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Computational Design, Freeform Fabrication and Testing of Nylon-6 Tissue Engineering Scaffolds

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ABSTRACT

Advanced and novel fabrication methods are needed to build complex three-dimensional scaffolds that incorporate multiple functionally graded biomaterials with a porous internal architecture that will enable the simultaneous growth of multiple tissues, tissue interfaces and blood vessels. The aim of this research is to develop, demonstrate and characterize techniques for fabricating such scaffolds by combining solid freeform fabrication and computational design methods. When fully developed, such techniques are expected to enable the fabrication of tissue engineering scaffolds endowed with functionally graded material composition and porosity exhibiting sharp or smooth gradients. As a first step towards realizing this goal, scaffolds with periodic cellular and biomimetic architectures were designed and fabricated using selective laser sintering in Nylon-6, a biocompatible polymer. Results of bio-compatibility and *in vivo* implantation studies conducted on these scaffolds are reported.

INTRODUCTION

Tissue engineering [1, 2] is an interdisciplinary field that combines engineering and life sciences to develop techniques that enable the restoration, maintenance or enhancement of living tissues and organs. A majority of these techniques utilize three-dimensional scaffold structures composed of natural or synthetic polymers [3-10]. These scaffold structures are typically endowed with complex internal architecture, channels and porosity that provide sites for cell attachment and proliferation, as well as for conveying cells, growth factors and biomolecular signals to promote tissue regeneration at an implantation site. The composition of most tissue engineering scaffolds is such that the scaffolding material is biodegradable, and it erodes away over time after implantation, eventually being replaced by newly formed tissue.

Recently, solid freeform fabrication (SFF) [11] methods have been employed for fabricating bioimplants and tissue engineering scaffolds [12-20]. In principle, SFF methods are capable of constructing three-dimensional scaffolds with complex architectures incorporating multiple, functionally graded bio-materials and porosity. The overall goal of our research is to develop homogenization theory based computational design techniques and laser sintering based freeform fabrication methods for constructing such heterogeneous tissue engineering scaffolds using multiple biomaterials. This goal is to be achieved via the following three research objectives. First, computational techniques are being developed to locally optimize scaffold architecture, material composition and mechanical properties yielding three-dimensional digital representations of functionally tailored scaffolds. Second, solid freeform fabrication techniques

based on laser sintering are being developed that can construct such scaffolds using multiple biomaterials possibly with the incorporation of drugs or bioactive factors *in-situ*. Finally, mechanical and biological (*in vivo, in vitro*) testing and CT/MRI image analysis is being conducted to evaluate structure and function of both scaffold materials and regenerate tissue. As a first step towards realizing this goal, we have chosen to investigate monolithic Nylon-6 as a scaffold material for tissue engineering applications.

METHODS AND MATERIALS

Scaffold Design Methods

Scaffold design requirements should be addressed on both macroscopic and microscopic scales. On the microscopic scale (100 μm-1 mm), the scaffold internal architecture must fulfill temporary tissue function, enhance tissue regeneration and vascularization, and facilitate nutrient/biofactor delivery. On the macroscopic scale (> 1 mm), the scaffold's external shape must replicate human anatomy. These two scales must be integrated to produce a single design that can be embodied in a format appropriate for SFF. In our studies, we have used both periodic cell-based designs and biomimetic designs to construct scaffolds using SLS.

Periodic Cell-Based Designs

In periodic cell-based designs, a unit cell with specific microstructure is repeated to create an entire scaffold. This technique can be used in combination with topology optimization methods to design microstructures with effective physical properties matching native tissue properties. Additional details of homogenization theory based topology optimization techniques for bioimplants are available elsewhere [21,22]. Figures 1a,b show 8 mm cubic and 8 mm diameter cylindrical periodic scaffolds with 800 μm orthogonal channels and 1200 μm pillars that were designed using such optimization methods. These designs result in scaffolds with a pore or void fraction of approximately 53.7%.

