Stem Cell-Based Meniscus Tissue Engineering Using a Hydrogel Form of Decellularized Matrix

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Statement of Purpose: The menisci are fibrocartilaginous tissues in the knee with high load-bearing properties due to their unique extracellular matrix (ECM), composed of collagenous proteins and glycosaminoglycans (GAGs)[1]. The limited extent of innate meniscal repair after injury has led to efforts towards engineered tissues. Recently, promising new approaches have incorporated natural scaffolds made from the ECM of decellularized tissues, which preserves tissue-specific molecules that guide the behavior of new cells and facilitate tissue development, without the immunogenic components[2]. A decellularized whole meniscus was developed previously, but cell migration into the dense tissue was limited to ~150 µm over seven days *in vitro*, impeding robust tissue formation[3]. To overcome this limitation, this study sought to create a novel hydrogel from meniscus ECM, supporting the delivery of stem cells for the growth of viable repair tissue.

Methods: The menisci of bovine calves were dissected, minced to 1-2 mm^3 pieces, and lyophilized for 24 h, prior to decellularization at 25 °C. The pieces underwent three cycles of 24 h each in 2% SDS with 10 mM Tris with agitation, followed by 2 h in 0.1% peracetic acid with agitation. Tissue was then washed with three cycles of sterile water and PBS, before lyophilization for 24 h and further biochemical analysis [DNA, sulfated GAG, collagen via hydroxyproline (OHP)]. The decellularized pieces were digested in 0.1% pepsin in 0.01 M HCl for 12 h at 25 °C, and the resulting decellularized ECM (dECM) digestion solution was allowed to repolymerize, using a previously established protocol[2]. Human mesenchymal stem cells (hMSCs; 30×10^6 cells/mL) were encapsulated in either dECM (7.5 mg/mL) or type I collagen (3 mg/mL) hydrogels (25 µL/sample), and cultured for 28 days in chondrogenic media supplemented with 10 ng/mL TGF-β3. Samples were collected at days 0, 14, and 28 for biochemical (DNA, GAG, OHP) and histological (H&E, Alcian blue, Picrosirius red) analyses. Unpaired *t*-tests and ANOVA with Bonferroni *post-hoc* tests were performed using Prism. Data are shown as mean ± SEM.

Results: Decellularization of the meniscus tissue produced a significant decrease in DNA content per dry weight from 0.13 ± 0.014% to 0.034 ± 0.0055% ($p < 0.0001$; n = 7-32), but no significant differences in GAG or OHP content per dry weight. Encapsulation of hMSCs in the resulting dECM hydrogel led to enhanced chondrogenesis, compared to cells in type I collagen alone. By day 14 of culture, cell numbers were greater in dECM hydrogel than in collagen (**Figure 1**). The sulfated GAG and OHP content were both significantly higher in dECM constructs relative to collagen alone at day 28 (**Figure 1**). Histological staining of dECM constructs at day 28 demonstrates the production of a matrix rich in sulfated GAGs and collagens (**Figure 2**). While the OHP content of dECM constructs at day 28 is not significantly different from initial values (**Figure 1**), the stronger intensity of Picrosirius red staining at day 28 suggests the presence of more mature collagen (**Figure 2**). Moreover, the OHP content of collagen constructs decreases throughout the culture period, in contrast to dECM constructs, in which it recovers between days 14 and 28.

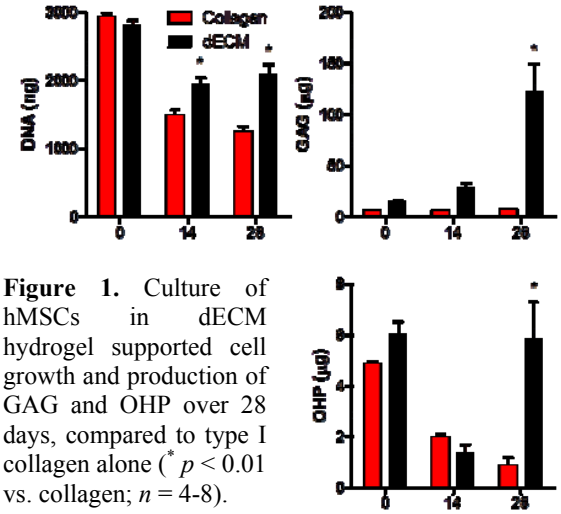

Figure 1. Culture of hMSCs in dECM hydrogel supported cell growth and production of GAG and OHP over 28 days, compared to type I collagen alone ($^*p < 0.01$ vs. collagen; n = 4-8).

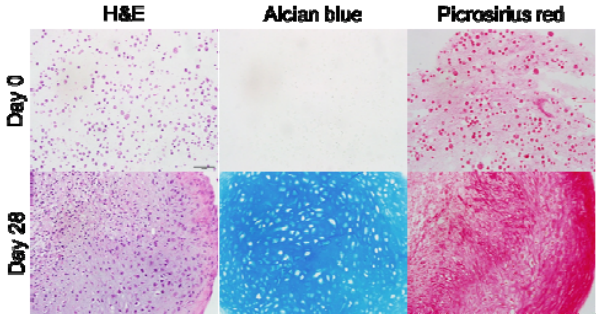

Figure 2. Histological staining of hMSCs in dECM hydrogel at days 0 and 28 for cells (H&E), sulfated GAG (Alcian blue), and collagens (Picrosirius red). 20× magnification, scale bar = 100 µm.

Conclusions: Decellularized meniscus ECM hydrogel retains native, tissue-specific molecules that provide cues to stem cells towards forming new meniscus tissue, as demonstrated by superior cell growth and enhanced GAG and collagen content. dECM hydrogel is therefore a promising new material for meniscus tissue engineering and for future studies to promote meniscal repair.

References: 1) Sweigart MA & Athanasiou KA. Tissue Eng. 2001;7: 111-29. 2) Freytes DO, *et al.* Biomaterials. 2008;29:1630-7. 3) Stapleton TW, *et al.* Tissue Eng. 2008;14:505-18.