. For example, Bryant and Anseth showed that degrading gels, with increasing pore size, demonstrated enhanced cellular proliferation and increased matrix diffusion (28).

Hydrogels can also efficiently encapsulate and maintain viable cells. In one study, Elisseeff *et al.* created poly(ethylene oxide)-based hydrogels that were injected with bovine chondrocytes into mice. The chondrocytes survived for a period of seven weeks and produced neocartilage (29). Total collagen contents increased over the culture period and histology indicated mitotically active cells in tissue structures characteristic of cartilage, including ovoid cells surrounded by extracellular matrix. In another study, Mosahebi *et al.* showed that alginate gels promoted the viability and function of Schwann cells for neural applications (30). Neurite growth *in vitro* was enhanced by alginate gels encapsulating Schwann cells over plain alginate gels and demonstrated the potential for reconstructive tissue engineering of peripheral nerves. Hydrogels formed from synthetic polymers can therefore offer the advantages of biocompatibility and stability for cell encapsulation and tissue development.

Injectable Hydrogel Systems

Tissue engineering therapies would benefit from minimal surgical procedures to decrease patient morbidity. *Ex vivo* engineered tissues are created in controllable environments with the ability to optimize culture conditions. However, the *ex vivo* setting may also produce difficulties in shaping the construct to fit into complicated or irregular-shaped defect sites (31). In addition, invasive surgery may be required to transplant preformed tissue-engineered constructs into defects. In contrast, injectable systems provide the advantages of moldability to fill irregular-shaped defects, simple incorporation of bioactive factors, and limited surgical invasion (32, 33). A liquid solution of polymer and cells is injected and polymerized *in situ* to form a solid scaffold. Tissues engineered *in vivo* are surrounded by biological and mechanical signaling that may enhance tissue development and are provided with regulatory mechanisms that cannot be duplicated in an *in vitro* setting. However, the reproducibility of *in vivo* engineered tissues may be difficult to maintain due to uncontrolled biological processes.

Hydrogels represent a class of materials that can be applied to injectable cell-based systems and *in situ* gel formation. Hydrogel formation obtained through macromer crosslinking can be accomplished by several methods including temperature change, chemical crosslinkers, or radiation exposure (18, 25). For thermal crosslinking, certain polymers possess phase transition properties that can be utilized to produce hydrogels with small changes in temperature (34). Reaching the lower critical solution temperature (LCST) of a polymer by increasing the polymer temperature causes a liquid solution to change phase and become solid. However, some gels change from a liquid state to a solid state by increasing the temperature and are also reversible (35). One example of a thermoreversible gel is a copolymer of poly(N-isopropylacrylamide) and acrylic acid poly(NiPAAm-co-Aac), within which encapsulated chondrocytes have been shown to regain their phenotype after monolayer culture (36). Thermoresponsive gels can also be produced from polymer solutions containing block copolymers of poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO-PPO-PEO, Pluronics). Pluronic 127, which gelates at 25^{o}C demonstrated the ability to encapsulate chondrocytes and facilitate cartilage formation *in vivo* (9).

Chemical crosslinking occurs when a radical initiator causes a cross-linking agent with difunctional or multifunctional groups to link two or more monomer chains (26). Peter *et al* used poly(propylene fumarate) (PPF) successfully to produce chemically crosslinked hydrogels *in vivo* capable of acting as an injectable bone cement (32, 37).

A third technique to crosslink monomers *in vivo* is photopolymerization, which uses light radiation. Visible or ultraviolet light causes photoinitiators, light-sensitive compounds, to produce free radicals that initiate polymerization through active sites on macromer chains (38, 39). Photopolymerization provides several advantages over other forms of polymerization. First, the spatial and temporal dimensions of the polymerization process can be controlled (29). The light intensity and exposure time can be adjusted to produce a specified depth of gelling. Since the polymer solution is a liquid prior to polymerization it can be placed in a mold or defect of choice to produce the desired construct shape. Like temperature and chemical crosslinking, photocurable hydrogels can also be injected using a syringe with the appropriate mixture of cells and bioactive factors. Photopolymerization has been used in orthopedic applications for cartilage and bone with success in generating viable tissue (12, 28, 29, 31, 40).

