ABSTRACT Cartilage tissue engineering aims to replace damaged or diseased tissue with a functional regenerate that restores joint function. Scaffolds are used to deliver cells and facilitate tissue development, but they can also interfere with the structural assembly of the cartilage matrix. Biodegradable scaffolds have been proposed as a means to improve matrix deposition and the biomechanical properties of neocartilage. The challenge is designing scaffolds with appropriate degradation rates, ideally such that scaffold degradation is proportional to matrix deposition. In this study, we developed a bioresponsive hydrogel with cell-mediated degradation aligned to the chondrogenic differentiation of human mesenchymal stem cells (hMSCs). We identified matrix metalloproteinase 7 (MMP7) as an enzyme with a temporal expression pattern that corresponded with cartilage development. By embedding MMP7 peptide substrates within a poly(ethylene glycol) diacrylate backbone, we built MMP7-sensitive hydrogels with distinct degradation rates. When MMP7-sensitive scaffolds were compared with nondegradable scaffolds in vitro, photoencapsulated hMSCs produced neocartilage constructs with more extensive collagenous matrices, as demonstrated through immunohistochemistry and biochemical quantification of matrix molecules. Furthermore, these changes translated into an increased dynamic compressive modulus. This work presents a practical strategy for designing biomaterials uniquely tuned to individual biological processes.

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A bioresponsive hydrogel tuned to chondrogenesis of human mesenchymal stem cells

Biodegradable scaffolds for tissue engineering applications have been proposed to improve formation of developing tissues by removing the interference to extracellular matrix elaboration imposed by a nondegradable scaffold (see reviews in refs. 1, 2). The advantage offered by degradable scaffolds is their capacity to impart immediate functional support to encapsulated cells in a structure that is eliminated as the cells begin to produce and assemble matrix proteins. The rate of scaffold degradation is critically important to the success of this system, and the ideal rate would be intimately tied to matrix production and assembly by the cells. This concept is of particular importance for structural tissues such as hyaline cartilage in which biomechanical function is dependent on the expression and assembly of extracellular matrix components (3, 4).

Recreating the native structure of the extracellular matrix within neocartilage remains a significant challenge in the field of cartilage tissue engineering. Synthetic polymers such as poly(ethylene glycol) diacrylate (PEGDA) are promising scaffolds for cartilage engineering applications, since they can be formed in situ under cyto-compatible conditions to enable uniform encapsulation of cells (5–9). PEGDA scaffolds are also highly amendable to physical (10–12) and chemical modifications that can enhance chondrogenesis by stimulating extracellular matrix production (13–17). However, PEGDA is chemically stable under physiologic conditions and degradation is not relevant within the time scale of tissue regeneration. A consequence of these minimally degradable scaffolds is that the extracellular matrix elaborated by the encapsulated cells remains restricted to the pericellular domain, producing a neocartilage construct with mechanical properties limited to those of the scaffold.

A number of approaches have been used to create degradable synthetic biomaterials. Most commonly, hydrolytic or enzymatic segments are built into the polymer backbone to enable degradation. Hydrolytically degradable scaffolds have shown some promise for cartilage engineering applications and typically dissolve through hydrolysis of an ester linkage when exposed to an aqueous environment (18–21). A limitation with this system is that the degradation kinetics are tied to macromer composition rather than cellular behavior, thus not allowing for variation in differentiation or matrix elaboration between cells of different patients.

Key Words: biodegradable scaffold • cartilage tissue engineering • matrix metalloproteinase • poly(ethylene glycol) diacrylate • matrilysin

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Alternatively, peptide substrates can be engineered into the polymer in a fashion that exploits cellular activity to locally degrade the scaffold. In this study, our goal was to produce a PEGDA-based degradable scaffold for cartilage engineering that was specifically tuned to chondrogenesis of human mesenchymal stem cells (hMSCs). We hypothesized that degradation driven through a cellular response concomitant with cartilage matrix deposition would improve the intracellular distribution of proteins and produce a stronger neoarticular construct.

