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 persist with regards to insufficient donor tissue supply, disease transmission and inadequate host-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 integration through the use of a novel elastomeric filament for fused deposition modeling (FDM) fabrication of bioactive scaffolds. The current system is expected 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 (Get-Lay, Por-Lay, Matternickers, Lake Forest, California) was used to manufacture porous scaffolds. A 35 mm × 35 mm × 2.5 mm model was designed in Rhinoceros 3D (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 3 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°C for three 30 minutes 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 fluid (pH 7.4) at 37°C with agitation at 50 RPM for 24, 72, and 120 hour nucleation times. Air-dried nucleated scaffolds and a non-nucleated control were trimmed into 5 mm × 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 and non-nucleated scaffolds. (µm, p<0.05).

Figure 2. Contact angle analysis of SBF nucleated scaffolds, (µm, p<0.05).

Figure 3. Effects on osteoblasts on 3D printed thermoplastic polyurethane/polyvinyl alcohol composite. All nucleated samples showed a significant increase in mechanical properties when compared to non-nucleated control. (n = 6, p < 0.05).

Figure 4. Human fetal osteoblast attachment on nucleated 3D printed thermoplastic polyurethane/polyvinyl alcohol composite. A noticeable increase in cell attachment was observed at a factor of nucleation time with 120 hour nucleation resulting in a 35% percent increase. (n = 3, p < 0.05).

Figure 5. A significant increase in cell number was observed on 24 and 72 hour samples when compared to control with an increase of 34% and 24% after 1 day, respectively, in 24 and 72 hour nucleation times. A significant increase after 3 days 72 and 72% respectively (n = 3, p < 0.05) when compared to 24 hour control (p < 0.05).

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

Figure 8. Alkaline phosphatase activity on 3D printed thermoplastic polyurethane/polyvinyl alcohol composite. 72 and 120 hour nucleation scaffolds showed a significant increase in ALP activity at 1, 2, and 3 weeks (p < 0.05). 72 and 120 hour nucleation time resulted in a 33% and 45% increase after one week and 8.9% and 10%, increase after two weeks, respectively. All nucleated scaffolds showed a dramatic increase in ALP activity after three weeks (*** with a 5-fold increase over control, (n = 3, p < 0.05).

Figure 9. Deposited extracellular calcium on 3D printed thermoplastic polyurethane/polyvinyl alcohol composite. All nucleated scaffolds showed a significant increase in extracellular mineralization when compared to control (**p < 0.05). In addition, nucleated scaffolds showed a significant increase in ALP activity when compared to control (**p < 0.05). After two weeks, all nucleated samples exhibited a 5-fold increase and after three weeks a greater than 10-fold increase. (**p < 0.05, ***p < 0.001).

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.

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