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Combination of PLA Micro-fibers and PCL-Gelatin Nano-fibers for Development of Bone Tissue Engineering Scaffolds

Naghieh S[1]*, Badrossamay M[2], Foroozmehr E[2] and Kharaziha M[3]

[1]Department of Biomedical Engineering, University of Saskatchewan, Canada
[2]Department of Mechanical Engineering, Isfahan University of Technology, Iran
[3]Department of Materials Engineering, Isfahan University of Technology, Iran

Abstract

In tissue engineering, biodegradable porous scaffolds have been employed to replace damaged tissues. These scaffolds are fabricated by conventional techniques such as fiber-bonding, solvent casting, particulate leaching and advanced ones like selective laser sintering, 3-dimentional printing and fused deposition modeling. The main issues limiting conventional techniques are inability to fabricate scaffolds with a highly interconnected porous structure and favorably regular construction with reproducible morphology. Hence researchers go towards advanced techniques with more flexibility. In this study, FDM and electrospinning (ES) techniques have been applied in order to develop multi-layered scaffolds consisting of micro- and nano-fibers for bone tissue engineering applications. While micro-fibrous layers were fabricated via FDM process, nano-fibrous layers were developed using ES technique. Although FDM technique has unique features, its fiber size limits to micro sizes. One of the trends that are useful for eliminating this issue is getting benefit of adding nano-fibers to scaffold's construction. These nano-fibers not only reduce total pore size of the scaffold, but also could improve cell functions. While poly (lactic-acid) (PLA) was used for FDM process, a mixture of poly (caprolactone) (PCL) and gelatin (PCL-gelatin) were applied for electrospinning process to develop interconnected pores with appropriate mechanical properties and degradation rate. The multi-layered scaffolds were examined using electron microscopy (SEM) and their mechanical properties were evaluated. The porosity of the scaffolds was about 40% and results also demonstrated that nano-fibers with size of 200 nm in diameter had good adhesion to micro-fibers and may provide better cell attachment and proliferation.

Keywords: Additive manufacturing; Fused deposition modelling; Bone scaffolds; Nano-fibers; PCL-gelatin

Introduction

Over the past ten years, tissue engineering has been identified as a treatment method with high potential in clinical fields [1]. One of its applications is healing of the damaged tissues by biodegradable bone scaffolds. Various conventional techniques are used for fabrication of bone scaffolds like electrospinning (ES) [2,3]. Additionally, there are other novel methods for fabricating bone scaffolds like additive manufacturing techniques (AM) have made a huge amount of attention in the last years [4].

AM is a group of emerging technologies that generate items by adding cross-sectional layer at a time and have different benefits like "design freeform" [5]. Diffusion of cells has many limitations in conventional approaches and this is where AM techniques show their decisive role for tackling with this issue. It means that they can eliminate the limitations of the conventional methods and facilitate the transportation of oxygen and nutrients throughout of the scaffold [6]. One of the major limitations of AM for tissue engineering applications is its large pore size in comparison with cell size and its surface properties which could be affected on the cell adhesion and tissue regeneration [7]. Moreover, despite the fact that conventional methods like ES, solvent casting, particulate leaching, gas foaming, phase separation, freeze drying, etc. could provide interesting topographical cues, they have the inability to accurately control scaffold pore size, geometry and its interconnectivity [8]. Hence, integration of additive manufactures methods and conventional techniques like ES is a new trend that many researchers go toward it in order to create a scaffold that has benefits of both methods so that eliminates limitations of mentioned techniques.

In this study, a new hybrid scaffold consisting nano-fibers of PCL-gelatin and micro-struts of biodegradable PLA were combined in one structure in order to have a construction with arranged shape by FDM technique and reduce the size of such building via ES process to enhance initial cell attachment. Moreover being of gelatin nano-fibers could increase scaffold hydrophobic feature. Fabricated scaffolds were tested from mechanical points of view.

Materials and Methods

Materials

PCL (MW 80.000), gelatin and Hexafluoroisopropanol (HFIP) applied for ES section, were supplied by Sigma Aldrich. Also PLA which was used in the FDM machine was supplied by Shenzhen Esun Industrial Company as 3 mm diameter filaments.

Scaffold fabrication

All bone scaffolds were printed with a double head FDM machine with 0.5 mm diameter (Byts by bits, UK). After designing of bone scaffold and generating its gcode file, this file determining the dimension, strut diameter and pore size of scaffolds was transferred to FDM machine and construction of scaffold with micro-fibers was performed with PLA filaments. Moreover, slicing which is necessary for generating gcode file

***Corresponding author:** Naghieh S, Division of Biomedical Engineering, College of Engineering, University of Saskatchewan, Saskatoon, SK, Canada, Tel: +13069664343; E-mail: san908@mail.usask.ca

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was performed using Axon 2.0 (Bits from Bytes) and scaffolds were designed with 0-90 lay down pattern and layer thickness 0.5 mm was selected for FDM machine. Also linear speed of the FDM machine substrate was 7 mm/s and its head temperature was set to 195ºC.