The first requirement in the design process was to identify an enzyme with a temporal expression profile that corresponded with chondrogenesis of encapsulated hMSCs. We chose to focus on matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) enzymes that have previously been characterized in skeletal development to either support collagen biosynthesis (ADAMTS-2,-3, and -14 and MMP-7; refs. 22–24) or cleave extracellular matrix components during remodeling (MMP-1,-2, and -13 and ADAMTS-4 and -5; refs. 25, 26). By evaluating mRNA and protein expression patterns in hMSC-laden hydrogels, we identified MMP-7 as a secreted enzyme with an expression pattern that corresponds to chondrocyte differentiation. MMP-7 substrates were embedded into a PEGDA backbone, and the resulting enzymatically degradable hydrogels provided for an intercellular expansion of the extracellular matrix produced by photoencapsulated hMSC differentiating to chondrocytes. This improved the dynamic compressive modulus of neocartilage constructs. This study highlights a successful technique for tailoring scaffold degradation for a desired biological process.

MATERIALS AND METHODS

Preparation of PEGDA

PEGDA (6 kDa) was synthesized as described previously (27). PEGDA was prepared by combining 0.1 mM dry PEG with 0.4 mM acryloyl chloride and 0.2 mM triethylamine in anhydrous dichloromethane and stirred under argon overnight. The mixture was then precipitated in cold ethyl ether, vacuum-dried overnight, lyophilized, and stored at 4°C. PEGDA-based semi-interpenetrating networks (sIPNs) were made by mixing a noncrosslinking PEG-ndimethyl ether (PEG; n=2000; molecular mass 88 kDa) with the crosslinking PEGDA such that the final concentration was 16% (w/v) PEGDA and 32% (w/v) PEG dissolved in PBS (11).

Peptide synthesis

Peptides were synthesized with an ABI 433A synthesizer (Applied Biosystems, Foster City, CA, USA). Couplings were carried out on an H-L-trans-4-hydroxyproline-2-chlorotritol resin (AnaSpec, San Jose, CA, USA). Fmoc-amino acids [Fmoc-Gly-OH and Fmoc-Pro-OH (Applied Biosystems) and Fmoc-4(R)Hyp/(Bu)-OH (Novabiochem; EMD Biosciences, Inc., San Diego, CA, USA)] were purchased and used without further purification. O-(7-azabenzotriazol-1-yl)-1.1.3.3-tetramethyluronium hexafluorophosphate (HATU; Perseptive Bio-systems, Foster City, CA, USA) and diisopropylamine were used as the coupling reagent. The peptide was cleaved from the resin with reagent R (trifluoroacetic acid/thioanisole/1,2-ethanedithiol/anisole; 90:5:3:2) at room temperature for 3 h. Peptides were isolated by precipitation from the cleavage cocktail with diethyl ether at 4°C, diluted with 0.1% TFA, and purified by preparative HPLC (Vydac C18; 5 μm, 300 Å; 218TP101550 50×250 mm, and the guard column, 218TP15202503; W. R. Grace & Co., Columbia, MD, USA) with a flow-rate of 36 ml/min and elution with 0–50% acetonitrile gradient in 0.1% trifluoroacetic acid. The peptide was characterized by electrospray quadrupole time-of-flight mass spectrometry (Q-Tof micro; Waters, Milford, MA, USA) and amino acid analysis. The peptides were stored at −20°C before stock solutions were made.

Bioresponsive hydrogels

To generate bioresponsive hydrogels, peptides containing either a MMP-7 substrate (PLE-LRA, ref. 28, and VPLS-LTMG, ref. 29, where a hyphen indicates a cleavage point) or a nondegradable scrambled peptide control (MLLVTPSG) were synthesized with short linker domains GGWGG and GGK at the N and C termini, respectively, to both facilitate enzyme accessibility to the substrate sequence and enable degradation testing using the spectrophotometric release of tryptophan (W; see Fig. 2). Peptides were conjugated to acrylate-PEG-succinimidyl carboxymethyl (acrylate-PEG-SCM; 3.4 kDa; Laysan Bio Inc., Arab, AL, USA) individually at a 1:2.1 (peptide/PEG) molar ratio in DMSO (Sigma-Aldrich, St. Louis, MO) for 24 h at room temperature to form an ABA block polymer. Diisopropylamine (DIPEA; Sigma-Aldrich) was added as a base catalyst at a 2:1 (DIPEA/PEG) molar ratio into the reaction. The resulting solutions were then diluted in 10 ml of ultrapure water and purified by dialysis (molecular mass cutoff 3.5 kDa; Fisher Scientific, Pittsburgh, PA, USA) against deionized water for 24 h. The purified polymers were then lyophilized and stored under argon at −20°C. Polymer solutions were sterilized via filtration (0.2-μm filter; Gelman Sciences, Ann Arbor, MI, USA) before use. The conjugations of products were analyzed by gel permeation chromatography (Polymer Laboratories, Amherst, MA, USA) with UV-vis and evaporative light scattering detectors.

