

# Airbrushed Amorphous CaP-Polymer Nanofiber Scaffolds with Enhanced Cell Penetration for Bone Tissue Regeneration

W. Tutak<sup>1</sup>, K. Hoffman<sup>1</sup>, D. Skrtic<sup>1</sup>

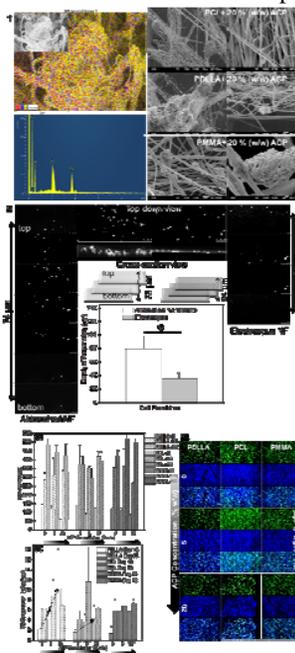
1. American Dental Association Foundation, Gaithersburg, MD, USA

**Statement of Purpose:** Polymer nanofibers are promising materials for bone tissue regeneration because their ability to bio-mimic extracellular matrix and degrade under physiological conditions. Recent studies indicate that the nanofiber mats can modulate human bone marrow stromal cell (hBMSC) response *in-vitro* without the presence of osteogenic supplements [1]. However, the commonly used electrospun nanofiber mats have poor cell penetration depth due to small pore size and provide only limited 3D environment. More open scaffold design that can provide improved bio-mimetic environment is important aspect of a scaffold design for *in-vitro* and *in-vivo* applications. To create the open scaffold architecture (larger pore size) we used a polymer fiber airbrushing method to synthesize the nanofiber scaffolds. The mats were formulated using three biodegradable polymers used in medical applications. The polymer solutions were mixed with amorphous calcium phosphate (ACP, to deliver Ca and P ions) prior to airbrushing. The purpose of this study was to evaluate: (i) the ability to incorporate ACP within various polymeric systems to form composite nanofibers using the airbrush method (ii) evaluate cell penetration within the novel airbrushed scaffolds (iii) evaluate hBMSC response (DNA and Osteocalcin (OC) biomarker) to ACP-polymer fiber composite.

**Methods:** Three biodegradable polymers were used: poly-caprolactone (PCL, 5% (w/w) in 50:50 chloroform/acetone, poly-D,L-lactic acid (PDLLA, 8% (w/w) in acetone and poly(methyl-methacrylate) (PMMA, 10% (w/w) in 50:50 chloroform/acetone. ACP was incorporated into the polymers at a level of 0%, 5% and 20% (dry w/w) [2]. A commercial airbrush (Master Airbrush, G222-SET) with a d= 0.3 mm nozzle was adapted to deposit nanofiber (30-40 PSI of air, 20 cm distance between nozzle and target). Scaffolds for cell penetration study were either electrospun using PCL 10% (w/w) in 75:25 chloroform/methanol at 2 ml/h, 15 cm away from an aluminum target at 16.5 kV voltage or airbrushed (see above setting). All mats were characterized using scanning electron microscopy (SEM) (Hitachi S-4700-II FE-SEM). Energy dispersive spectroscopy (EDS, AZtecEnergy) was used to detect Ca and P ions from the ACP. Primary hBMSCs were seeded at 10000/cells in 48 well plates (n=3) and cultured under standard condition (for up to 50 days) without osteogenic supplements. Cell penetration within the scaffolds was measured using confocal microscope (Leica TCS SP5, 10x, 0.7  $\mu$ m step size, pinhole size 1AU) and analyzed on fluorescently labeled, fixed cells (day 1, n=3) with Image J (NIH). Cell growth (day 16 and 50) was quantified using Picogreen DNA assay (n=3) and enzyme-linked immunosorbent assay (ELISA, BT-460) kit was used to measure osteocalcin (OC) biomarker levels (n=3).

**Results:** All polymers examined in this study were successfully loaded up to 20% (w/w) with ACP, as

confirmed by EDS and TGA (data not shown), and then airbrushed to form non-woven scaffolds (Fig.1). The polymer matrices were then tested for their ability to host the hBMSC cells. Specifically, we assessed cell penetration rate within the electrospun and airbrushed scaffolds.



Based on confocal imaging results it can be seen that hBMSCs penetrated almost twice as deep in the airbrushed scaffold than electrospun mats (Fig. 2). These results are most likely due to the larger pore size between airbrushed nanofibers [3]. We also investigated the composite CaP-polymer matrix ability to support the cell growth and osteogenesis. Obtained results indicate that cells have grown well on all airbrushed scaffolds and cell growth rate was unaffected by the ACP content (Fig. 3A). Projected images of fluorescent cells (at day 16)

show similar cell nuclei and actin distribution for all sample types (Fig. 3B). Comparison of OC biomarker expression levels (day 16 & 50) for polymers with and without ACP didn't yield statistical difference. However, an increase in ACP content caused relative increase in detected OC levels (in PDLLA and PCL, day 16). This suggests (corroborated by ion release and scaffold degradation study-data not shown) that selected polymers likely did not release enough ACP (either by ACP diffusion or polymer degradation) to significantly affect hBMSC response. **Conclusions:** This study revealed that (i) airbrushed nanofibers can be easily loaded with CaP biomolecules (ACP) (ii) airbrushed nanofiber scaffolds allow for a greater cell penetration, and therefore provide more of a 3D-like environment (iii) hBMSCs demonstrated a limited response to ACP biomolecules (iv) the amount of ACP released from fibers is likely restricted by the type of polymeric system. Future studies will be focused on adjusting polymer degradation rates to control and enhance ACP release rate, and determining pore size distribution, tortuosity and mechanical properties of the airbrushed polymer networks.

**References:** (1) Kumar, G., et al. *Biomaterials*, 2011. 32(35): p. 9188-9196. (2) Skrtic et al., *Journal of Research-National Institute of Standards and Technology*, 2003. 108(3): p. 167-182. (3) Tutak, W., et al., *Biomaterials*, 2013. 34(10): p. 2389-2398.