Polymers for Injectable Hydrogel Systems

Poly(ethylene glycol)

Poly(ethylene glycol) (PEG), or poly(ethylene oxide) (PEO), has a number of qualities that make it a desirable polymer for biomedical applications, including hydrophilicity and biocompatibility (25, 41). In addition, its properties of limited immunogenicity, antigenicity, and minimal protein and cell adhesion make PEG an attractive candidate for scaffolds in cell-based tissue engineering systems (18, 42). PEG-based hydrogels have utilized photocurable methods to encapsulate various cell types (12, 28, 29, 31, 40, 43-46). Figure 1 shows the chemical structures of select polymers that can be used to synthesize hydrogels.

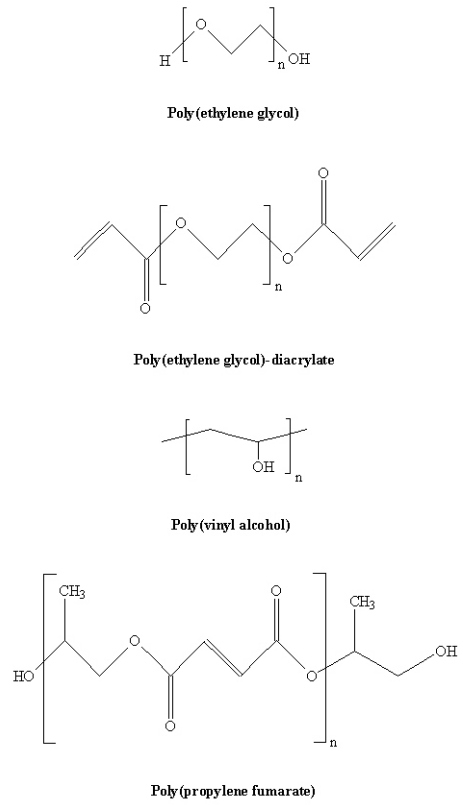

Fig. 1. Chemical structures of selected synthetic polymers

Elisseeff *et al.* produced cartilage tissue *in vivo* using methacrylated PEO to encapsulate bovine chondrocytes subcutaneously in athymic mice (43). Transdermal photopolymerization after subcutaneous injection of the polymer/cell suspension resulted in hydrogels that were incubated for 7 weeks. Histological analysis of the constructs and biochemical assays for glycosaminoglycan (GAG) and total collagen demonstrated tissue that resembled neocartilage. Williams *et al.* photoencapsulated acrylated PEG with goat bone marrow-derived mesenchymal stem cells (MSCs) to produce chondrogenesis *in vitro* (40). The addition of transforming growth factor-beta 1 (TGF-β1) stimulated type II collagen and aggrecan gene expression over gels without the growth factor, while type I collagen expression decreased. Over the 6 week culture period, the production of GAG and total collagen also increased in gels with TGF-β1 over gels without the growth factor. Figure 2 demonstrates that the addition of TGF-β3 increases MSC synthesis of cartilage matrix.

