

PRECISION EXTRUSION DEPOSITION OF POLYCAPROLACTONE/ HYDROXYAPATITE TISSUE SCAFFOLDS

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Abstract

Freeform fabrication provides an effective process tool to manufacture advanced tissue scaffolds with specific designed properties. Our research focuses on using a novel Precision Extrusion Deposition (PED) process technique to directly fabricate Polycaprolactone (PCL) and composite PCL/ Hydroxyapatite (HA) tissue scaffolds. The scaffold morphology and the mechanical properties were evaluated using SEM and mechanical testing. In vitro biological studies were conducted to investigate the cellular responses of the composite scaffolds. Results and characterizations demonstrate the viability of the PED process as well as the good mechanical property, structural integrity, controlled pore size, pore interconnectivity, and the biological compatibility of the fabricated scaffolds.

1. Introduction

Three-dimensional scaffolds play important roles in scaffold guided tissue engineering because they provide critical functions as extra-cellular matrices onto which cells can attach, grow, and form new tissues. To design scaffolds for load bearing tissue replacement, researchers often need to address multiple biological, mechanical and geometrical design constraints. With precise control of the scaffold external and internal geometry, porosity, pore size and interconnectivity, the needed structural integrity, strength, and transport properties, can be provided for and an ideal micro-environment for cell and tissue in-growth and healing [1-3]. Most available scaffold fabrication methods, such as solvent casting, fiber bonding, phase separation, gas induced foaming, and salt leaching, are either limited to producing scaffolds with simple geometry, or depend on in-direct casting method for scaffold fabrication [4, 5], therefore they are impractical for the manufacturing of scaffolds with complex structural architectures. To overcome this, solid freeform fabrication techniques, such as 3D Printing, Multi-phase Jet Solidification, and Fused Deposition Modeling (FDM) have been widely adopted for scaffold fabrication [6, 7].

The SFF technique offers a unique opportunity to study the influence of the micro-architecture upon cell proliferation and ECM generation. Traditional scaffold fabrication methods result in structures of random internal architecture and have great variation from part to part. Due to the repeatability of the PED process, a more thorough investigation into the influence of the internal micro-architecture on cellular responses is available. Employing a computer-aided tissue engineering approach, with a novel precision extruding deposition process composite Polycaprolactone/ Hydroxyapatite scaffolds for bone tissue engineering applications were fabricated.

2. Precision Extrusion Deposition

Previous research has focused on Fuse Deposition Modeling (FDM) for the fabrication of

ceramic components. It has been shown that the main obstacle for FDM, in the application of new materials with specific characteristics often comes from the use of intermediate precursor filament [8]. Furthermore, during the extrusion phase, the frequent buckling failures cause interruption of the process (with the necessary cooling down and warming up of the liquefier) and necessitates frequent operator intervention [9]. Consequently, this problem prevents an automatic and continuous process diminishing the main advantage of a filament-based system. In addition, the backpressure encountered during deposition limits the powder volume fraction in the filament [10] reducing the possibility of higher particle loading.

The new system, called Precision Extrusion Deposition (PED) and consisting of a mini-extruder mounted on a high-precision positioning system (see figure 1), operates using with bulk material in granulated form, which avoids most of the material preparation steps in a filament-based system. This configuration opens up the opportunity for the use of a wider range of materials, making the PED a viable alternative manufacturing process for composite materials.

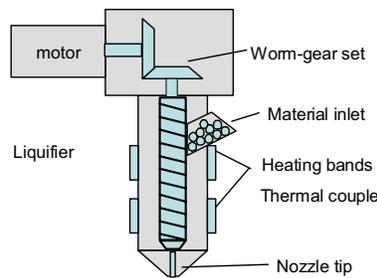


Figure 1: Schematic of mini-extruder in precision extrusion deposition system

3. Generation of Scaffold Geometry

The fabrication of scaffolds for tissue engineering by means of rapid prototyping has many advantages, one of which is the ability to create patient specific implants with a controlled internal design. Through the use of commercially available software packages such as MIMICS, tissue geometry can be extracted from CT or MRI data and reconstructed as a 3D model. The model is then refined to fit the overall macro-structural requirements of the implant, while the micro-structure is governed by the designed tool path of the individual 2-D “slices” of the model. The pore size (L) and porosity (ϕ) are controlled by the material extrudate diameter, orientation and spacing as seen in Figure 2.

