

## Methacrylated Hyaluronan and Poly(vinyl alcohol) Scaffolds for Periodontal Tissue Regeneration

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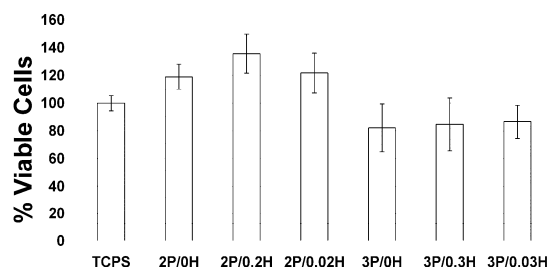
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**Statement of Purpose:** The pathogenesis of periodontal disease (PD) is intimately related to osteoimmunology, in which inflammatory mediators activate pathways leading to bone resorption. A major goal of periodontal therapy is the restoration of damaged tissues to their original form and function. For the purpose of regenerating periodontal tissue, a methacrylated hyaluronan /poly(vinyl alcohol) (HA-MA/PVA) scaffold was developed and fabricated with varying pore diameters. HA is a highly conserved macromolecule found in numerous species<sup>[1]</sup> and is an essential component of periodontal ligament (PDL) extracellular matrix.<sup>[2]</sup> HA plays an important role in both the early and later stages of bone formation, and has been shown to be osteoinductive<sup>[3]</sup>. In this preliminary study we examine human mesenchymal stem cell (hMSC) toxicity and adhesion. Our long term goal is to develop a bilayered scaffold for the regeneration of PDL and alveolar bone in order to treat periodontal defects.

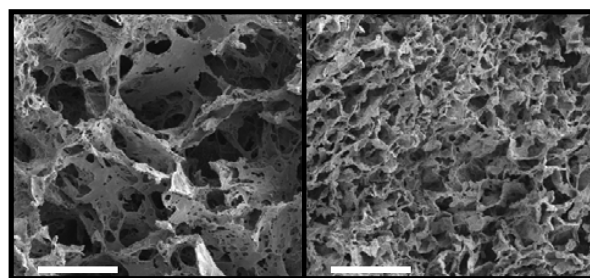
**Methods:** HA (MW=66K) was complexed with an ammonium salt and dissolved in dimethyl sulfoxide; methacrylic anhydride was added and reacted for 24 h. An excess volume of 0.4M NaCl was added followed by an excess of ethanol. The HA-MA precipitate was washed with ethanol and vacuum dried, then characterized by <sup>1</sup>H-NMR. Scaffolds were fabricated by freeze-drying solution blends of PVA (MW=100K) and HA-MA. PVA solutions of 2 and 3% (w/v) were blended with 1 and 10% (w/w) of HA-MA, which were frozen at -20°C for 12 h then lyophilized for 12 h. PVA solutions without HA-MA served as controls. To covalently crosslink the HA-MA constituent, dry scaffolds were placed in a 75:25 ethanol:1M CaCl<sub>2</sub> solution, followed by 100mM CaCl<sub>2</sub> solution. Scaffolds were rinsed in DI water, then placed in 0.5% aqueous photoinitiator (D-2959, Ciba) solution and exposed to UV radiation for 8 min. Scaffolds were soaked in DI water for 30 min; fresh DI water was replaced twice. Swelling ratios of the scaffolds was evaluated by immersion of dry scaffolds in PBS at 25°C. The retention of HA within the construct was verified by Toluidine Blue O staining. Cytotoxicity of the scaffolds was measured by an MTT assay after 24 h of culture with hMSCs. To demonstrate interactions of hMSCs with the material, scaffolds and controls were cultured with hMSCs for 3 days. Samples were then fixed, gold coated and characterized by SEM.

**Results:** The methacrylation conjugation efficiency was ~80% for the modified HA, as determined by <sup>1</sup>H-NMR. Freeze-drying resulted in porous cylindrical scaffolds that swelled upon hydration. After crosslinking, the HA-MA remained within the scaffold, as verified via Toluidine Blue O staining. The swell ratios of the PVA controls and scaffolds containing various weight percents of HA-MA were as follows: 2PVA control = 953±172%, 2PVA/0.2HA = 927±162%, 2PVA/0.02HA = 928±241%, 3PVA control = 1100±97%, 3PVA/0.3HA = 876±14%, 3PVA/0.03 = 1093±65%. hMSCs were viable on the HA-

MA/PVA scaffolds as determined by an MTT assay; the 3%PVA samples with and without HA-MA were slightly cytotoxic compared to the TCPS control and 2%PVA scaffolds (Fig.1). SEM images show hMSCs spreading on the HA-MA/PVA scaffolds. The porous structure of the scaffolds is also evident in the SEM images (Fig.2); 2%PVA scaffolds exhibit larger pore diameters (~120µm) compared to 3%PVA scaffolds (~30µm).



**Fig.1.** MTT assay results after 24 h of hMSC culture with control (tissue culture polystyrene – TCPS) and PVA/HA scaffolds. Cell viability was normalized to TCPS control.



**Fig.2.** SEM images of HA-MA/PVA scaffolds, 2P/0.2H (left) and 3P/0.3H (right); scale bar = 100µm.

**Conclusions:** The HA-MA/PVA scaffolds retain their structure and HA content after hydration and are non-cytotoxic. Adhesion of hMSCs was difficult to verify by SEM alone and histological sectioning and H&E staining will be required to verify cell adhesion to the scaffold; the HA-MA constituent appeared similar to hMSC cytoplasm which made distinction difficult. Future experiments will include degradation experiments and unconfined compression tests. Bilayered scaffolds will be fabricated by crosslinking two distinct scaffolds together, with different pore diameters and compressive moduli, to regenerate of connective tissue and alveolar bone together for the treatment of periodontal defects. PDL adult stem cells will be used to examine stem cell differentiation.

**References:** <sup>[1]</sup>Allison DD, Grande-Allen KJ. *Tissue Eng*, 2006;12:2131. <sup>[2]</sup>Mesa FL *et al.* *Histol Histopathol*, 2002;17:747. <sup>[3]</sup>Bastow ER *et al.* *Cell Mol Life Sci*, 2008;65:395.

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