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Spatial alignment of 3D printed scaffolds modulates genotypic expression in pre-osteoblasts

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Abstract

3D printing, an advent from rapid prototyping technology is emerging as a suitable solution for various regenerative engineering applications. In this study, blended gelatin-sodium alginate 3D printed scaffolds with different pore geometries were developed by altering the spatiotemporal alignment of even layered struts in the scaffolds. A significant difference in compression modulus and osteogenic expression due to the difference in spatiotemporal printing was demonstrated. Pore geometry was found to be more dominant than the compressive modulus of the scaffold in

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Declaration of interests

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regulating osteogenic gene expression. A shift in pore geometry by at least 45° was critical for significant increase in osteogenic gene expression in MC3T3-E1 cells.

Keywords

3D printing; gelatin; sodium alginate; gene expression; pore geometry

1. Introduction

The advent of 3D printing for developing hydrogels for bone tissue regeneration has shown tremendous promise [1]. Gelatin and alginate are widely investigated natural biomaterials for bone regeneration because of their biocompatibility, biodegradability, thermal stability [2,3]. Gelatin and sodium alginate blended hydrogels have been used in the past for 3D printing as addition of alginate maintains the shape fidelity of the printed hydrogels [4].

The structural properties like geometry, pore size, porosity and mechanical properties of the 3D printed scaffolds also play a pivotal role in modulating the gene expression profile [5,6]. Previous studies have shown that scaffold pore geometry regulates bone-related genes and the amount of mineralized ECM [7]. Thus controlling the pore geometry can have significant impact on osteogenesis and bone tissue regeneration. The goal of this study was thus to evaluate the critical shift in spatial geometry of 3D printed scaffolds in genotypic expression of MC3T3-E1 cells.

2. Materials and Methods

Gelatin Type A was purchased from (MP Biomedicals LLC, USA). Sodium alginate and all other chemical reagents were purchased from Sigma-Aldrich Inc. and Fisher Scientific, USA.

Blends of gelatin and sodium alginate were prepared by dissolving 25% w/v of gelatin and 3% w/v sodium alginate in PBS (pH 7.4). The dissolved solution was 3D printed using a pneumatic extrusion piston driven (EnvisionTEC, Germany). The solution was maintained in the printing head for 3–5 hours at 24–27° C and printed at a speed of 10 mm/s using 0.010 inches diameter nozzle on 12 well plates with the overhead pressure of 1.1 Pa. Three different scaffolds with strut angle orientation between each layer of 0–90°, 0–60° and 0–45° were printed with 8 layers of 10×10 mm boxes. The odd layers were designated as 0° and the even layers as 45°, 60° or 90°. The distances between the struts were maintained at 1 mm for the even layers. The 3D printed scaffolds were then cross linked by 0.5% w/v genipin solution in ethanol for 48 hours and washed with PBS several times to remove residual cross linker and ethanol. After crosslinking, shrinkage along the edges of the scaffold was observed which was rectified after washing in PBS.

Compression modulus of the 3D printed scaffolds (0.9×0.9×0.4 cm) was tested using a Dynamic Mechanical Analysis Machine (TA instrument, USA). Stress-strain measurements were carried out by equilibrating the chamber at 37 °C. The isothermal temperature was maintained for 2 minutes before a ramp force of 0.5N /min was applied to a maximum of

18N. Compression modulus of the scaffolds was calculated from the slope values obtained from the stress strain graphs.

MC3T3-E1 cells (ATCC, USA) were seeded on the 3D printed scaffolds at a density of 0.7×10^6 cells/scaffold and maintained in α -MEM α medium containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C and 5% CO₂. Cell viability was assessed using Live/dead assay (Thermo Scientific, USA) after 3 days. Gene expression analysis was done by quantitative real-time PCR using Bglap 1, Col1a1 and GAPDH Taqman assay kits (Thermo Scientific, USA). Threshold cycle values of target genes was standardized against GAPDH expression and normalized to the expression in the control culture. The fold change in expression was calculated using the Ct comparative threshold cycle method.

All experiments were performed in triplicate and values were expressed as mean $\pm$ standard deviations. All data were compared using Student's t-test with $p < 0.05$ considered to be statistically significant.