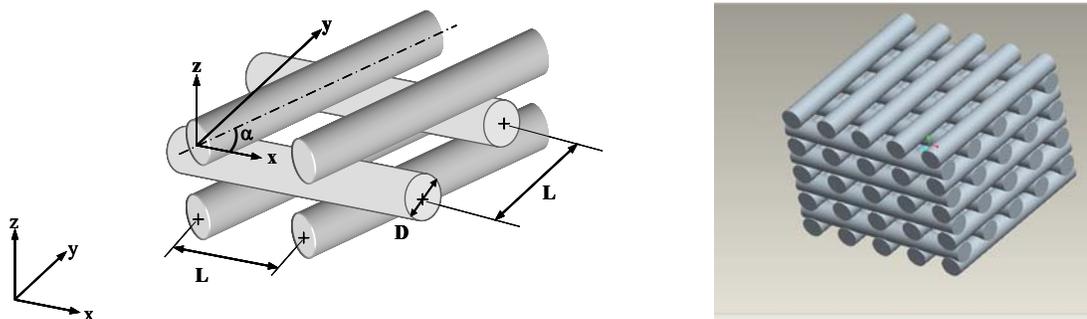


Figure 2: Schematic of strand layout and porosity calculation

The porosity can be calculated from the following equation:

$$\phi = 1 - \frac{\pi D}{8(L + D)} \left(1 + \frac{1}{\sin \alpha} \right)$$

4. Materials

Hydroxyapatite (HA, Clarkson Chromatography Products Inc, South Williamsport, PA), a calcium phosphate ceramic, has been widely investigated for bone tissue engineering applications because of its chemical similarity to the mineral constituent of natural bone. It is both mechanically strong and osteoconductive. However, because of its brittle characteristic and material properties, it is often difficult to process.

Polycaprolactone (PCL, Sigma Aldrich Inc, Milwaukee, Wisconsin) a biodegradable and biocompatible polymer was combined with the HA. PCL is a semi-crystalline aliphatic polymer that has a slower degradation rate than most biopolymers in its homo-polymeric form. It has a low glass transition temperature at -60°C , a melting temperature at about 58°C - 60°C , and a high thermal stability. It has a high decomposition temperature T_d of 350°C . The mechanical properties of Bulk PCL ($M_w = 44,000$) with a tensile strength of 16MPa, tensile modulus of 400MPa, flexural modulus of 500MPa, elongation at yield of 7.0%, and elongation at break of 80% have been reported. The operational temperature of the system ranged from 85°C - 110°C , well below the decomposition point of PCL. A composite PCL/HA material would provide increased osteoconductivity and mechanical strength over scaffolds constructed of PCL alone.

5. Results of Scaffold Fabrication

Hydroxyapatite powder, with particles ranging in size from 10-25 microns, was melt blended with PCL, with 25% HA by weight. Two sets of cylindrical scaffolds, measuring 20mm in diameter, were fabricated with 450 micron roads and porosities of 60% and 70% respectively.. The liquefier temperature was set to 90°C , and a .245 mm exit diameter nozzle was used. Each layer was filled with the designed scaffold pattern of a 0/90 degree orientation to generate the porous structure.

5.1 Evaluation of Morphology, Mechanical and Biological properties

The scaffold service topography and architecture was evaluated using an Environmental Scanning Electron Microscope (FEI-Phillips XL30 ESEM-TMP).

