Simulated Body Fluid Nucleation of 3D Printed Elastomeric Scaffolds for Bone Regeneration

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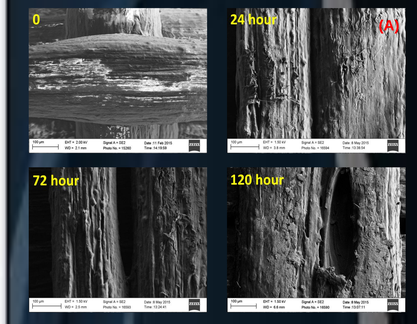
Introduction

Osseous tissue defects caused by trauma present a common and serious clinical problem. Although traditional clinical procedures including autografts and allografts have been successfully employed several limitations persists with regards to insufficient donor tissue, disease transmission and inadequate host-implant integration. Therefore, better strategies are necessary for the treatment of osseous tissue defects. Thus, the current work aims to address current limitations with regards to inadequate host tissue integration through the use of a novel elastomeric filament for fused deposition modeling (FDM) fabrication of bioactive scaffolds. The current system served to utilize several advantages of rapid prototyping as well as explore the use of biomimetic nanobiomaterials for osteogenic modulation of human fetal osteoblasts (hFOBs).

Materials and Methods

A novel poly(vinyl alcohol) (PVA)-based thermoplastic polyurethane (TPU) elastomeric composite filament (Gel-Lay, Poro-Lay, Matterhackers, Lake Forest, California) was used to manufacture porous scaffolds. A 35 mm × 35 mm × 2.5 mm model was designed in Rhino3D (McNeel North America, Seattle, Washington) and the resulting CAD file was prepared for 3D printing by conversion to a computer numerical control file with the open source software package Slic3r. Next, the models were printed using a Solidoodle© table-top fused deposition modeling printer (Solidoodle©, Brooklyn, New York) with Gel-Lay Porous 3D Printing Filament. 3D printed TPU/PVA composite scaffolds were immersed in ultrapure water and ultrasonicated at 60℃ for three 90 minute cycles to fully dissolve the PVA component. Subsequently, scaffolds were washed with distilled water and air-dried at room temperature. TPU scaffolds were nucleated in simulated body in a polypropylene beaker with a magnetic stir bar at 36.5±1.5℃ for 24, 72 and 120 hour nucleation times. Air dried nucleated scaffolds and a non-nucleated control were trimmed into 5 mm X 5 mm squares, placed in 96-well cell culture plates and sterilized under ultraviolet light for 20 minutes prior to hFOB cell studies.

Results



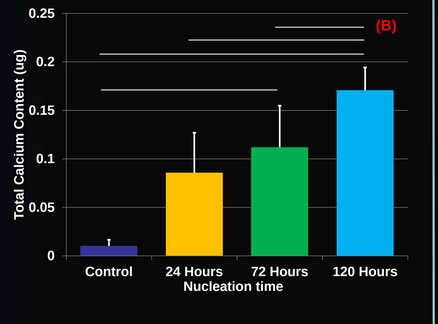


Figure 1. SEM of nucleated TPU 3D printed scaffolds (A) with corresponding quantified Ca2+ (B) as a function of nucleation time in SBF. (n=5, p<0.05).

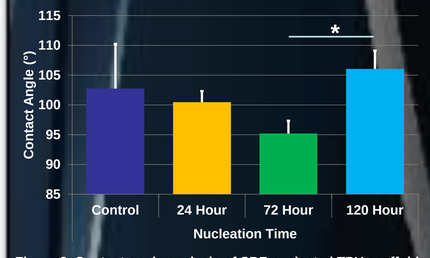


Figure 2. Contact angle analysis of SBF nucleated TPU scaffolds. (n=5, p<0.05).

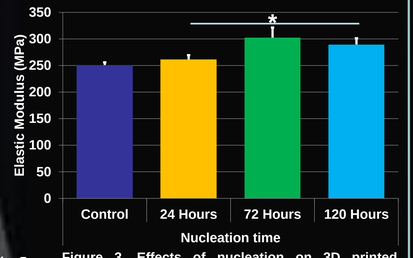


Figure 3. Effects of nucleation on 3D printed thermoplastic polyurethane/polyvinyl alcohol composites. All nucleated samples showed a significant increase in mechanical properties when compared to non-nucleated control. (n = 5, p < 0.05).

