3D Fabrication of Biomimetic Nanocomposite Scaffolds for Tissue Interface Engineering and Regenerative Medicine

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Introduction

Articular joint repair and regeneration continue to be largely intractable due to the poor regenerative properties of this tissue. Consequently, once injured, cartilage is much more difficult to self-heal. Further, once a cartilage lesion becomes deep enough, it continues to wear into the bone, causing a full osteochondral injury. These types of wounds are even harder to treat because they encompass two different types of tissue, and require special mechanical and hierarchical tissue structure considerations. Although traditional methods such as autografts and allografts have been clinically employed to treat various osteochondral lesions, there still exist many shortcomings associated with these therapies including insufficient donor tissue, donor site morbidity, infection and transmission of disease.

More advanced scaffold techniques also fail, due to insufficient material strength at the material interface.

The osteochondral region has a 3D hierarchical fibrous structure and a porous, randomly oriented porous structure that provides a cell-favorable nano-to-micro environment, and a strong interface between cartilage and bone. Therefore, the objective of this project is to create a novel biomimetic 3D tissue engineered construct via an 3D printing / rapid prototyping, and to employ biomimetic multi walled carbon nanotubes (MWCNTs) and acetylated collagen for osteochondral regeneration. Specifically, Fused Deposition Modeling (Figure 1) is a proven fabrication technique to design biocompatible tissue engineered scaffold with defined parameters specific to an engineering design or computer model. Biomimetic (~50 nm) MWCNTs and collagen can also be incorporated into the 3D printed constructs via several coating techniques. MWCNTs and other species of carbon nanotubes have recently been explored for bone and cartilage applications. Furthermore, CNTs have several exceptional features that make them ideal for Tissue Engineered scaffold: **(a) excellent mechanical properties** to significantly strengthen scaffolds; **(b) biomimetic nanostructure size and tubular shape** similar to natural ECM collagen components; **(c) high electrical conductivity** to enhance cellular interaction pathways and eventual tissue formation and **(d) extremely flexible design** via surface functionalization. Furthermore, human bone marrow-derived mesenchymal stem cells (MSCs) will be seeded into the biomimetic 3D printed scaffold for efficiently improving their adhesion and directing their chondrogenic differentiation.

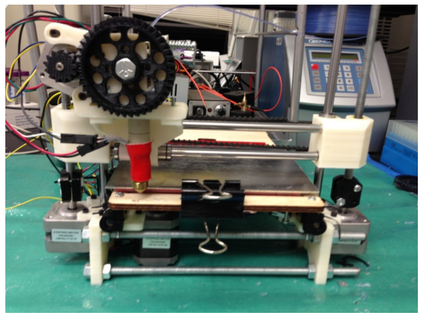


Figure 1The lab's FDM 3D printing setup

Materials and Methods

In this study, we will seek to explore the effects of varying physical, mechanical and chemical properties of 3D biomimetic bi-phasic 3D printed polymer MWCNT and collagen coated scaffolds on human bone marrow-derived MSC behavior and phenotypic expression.

3D Printed scaffold fabrication and characterization: A series of biocompatible poly-lactic acid (PLA) scaffolds with varying internal feature sizes were manufactured by designing different 3D models using the Rhinocerous 3D modeling package. Scaffolds were then printed in groups of six using a PrinterBot 3D printing system , modified with a 347 µm diameter nozzle, and a spool of 1.75 mm diameter Poly lactic acid (PLA) polymer. 3D models were converted into a gcode instruction file using Slic3r, and then used to instruct the printer via the Pronterface software package. There were a total of six experimental groups designed: (1) homogenous cross-hatched structures; (2) bi-phasic structures consisting of a cross hatched pattern and an intersecting rings structures; and (3) biomimetic bi-phasic structures with key features; each of the structure with large and small pore features.

In addition, we applied a collagen type II coating on the printed scaffolds to further improve their cytocompatibility properties. A protocol for chemically functionalized attachment known as acetylation was utilized

As opposed to a chemical process, hydrogen-treated MWCNT were also attached to scaffolds using poly-L-lysine absorption.