Degradation testing

Degradation of the peptide-modified PEGDA scaffolds was determined by measuring tryptophan release from cell-free scaffolds exposed to recombinant human MMPs. Macromer was dissolved at 10% (w/v) in a sterile PBS solution containing a photoinitiator system of 0.75% triethanolamine, 0.1 mM eosin Y, and 37 mM 1-vinyl-2-pyrrolidinone. Droplets (10 μl) were photopolymerized and then swollen in PBS overnight at 37°C. Hydrogels were then incubated in protease solution at 37°C for up to 48 h, and spectrophotometric measurement of the solution at 285 nm was taken at intervals as an indication of tryptophan release. Degradation by recombinant human MMP-1, -2, -7, and -13 (AnaSpec, San Jose, CA, USA) was tested against negative control (Tris buffer), or positive control (0.2 μg/ml proteinase K). Percentage tryptophan release was normalized to complete dissolution (proteinase K digestion, 24 h); values represent means ± s.d.

MSC isolation and culture

hMSCs were isolated and expanded from iliac crest bone marrow aspirates as described previously (11, 30, 31). Briefly, human bone marrow aspirates, obtained from the iliac crests...
of consenting donors, were fractionated on a Percoll density gradient and plated in DMEM with 10% FBS (Invitrogen, Carlsbad, CA, USA; lot selected for optimal cell growth and differentiation). Adherent cells were cultured at 37°C, 5% CO₂, with medium changes every 4 d. Once primary cells were confluent, serum-containing DMEM was supplemented with fibroblast growth factor (FGF-2; 10 ng/ml) to facilitate expansion with retention of chondrogenic potential. Expanded hMSCs at passages 1 to 3 were used for all experiments.

Cellular encapsulation and culture

Expanded hMSCs were mixed with 1 of 5 different PEG-based monomers (Table 1). Cells were photoencapsulated into disk-shaped hydrogels (8 mm diameter × 2 mm height) in a stainless steel mold. UV photopolymerization (365 nm, 6 mW/cm², 6 min exposure; Spectroline UV lamp; Spectrosciences Corp., Westbury, NY, USA) was used with the PEG based sIPN for initial characterization experiments; this reaction used Corp., Westbury, NY, USA) was used with the PEG based sIPN and was initiated using the photosensitizer eosin Y (peak absorbance 510 nm, 0.1 mM), initiator triethanolamine (37.5 nm).

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TABLE 1. Summary of hydrogel design

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Monomer chemistry</th>
<th>Embedded peptide</th>
<th>Proteolytic degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEGDA</td>
<td>10% (w/v) PEGDA</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>sIPN</td>
<td>16% PEGD + 32% PEG sIPN</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>sc-PEGDA</td>
<td>10% aPEG-peptide-PEGa</td>
<td>GGWGGMLLVLTPSGGGGK</td>
<td>MMP-7</td>
</tr>
<tr>
<td>VPLS-PEGDA</td>
<td>10% aPEG-peptide-PEGa</td>
<td>GGWGGVPDSLTMGGGK</td>
<td>MMP-7</td>
</tr>
<tr>
<td>PLE-PEGDA</td>
<td>10% aPEG-peptide-PEGa</td>
<td>GGWGGPLELRAGGK</td>
<td>MMP-7</td>
</tr>
</tbody>
</table>

by normalizing to the housekeeping gene (18S) and the hMSC expression before encapsulation (ΔΔCₜ). Graphs represent means ± 95% confidence.

Histology and immunohistochemistry

Hydrogels were fixed in 10% neutral buffered formalin and embedded in paraffin, and 5-μm sections were cut onto slides. Representative slides were deparaffinized and stained with toluidine blue to visualize sulfated proteoglycans. For immunohistochemistry, slides were deparaffinized and then blocked in 5% BSA for 1 h at room temperature. For collagen II detection, sections were exposed to Promace (1 μg/ml in PBS, 20 min) at room temperature before reaction with a 1:200 dilution of monoclonal anti-collagen II antibody (II-H6B3; National Institutes of Health Hybridoma Bank, University of Iowa, Iowa City, IA, USA) in 1% BSA, overnight at 4°C. MMP-7 in hydrogels was detected with a prediluted mouse monoclonal MMP-7 antibody recognizing both the pro- and active form of human MMP-7 (GTX17B54; GenTex, Irvine, CA, USA). Detection was done using goat-anti-mouse AlexaFluor 594-linked secondary antibody. Images were converted to grayscale in Photoshop (Adobe Systems, San Jose, CA, USA).

