Effect of hydroxyapatite-coated PLLA and PCL porous scaffolds on bone formation *in vivo*

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Statement of Purpose: Ideal porous scaffolds should support new tissue formation and degrade in concert with newly generating tissue. Poly (L-lactic acid) (PLLA) and poly (ε-caprolactone) (PCL) are FDA approved biodegradable polymers and have been studied for bone tissue engineering applications. However, these polymers have poor osteoconductivity to support bone growth. To improve osteoconductivity, biomineral coatings have been developed and characterized their osteoconductivity *in vitro*. The goal of this study was to determine the effects of mineral coated porous scaffold made of two different biodegradable polymers on bone formation *in vivo*. We computationally designed and fabricated identical PLLA and PCL porous scaffolds which were mineral-coated using a modified simulated body fluid (mSBF). Four groups of the scaffolds (coated or uncoated PLLA and PCL) were seeded with bone morphogenic protein-7 (BMP-7) transduced fibroblasts and implanted subcutaneously into mice to characterize bone formation.

Methods:

Scaffold Design and Fabrication: PLLA and PCL porous scaffolds with 5mm diameter and 3mm height (pore size = 550μm) were designed using image-based technique, and were fabricated using indirect solid freeform fabrication (SFF) technique.

Biomineral Coating: The fabricated PLLA and PCL scaffolds were hydrolyzed in a 0.1M NaOH for 60 minutes and incubated at 37 °C in mSBF for 14 days.

Surface Analysis: The morphology and composition of the biomineral coatings on the scaffolds was investigated by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and X-ray diffraction (XRD).

Cell Seeding and Animal Implantation: Human gingival fibroblasts were cultured and transduced with adenovirus BMP-7. The fibroblasts were seeded into the sterilized scaffolds with collagen gel and incubated for 24 hours. The scaffolds were subcutaneously implanted into immuno-compromised mice for 3 and 10 weeks.

Micro-Computed Tomography: The scaffolds were scanned using a high resolution Micro-CT Scanner (GE Healthcare Inc., CAN) before and after implantation. The region of interest (ROI) (5mm diameter x 1.8mm height) was selected at the center of a scaffold, and bone ingrowth was quantified by subtracting bone volume before implantation from after implantation. Tissue mineral density (TMD) within ROI was also calculated. One-way ANOVA ($p < 0.05$) was performed (N = 6-8).

Results/Discussion: PLLA and PCL porous scaffolds with the identical architectures were successfully fabricated (Fig.1 (b, d, f and h)). The presence of biomineral coatings within the coated scaffolds were observed with micro-CT (Fig.1 (e, g), pointed by green arrow). SEM micrographs show a continuous coating over the entire pore surfaces of the coated scaffolds with a spherulitic morphology and a plate like morphology (Fig.1 (i, j)). EDS and XRD analysis confirmed the coatings was composed primarily of Ca and P with a Ca/P ratio of 1.58 for PLLA coatings and 1.56 for PCL coatings, and characteristic peaks of hydroxyapatite at $2\Theta = 25.9°$ and 31.95°.

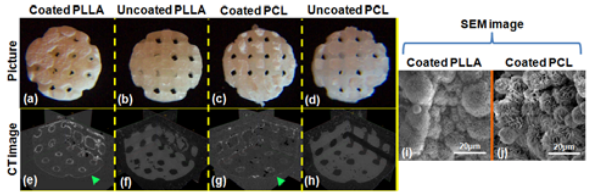

Fig 1: The fabricated Coated PLLA, PLLA, Coated PCL and PCL scaffolds; Pictures (a – d), CT images (e – h), and SEM image of mineral (i, j)

Bone ingrowth was observed at both time points as shown as 3D rendering images (red) within ROI (yellow) (Fig.2). More bone formation was observed at the later time points. At 10 weeks, the coated scaffolds showed greater bone ingrowth than the uncoated scaffolds, and bone tissue grew following the designed scaffold architectures (Fig.2 (e, g)).

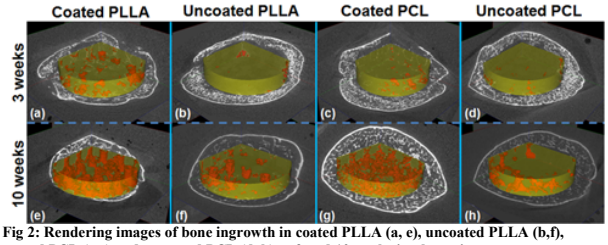

Fig 2: Rendering images of bone ingrowth in coated PLLA (a, e), uncoated PLLA (b,f), coated PCL (c,g) and uncoated PCL (d, h) at 3 and 10 weeks implantation.

There was no difference of bone ingrowth between the scaffold groups at 3 weeks (Fig.3 (a)). However, the coated scaffolds had significantly more bone ingrowth than the uncoated scaffolds at 10 weeks (Fig.3 (a)). The coated PLLA had significantly greater TMD than the uncoated PLLA at 10 weeks (Fig.3 (b)). The data suggest that the mineral coating may have an influence on later-stage bone tissue formation by differentiated osteoblasts rather than early-stage osteogenic differentiation of precursor cells.

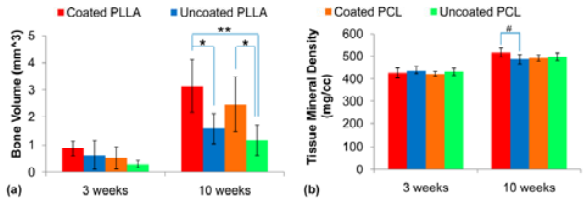

Fig 3: Bone volume (a) and Tissue mineral density (b) of the ROI

Conclusions: Biomineral coatings were successfully applied on designed PLLA and PCL scaffolds. Bone ingrowth was improved by biomineral coatings, and the shapes of the bone formation were controlled by computationally designed scaffold architectures. Current investigation of histology and mechanical property may reveal further details of the advantages of the coating.

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