3D Printed Bone Scaffolds with Biomimetic Nanomaterials and Vascular Mimicking Microchannels

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Statement of Purpose: Critical sized bone defects resulting from traumatic injury, cancer, degenerative diseases, or birth defects present a crucial clinical problem. The area of such defects is typically large, and is often debilitating to those afflicted. As a multifunctional tissue comprised of both a porous nano bone extracellular matrix (ECM) and an interconnected microstructure of blood vessels, it is hard to repair due to the need for an adequate vascular network [1]. Although various biomaterials and 3D fabrication approaches to address critical sized bone defects have been investigated, it is still very challenging to replicate the complex integration of vasculature within a bone structure. In this study, we aim to integrate 3D bioprinting and nanomaterials to create a vascularized bone scaffold.

Methods: Here we have designed and 3D printed a series of microstructured scaffolds, containing both a bone matrix and a microvascular network. The size of the bone microstructure was kept constant (i.e., 350 µm hexagonally shaped pores alternating with dense linear patterns, layer by layer, to adequately restrict fluid perfusion through the bone network itself). The sizes of the microvascular network were 500 µm (large vascular, Figure 1A) and 250 µm (small vascular, Figure 1B). Printed scaffolds were then conjugated with hydrothermally treated biomimetic nanocrystalline hydroxyapatite (nHA, bone minerals), using an acetylation chemical functionalization process. Scaffolds were then characterized by scanning electron microscope (SEM), compressive mechanical testing and experimental fluid flow measurement. Human bone marrow derived mesenchymal stem cell (hMSC) adhesion, proliferation and osteogenic differentiation were investigated in these scaffolds *in vitro*. Human umbilical vein endothelial cell (HUVEC) adhesion, proliferation and confocal imaging were also conducted.

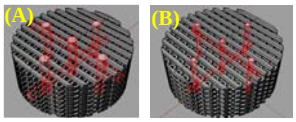

Figure 1; (A) 500 µm large vascular network and (B) 350 µm small vascular network in bone scaffolds.

Results: Compressive Young's modulus showed the scaffold with a smaller microvascular network has higher mechanical stiffness and more bone-like properties. Flow study also revealed that pressure and flow rate waves traveling through the microchannels behave like fluid flowing through a native blood vessel. For 4 hour cell adhesion study, our result demonstrated that 3D printed scaffolds with a smaller microvascular network and nHA had the greatest cell adhesion. In addition, 5 day hMSC proliferation result also showed an excellent cell growth on all scaffolds, with the greatest increase on small microvascular nHA scaffolds, at one and five days. More importantly, Figure 2 showed a greatly improved hMSC osteogenic differentiation (calcium deposition) on scaffolds modified with nHA and microvascular network. HUVEC adhesion, proliferation and confocal imaging also demonstrated positive results (Figure 3).

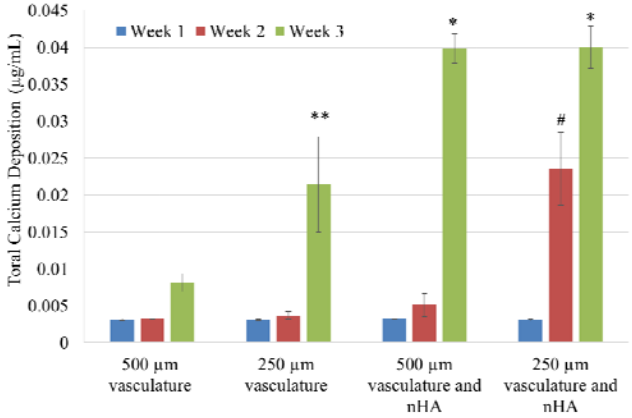

Figure 2; Enhanced calcium deposition on microvascular nHA modified scaffolds after 3 weeks. *p<0.05 when compared to 3D printed bone scaffolds with 500 and 250 µm vasculature at week 3; **p<0.05 when compared to 3D printed bone scaffolds with 500 µm vasculature at week 3; #p<0.01 when compared to all other scaffolds at week 2.

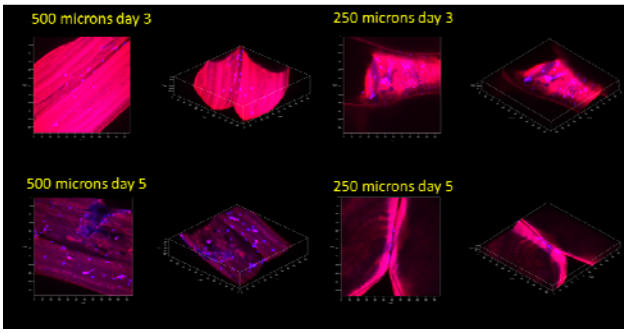

Figure 3; Enhanced HUVEC proliferation and morphology on nHA modified scaffolds after 5 days

Conclusions: In this study, we have designed and successfully 3D printed bone constructs with vascular mimicking microchannel networks and biomimetic nHA. The presence of a biomimetic nanostructured bone minerals and a microvascular network in a well-designed bone matrix has yielded a construct with both good mechanical properties and favorable biocompatibility properties for improved hMSC growth, osteogenic differentiation, as well as vascular cell activity.

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References:

1. Cooke MN, et al. J Biomed Mater Res B Appl Biomater, 2003;**64**(2):65-69.