After this step, the procedure of fabricating hybrid scaffold continued with addition of nano- fibers using ES process on the top of the fabricated scaffolds. For ES process, PCL and gelation polymers with weight ratio of 1:1 were mixed in HFIP solvent in order to prepare 10 wt.% solutions. As prepared solutions were injected into 1 ml syringe having a 23G blunted stainless steel needle a syringe pump. While the voltage was kept at 20 kV, the distance between collector and needle was set to 17 cm. Additionally, the flow rate was set to 1 mL/h during the electrospinning process. The cycle of micro-nano fiber formation was repeated until the required height obtained. Both hybrid and pure scaffolds were fabricated with geometrical size of 35 × 35 × 4 mm^3 and then for mechanical tests were cut to smaller dimensions. The morphology of nano-fibers was investigated by scanning electron microscopy (SEM) (Philips XL30). Sample was sputter-coated with gold before investigation and preparation and measurements were done according to SEM machine instructions.

Compression tests

One of the significant requirements for bone scaffolds is their mechanical property. If the scaffolds do not have appropriate mechanical support, tissue formation and bone healing will fail [9]. As many researchers studied, mechanical properties of a bone scaffolds follow from different factors like the pore structure strut diameter and its lay down patterns.

According to ISO 604/B/1, evaluation of the mechanical properties of the fabricated bone scaffolds were done and samples were prepared in 10 mm × 10 mm × 4 mm right prism shapes (large samples were cut into small specimens).

HOUNSIFELD (H50KS) machine utilized for all the compression tests. Figure 1 shows the setup of compression tests. Initially, each sample was set 4 mm apart from the compression plates and compression speed of 1 mm/min was applied. It is worth pointing out that compression load was fixed to zero each time after the primary compression plate setup (Figure 1).

Results and Discussion

The fabricated pure bone scaffold construction captured by an optical microscope (Figure 2).

Also, the side view of the fabricated pure scaffold is indicated (Figure 3).

FDM and ES machines were combined in order to fabricate hybrid scaffolds (Figure 4).

Results demonstrated that nano-fibers with size of 200 nm in diameter had good adhesion to micro-fibers. The nano-fibers of PCL-gelatin located among the micro-struts of FDM fabricated scaffold has been shown in Figure 5.

All the scaffolds (hybrid and pure ones) had mean strut diameter and pore size of 600-650 and 350 micron, respectively. The porosity of the scaffolds measured by Archimedes principle was about 40% [10].

Figure 1: The front view of the compression test setup.

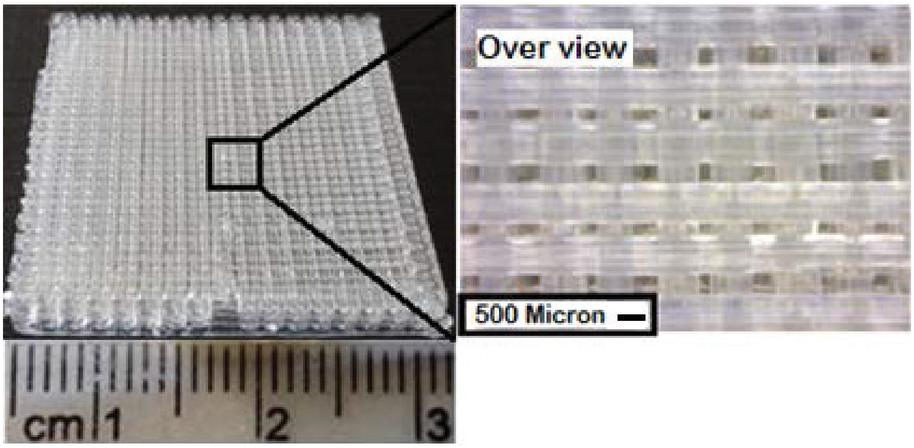

Figure 2: Overview of the prepared pure bone scaffold via FDM technique and its microscopic view.