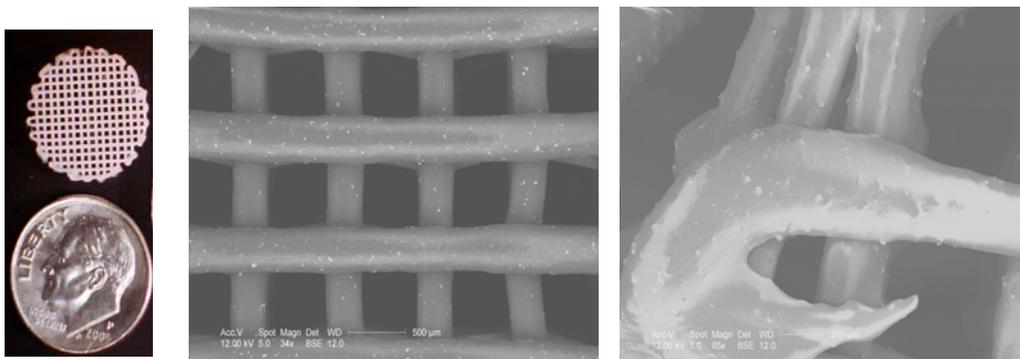


Figure 3: (a): PCL/HA Scaffold ; (b): PCL/HA backscatter image at 65x.

©: PCL/HA backscatter image at 34x

The SEM images show uniform dispersion of HA particles with no visible areas of agglomeration. In addition, HA particles are seen protruding from the surface of the scaffold. The required architecture for tissue engineering scaffolds could be achieved at the micron scale level. The uniformity of the pores and the depositing roads shown demonstrate the applicability of using the PED process to fabricate composite scaffolds at micro-scale level.

5.2 Mechanical Testing

Compression tests were conducted on scaffolds of PCL and PCL/HA of a 25% concentration by weight of HA. Each sample is 20mm in diameter and 20mm in height. Scaffolds of 60% and 70% porosity were tested where the internal pore structure is of the 0/90 pattern with pore sizes of 450 microns and 750 microns respectively, and the diameter of the extrudate is 450 microns. The tests were conducted on the MTS insight 40 mechanical, with a cross-head displacement speed of 2 mm/min. Stress-Strain data was computed from Load-Displacement measurements, and the compressive modulus was determined from the elastic region of the curve. The stress-strain curves derived from the testing data are plotted in Figure 4. The calculated compressive modulus from the stress-strain data are listed in Table 1.