To detect MMP-7 in embryonic mouse limbs, sections were deparaffinized, blocked with 5% BSA for 1 h, and then treated with 2 μg/ml type V hyaluronidase for 30 min at 37°C. Sections were exposed to a 1:200 dilution of rat monoclonal anti-MMP7 antibody, kindly provided by Dr. Lynn Matrisian (Vanderbilt University, Nashville, TN, USA; clone 33B; refs. 32, 33). Detection was done using a 1:100 dilution of goat-anti-rat horseradish peroxidase and peroxidase substrate DAB plus nickel (SK-100; Vector Laboratories, Burlingame, CA, USA). Staining of a colon tumor metastasis in liver was used as a positive control, and staining was consistent with the literature (34–36).

mRNA isolation and quantitative RT-PCR

Triplicate samples of hydrogels were harvested weekly into 1 ml TRIzol reagent (Invitrogen) and homogenized using the Ultra-Turrax IKA-T10 basic homogenizer (IKA, Wilmington, MA, USA). Homogenates were left at room temperature for 5 min to facilitate mRNA extraction and then centrifuged at 12,000 g for 15 min. The supernatant was removed and stored at −80°C until all samples had been collected. mRNA was extracted per manufacturer’s instructions. cDNA was reverse transcribed using Quanta qScript cDNA SuperMix (cat. no. 95048; Quanta Biosciences, Gaithersburg, MD, USA) with 1 μg mRNA/20-μl reaction. Quantitative real-time RT-PCR analysis was done on the Bio-Rad MyiQ iCycler with cycle number set to 40. Taqman assay primer/probes were used with Taqman PCR master mix (Applied Biosystems; Table 2).

Relative gene expression was calculated for each experiment by normalizing to the housekeeping gene (18S) and the hMSC expression before encapsulation (ΔΔCₜ). Graphs represent means ± 95% confidence.

TABLE 2. Taqman primer/probe assays

<table>
<thead>
<tr>
<th>Gene</th>
<th>Taqman assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>hs0999999901_s1</td>
</tr>
<tr>
<td>COL1A1</td>
<td>hs00164004_m1</td>
</tr>
<tr>
<td>COL2A1</td>
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</tr>
<tr>
<td>MMP2</td>
<td>hs00234422_m1</td>
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<tr>
<td>MMP7</td>
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<tr>
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<td>hs00239358_m1</td>
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<tr>
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<tr>
<td>ADAMTS3</td>
<td>hs00610744_m1</td>
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<tr>
<td>ADAMTS4</td>
<td>hs00192708_m1</td>
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<tr>
<td>ADAMTS5</td>
<td>hs00199841_m1</td>
</tr>
<tr>
<td>ADAMTS14</td>
<td>hs003565506_m1</td>
</tr>
</tbody>
</table>
Mechanical testing

The elastic modulus of compression for the hydrogel scaffolds was measured with a custom apparatus that imposed unconfined dynamic compression. Specimens were compressed by a voice-coil force actuator (model Cal36; SMAC, Carlsbad, CA, USA) that was controlled using data acquisition software and hardware (LabView 8.0; PCI 6221; National Instruments, Austin, TX, USA). The actuator applied an upward force to a rigidly connected plunger to compress specimens into an impermeable aluminum platen (15 mm diameter). Compressive forces were measured by connecting the platen to a rigidly fixed load cell (model 31, resolution 0.005 N; Sensotec, Morristown, NJ, USA). Specimen displacement was measured with a glass-scale encoder integrated into the voice-coil actuator (resolution 1 μm). The actuator was powered with a linear current amplifier (LCAM; Quanser, Markham, ON, Canada), and the load cell was powered by a signal conditioner with a low-pass filter (PMD-463WB; Omega, Stamford, CT, USA). In the absence of a testing specimen, the test system yielded a dynamic stiffness of 1 N/μm, which is 2 orders of magnitude greater than the dynamic stiffness of standard hydrogels (37). A force-controlled testing system was selected to ensure that the platen would not lift off the specimen during testing (38).

