3D Printed Bone Scaffolds with Microvascular Network and Nano Hydroxyapatite for Improved hMSC Functions

Benjamin Holmes[1], Kartik Bulusu[1], Michael Plesniak[1] and Lijie Grace Zhang[1,2]*

1. Department of Mechanical and Aerospace Engineering, and 2. Department of Medicine, The George Washington University, Washington, DC 20052.

Introduction

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.

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 addition, it is difficult to create large engineered bone constructs that replicate macroscopic patient specific injuries, while also adequately incorporating biomimetic nano and micro architecture. In this study, we will integrate 3D bioprinting and nanomaterials to create a series of bone scaffolds with microvascular channels, for efficient and enhanced vascularized bone growth.

Materials and Methods

Scaffolds with 500 and 250 µm radius vascular channels within a hexagonal bone matrix were designed using the Rhino 3D modeling package and printed on a solidoodle 3D printer (Figure 1). Printed scaffolds were then conjugated with nanocrystalline hydroxyapatite (nHA, bone minerals), using an acetylation chemical functionalization process

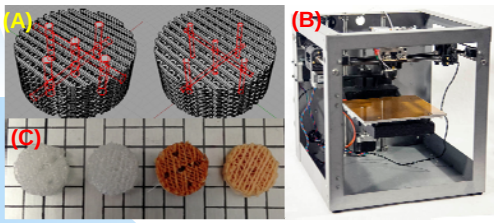

Figure 1; (A) 3D CAD designs (B) our printer (C) 3D printed and nHA modified scaffolds.

Scaffolds were then characterized by scanning electron microscope (SEM) and a pulsatile blood flow setup. Human bone marrow derived mesenchymal stem cell (hMSC) 4 h adhesion, 5 day proliferation and 3 week osteogenic differentiation were investigated *in vitro*. Human umbilical vein endothelial cells (HUVECs) adhesion and proliferation were also evaluated and imaged using a confocal microscope.

Results

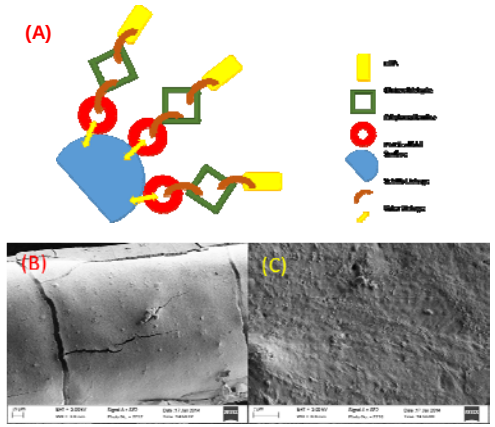

Figure 2; (A) Schematic illustration of an acetylation chemical functionalization process and (B-C) SEM images of a printed scaffold with nHA at low and high magnification.

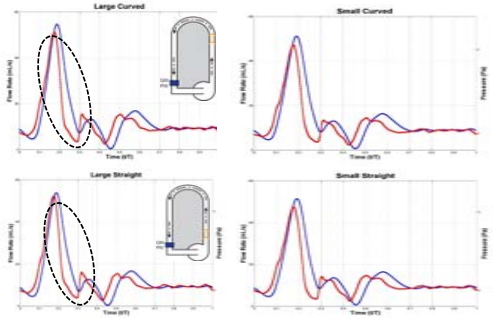

Figure 3; Experimental flow mechanics, pressure and flow analysis.

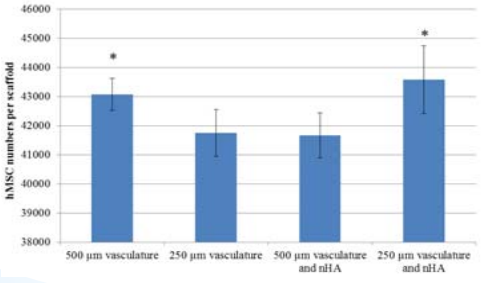

Figure 4; hMSC adhesion on 3D printed scaffolds; *p<0.05 when compared to scaffolds with 250 µm vasculature and with 500 µm vasculature and nHA.

