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 bone supply, 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 nanostructure with a porous, randomly oriented pore structure that provides a cell-friendly nano- to micro-environment, and a strong interfacial bond 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 biomechanics multi walled carbon nanotubes (MWCNTs) and acetylated collagen for osteochondral regeneration. Specifically, Fused Deposition Modelling (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 (~6 mm) MWCNTs and collagen can also be incorporated into the 3D printed constructs via sintering coating techniques. MWCNTs and other species of carbon nanotubes have recently been explored for bone and cartilage applications. CNTs are excellent electrical conductors that can be easily coated with biocompatible polymers, such as collagen, to create a 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 (hMSCs) will be seeded onto the biomimetic 3D printed scaffolds for efficiently improving their adhesion and directing their chondrogenic differentiation.

Materials and Methods
In this study, we will seek to explore the effects of varying physical, mechanical and chemical properties of 3D printed biocomposite scaffold behavior and phenotypic expression. In vitro MSC proliferation study: Human bone marrow derived MSCs were expanded up to passage 5 under standard cell culture conditions (5% CO2, humidified, 37°C, 95% air), and MSCs were then seeded at 200,000 cells/cm², into the fabricated scaffolds and cultured in a standard medium, for 5 days total. After 1 day, 3 days and 5 days, attached stem cells were fixed using 4% paraformaldehyde, and counted using a Multiscan microplate reader at 560 nm wavelength. The cell study was performed in triplicate for a total of n=9 per variable.

Discussion
In the 3D printing of scaffolds, we have demonstrated excellent mechanical properties similar to or exceeding cartilage (~75 to 1500 Pa) and subchondral bone (~30 to 5000 Pa) in human osteochondral tissue. Under compressive loading, the biocompatible model and hard feature have the highest modulus when compared to the homogeneous controls and the bi-phasic models. The biocomposite scaffolds with large features performed better than the similar constructs with small features and large feature have the highest modulus when compared to the homogeneous controls and the bi-phasic models. 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 MWCNTs were also attached to scaffolds using poly-L-lysine absorption. The biophasic scaffolds with large features and collagen coating showed excellent mechanical properties similar to or exceeding cartilage and subchondral bone. 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 bone supply, 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 nanostructure with a porous, randomly oriented pore structure that provides a cell-friendly nano- to micro-environment, and a strong interfacial bond 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 biomechanics multi walled carbon nanotubes (MWCNTs) and acetylated collagen for osteochondral regeneration. Specifically, Fused Deposition Modelling (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 (~6 mm) MWCNTs and collagen can also be incorporated into the 3D printed constructs via sintering coating techniques. MWCNTs and other species of carbon nanotubes have recently been explored for bone and cartilage applications. CNTs are excellent electrical conductors that can be easily coated with biocompatible polymers, such as collagen, to create a 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 (hMSCs) will be seeded onto the biomimetic 3D printed scaffolds for efficiently improving their adhesion and directing their chondrogenic differentiation.

Results and Discussion
Characterization of 3D printed bi-phasic PLA scaffold
Six cylindrical osteochondral construct designs, with different internal structures. The first group was a homogeneous 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 24 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 feature was also designed (Figure 2A). A Stratasys Fortus 250 in 3D printing system was used to fabricate the full 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 PLA polymer (Figure 2B) in 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 bi-phasic scaffolds with PLA, and can be seen here in Figure 23. 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 1500 Pa) and subchondral bone (~30 to 5000 Pa) in human osteochondral tissue. Under compressive loading, the biocompatible key models both in small and large feature have the highest modulus when compared to the homogeneous controls and the bi-phasic models. The biocomposite scaffolds with large features performed better than the similar constructs with small features and large feature have the highest modulus when compared to the homogeneous controls and the bi-phasic models. 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 MWCNTs were also attached to scaffolds using poly-L-lysine absorption.

Stem Cell Proliferation and Differentiation Study
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.

Conclusion and Discussion
In the 3D printing project, we created a series of biocomposites and bi-phasic constructs that had excellent mechanical properties, cyocompatibility 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 directly manipulated to yield desirable MSC activity. It was also demonstrated that the design of both a bi-phi and the homogenous scaffold enhanced the mechanical characteristics of the scaffold when compared to homogeneous control scaffolds. Finally, chemical and nano-constituent modification established in the electronbeam 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.

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References