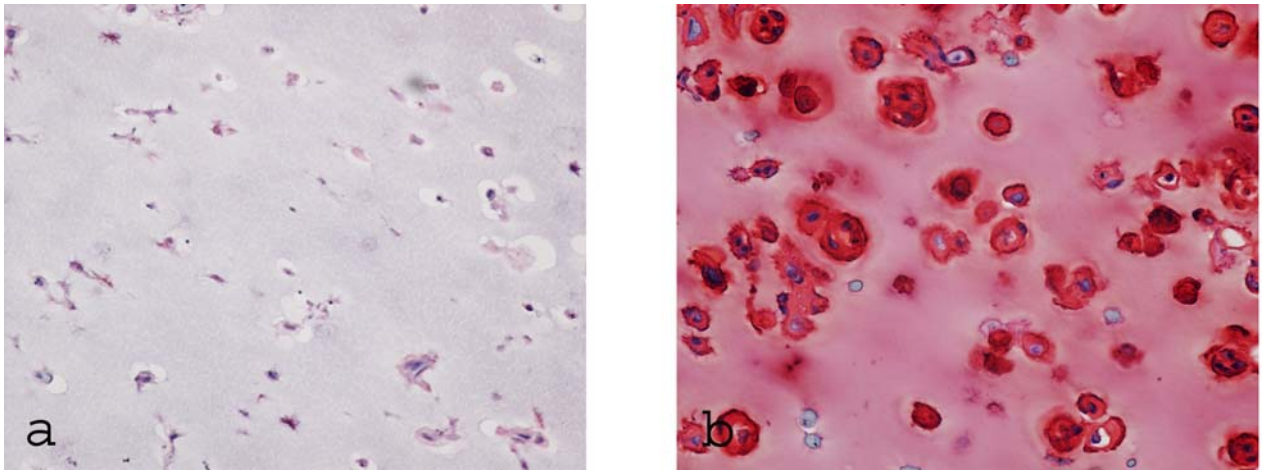

Fig. 2. Safranin-O staining (20x) at 3 weeks of photoencapsulated MSCs in PEGDA hydrogels (a) without TGF-β3 and (b) with TGF-β3

Due to the inert nature of PEG, there is no interaction between the polymer and encapsulated cells. However, the addition of bioactive peptides to PEG chains incorporates a biological component to the hydrogel that can alter the nature of the scaffold to induce cellular behavior. For example, Drumheller and Hubbell incorporated the adhesion peptide RGD into PEG hydrogels and determined the necessary amino acid concentrations to induce fibroblast cell spreading (47).

PEG is not naturally degradable, but can be altered to possess degradation properties. In one study, Sawhney *et al.* synthesized macromers of PEG with hydrolytically degradable PLA and PGA units (48). The macromers were endcapped with acrylate groups and photopolymerized. The PEG-*co*-polyester hydrogels were placed in PBS and incubated at 37°C *in vitro* to determine hydrolytic degradation. The authors determined that lower molecular weight PEG monomers formed tighter crosslinks and exhibited slower degradation times from hydrogels created using higher molecular weight precursors.

Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) gels have been formed through chemical crosslinking with aldehydes or photopolymerization. Since chemical crosslinking can create harsh environments that are potentially toxic to cells, researchers have turned towards the use of photocuring PVA to produce hydrogels (49). PVA has the benefits of pendant alcohol groups that provide attachment sites for biological molecules and are an alternative to PEG hydrogels that have less availability of functional groups (18, 49). PVA also has elastic properties that may be beneficial to seeded cells, such as enhancing the transmission of mechanical stimuli to induce cell orientation or matrix synthesis (49). Like PEG hydrogels, PVA is non-adhesive to cells and proteins, but can be covalently modified with cell-attachment peptides for bioactive regulation and has been the focus of some studies. Matsuda *et al.* incorporated GRGDSP peptides onto PVA films and demonstrated enhanced adhesion of bovine endothelial cells to treated PVA surfaces over plain PVA films (50). In another example using the RGD adhesion peptide sequence, Schmedlen *et al.* altered PVA hydrogels with RGDS to support the adhesion and spreading of human dermal fibroblasts (49). Cell viability was maintained homogenously throughout the gel for 2 weeks in culture. The degree of spreading and adhesion was dose-dependent and increased with greater RGDS concentrations. Kawase *et al.* immobilized glycl-L-

histidyl-L-lysine (GHK) to PVA-derived surfaces for use as substrates for hepatocyte culture (51). The cells initiated aggregation after 24 hours and developed multicellular spheroids that attached firmly to the PVA-derived gels during the 5 day incubation period.

Copolymers using PVA have also been fabricated. Martens *et al.* designed degradable, crosslinked hydrogels by copolymerization of PEG and PVA macromers through photoinitiated chain polymerization (41). Degradation studies indicated a shorter degradation time for the PEG/PVA copolymer at 28 days compared with PEG homopolymer gels that did not fully degrade until 34 days, whereas PVA homopolymer gels degraded within 1 day. Chondrocytes were also encapsulated in the PEG/PVA copolymer hydrogel for a period of 6 weeks *in vitro*. During that time, GAG and total collagen content steadily increased while DNA content increased between weeks 4 and 6, indicating proliferation during the later stages of the culture period.

