Poly(Vinyl Alcohol)-Gelatin Interpenetrating Network Hydrogels for Tissue Engineering Applications

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Statement of Purpose: Erosion of articular cartilage results in subsequent destruction of subchondral bone, generating an osteochondral defect. Osteochondral tissue engineering would benefit from the use of multi-phasic biomimetic scaffolds to promote simultaneous regeneration of articular cartilage and subchondral bone. In the present study, poly(vinyl alcohol) (PVA)-gelatin interpenetrating network hydrogels were fabricated in the presence of poly(ethylene glycol) (PEG) molecules.

Methods: PVA-PEG-gelatin hydrogels were prepared by varying molecular weights and concentrations of PVA, PEG and gelatin. PVA (Mw=145kg/mol) was combined with PEG (Mw=400 or 600g/mol) in a 1:1 weight ratio to form solutions 18% (w/v) PVA and 18% PEG. 5% and 7% (w/v) gelatin was then added to some solutions. The polymer blend solutions were autoclaved for 1h then transferred to a glass mold pre-heated at 90°C and allowed to cool to room temperature for 24h. The hydrogel was then dialyzed in DI water for 3 days to remove PEG. Samples were lyophilized for scanning electron microscopy (SEM) characterization and swell ratio experiments. Swell ratio was calculated as the percentage of wet weight divided by dry weight after hydrating in buffered saline pH 7.4 for 24h and lyopholizing. Hydrogels were tested in unconfined compression up to 20% strain using a TA AR2000 Rheometer. The compressive elastic moduli were calculated via linear regression of 5-15% strain (n=4). Scaffolds were stained with Von Geison to verify retention of gelatin within the hydrogels. Hydrogel cytotoxicity was determined using a MTT assay after 24h of primary human mesenchymal stem cell (hMSC) culture with scaffolds.

Results and Discussion: Increasing Mw of PEG resulted in larger pores within scaffolds. Adding gelatin increased pore size further, confirmed through SEM (Fig.1). Increasing concentration gelatin allowed for a more solid and stiffer hydrogel (Fig.2). The control hydrogels that did not contain gelatin were very soft and compressive moduli were not obtained. PVA hydrogels with and without gelatin did not exhibit significant differences in swell ratio, between 500 and 720%. Von Geison staining verified that gelatin was retained in the hydrogels after dialysis (gelatin samples stained red, PVA controls did not; Fig.3). All of the PVA hydrogels, with and without gelatin, maintained cell viability (>100%) (Fig.4).

Conclusions: Through a systemic solidification process, interpenetrating network hydrogels were obtained from the physical crosslinking of PVA and gelatin and the diffusion of PEG for pore formation. Pore size was optimized by varying PEG Mw and concentration of gelatin. The bio-synthetic hydrogels are promising candidate for osteochondral tissue engineering scaffolds and warrant further investigation. Further work will include examination of PVA Mw, concentrations and strength of collagen, and PEG Mw. Future *in vitro* experiments will examine hMSC chondrogenic and osteogenic differentiation on bi-layered scaffolds for the regeneration of osteochondral tissue. Individual layers will vary in pore size, collagen type and content, and mechanical properties.

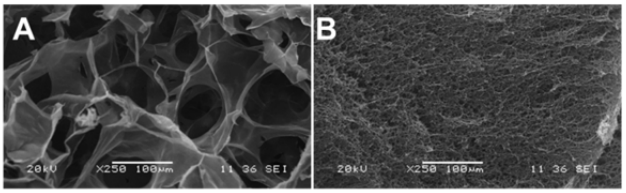

Fig.1. SEM images of 18-PEG600-7G (A) and 18-PEG600-0G (B) hydrogels. Magnifications 250x, scale bar = 100µm.

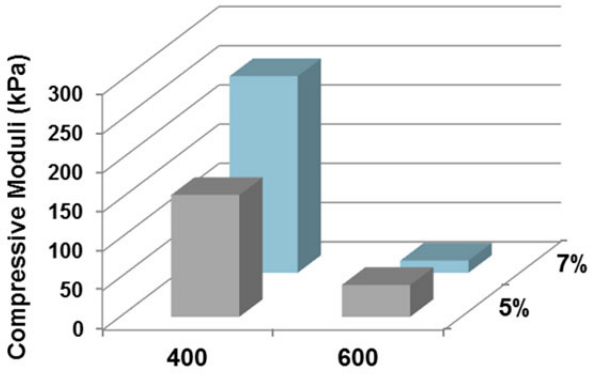

Fig.2. Unconfined compressive elastic moduli of PVA-gelatin hydrogels, for various PEG Mw and concentration of gelatin.

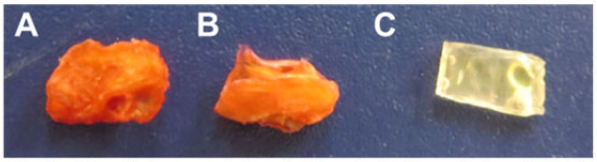

Fig.3. Von Geison staining (red color) for gelatin: (A) 18-PEG400-7G, (B) 18-PEG400-5G, (C) 18-PEG400-0G.

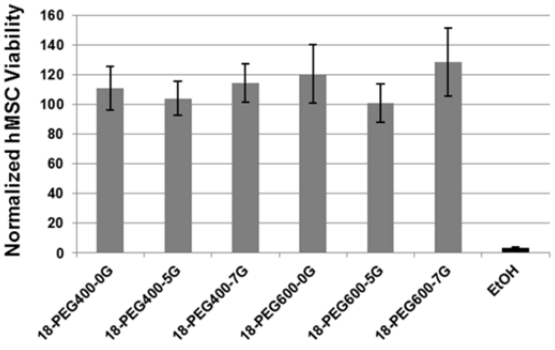

Fig.4. Primary hMSC viability after 24h culture with PVA hydrogels, including a negative control (ethanol, EtOH).