3. Results and Discussion

Gelatin was used as a model polymer for assessing the spatiotemporal effect on gene expression of MC3T3-E1 cells. 3D-printing of gelatin at various concentrations either led to discontinuous or uneven printing of struts due to higher viscosity (data not shown). To overcome this hurdle, alginate used in the past for reducing the shear stress for ease in printing was utilized. Different weight percentages of alginate were blended with different compositions of gelatin. The printing parameters were optimized to 1:1 ratio of gelatin (25% w/v) and alginate (3% w/v). Since the stability and mechanical properties of gelatin and alginate are quite poor without crosslinking, post-processing methods are needed to improve the stability and mechanical properties of hydrogel scaffolds. Genipin, an alternative chemical crosslinking agent with similar physical, mechanical, and biochemical characteristics to aldehyde based crosslinking agents was used [8]. Figure 1 A-C shows the representative 3D printed scaffold of 45°, 60° and 90° orientations. All scaffolds displayed well defined structures with geometries similar to those designed in the Bioplotter software. The two dimensional pore geometries of the 3D printed scaffolds shifted from square shape in 90° to a parallelogram in 60° and rhombic shape in the 45° orientation. Swelling properties of the 3D printed scaffolds due to water absorption elucidates the permeation and transport of nutrients and waste [9]. All scaffolds, irrespective of their initial dry weight after crosslinking, swelled 5 minutes after immersion in water or PBS (pH 7.4) (Supporting information Figure 1). The high water uptake properties of the 3D printed scaffolds are due to the interconnected pores [10]. Swelling was more pronounced around the edges of all scaffolds and led to the reduction in interconnected pore size along the edges alone.

Young's modulus of the 3D printed scaffolds were obtained from the slope of stress-strain curves (Figure 1D). The Young's modulus of 45°, 60° and 90° was 1.02 ± 0.04 kPa, 1 ± 0.07 kPa and 1.2 ± 0.03 kPa, respectively. The 90° orientation had more contact area between the strands from adjacent layers, thereby, leading to a decrease of the local stress and contributing to significantly higher moduli when compared to 45° and 60° orientations [11].

All 3D printed hydrogel scaffolds supported the adhesion and growth of MC3T3-E1 cells on its surface, indicating an excellent cytocompatibility of the scaffolds (Fig. 2 A-C, (Supporting information Figure 2)). Gene expressions have been found to vary with a significant difference in mechanical properties and pore geometries [6,12]. Expression of collagen 1a and osteocalcin genes were found to vary in 3D printed scaffolds with all orientations (45°, 60° and 90°). The 90° orientation which produced a square geometry had the least expression while the 45° orientation which produced the rhombic geometry had the highest. The trend was found to correspond more with the pore geometry shift rather than due to the compression moduli shifts of the 3D printed scaffolds. Recently, a study has shown that geometry shift from 90° to 60° has led to significant higher expression and function in the hepatocyte cell line [HUH 7] [6]. Although a higher expression of Bglap1 and Col1a1 genes are observed with an orientation shift from 90° to 60°, a critical shift of 45° was required to show a significantly higher expression of Bglap1 and Col1a1 in case of MC3T3-E1. This is because of the smaller vertical space between the struts aligned at 45°. Previous studies have also shown that at the corners of a pore, the small angle of struts provides a suitable environment for cells to interact, thereby upregulating osteogenic genes and promoting osteoblast differentiation [12].

Hence, optimization of pore geometry for each cell type proves to be a major aspect for construction of ideal 3D printed scaffolds for specific regenerative engineering applications.

Conclusion

We have developed spatiotemporal configurations of 45°, 60° and 90° with blended gelatin-sodium alginate constructs with different pore geometries by 3D-printing and chemically crosslinked using genipin. The difference in contact areas between the strands from adjacent layers of the various configurations of the 3D printed scaffolds led to the significant difference in compression modulus. 3D-printing gelatin-sodium alginate blended constructs significantly influences the genotypic expression of pre-osteoblasts. The gene expression corresponds to the corresponding shift in pore geometry and a critical spatiotemporal shift of 45° is necessary for significant higher expression of Bglap1 and Col1a1 in MC3T3-E1 cells. The future study will focus on optimizing spatiotemporal alignments with various cell types for regenerative engineering applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Gelatin was 3D printed using sodium alginate for co-printing
- Different pore geometries were created by altering the alignment during printing
- Compression modulus varied with printing alignment
- Pore geometry plays an important role in regulating osteogenic gene expression.
- Shift in pore geometry of 45° was critical for significant difference in osteogenic gene expression

Figure 1 A-C. Genipin crosslinked 3D printed scaffolds of a) 45°, b) 60° and c) 90° orientations D. Compression stress-strain curves of a) 45° b) 60° and c) 90° orientations.

Figure 2 A-C.

A-C. Confocal microscopy images of 3D printed scaffolds A. 0–45, B. 0–60 and C. 0–90.

Scale bar is 50 microns D. Fold expression in different orientations.