Poly(propylene fumarate)

Poly(propylene fumarate) (PPF) is an unsaturated linear polyester that has been applied as a biomaterial for orthopedic applications such as bone cements (52-55). Crosslinking performed with a vinyl monomer produces a mechanically strong polymer network with applications for bone tissue (18). Recently, PPF has been employed as cell encapsulation scaffolds. Suggs and Mikos investigated poly(propylene fumarate-*co*-ethylene glycol) as a scaffold carrier for endothelial cells (56). In that study, cell viability was not compromised and a normal wound-healing response was observed without further adverse biocompatibility issues when implanted *in vivo*. In another study, He *et al.* fabricated composite polymers of PPF crosslinked with PEG-dimethacrylate (PEG-DMA) and the incorporation of the particulate ceramic β-tricalcium phosphate (β-TCP) (57). Mechanical studies indicated an increase in the compressive strength at yield and compressive modulus of PEG-DMA/PPF composites as the double-bond ratio of PEG-DMA/PPF increased, due to a higher crosslinking density. Mechanical properties were augmented with the addition of β-TCP. The equilibrium water content also increased with an increase in the double-bond ratio. The time of gelation of the composites ranged from 8.0 to 12.6 minutesh is, whic within the desired range of clinical application. This study demonstrated the ability to develop biodegradable, injectable hydrogels having adjustable parameters by varying the ratio of PEG-DMA to PPF.

Alginate

Alginate is a natural polymer found in brown algae, a form of seaweed, and has been applied as a biomaterial for drug delivery vehicles and cell-encapsulating scaffolds (34, 58-63). Alginate is a block copolymer that forms a gel through the interaction of divalent cations with blocks of guluronic acid on the polysaccharide chains (18). Alginate is also degradable, but the process is not easily controlled and can be unpredictable (34).

Alginate alone does not interact with cells and has limited bioactivity. However, research has been performed to modify alginate to overcome the potential limitations and utilize its benefit as a biocompatible material. In one study, Sultzbaugh and Speaker modified the surface of alginate with lectin, a protein possessing carbohydrate specific binding properties (64). The ability of lectin to retain ligand-specific properties was validated through the uptake of radiolabelled ligands when incubated with the lectin modified alginate gels. In another study, Alsberg *et al.* attached peptide sequences containing RGD to alginate hydrogels, promoting increased osteoblast adhesion and spreading over unmodified hydrogels (65). Up-regulation of bone markers in MC3T3-E1 cells encapsulated in RGD-modified hydrogels indicated osteoblast differentiation of the cells. The authors also demonstrated that rat calvarial osteoblasts seeded onto G4RGDY-modified alginate hydrogels produced increased *in vivo* bone formation compared with cells seeded onto plain alginate hydrogels at 16 and 24 weeks. Halberstadt *et al.* injected alginate-RGD hydrogels encapsulated with preadipocytes in the subcutaneous region of sheep (66). The construct maintained shape and cell viability for a period of 3 months with evidence of minimal inflammatory reaction.

Previous research has demonstrated feasibility for using alginate as injectable cell carriers. Atala *et al.* seeded autologous chondrocytes on alginate and injected the polymer solution subcutaneously in mice (67). Histological analyses indicated that the injected constructs remained localized and there was also evidence of cartilage formation that slowly replaced the gels over time. Marler *et al.* incorporated syngeneic fibroblasts in alginate gels that were injected subcutaneously in rats (68). Alginate gels were either preformed or gelled after injection. Constructs polymerized after injection retained their geometry and more of their initial volume than preformed constructs.

Study of an Injectable Synthetic-Biological Composite Hydrogel System

While purely synthetic hydrogels support tissue development, their bioactivity is limited and prevents interaction with encapsulated cells. However, cellular regulation can be accomplished through incorporation of biological molecules into hydrogels. One strategy used to incorporate bioactivity into scaffolds employs components of the extracellular matrix (ECM), which are known to influence cell behavior (69-72). The integration of ECM components such as growth factors, proteins, and proteoglycans into hydrogels therefore augment an environment proven to promote cell function and can potentially direct the behavior of encapsulated cells. The remainder of this chapter presents a synthetic-biological composite hydrogel developed by the authors. As described previously, moldable, injectable PEG scaffolds provide a convenient method for cell encapsulation and a suitable environment for chondrogenic development of MSCs. Incorporating Cartrigel, an ECM extract of cartilage, into photopolymerizing PEG-derived scaffolds may provide biological cues and direction for cellular differentiation. It was hypothesized that Cartrigel would promote cell-material interactions and modulate the MSC response to the chondrogenic stimulus TGF-β3.