Results

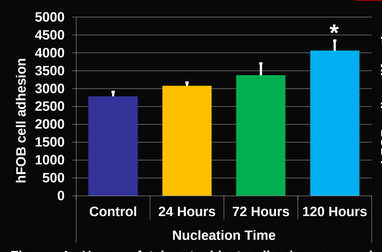


Figure 4. Human fetal osteoblast adhesion on nucleated 3D printed thermoplastic polyurethane/polyvinyl alcohol composites. A noticeable increase in cell adhesion was observed as a factor of nucleation time with 120 hour nucleation resulting in a 31% percent increase. (N = 3, p < 0.05).

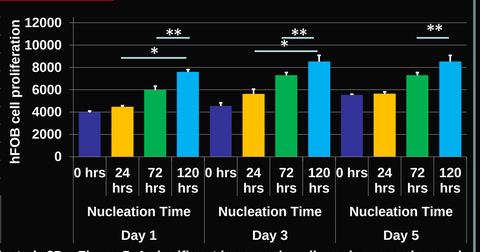


Figure 5. A significant increase in cell number was observed on 72 and 120 hour samples when compared to control with an increase of 34% and 48% after 1 day; a 38% and 47% increase after 3 days; and 24% and 35% respectively. (N = 3, * when compared to control; ** when compared to 0 and 24 hours p < 0.05)

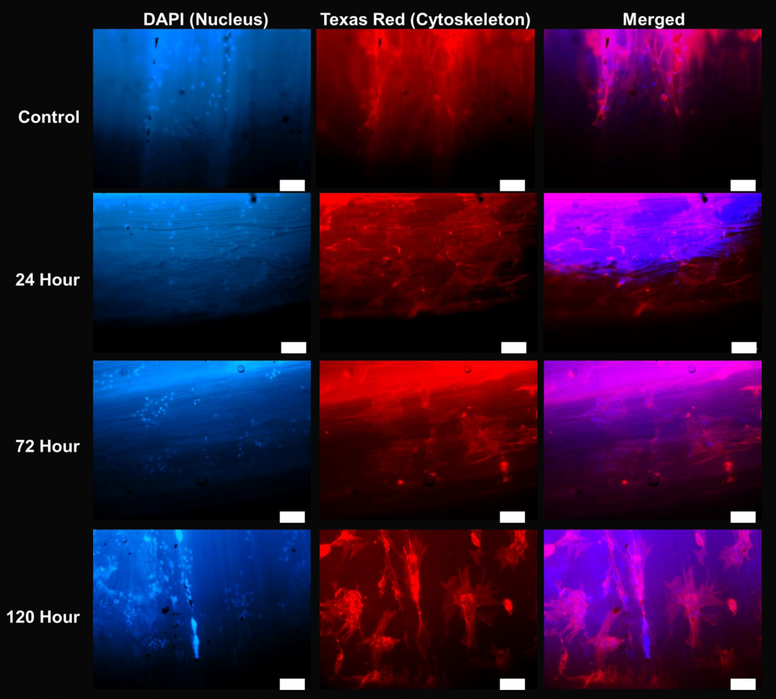


Figure 6. Fluorescence microscopy analysis of 24 hour hFOB cell adhesion on nucleated 3D printed TPU scaffolds. Excellent cell attachment and spreading was observed on all samples including non-nucleated control. Scale bar = 100um

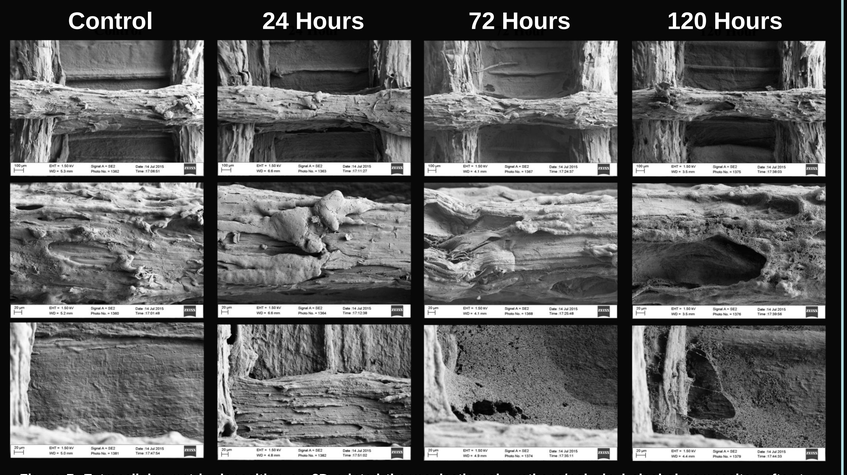


Figure 7. Extracellular matrix deposition on 3D printed thermoplastic polyurethane/polyvinyl alcohol composites after two weeks. All nucleated samples showed noticeable extracellular matrix deposition when compared to non-nucleated control after two weeks.