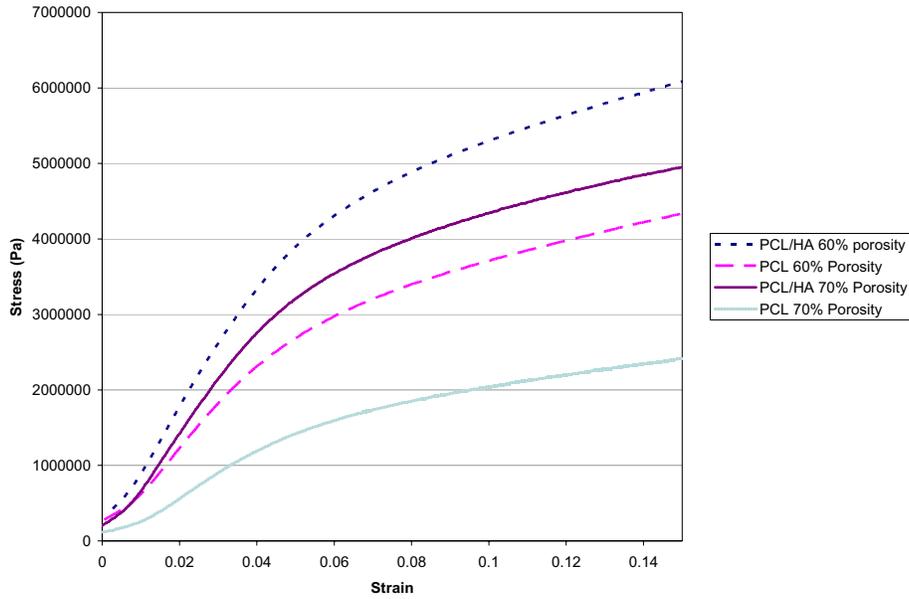


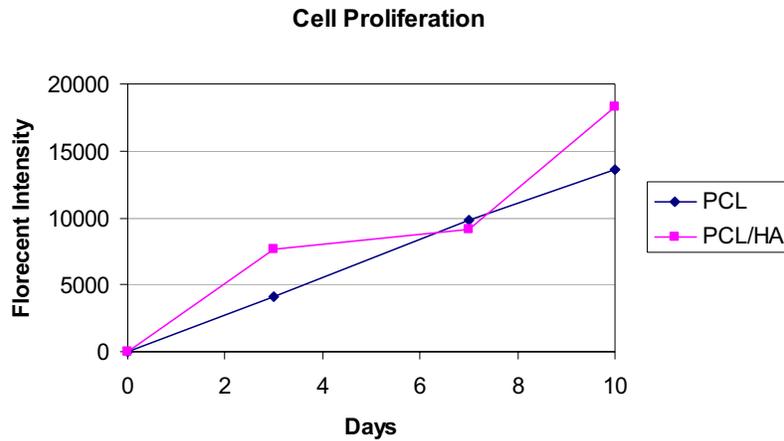
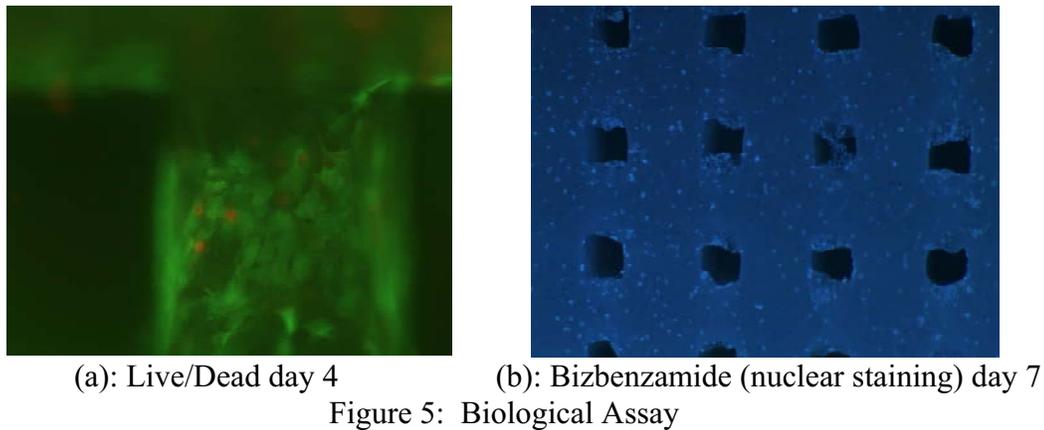
Figure 4: Stress-Strain curve for PCL and composite PCL/HA scaffold

Table 1: Calculated Compressive Modulus from Stress-Strain Data

Material	Porosity	Compressive Modulus (MPa)
PCL/HA	60%	84
PCL	60%	59
PCL/HA	70%	76
PCL	70%	30

5.3 Biological Characterization

An in vitro initial cell viability study was conducted on HA/PCL composite scaffolds. Osteoblast cells (7F2) were seeded onto cylindrical samples with 300 micro pores and a porosity of 65%, and left to incubate for 7 days. A live/dead and bizbenzamide assays were performed to determine the location and approximate number of living cells. In addition a fluometric assay, alamar blue, was used to determine cell proliferation over the 7 day period. Readings were taken at days 0, 3, 7 and 10. Figure 5(a) show cellular attachment covering the top surface after only 4 days. The experiment indicated that the composite material was, biocompatible with positive cell attachment and proliferation on the entire top surface of the scaffold structure, as shown in Figure 2.



6. Conclusion

The ability to fabricate scaffolds of composite biomaterials using the PED system has been demonstrated. Biological testing has show that the fabrication process has no adverse cytotoxic effect on the process PCL and HA biomaterials. However, success in scaffold guided tissue engineering requires a greater understanding of the cellular response to the constructed micro environment. PED has the advantage of high precision on the micro scale as well as repeatability not available using more traditional scaffold manufacturing methods.

References

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