The ECM plays an important role in directing cellular activity. Cellular interaction with ECM proteins can aid in regulating adhesion, proliferation, and differentiation of the cells (73-76). For example, chondrocytes in cartilage tissue are prompted by regulatory signals from the extracellular environment to produce collagens and proteoglycans to maintain the ECM. The degeneration of cartilage tissue and subsequent alteration to the ECM causes chondrocytes to attempt repair by proliferating and producing more collagens and proteoglycans. In order to take advantage of cell-matrix interactions, tissue extracts have been developed.

One example of a tissue extract is Matrigel, created from the Englebreth-Holm-Swarm (EHS) sarcoma. Matrigel is rich in basement membrane molecules that include laminin, heparin sulfate proteoglycans, and type IV collagen (77). Cultures of various cells types with Matrigel have shown increased levels of differentiation or tissue production. For example, Grant *et al.* demonstrated that human umbilical vein endothelial cells cultured with Matrigel expressed thymosin β4, a gene implicated in tube formation of blood vessels (78). Levenberg *et al.* cultured human embryonic

stem cells on Matrigel and produced cellular differentiation as well as the formation of tube-like structures (79). In collaboration with H. Kleinman of the National Institute of Dental and Craniofacial Research, we have developed another tissue-derived extract, Cartrigel, which is obtained from cartilage. Cartrigel contains collagens and proteoglycans, proteins that have been individually shown to affect cell behavior *in vitro*. For example, Qi and Scully increased chondrocyte expression of aggrecan by modulating its response to TGF-β1 through type II collagen interaction (80). Dinbergs *et al* demonstrated that proteoglycans can bind and retain growth factors, localizing bioactive molecules for potential cell presentation (81).

Materials and Methods

MSCs derived from goat bone marrow were isolated and photoencapsulated in Poly(ethylene glycol)-diacrylate hydrogels (Acrylate-PEG-Acrylate, 3400MW, Nektar Therapeutics, Huntsville, AL) as previously described (40). PEGDA-Cartrigel hydrogels were fabricated in the same fashion and PEGDA-Cartrigel-acellular constructs were made as control constructs. Briefly, the polymer solutions were prepared by thoroughly mixing PEGDA polymer, TGF-β3, and photoinitiator (Irgacure 2959, Ciba Specialty Chemicals, Tarrytown, NY) in sterile PBS or Cartrigel solutions. MSCs were suspended in the polymer solution and aliquots were distributed to cylindrical molds. The samples were photopolymerized by exposure to long-wave ultraviolet light for several minutes and then transferred to culture plates for *in vitro* culture in chondrogenic media (82) with or without TGF-β3 (10 ng/ml, Research Diagnostics, Flanders, NJ) and harvested after 1 and 3 weeks. Cartrigel was made using the protocol for Matrigel production, substituting juvenile bovine articular cartilage for the EHS sarcoma (83).

Protein release studies in culture media were performed using a BCA protein assay, (Pierce Biotechnology, Rockford, IL) to determine the ability of hydrogels to retain the Cartrigel components. Gene expression of cartilage-specific molecules was evaluated at 1 and 3 weeks, and histology was performed at 3 weeks to observe cell morphology and matrix production. For gene expression, hydrogels were first homogenized and RNA extracted using methods previously described (84). cDNAs were made from RNA using random hexamers using the Superscript First-strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Real-time RT-PCR using the SYBR Green PCR Master Mix kit and Sequence Detector 7700 (Applied Biosystems, Foster City,

CA) was performed to determine relative quantification of type I collagen, type II collagen, aggrecan, and β-actin. Primer sequences were as follows: type I collagen (5′ TGA CGA GAC CAA GAA CTG 3′, 5′ CCA TCC AAA CCA CTG AAA CC 3′), type II collagen (5′ GTG GAG CAG CAA GAG CAA GGA 3′, 5′ CTT GCC CCA CTT ACC AGT GAG 3′), aggrecan (5′ GTG GGC GGT GAG GAG GAC ATC AC 3′, 5′ GGG CCG GGT GGC CTC TTC AGT C 3′), β-actin (5′ TGG CAC CAC ACC TTC TAC AAT GAG C 3′, 5′ GCA CAG CTT CTC CTT AAT GTC ACG C 3′). To evaluate tissue morphology, intact hydrogels were fixed, sectioned, and stained with Safranin-O (Histoserv, Germantown, MD).