Results

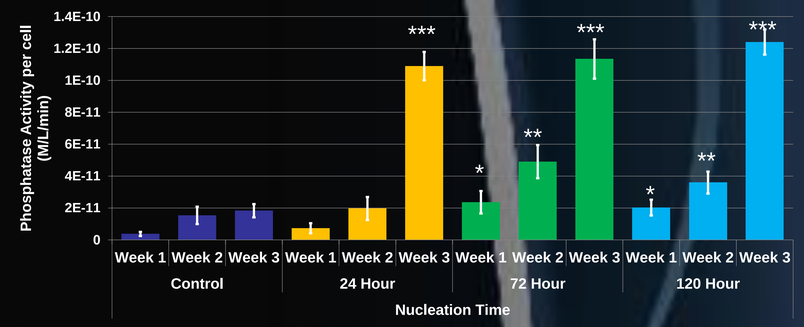


Figure 8. Alkaline phosphatase activity on 3D printed thermoplastic polyurethane/polyvinyl alcohol composites. 72 and 120 hour nucleated samples showed a significant increase in alkaline phosphatase activity when compared to 24 hour and non-nucleated control after one (*) and two weeks (**). 72 and 120 hour nucleation time resulted in a 533% and 443% increase after one week and a 219% and 134% increase after two weeks, respectively. All nucleated samples showed a dramatic increase in ALP activity after three weeks (***) with a >5-fold increase over control. (n = 5, p < 0.05)

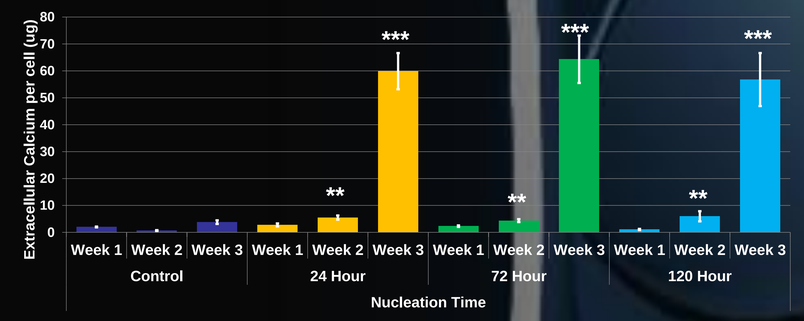


Figure 9. Deposited extracellular calcium on 3D printed thermoplastic polyurethane/polyvinyl alcohol composites. All nucleated samples showed a significant increase in extracellular calcium deposition when compared to non-nucleated control after two (**) and three weeks (***). After two weeks, all nucleated samples exhibited a > 5-fold increased and after three weeks a greater than 14-fold increase. (n = 5, p < 0.05)

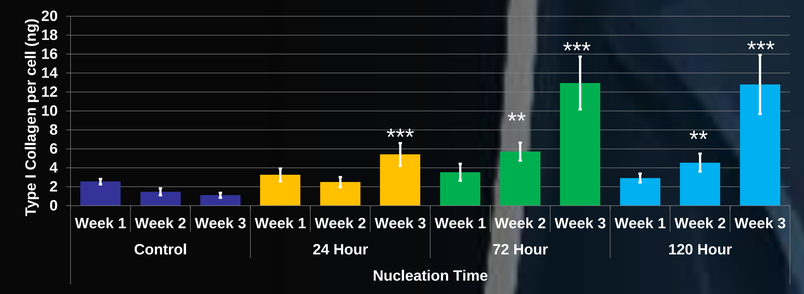


Figure 10. Type I collagen deposition on 3D printed thermoplastic polyurethane/polyvinyl alcohol composites. 72 hour and 120 hour nucleated samples resulted in a 290% and 211% increase in type I collagen when compared to control (**). In addition, all nucleated samples showed a significant increase in type I collagen when compared to non-nucleated control after three weeks (***). 24 hour nucleation time resulted in a near 4-fold increase with 72 hour and 120 hour nucleation resulting in an 11-fold increase, respectively. (n = 5, p < 0.05)

Conclusions

The aim of the current work was to evaluate a novel 3D printable elastomeric composite for bone tissue regeneration. In addition, SBF nucleation was performed to further enhance cell performance and osseous tissue formation of hFOBs. SBF nucleation and scaffold geometry were well defined with clear nucleation and nanotexturization under SEM examination with increases in elastic modulus with minimal effects on hydrophilicity. hFOB cell adhesion, proliferation and osteogenesis demonstrate that SBF nucleation can further render the 3D printed scaffolds more bioactive. Therefore, SBF nucleation of 3D printed TPU scaffolds provides an excellent tool and promising material for bone regeneration.

Acknowledgements

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