Biomimetic Designs

The design of biomimetic scaffolds relies on micro-CT, micro-MRI or confocal microscopy data to assemble scaffold architectures. In biomimetic designs, scaffolds mimic natural tissue structure and seek to replicate all aspects of tissue structure and function. We used micro-CT derived architecture of human proximal femur trabecular bone as the basis for creating biomimetic scaffolds in Nylon-6. Figures 1c,d show a volumetric rendering of human trabecular bone micro-CT data along with a faceted representation appropriate for use in SFF machines.

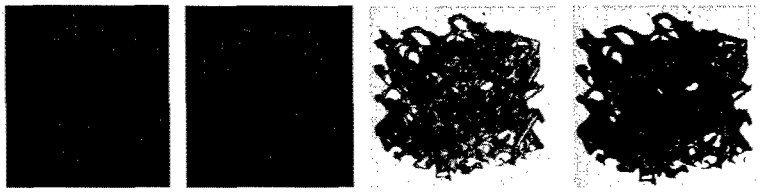

Figure 1. (a) Cubical and (b) Cylindrical periodic scaffold architectures. (c) Volumetric rendering of trabecular bone microstructure derived from micro-CT image data for biomimetic design. (d) Corresponding triangular facet data for SFF.

Scaffold Fabrication

The choice of Nylon-6 as the material for fabricating scaffolds during this study was prompted by Risbud and Bhonde's recent data [9] on the biocompatibility of polyamide 6. Their aim was to develop polyamide 6 membranes blended with gelatin (a natural polyamide) and chondroitin sulfate (a biopolymer) using the phase precipitation method and to evaluate their *in vitro* biocompatibility. A large collection of biocompatibility test data demonstrated that such polyamide 6 composite membranes are biocompatible and prospective candidates for tissue engineering. Several other studies have documented the use of Nylon in suture materials and for dialysis membranes [23], in burn dressings [24], and as cell culture substrata for a variety of cell types [25-27].

Two types of Nylon-6 powders were used in our study. Honeywell Capron 8202 (10-100 μm particle size) was used for fabricating periodic cell-based designs while Atofina Orgasol 1002 ES4 (38-42 μm particle size) was used for fabricating biomimetic structures as well as disks for biocompatibility testing. These powders were processed in a Sinterstation 2000 commercial SLS machine using 200º C preheat, 7 Watts laser power, 49.5 in/s scan speed and 100 μm layer thickness. Porous specimens of both cylindrical and cubical periodic geometry (figure 1), as well as biomimetic architectures (figure 3b) derived from micro-CT data were fabricated. Solid disks (11 mm diameter, 2 mm thick) were also fabricated for biocompatibility tests. The parts were cleaned post-process by simple brushing and careful removal of powder trapped inside the porous channels where necessary.

Biocompatibility Testing

In vitro biocompatibility was determined using the CellTiter 96® AQ_{ueous} One (Promega Corp, Madison, WI) assay, which provides a measure of cell viability related to the level of mitochondrial respiration. This assay is analogous to the MTT assay commonly utilized in biocompatibility studies [28], with the exception that the colored product produced is water soluble [29]. The cells utilized in this study were porcine bone marrow stromal cells (isolated from marrow extracts) and were limited to passage 5 through 8. Additional details of the biocompatibility test procedures are available elsewhere [30].

RESULTS AND DISCUSSION

Figure 2a shows an 8 mm cube with 800 μm channels and 1200 μm pillars fabricated in Nylon-6 by SLS. These specimens will be used for conducting uniaxial tests in unconfined

compression inside a micro-CT machine. Complete 3D strain fields in the scaffold under testing will be computed by comparing images before and after deformation. These tests will provide effective failure stress and local strain values at failure. The results of these studies will be published elsewhere [31]. Figure 2b shows an 8 mm diameter, 6 mm high cylinder with 800 μm channels and 1200 μm pillars fabricated in Nylon-6 by SLS. This scaffold geometry was designed for surgical implantation and histology assessment. Figure 2c shows implantation of cylindrical scaffolds into a Yucatan minipig mandible. These scaffolds were subsequently removed after 6 weeks and assessed for mineralized tissue formation by micro-computed tomography (micro-CT). Figure 3a shows a volumetric rendering of the micro-CT scan conducted on the removed scaffold, showing the ingrowths of mineralized bone tissue into the pore channels of the scaffold. Quantitative image analysis of the micro-CT data revealed that new bone tissue occupied 43.2% of the pore volume. This value is consistent with the range of pore occupation fractions (50.3-65%) attained with hydroxyapatite scaffolds of identical pore geometry implanted for the same 6 week time period at the same site [32]. The lower value of pore occupation for Nylon-6 is likely due to loosely bonded particles on the pore walls causing an adverse cellular response as discussed below, although further experiments are yet to confirm this hypothesis.