Results

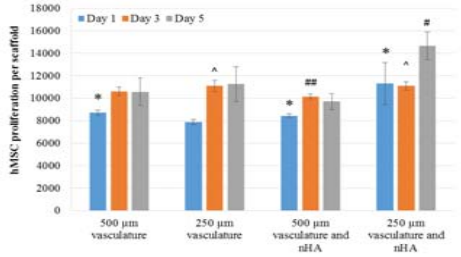

Figure 5; Improved hMSC proliferation on 3D printed scaffolds with nHA and small vasculature; #p<0.01 when compared to all other scaffolds at day 5 and ##p<0.05 when compared to 500 and 250 µm vasculature without nHA; ^p<0.05 when compared to scaffolds with 500 µm vasculature and nHA at day 3; and *p<0.05 when compared to scaffolds with 250 µm vasculature at day 1.

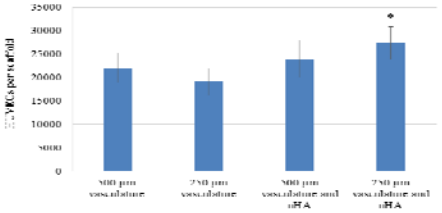

Figure 6; HUVEC adhesion; *p<0.05 when compared to scaffolds with 250 µm vasculature

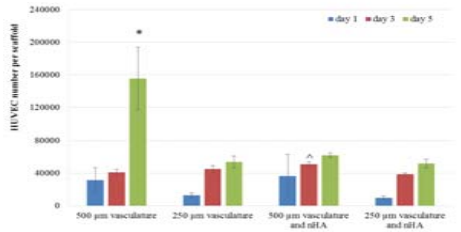

Figure 7; HUVEC proliferation on 3D printed scaffolds; *p<0.05 when compared to all other scaffolds at day 5; and ^p<0.05 when compared to scaffolds with 500 µm vasculature and 250 µm vasculature with nHA at day 3.

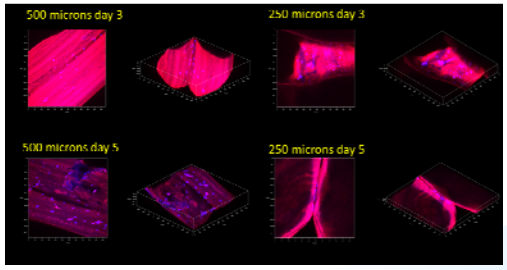

Figure 8; HUVEC 3 and 5 day confocal imaging on nHA coated scaffolds

Results

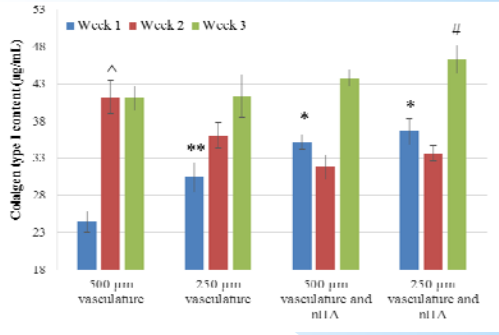

Figure 9; Enhanced type I collagen synthesis on microvascular nHA modified scaffolds after 3 weeks. *p<0.01 when compared to scaffolds with 500 and 250 µm vasculature at week 1; **p<0.05 when compared to scaffolds with 500 vasculature at week 1; ^p<0.05 when compared to all other scaffolds at week 2; #p<0.05 when compared to scaffolds with 500 µm vasculature at week 3

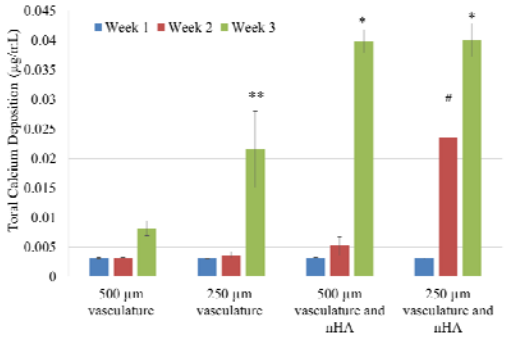

Figure 10; Enhanced calcium deposition on microvascular nHA modified scaffolds after 3 weeks. *p<0.05 when compared to 3D printed bone scaffolds with 500 and 250 µm vasculature at week 3; **p<0.05 when compared to 3D printed bone scaffolds with 500 µm vasculature at week 3; #p<0.01 when compared to all other scaffolds at week 2.

Conclusion

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 favorable biocompatibility properties for improved hMSC growth, osteogenic differentiation, as well as vascular cell activity.

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