In vitro MSC proliferation study: Human bone marrow-derived MSCs were expanded up to passage #3 under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air). MSCs were then seeded at 200,000 cells/mL into the fabricated scaffolds and cultured in a standard MSC medium for 5 days total. After 1 day, 3 days and 5 days, attached stem cells lifted using EDTA, and counted using a MultiScan microplate reader at 560 nm wavelength. The cell study was performed in triplicate for a total n=9 per variable.

Mechanical Testing: All mechanical tests were done using and ATS axial tester, a 50 Newton load cell and compression placard. Samples from each of the experimental groups were also mechanically tested. Circular samples of 8 millimeters in diameter and about 1 to 2 mm in height were taken and tested in compression, at a strain rate of 0.2 mm per second. The force-deformation data was then used to calculate and compare the Young's Modulus of each sample.

SEM Imaging: Microscopy was done on samples coated in gold nanoparticles, which were then viewed using a Zeiss SigmaVP Scanning Electron Microscope (SEM). The surfaces of uncoated, MWCNT coated and collage type II coated scaffolds were viewed. Scaffolds that had been seeded and cultured for five days with MSCs were also viewed using SEM.

Intellectual Property: Some or all of the material in this presentation is Patent Pending

Results and Discussion

Characterization of 3D printed bi-phasic PLA scaffold

Six cylindrical osteochondral construct designs, with different internal structure. The first group was a homogenous cross-hatched structure, with features of 1 to 0.5 mm in size (Figure 2B). The second was a bi-phasic structure consisting of a cross hatched pattern and an intersecting rings structure. Figure 2A shows bi-phasic structures but with reinforced key feature in the interface. In addition to above samples printed for cellular study and imaging, a large construct, mimicking the structure and anatomical shape of a human knee with internal bi-phasic and key features was also designed (Figure 2c). A Stratasys Fortus 250 m 3D printing system was used to fabricate the full large model out of Acrylonitrile butadiene styrene (ABS), a common material used in rapid prototyping 3D printing, for demonstration purpose (Figure 2D). Furthermore, we 3D printed the cartilage layer of the model via biocompatible PLLA polymer (Figure 2E). This model also had superficial pores on the surface, to allow fluid perfusion in a theoretical *in vivo* scenario . In addition, a plain sample, a collagen coated sample and a CNT coated sample were produced using small featured bi-phasic key featured scaffolds, and can be seen here in Figure 23.

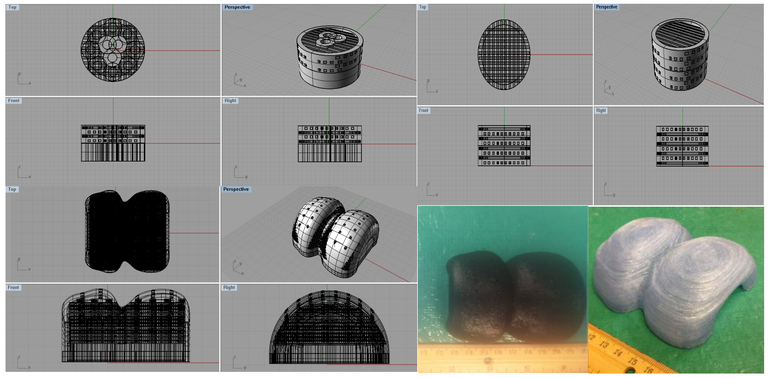


Figure 2. 3D models and images of fabricated constructs (A) "Key" bi-phasic, (B) control, (C) full knee (D) fabricated full knee and (E) fabricated PLA cartilage layer

Mechanical compression tests were also conducted on the six different scaffold construct designs (Figure 24). All of the scaffolds showed excellent mechanical properties similar to or exceeding cartilage (.75 to 1 MPa) and subchondral bone (30 to 50 Mpa) [4] in human osteochondral tissue. Under compressive loading, the biphasic key models both in small and large feature have the highest modulus when compared to the homogeneous controls and the bi-phasic models. The biophasic scaffolds with large features performed better than the similar constructs with small features