Results and Discussion

Release studies from the PEGDA-Cartrigel constructs demonstrated an initial release of protein after encapsulation. After the initial 24 hour period, little protein was observed outside the gels, while 71% of the initial protein remained in the constructs (Fig. 3). Figure 4 shows histological staining of PEGDA-Cartrigel-acellular constructs with protein visible throughout the gel after 1 week.

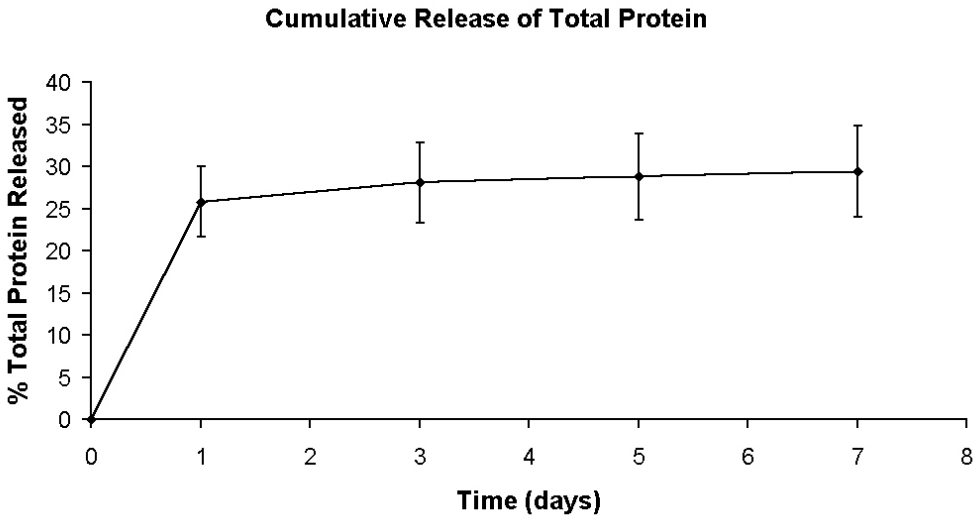

Fig. 3. Cumulative protein release from PEGDA-Cartrigel acellular hydrogels

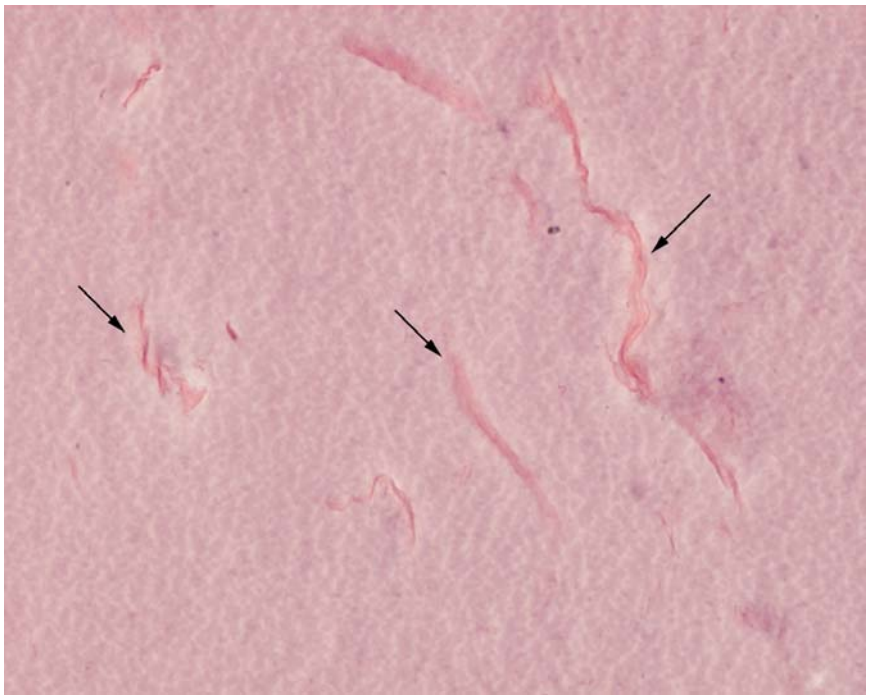

Fig. 4. Proteins visible (indicated by arrows) in PEGDA-Cartrigel acellular hydrogels stained with Safranin-O (20x) at 1 week.