Biomimetic architectures derived from CT/MRI data of bony structures are difficult to fabricate by SFF as resolved tissue structures (10-100 μm) are often smaller than the resolution of the SFF machine. Alternatively, these structures can be scaled up and then fabricated to have optimal porosity for bone tissue regeneration (typically 300-1200 μm) [32] while retaining biomimetic architecture. Shown in figure 3c,d are the volumetric rendering of a section of human proximal femur trabecular bone micro-CT data and the corresponding Nylon-6 replica scaled 4X fabricated using SLS. In addition to biological testing of these scaffolds by implantation, we will visualize 3D deformation and failure modes under compression during micro-CT and compare them with failure modes of real bone. These results will be published elsewhere [31].

Biocompatibility tests conducted on the SLS fabricated Nylon-6 disks and their leachable products indicated that cell viability was considerably higher for the cells subjected to the conditioned media containing leach-out products. The results indicated that neither early nor late leaching products were detrimental to the cells. However, cell viability relative to controls dropped when the cells were in direct contact with the media in the presence of disks (CoCulture group). While the level of cell viability was slightly less than 70%, on average, the result still indicates a relatively low level of cytotoxicity. One possible explanation for the reduction in cell viability in the CoCulture group is that the solid free form fabrication method utilized, SLS, resulted in some residual Nylon-6 particulates that were not fully bonded to the construct. This particulate matter may have initiated a detrimental cellular response in the CoCulture group. Particulate matter has been shown to cause osteolysis *in vivo* [33] although this is yet to be confirmed experimentally. The results are still very promising as the fabrication and post-fabrication preparation for biological usage can be further refined and improved. Further details on biocompatibility testing results can be found elsewhere [30].

Figure 2. (a) 8mm cubic periodic scaffold fabricated in Nylon-6. (b) 8mm diameter, 6mm high periodic cylindrical scaffold fabricated in Nylon-6. (c) Scaffold implantation into Yucatan minipig mandible.

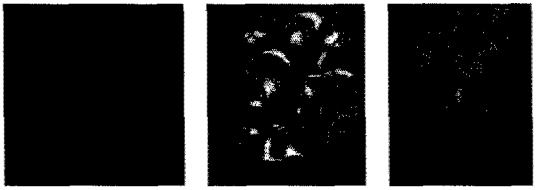

Figure 3. (a) Volumetric rendering of scaffold micro-CT scan. (b) Volumetric rendering of human trabecular bone micro-CT data. (c) 4X scaled replica fabricated in Nylon-6 by SLS (scale in mm).

CONCLUSIONS

This work demonstrates an approach combining computational design, freeform fabrication and testing of tissue engineering scaffolds. Scaffolds with periodic and biomimetic internal architecture were fabricated in Nylon-6 using SLS. Implantation and subsequent histology of scaffolds show presence of regenerate mineralized tissue, consistent with pore occupation fractions attained using monolithic hydroxyapatite scaffolds. The biocompatibility tests show that Nylon-6 scaffolds fabricated via SLS support cell viability quite well. Biocompatibility may be improved by better methods of post-fabrication cleaning or treatment of SLS fabricated scaffold constructs to eliminate loosely bonded polymer particles. Although not bioresorbable, such Nylon-6 scaffolds are biocompatible and could serve as drug/cell delivery devices as well as models for future work on bioresorbable polymers. This work sets the stage for construction of functionally tailored tissue scaffolds in a single step via SLS of multiple materials. Scaffolds incorporating graded architectures with multiple biopolymers and their composites with calcium phosphate ceramics are being explored using a newly designed multiple powder deposition system.

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