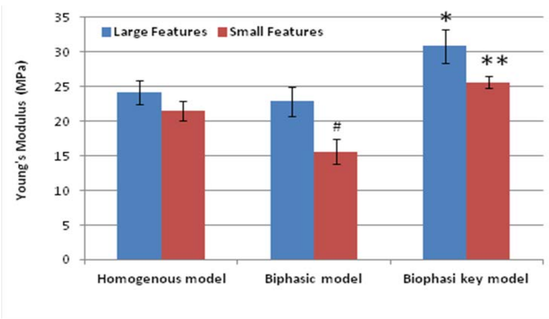
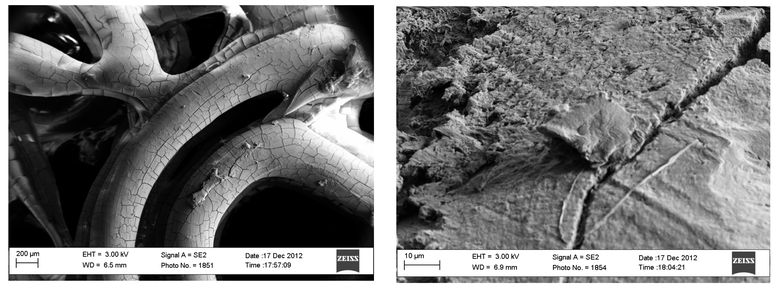


Figure 3. Figure 24; Young's modulus data for 3D printed scaffolds. Data are ± SEM, n=5; *p<0.05 when compared to all homogenous and biphasic scaffolds; **p<0.05 when compared to all other scaffolds with small features; and #p<0.05 when compared to all other scaffolds.

Figure 26 shows the surface topography of these surface modified scaffolds when compared to controls. It was also observed that all scaffolds showed a decreased MSC proliferation on day three, which may be due to the fact that these constructs had very large internal features, and cells have been shown to cease proliferative activity when migrating through a construct.



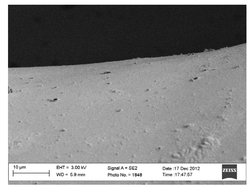


Figure 4 shows SEM images of (A) uncoated scaffold(B) collagen coated scaffold and (C) Control scaffold

Stem Cell Proliferation and Differentiation Study

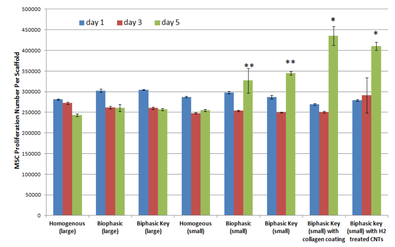


Figure 4, MSC proliferation in a variety of 3D printed PLLA scaffolds with different internal structure and surface modification. Data are ±SEM, n=9; *p<0.05 when compared to all other scaffolds and **p<0.05 when compared to all scaffolds with large features and homogenous controls with small features at day 5.

The proliferation study result showed on day one there was slightly greater cellular activity on bi-phasic scaffolds when compared to homogenous control groups. More importantly, our result shows that all of the biphasic scaffolds with small features can significantly promote MSC proliferation after 5 days. Based on table 4, these biphasic scaffolds with smaller feature attain increased surface area and greater feature density, thus providing a more advantageous environment for cellular growth. Furthermore, the scaffolds with acetylated collagen and poly-L-lysine coated H2 treated MWCNTs outperformed all other groups, which shows that nanostructured surface morphology and chemical modification can greatly increase
MSC proliferation

Conclusion and Discussion

In the 3D printing study, we created a series of biomimetic and bi-phasic constructs that had excellent mechanical properties, cytocompatibility and anatomical shape for musculoskeletal tissue engineering applications. This study showed that, through modification of the initial design parameters, the surface area, pore density and number of internal features could be easily controlled to yield desirable MSC activity. It was also demonstrated that the design of both a bi-phasic construct and that of a mechanically enhanced key structure increased cellular activity, and the addition of an internal key feature enhanced the mechanical characteristics of the scaffold when compared to homogenous control scaffolds. Finally, chemical and nano-constituent modification established in the electrospinning project were applied to 3D printed constructs, showing that the addition of collagen and poly-L-lysine coated MWCNTs further enhanced MSC proliferation in vitro.

Acknowledgements

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