STRUCTURAL EVALUATION OF PLA SCAFFOLDS OBTAINED BY 3D PRINTING VIA FUSED DEPOSITION MODELING (FDM) TECHNIQUE FOR APPLICATIONS IN TISSUE ENGINEERING

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Abstract. Fused Deposition Modeling (FDM) is an efficient method to produce porous scaffolds for tissue engineering, since is possible to print parts with different geometry and porosity. Polylactic acid (PLA) is currently applied in FDM. The aim of this work was to evaluate the effect of strut spacing on the morphology and mechanical properties of PLA scaffolds and to evaluate the cytotoxicity and efficiency of cell seeding of them. Cubic PLA scaffolds were manufactured with 0.8, 1.0 and 1.2 mm of strut spacing. Porosity and mechanical properties were assessed by Archimedes principle and Compression tests. To evaluate the cell viability, Live/Dead® and DNA Hoechst Assay were performed. Scaffold porosity interfere, directly, in compressive modulus, part with 0.8 mm of strut spacing shown the highest compressive strength in comparison with the others. PLA scaffolds were non-toxic for cells and the strut spacing is not affecting the cell deposition onto the structure. This study suggest that FDM allow to produce PLA scaffolds with structural properties in the same range as cancellous bone, may be considered a potential device for bone tissue regeneration.

Palavras-chave: Tissue Engineering, 3D printing, PLA, FDM.

1. INTRODUCTION

Additive manufacturing techniques, such as Fused Deposition Modeling (FDM), have been employed in Tissue Engineering to produce porous scaffolds with complex geometries and controllable porosity (Giannitelli et al., 2014). Polylactic acid (PLA) is a biodegradable, synthetic polymer that has been widely used for scaffolds fabrication mainly due to its biocompatibility and good thermal and physical properties (H.-Y. Mi et al., 2013).

Considering that the scaffold architecture plays a key role in the tissue regeneration, the aim of this work was to evaluate the effect of strut spacing on the morphology and mechanical properties of PLA scaffolds and to evaluate the cytotoxicity and efficiency of cell seeding of them.

2. EXPERIMENTAL METHODS

Digital models with layer orientation of 0-90° and strut spacing varying from 0.8 to 1.2 mm were created in SolidWorks® 2014 software and were printed with PLA white filament (eSun, China) in a 3D Cloner FDM printer (Microbrás, Brazil). Porosity was estimated using Archimedes principle (standard ASTM F2450-10). Mechanical characterization of the samples was carried out using a universal testing machine (compression mode, Zwick Z005), 2.5 kN load, cross-head speed of 1 mm/ min. In vitro cell viability was assessed using a
Live/Dead® viability/cytotoxicity assay kit (Invitrogen). MC3T3-E1 cells were directly cultivated on PLA scaffolds for 24 h. Scaffolds were seeded with $5 \times 10^5$ cells and the efficiency of cell seeding was evaluated by DNA Hoechst Assay after 24 h of culture.

3. RESULTS AND DISCUSSION

The scaffolds porosity was 55%, 60% and 66%, to 0.8, 1 and 1.2 mm of strut spacing, respectively (Fig. 1A-C).

![Figure 1. Image of PLA scaffolds with three different strut spacing, 0.8 mm (A), 1.0 mm (B) and 1.2 mm (C).](image)

This result was expected, once the increase in the filament spacing becomes the structure more open and, consequently, with a higher porosity degree. Structures with high porosity are more suitable for nutrient and oxygen flow and to permit cell survivor through the material. However, the increase in porosity ultimately reduce the material mechanical strength.

![Figure 2. Cell viability in PLA scaffold by Live/Dead Technique.](image)

The live/dead results (Fig. 2) indicated that PLA white filament had good cell viability, despite having commercial additives.

Scaffolds used to regenerate a load-bearing tissue must have mechanical properties similar than the target tissue. The results of compression tests showed that, with the increase in strut spacing, there was a decrease in the modulus and strength values (Table 1). The compressive strength of each printed group was compatible with that of human cancellous bone (Hutmacher et al., 2007). However, only scaffolds produced with lowest strut spacing showed compressive modulus compatible to the lower range of bone.

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Table 1. Values of the mechanical tests.

| Strut spacing | Compressive strength (MPa) | Young modulus (GPa) |
|---------------|---------------------------|---------------------|
| 0.8 mm        | 13.25 ± 1.6               | 0.53 ± 0.1          |
| 1 mm          | 9.47 ± 0.47               | 0.40 ± 0.006        |
| 1.2 mm        | 5.75 ± 0.27               | 0.46 ± 0.06         |
| Cancellous Bone | 02 – 12                  | 0.5 – 0.05          |

Cells were seeded onto PLA scaffolds with different strut spacing and a DNA Hoechst Assay was conducted in order to evaluate the scaffold capability to retain an adequate amount of cells (Table 2).

Table 2. Total DNA after 24h (ng/scaffold)

| Strut spacing | DNA              |
|---------------|------------------|
| 0.8 mm        | 732.10 ± 141.25  |
| 1.0 mm        | 678.17 ± 160.44  |
| 1.2 mm        | 747.92 ± 30.82   |

After 24 hours in culture, the total amount of DNA for PLA scaffolds was in the same range as the cell control (5x10^5 cells) which indicate that scaffolds were able to hold the cells into its architecture. Furthermore, there was no significant difference in DNA amount for all the groups after 24 h.

3. CONCLUSION

The results showed that mechanical properties and morphology of PLA scaffolds were, significantly, influenced by strut spacing. Despite this, all the groups owned porosity and mechanical properties in the same range as cancellous bone. All the scaffolds were considered non-cytotoxic. Cells were efficiently seeded on to the surface of each group and the variation of strut spacing did not affect the cell attachment after 24h. Accordingly, these materials can be considered potential candidates for bone regeneration applications.

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