Results from realtime RT-PCR demonstrated differences in gene expression between MSCs encapsulated in PEGDA and PEGDA-Cartrigel hydrogels. Chondrogenic markers of type II collagen and aggrecan were expressed at higher levels in PEGDA gels compared to PEGDA-Cartrigel composites after 1 and 3 weeks (Fig.5). Histology also indicated greater Safranin-O staining for ECM matrix in PEGDA constructs over PEGDA-Cartrigel composites (Fig. 6). A higher intensity and homogenous distribution of pericellular staining was observed throughout the PEGDA gels.

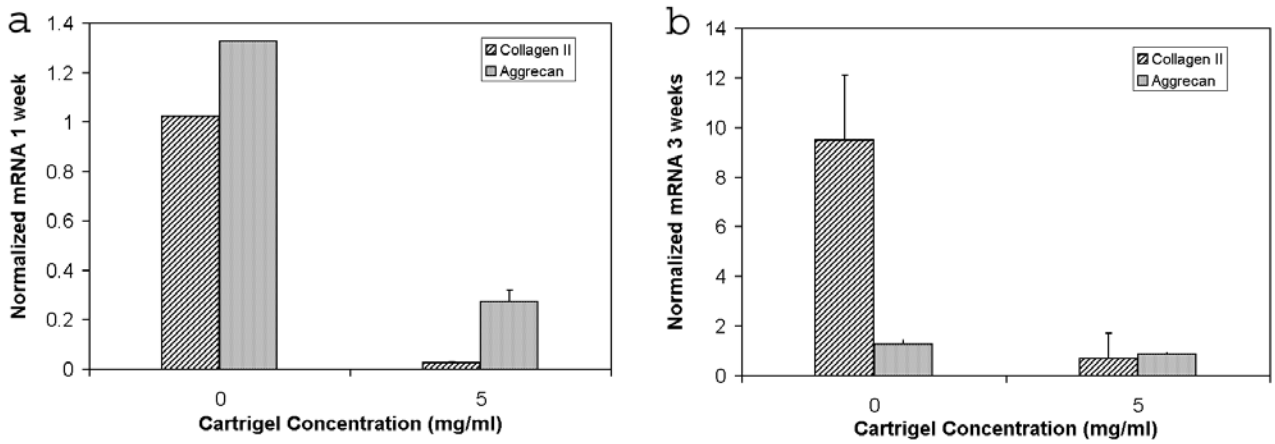

Fig. 5. Normalized levels of type II collagen and aggrecan mRNA from MSCs in PEGDA hydrogels with two concentrations of Cartrigel (0 and 5 mg/ml) at 1 (a) and 3 (b) weeks.