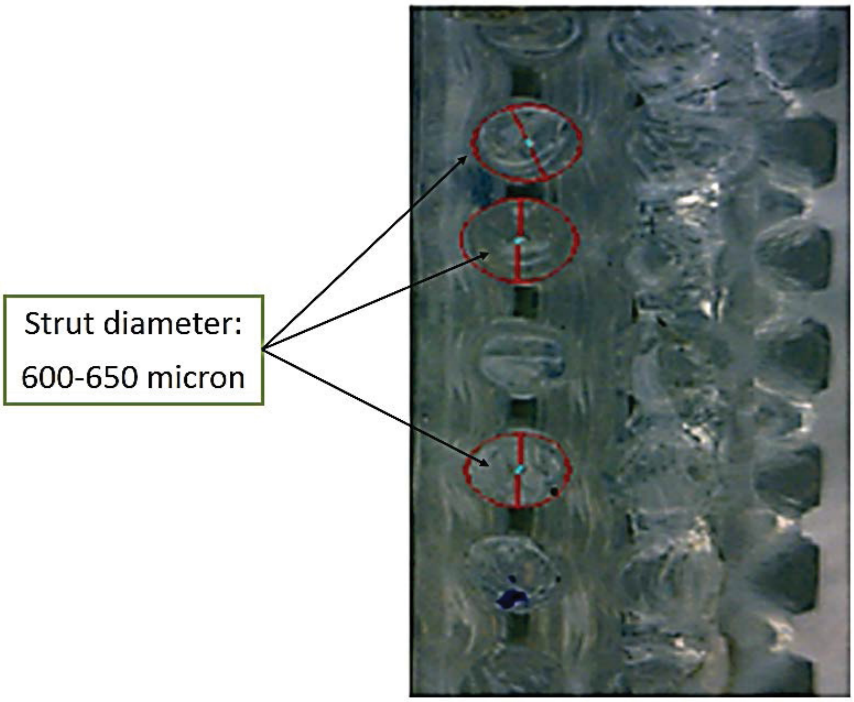

Figure 3: Side view of the pure fabricated bone scaffold fabricated by FDM technique.

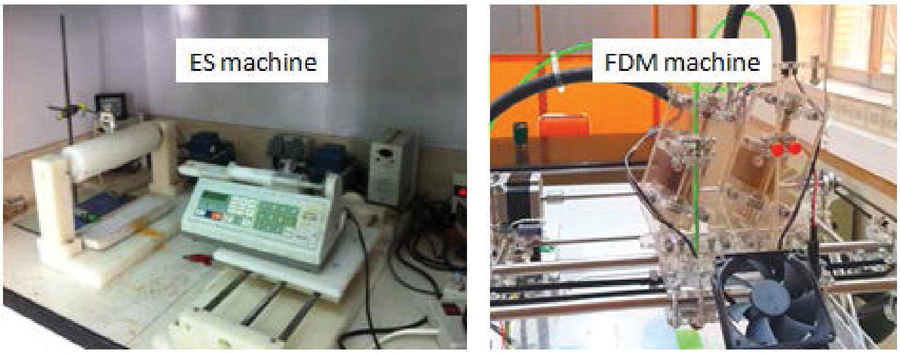

Figure 4: FDM and ES machines applied for fabrication of hybrid scaffolds.

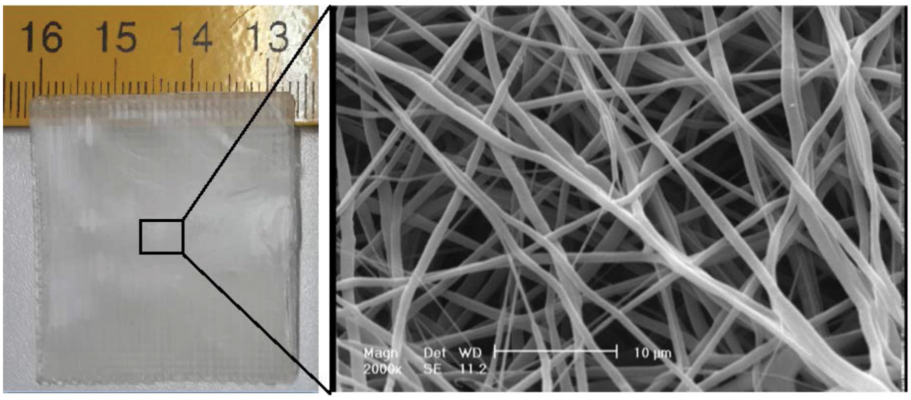

Figure 5: The fabricated hybrid bone scaffolds and its closed view.

Conclusion

The aim of this research is to combine various micro-fabrication techniques of FDM and ES for bone tissue engineering application. In this study, nano-fibrous layer of PCL-gelatin were electrospun on the PLA scaffold fabricated using FDM process in order to reduce the hydrophobic feature of the matrix polymer and increase the initial cell attachment. Results demonstrated that nano-fibers had good adhesion to the matrix polymer fabricated by FDM technique and based on comparison between fabricated pure and hybrid scaffolds from mechanical points of view, the amount of elastic modulus of hybrid ones had considerable increase. So not only is the proposed fabrication method appropriate biologically, but also it improved the mechanical properties of fabricated bone scaffolds in comparison with pure ones.

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