Human Mesenchymal Stem Cell Response to Dynamic Loading of Poly(Vinyl Alcohol) Cryogels

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Statement of Purpose: The prevalence of osteochondral tissue degradation due to injury and disease is increasing; however, these ailments may be mitigated using a tissue engineering approach. Synthetic hydrogels are excellent candidates for creating biomimetic microenvironments for tissue regeneration. Poly(vinyl alcohol) (PVA) is a synthetic, biocompatible, nondegradable polymer that forms hydrogels of varying stiffness by physical or chemical crosslinking and various design parameters.[1,2,3] Mechanical stimulation of various cell-seeded scaffolds has been shown to influence stem cell differentiation, proliferation, and tissue growth.[4,5] Thus, the goal of this study is to optimize the mechanical properties (*i.e.* porosity and compressive elastic modulus) of PVA cryogels by varying molecular weight, concentration, and cryogel cycles in order to attain stiffnesses that, when combined with dynamic loading, will influence mesenchymal stem cell (MSC) mechanotransduction towards osteochondral lineages.

Methods: Neat PVA (M_w=145 kg/mol and M_w=95 kg/mol) was dissolved in DI water to make 3 and 6% (w/v) solutions. The polymer solutions underwent repeated freeze/thaw (F/T) cycles (1 cycle = -20°C 22h, ice water 1h, RT 1h). Monolayered and bi-layered scaffolds (each layer exhibiting different numbers of F/T cycles) were prepared. Scaffolds were disinfected and hydrated in non-inductive α-MEM media (10% fetal bovine serum, 1% penicillin-streptomycin) then incubated for 24h at 37°C, 5% CO_2. Scaffolds were tested in unconfined compression up to 20% strain using a TA AR2000 Rheometer. The compressive elastic moduli were calculated via linear regression of 4-20% strain (n=3). Primary human MSC (20,000 cells/well, passage 5) viability was examined in the presence of cryogels after 24h culture via a MTT assay. In addition, cell-seeded scaffolds ($1.7x10^6$ cells/mL) were pre-cultured for 24h prior to dynamic loading. A 20% cyclic compressive strain was applied for 5 minutes and cell viability was evaluated 24h later via a MTT assay.

Results: Scanning electron microscopy (SEM) confirmed scaffold porosity. A range of stiffness values (0.11 kPa – 55 kPa), and thus porosity, was obtained by varying the concentration, the number of F/T cycles, and the M_w for the PVA monolayer and bi-layer scaffolds. The moduli of the bi-layer scaffolds were intermediate between that of the individual layers, but closer to that of the stiff layer. PVA scaffolds were not cytotoxic (cell viability > 100%). Dynamic loading showed an increase in cell viability for cell-seeded scaffolds (change in cell viability ≥ 27%).

Conclusions: F/T cycles, concentration, and M_w can be used to control scaffold compressive moduli. Furthermore, the PVA scaffolds were not cytotoxic to cells as confirmed by the MTT assay. Dynamic loading increased hMSC viability on PVA scaffolds. Future work will include long-term dynamic loading sequences of 2 weeks in order to investigate MSC differentiation in response to the mechanotransductive cues being provided by stiffness and dynamic loading.

References: 1. Hassan CM. Adv. Polym. Sci. 2000; (153:37-65). 2. Bray JC. J Biomed. Mater. Res. 1973; (7:431-443). 3. Schmedlen RH. Biomaterials. 2002; (23:4325-4332). 4. Bian L. Tissue Eng Part A. 2012; (18:715-724). 5. Pelaez D. Stem Cells Dev. 2009. (18:93-102).

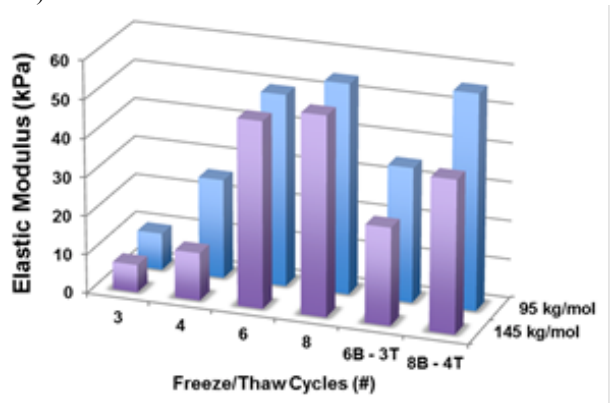

Fig.1. Unconfined compressive elastic modulus increases with increasing number of F/T cycles for mono- and bi-layered scaffolds and decreasing PVA molecular weight.

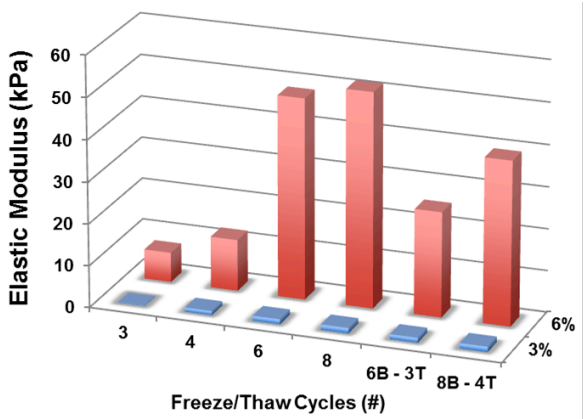

Fig.2. Unconfined compressive elastic modulus increases with increasing number of F/T cycles and increasing PVA concentration.

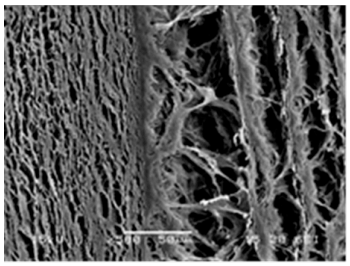

Fig.3. SEM micrograph of 3% PVA (M_w = 145 kg/mol) bi-layered scaffold with 3 F/T (left) and 6 F/T (right).