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Fabrication of Integrated Multi-Phasic MSC-Laden Composite Scaffolds for Osteochondral Repair

George W Fryhofer, MD, MTR[1,2]
Hannah M Zlotnick, BS[1,2,3]
Brendan D Stoeckl, MSE[1,2]
Megan J Farrell, PhD[1,2,3]
David R Steinberg, MD[1,2]
Robert L Mauck, PhD[1,2,3]

[1]Dept. of Orthopaedic Surgery
University of Pennsylvania
Philadelphia, PA, USA

[2]Translational Musculoskeletal Research Center
Philadelphia VA Medical Center
Philadelphia, PA, USA,

[3]Dept. of Bioengineering
University of Pennsylvania
Philadelphia, PA, USA

Introduction

Osteoarthritis (OA) is the most common degenerative joint condition in the United States.[1] In addition to OA in older patients that often requires total joint replacement, full thickness cartilage defects are present in the knees of as many as 36% of younger athletes.[2] While some biologic joint-sparing treatments do exist, their efficacy and long term durability in large defects are either limited or unknown. We recently developed, in a Yucatan minipig model, a rapid fabrication method to produce patient-specific engineered osteochondral hydrogel/porous PCL units that can be implanted to completely replace an entire articular surface.[3] That prior work represented only a proof of concept, however, given that it included a hydrogel alone—without cells to provide sustained matrix production. To extend this work, the purpose of this study was to couple a cell-laden hydrogel (methacrylated hyaluronic acid, MeHA) with the porous polycaprolactone (PCL) bone interating phase. Constructs were formed into cylindrical units and mesenchymal stem cells (MSC) viability, matrix elaboration, and construct mechanical properties were evaluated over time in culture in comparison to cell-free controls.

Methods

Construct fabrication

Poly(ε-caprolactone) (PCL) was dissolved in chloroform at 20% wt/vol and mixed with NaCl crystals sieved to ~106 μm. The slurry was poured into a polydimethylsiloxane (PDMS) cylindrical mold (height: 3.5mm, diameter, 5mm), and the solvent was evaporated. PCL units were salt-leeched, sterilized in ethanol, and placed into an array of cylindrical (diameter: 5mm) PDMS casting wells. Juvenile bovine MSCs (passage 3) were suspended ($20x10^6$/mL) in 1% methacrylated hyaluronic acid (MeHA) with 0.05% LAP crosslinker, and pipetted (~40μl) onto the surface of the PCL units. Constructs underwent UV-crosslinking within a nitrogen chamber. Cell-laden constructs were cultured in chemically defined media supplemented with 10ng/mL TGF-β3 (10mL of media per construct) changed two-three times weekly for up to 8 weeks.

MicroCT.

Cell-free constructs (n = 2) were also created using MeHA spiked with zirconium powder (2.5% w/v) and imaged by μCT (Scanco).

Live/dead assay

Constructs (n = 2) were diametrically halved, labeled with Calcein-AM (live), Ethidium Homodimer-1 (dead), and Hoechst stain, and imaged after 4 weeks of culture using a confocal microscope. Maximum projection images were compiled from multiple focal planes using FIJI.

Matrix staining

Following the live/dead assay, constructs (n = 2) were paraffin embedded, sectioned, and stained with Alcian blue (proteoglycans) and nuclear fast red.

Mechanical testing

Thickness and diameter of acellular (n = 6) and cell-laden constructs (n = 4, after 8 weeks of culture) were measured followed by unconfined compression testing. Hydrogels were separated from PCL constructs prior to testing, which consisted of: 1) ramp to 0.2 N; 2) stress relaxation to 10% strain; 3) and dynamic compression (1% strain at 0.5 Hz).

Biochemical analysis

Dimethylmethylene blue (DMMB) assay was used to determine GAG content following proteinase K digestion (n = 4).

Statistics

Data are presented as the mean ± SD. Significance was set at $p < 0.05$. Fig. 3 utilized an unpaired two-tailed t-test.

Results

MeHA/PCL composite scaffolds were reproducibly fabricated in PDMS molds. MicroCT demonstrated some interdigitation at the hydrogel/PCL interface (Fig. 1). Live/dead imaging at 4 weeks demonstrated good cell viability in the construct periphery but fewer viable cells in the center of the gel/PCL interface (Fig. 2A). Proteoglycan staining by Alcian blue identified subjectively greater matrix at the hydrogel periphery, in the area of greater cell

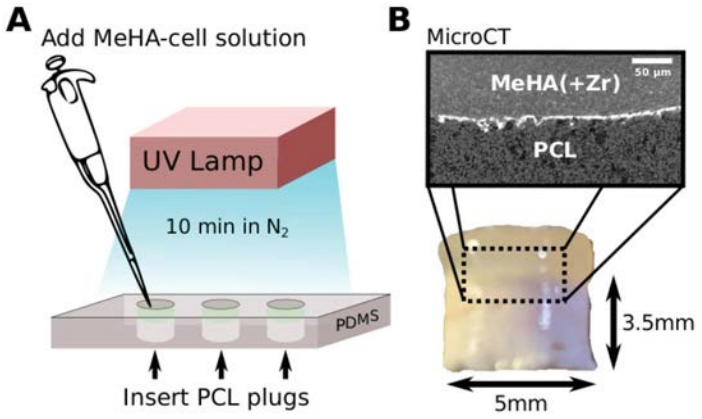

Figure 1. Fabrication of integrated MSC-laden hydrogel/poly-caprolactone composite scaffolds. **(A)** MeHA hydrogels are crosslinked on PCL cylinders under UV light in PDMS casting wells. **(B)** MicroCT of PCL-hydrogel construct with zirconium shows limited interdigitation at the hydrogel-PCL interface.