For each material test, specimens were centered in a culture dish filled with 1 ml PBS and loaded into the testing apparatus. To establish a consistent reference position for all samples, a 0.1-N preload was applied, and specimen thickness was recorded ($l_0$). The samples were then loaded to 0.4 N and allowed 90 s to equilibrate. This hold force corresponds to a 5–10% strain level in the constructs. After creep, sinusoidal force waves were applied for 30 cycles at 1 Hz to an amplitude of 0.5 N. The 0.5-N amplitude corresponded to a strain amplitude between 2 and 5%, which is the range of strain that hydrogel material properties are often measured (39, 40).

Dynamic modulus was calculated as the ratio of the first Piola-Kirchhoff stress (force in the present configuration to area in the reference configuration) and engineering strain $[(l - l_0)/l_0$, where $l$ is the current thickness and $l_0$ is the reference thickness]. These values were extracted by fitting the final 3 cycles of stress and strain data to a 4-parameter sine function in LabView (41). Dynamic modulus values represent means ± sd; significance was determined using the Mann-Whitney test, with significance set at $P < 0.05$.

Biochemical assays

After 6 or 12 wk of in vitro culture, triplicate hydrogels were removed from the culture medium and digested with 0.1 N NaOH overnight at 60°C. Samples were neutralized with 0.1 N HCl and then digested for and additional 18 h at 60°C with 125 μg/ml papain in 10 mM EDTA and 2 mM cysteine, pH 6.0 (Sigma). Sulfated proteoglycan content of the digested cell-polymer construct was assessed spectrophotometrically using the 1,9-dimethylmethylene blue dye assay (pH 3.0; Polysciences, Warrington PA, USA; ref. 42). Sample proteoglycan content was compared with shark cartilage chondroitin sulfate standards (Sigma-Aldrich). DNA content was determined spectrofluorimetrically using the PicoGreen DNA assay according to the manufacturer’s instructions (P11496; Invitrogen-Molecular Probes); sample fluorescence was compared with DNA standards included in the assay kit. Hydroxyproline content was used as a measure of total collagen content (43). This was determined by oxidation of hydroxyproline residues in collagen with chloramine T trihydrate (ICN Biomedicals, Irvine, CA, USA), developed with p-dimethylaminobenzaldehyde (Ehrlich’s reagent); ICN Biomedicals; ref. 43). Sample concentrations were compared with hydroxyproline standard solutions made from trans-4-hydroxy-L-proline (Sigma). All biochemical values represent means ± 95% confidence; significance was determined using the Mann-Whitney test, with significance set at $P < 0.05$.

RESULTS

Identification of MMP-7 as candidate enzyme for bioresponsive hydrogels

To develop a bioresponsive scaffold with degradation directly related to cartilage matrix deposition, it was necessary to identify an enzyme expressed by the encapsulated hMSCs that had a temporal profile that corresponded to chondrogenesis. To prevent premature scaffold degradation, it was also desirable for this enzyme to have very low expression in the hMSC before differentiation. We screened hMSCs for their temporal gene expression of candidate MMP and ADAMTS enzymes during chondrogenesis in a sIPN PEGDA-based hydrogel (11). MMP-7 was identified as the only metalloproteinase to have both a low expression in hMSCs (Fig. 1A) and a relative increase in expression during in vitro culture in chondrogenic medium (Fig. 1B). This temporal increase in MMP-7 gene expression positively correlated with chondrogenic markers collagen II and aggrecan (Fig. 1C).

Gene expression data for MMP-7 was validated at the protein level with immunohistochemistry. Representative sections from hydrogels cultured for 1, 4, and 6 wk indicated that increased MMP-7 protein expression corresponded with the deposition of proteoglycans and collagen II (Fig. 1D). Expression of MMP-7 was also detected in the developing cartilaginous anlagen of embryonic mouse limbs, suggesting that its expression in hydrogels is consistent with chondrogenesis during limb development (Fig. 1E).