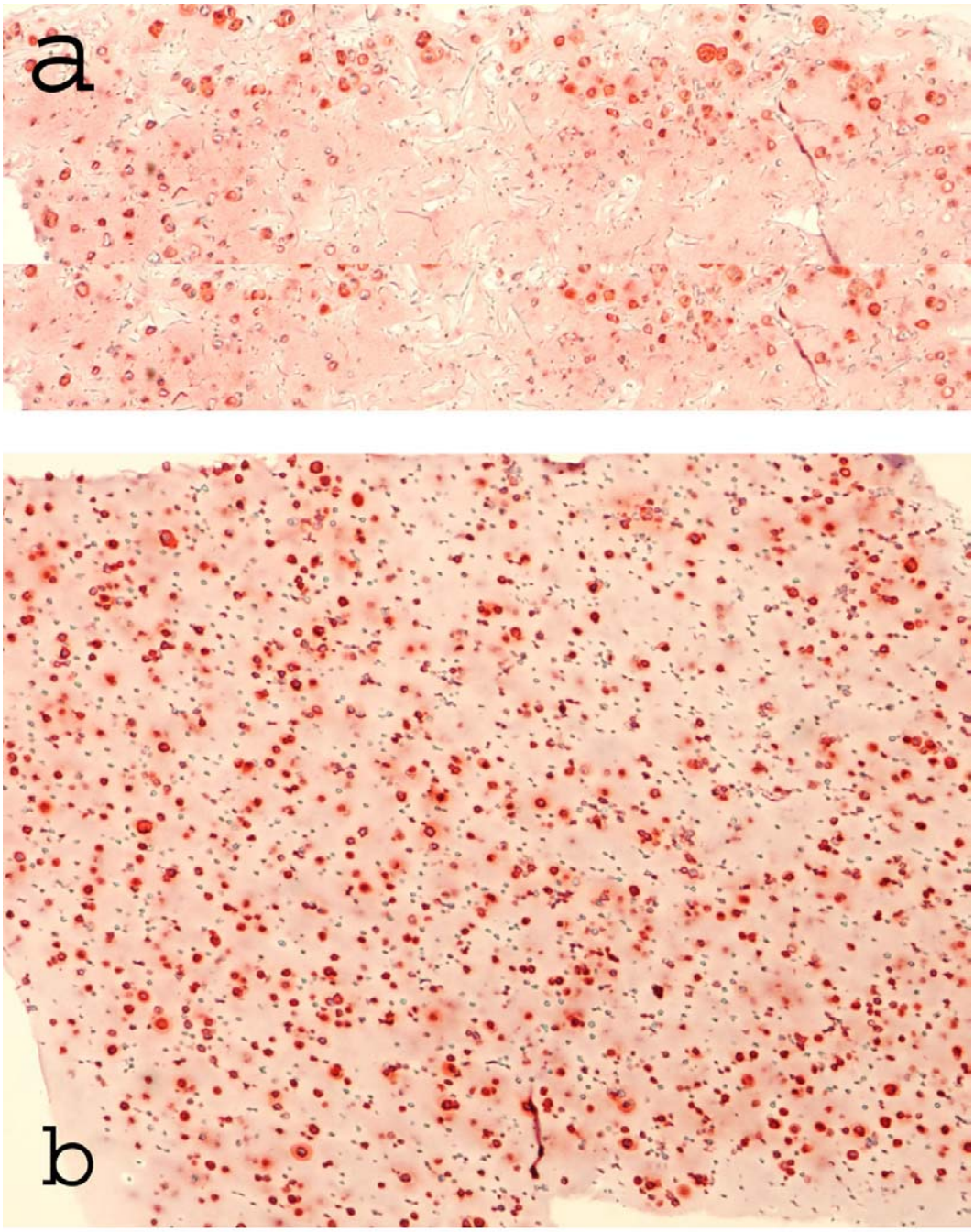

Fig. 6. Safranin-O staining (4x) at 3 weeks of photoencapsulated MSCs in PEGDA-Cartrigel (a) and PEGDA (b)

The greater expression of chondrogenic markers and increased Safranin-O staining in PEGDA hydrogels over PEGDA-Cartrigel composites indicate reduced chondrogenesis of MSCs in the presence of ECM at early timepoints. These results are similar to the response of chondrocytes to Cartrigel, in which there was a demonstrated decrease in type II collagen and aggrecan expression at 1 week (84). Previous studies have reported that proteoglycans in the ECM can bind growth factors (85-89). These interactions may lead to the sequestering of growth factors and the inability of cells to complex with the growth factors. Therefore, MSC chondrogenesis in PEGDA-Cartrigel composites may have been subdued due to Cartrigel sequestering of TGF-β3. An absence of ECM in PEGDA constructs allowed TGF-β3 to diffuse through the gel and interact with MSCs, stimulating and enhancing chondrogenesis.

The differences in type II collagen and aggrecan expressions between PEGDA gels and PEGDA-Cartrigel composites after 3 weeks were reduced from the differences in expressions after 1 week. Cells respond to their environment, manufacturing proteins to maintain the ECM. If the necessary proteins are present in the matrix, the cells may not be prompted to express the corresponding genes. The presence of ECM proteins in Cartrigel may have been another factor in the reduced expression of type II collagen and aggrecan in PEGDA-Cartrigel composites, whereas the lack of ECM in PEGDA gels coupled with the presence of TGF-β3 promoted and enhanced MSC chondrogenesis. Therefore at 1 week PEGDA gene expression of type II collagen and aggrecan were greater than PEGDA-Cartrigel composites. However, accumulation of protein surrounding MSCs in PEGDA gels during the culture period may have eventually signaled a reduction in expression, explaining similar aggrecan mRNA levels between PEGDA and PEGDA-Cartrigel gels at 3 weeks and less of a difference in type II collagen mRNA levels from 1 week expressions.

Cartrigel provides a biological dimension to synthetic hydrogels for interaction and regulation of cells. However, like most tissue extracts, Cartrigel is a complex material and may contain conflicting regulatory stimuli. Therefore, further studies are necessary to characterize and optimize its biological function for use in tissue engineering applications.

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