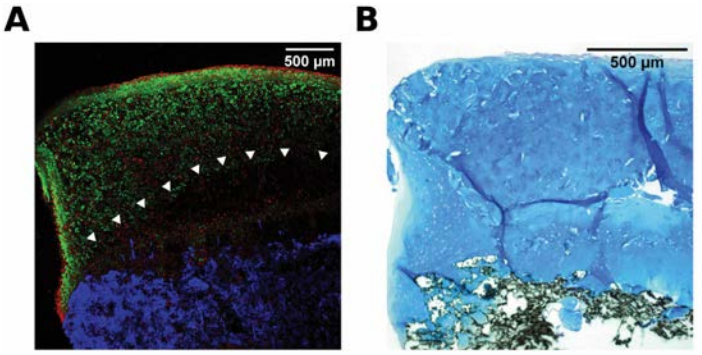

Figure 2. Cell viability and matrix production. **(A)** Live (green)-dead (red) staining with Hoecht's stain (blue) shows live cells in periphery with more dead cells centrally (region demaracated by white triangles). **(B)** Glycosaminoglycan production (Alcian blue) is subjectively more pronounced in peripheral compared to central regions.

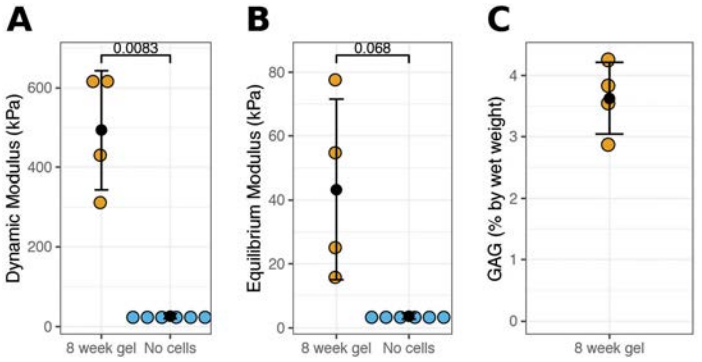

Figure 3. Mechanical and biochemical properties of 8-week cellular (n=4) and acellular (n=6) hydrogel constructs (mean ± SD). **(A)** Dynamic modulus was significantly elevated in cellular constructs after 8 weeks of growth, as was **(B)** equilibrium modulus (trend). **(C)** Mean GAG content after 8 weeks culture was 3.6 ± 0.6%.

viability (Fig. 2B). Mechanical and biochemical properties of the hydrogel portion were assessed after 8 weeks of growth. Mean hydrogel thickness was similar in hydrogels after 8 weeks of growth (2134 ± 337µm) compared to acellular constructs (2179 ± 198µm); however, gel diameter was greater (4.8±0.2mm vs. 3.7 ± 0.3mm, $p < 0.001$). Dynamic modulus was significantly greater at 8 weeks compared to acellular constructs (493.9 ± 149.8 kPa vs. 26.5±6.2 kPa, $p = 0.008$), and a similar trend was observed for equilibrium modulus. At this time point, mean GAG content as a proportion of wet weight was 3.6 ± 0.6%.

Discussion

In this study, we hypothesized that juvenile bovine MSCs could be reproducibly encapsulated within a hyaluronic acid hydrogel/PCL composite scaffold with relatively uniform cell viability and extracellular matrix production. Although reproducible composite scaffold fabrication was achieved, cell viability within the scaffold was not uniform, and instead demonstrated a predilection for greater cell viability in peripheral regions, perhaps driven by greater ease of nutrient diffusion from the culture media at the scaffold periphery. As expected, a GAG-rich matrix was evident after 4 and 8 weeks of growth, accompanied by a significant increase in mechanical properties relative to acellular hydrogels. The dynamic and equilibrium moduli observed here agree with previous results of unconfined compression testing of cell-laden 1% MeHA hydrogels at similar time points.[4] While these results support the overall approach, only one time point was assessed here for histological and mechanical assessment. This prevents assessment of growth trajectory, a factor known to vary among constructs and cell populations.[5] Future studies will evaluate construct maturation at multiple time points and over a longer time course. Additionally, while compression is one of the primary modes of mechanical loading for articular cartilage, shear at the bone-cartilage interface is also critical for function of these implants. Future work will implement "push-off" evaluation of the forming interface with time in culture.[6]

Significance

This study demonstrated the feasibility of reproducibly fabricating viable cell-laden hydrogel/PCL scaffolds with mechanical properties significantly greater than those of cell-free constructs. These findings support the further development of hydrogel/PCL molds with patient-specific geometry, with the ultimate goal of recapitulating native articular geometry and mechanical properties in an implantable osteochondral composite scaffold.

Acknowledgments

This worked was supported by the Department of Veterans' Affairs, and the NIH/NIAMS (R01 EB008722, T32-AR007132, and P30AR069619).

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