Design and degradation kinetics of bioresponsive hydrogels

Two MMP-7 substrates, PLE-LRA and VPLS-LTMG (hyphens indicate cleavage points), were synthesized with short linker domains included at the N (GGWGG) and C (GGK) termini. A scrambled version of VPLS-LTMG (MLLVTPSG) was used as a control. These peptide sequences were embedded within PEGDA by reacting the primary amines at both ends of the peptide with aPEG-NHS to generate the macromer foundation for 3 bioresponsive hydrogels: PLE-PEGDA, VPLS-PEGDA, and the MLLVTPSG scrambled control (sc-PEGDA; Table 1 and Fig. 2). Degradation for each of the peptide-containing PEGDA scaffolds was quantified by tryptophan (W) release from 10% (w/v) cell-free hydrogels following treatment with human recombinant MMP-1, -2, -7, or -13 (Fig. 3). Degradation from MMP exposure was compared with proteinase K and Tris buffer as positive and negative controls, respectively. Both MMP-7-sensitive PEGDA scaffolds showed a dose-
dependent response to the human recombinant MMP-7, but PLE-PEGDA was more rapidly degraded (Fig. 3A, B). To test the specificity of the peptide sequences, cell-free scaffolds were exposed to 2 nM MMP-1, -2, -7, or -13. Degradation of PLE-PEDGA was less specific than VPLS-PEGDA (Fig. 3C, D). No degradation of sc-PEGDA was detected on exposure to any of the MMPs, validating the MLLVTPSG sequence as a nondegradable control (Fig. 3E).

**In vitro chondrogenesis of hMSCs in MMP-7 bioresponsive hydrogels**

hMSCs were photoencapsulated into the 2 MMP-7 bioresponsive hydrogels (PLE-PEGDA and VPLS-PEGDA) or 1 of 3 different nondegradable scaffolds (Table 1). Following in vitro culture for 6 and 12 wk, immunohistochemical staining detected collagen II deposition restricted to the pericellular domain in all nondegradable scaffolds (Fig. 4A, B). In contrast, interterritorial deposition was observed in both the VPLS-PEGDA and PLE-PEGDA MMP-7-sensitive scaffolds. Consistent with the faster and more permissive degradation of PLE-LRA (Fig. 3), greater interterritorial deposition of collagen II was observed earlier in PLE-PEGDA than in VPLS-PEGDA hydrogels. Degradation of the PLE-PEGDA scaffold resulted in increased total collagen accumulation compared with the sc-PEGDA (Fig. 4C). Together with the change in collagen distribution, this translated into a significantly increased dynamic compressive modulus after 12 wk of culture (Fig. 4D; \( P < 0.05 \)). The initial compressive modulus of the 3 peptide containing PEGDA hydrogels was similar at 82.9 ± 4.5 kPa and therefore intrinsic material properties would not be expected to affect differentiation capacity.

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**Figure 1.** Identification of MMP-7 as candidate enzyme for bioresponsive hydrogels. A) Gene expression from expanded hMSCs before encapsulation, as measured by quantitative real time RT-PCR. B) Changes in metalloprotease gene expression for hMSCs photoencapsulated in sIPN hydrogels over 12 wk. C) MMP-7 gene expression relative to chondrogenic markers, collagen II (COL2A1) and aggrecan (ACAN). D) Toluidine blue staining for proteoglycans and immunohistochemistry for collagen II and MMP-7 at 1, 4, and 6 wk. E) Toluidine blue staining and MMP-7 immunohistochemistry on E15.5 mouse digits. Error bars represent 95% confidence intervals.
Presumably due to the smaller size of proteoglycans, their deposition was observed throughout the degradable and nondegradable scaffolds at both 6 and 12 wk (Fig. 5A–C). However, proteoglycan content was decreased in the PLE-PEGDA hydrogels by 12 wk (Fig. 5D), and toluidine blue staining appeared less intense at the peripheral region of both MMP-7-sensitive hydrogels at this time (Fig. 5C). There was no significant change in the gene expression of metalloproteinase enzymes, indicating that the presence of peptide substrates does not alter catabolism in the bioresponsive hydrogels. Similarly, gene expression of COL2A1 and ACAN was not significantly different between the MMP-7-sensitive hydrogels and their nondegradable counterparts, suggesting that a change in extracellular matrix deposition is not the consequence of changes to the anabolic state of the cells (data not shown).

Relative cell content was quantified in the hydrogels by DNA measurements after 6 and 12 wk of culture (Fig. 5E). After 6 wk of culture, DNA content was significantly higher in the MMP-7-sensitive hydrogels compared with the nondegradable sc-PEGDA hydrogels, but at 12 wk this effect was reversed such that DNA content was significantly lower in PLE-PEGDA hydrogels.

**DISCUSSION**

Cartilage engineering is a therapeutic strategy that aims to produce a tissue regenerate competent to restore
normal joint function following focal lesions to the articular cartilage. The clinical success of a neocartilage construct depends on its ability to restore the metabolic and mechanical function of native tissue, thereby preventing or abating cartilage degeneration in diseases such as osteoarthritis. In this study, we present the design of a biodegradable scaffold for cartilage engineering that relies on intrinsic cellular mechanisms involved in hMSCs chondrogenesis to mediate degradation. We demonstrate that MMP-7 has a temporal expression pattern in hydrogels that corresponds to cartilage matrix elaboration and that MMP-7 substrates can be effectively incorporated into a PEGDA backbone to facilitate cell-mediated degradation that enables expansion of the collagen II matrix and increases elastic modulus of compression in neocartilage constructs.

The best cell type for cartilage engineering applications has not yet been determined and will depend on the design of the supporting scaffold (44). Chondrocytes, MSCs, and more recently embryonic stem cells (45–47) have been incorporated into a variety of different scaffolds, and each cell type has advantages and disadvantages (48–50). MSCs are well established as chondroprogenitor cells and offer the advantage that they are easily harvested and expanded. Cell choice was important to our design process, since our study aimed to optimize scaffold degradation to chondrogenesis of the encapsulated cells. The importance of biological processes driving the evolution of biomaterials has recently been highlighted in the literature (51–54) but has never been specifically applied to tuning scaffold degradation for cartilage engineering.

Degradable scaffolds offer an advantage over permanent biomaterials, since they can be completely replaced by an extracellular matrix made by the encapsulated cells. This is important, since hydrogels formed in situ for cartilage repair require a high cross-linking density to impart mechanical functionality and restore the proper distribution of forces during joint movement. However, high cross-linking density corresponds to smaller pore size within the scaffold, and these factors have previously been associated with reduced chondrogenesis and pericellular restriction of matrix proteins, specifically collagen (10, 11, 55, 56). Tailoring the degradation rate such that it corresponds to the elaboration of cartilage matrix is one of the main challenges in designing a biodegradable scaffold. Degradation that occurs too quickly could release cells and matrix components, whereas scaffolds that degrade too slowly inhibit matrix production and assembly. Hydrolytically degradable segments are one technique to incorporate degradation into a scaffold, and recent design efforts show that incorporating both fast and slow degrading elements allows for better control of degradation kinetics (20, 57). Enzymatically degradable

![Image of collagen deposition](image-url)

**Figure 4.** Collagen deposition. **A, B** Collagen II immunohistochemistry following 6 wk (**A**) or 12 wk (**B**) of *in vitro* culture. **C** Total collagen content in hydrogels at 6 wk (gray bars) or 12 wk (black bars). **D** Elastic modulus of compression for hydrogels following 12 wk of *in vitro* culture. *P < 0.05.
scaffolds offer greater spatiotemporal control over hydrolytic degradation. Using exogenously triggered degradation of lipase-sensitive poly(ethylene glycol)-b-polycaprolactone dimethacrylate scaffolds, Rice and Anseth (58) were able to explicitly control the timing of scaffold degradation. They found that adding lipase after allowing encapsulated chondrocytes to produce some matrix was preferred to stimulating early scaffold degradation, but in their system the exogenously driven degradation was not directly linked to cellular behavior.

Bioresponsive materials include those with cell-mediated degradation properties. The term “bioresponsive” was adopted to convey a distinct physical or chemical response occurring within the biomaterial as a consequence of cellular behavior (59). The concept of engineering synthetic hydrogel scaffolds with cell-mediated degradation features was first introduced by West and Hubbell in 1999 (60). Collagenase and plasmin specific peptide substrates were incorporated into a PEGDA-based hydrogel to facilitate cellular migration for wound healing. These peptide sequences and polymer chemistry were later modified for faster degradation kinetics to increase the efficiency of fibroblast migration (61). Park et al. (62) explored the utility of a collagenase-sensitive scaffold for cartilage engineering with encapsulated chondrocytes and suggested that matrix was less constrained in the degradable hydrogel. However, the peptide sequence they incorporated into their hydrogels is cleaved by multiple matrix metalloproteinases (61, 63) and thus was not optimized for chondrogenesis. Our work represents a progression of this concept in that the bioresponsive scaffold is specifically linked to hMSCs chondrogenesis and cartilage matrix elaboration through MMP-7 expression and activity. MMP-7 has a well-characterized role in tumor metastasis (see reviews in refs. 35, 36), yet recent data suggest
that it may also play a role in chondrogenesis (64). Evidence suggests MMP-7 supports cartilage development by both facilitating collagen II maturation (22) and modulating bioavailability of chondrogenic factors (65–67). During collagen maturation, MMP-7 can cleave the NH2-propeptide from the native type IIA procollagen, the alternatively spliced form of collagen II synthesized by chondroprogenitors (22). Both BMP-2 and TGF-β1 can bind to the extrahelical region of the collagen IIA propeptide, supporting a role for MMP-7 in growth factor mobilization (65). MMP-7 can also cleave all 6 of the insulin growth factor binding proteins (IGFBP) responsible for mediating IGF activity (66) and activate TGF-β indirectly through the activation of MMP-9 (67). The physiological activation of MMP-7 is not well understood, but activity can be regulated through gene expression, enzyme localization, and activation of the proenzyme. Recently, it was suggested that highly sulfated glycosaminoglycans may regulate MMP-7 activity by promoting proenzyme activation through autolytic cleavage of the prodomain or by providing an anchor to localize substrate activity (68).

Our data from both hydrogels (Fig. 1A, C, D) and embryonic mouse limbs (Fig. 1E) provide further evidence that MMP-7 is involved in chondrogenesis. MMP-7 was chosen from a screen of MMP and ADAMTS enzymes reported to be involved in skeletal development (26). Using RT-PCR and immunohistochemistry, we found that only MMP-7 and MMP-13 had temporal expression patterns that positively correlated with those of collagen II and aggrecan (Fig. 1). MMP-13 was not chosen for further evaluation, since its expression is associated with the final maturation state of chondrocytes during endochondral ossification. It is well established that MSCs express markers of the hypertrophic chondrocytes seen in endochondral ossification during in vitro culture (30, 69), and this has led to the concern that bone could form during in vivo implantation (70, 71). Although beyond the scope of this work, we believe encapsulated MSCs can be driven to a permanent cartilage chondrocyte phenotype, which would therefore make MMP-13 an irrelevant choice. Furthermore, MMP-13 was expressed by expanded hMSCs at the time of encapsulation (Fig. 1B) and therefore creates the potential for premature degradation of the scaffold.

Validation of MMP-7 as a good candidate enzyme to mediate scaffold degradation during neocartilage formation was shown with long-term in vitro studies of photocapsulated hMSCs (Figs. 4–5). We found significantly improved distribution of the collagen II matrix (Fig. 4A, B) and a higher dynamic compressive modulus (Fig. 4D) in the MMP-7-sensitive hydrogels when compared with nondegradable controls. PLE-PEGDA had a more extensive collagen II matrix earlier than the VPLS-PEGDA scaffold (Fig. 4A, B), presumably due to the faster degradation kinetics and lower specificity of the PLELRA substrate (Fig. 3). The MLL-VTPSG sequence has not been reported as degradable by any MMPs (63) and was not degraded using recombinant MMP-1, -2, -7, or -13 (Fig. 3E).

Although we significantly improved collagen distribution within the bioresponsive hydrogels, we did get less proteoglycan retention in the PLE-PEGDA than in nondegradable controls after 12 wk of culture (Fig. 5A–D). ACAN mRNA expression at 12 wk was equivalent in both the degradable and nondegradable hydrogels (data not shown), suggesting aggrecan potential is similar per cell. Furthermore, there was no change to collagen or metalloproteinase expression in the bioresponsive scaffolds, suggesting that the presence of a peptide substrate does not inherently increase enzyme production or matrix catabolism (data not shown).

Together these data suggest that degradation of the bioresponsive scaffold results in decreased retention of proteoglycans and cells (Fig. 5E). However, we postulate that this loss would decrease as the collagenous matrix is further elaborated and organized, but this remains to be tested. That the elaborated extracellular matrix in the bioresponsive PLE-PEGDA hydrogel achieved greater biomechanical properties with a lower proteoglycan content indicates that removing the scaffold is allowing the assembly of a matrix with characteristics of cartilage; the collagenous framework is structured such that a swelling pressure is developed by the constrained proteoglycans.

CONCLUSIONS

The design process used to develop this MMP-7-sensitive scaffold represents a biology-focused approach for improving biomaterials for tissue engineering. In this study, we demonstrate how the temporal characterization of hMSC chondrogenesis within scaffolds can be effectively used to tune scaffold degradation to cellular mechanisms associated with chondrogenesis and matrix elaboration. This led to an improved intercellular distribution of the type II collagen matrix and increased the dynamic modulus in neocartilage constructs. Using a similar approach to tailoring degradation kinetics to chondrogenesis, we can similarly modify the PEGDA scaffold to contain biomimetic (13–15, 47) and bioactive (27) features with temporally relevant presentations.

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