Biomimetic Biphasic Nanocomposite Scaffold for Osteochondral Regenration

N. Castro,[*] C. O'Brien,[*] and L.G. Zhang[*, **]

[*]Department of Mechanical and Aerospace Engineering
[**]Department of Medicine
The George Washington University, Washington, DC, 20052
Email: lgzhang@gwu.edu

ABSTRACT

Due to the disparity in composition and mechanical properties of the osteochondral interface, tissue engineering approaches to the regenration of the osteochondral site face unique challenges that are both biochemical and mechanical in nature. The current work has developed a novel biomimetic biphasic nanocomposite osteochondral scaffold integrating two biocompatible polymers each containing tissue-specific growth factor-encapsulated core-shell nanospheres. Specifically, a poly(caprolactone) based bone layer was successfully integrated with a poly(ethylene glycol) hydrogel cartilage layer. The current work also developed a novel nanosphere fabrication technique for efficient growth factor encapsulation and sustained delivery via wet co-axial electrospray. Human bone marrow mesenchymal stem cell adhesion, osteogenic and chondrogenic differentiation were evaluated in our constructs and showed significantly improved hMSC adhesion and differentiation *in vitro*.

Keywords: osteochondral, core-shell nanosphere, growth factor, biomimetic, scaffold

1 INTRODUCTION

As the leading cause of disability in the United States, osteoarthritis (OA) is a degenerative disease that manifests clinically as a loss of the articular surface in synovial joints resulting in discomfort, chronic pain, and loss of movement. As a disease, OA is significant based on the extent by which it affects so many individuals. Currently, 48 million Americans suffer from OA and that number is projected to rise to 67 million by 2030 [1]. As a result, the development of novel biomimetic tissue engineered (TE) constructs for the treatment of OA is of pressing interest. Tissue engineering and regenerative medicine approaches have been extensively employed in addressing single-tissue orthopedic defects through the use of functional biomimetic nanomaterials with more emphasis directed towards the interface [2]. The interdisciplinary field of TE holds great promise for the development of novel therapeutic approaches for the treatment of traumatic injuries, diseases and congenital defects that overcome the body's natural healing capacity [3]. The regeneration of cartilage by TE approaches has proven to be challenging since cartilage has a very limited regenerative capacity. Articular cartilage in joints is relatively avascular, contains few native mature cells (chondrocytes) and possesses a gradient of properties from the synovial surface to the subchondral bone. These characteristics are distinctly different from the highly vascularized and heavily cell populated composition of bone. In addition, there is a complex interface between cartilage and subchondral bone. The treatment of damaged tissues at interfaces, like the osteochondral interface, is particularly difficult due to the presence of biological and chemical gradients, namely: cell population(s), tissue type, and extracellular matrix (ECM) proteins are often present and difficult to recapitulate. The osteochondral interface is of great importance since it is a site of attachment between two distinct tissues while providing the necessary structure and mechanical integrity for energy transfer.

Interfacial tissue engineering (ITE) is one approach to address the complex bi- or multiphasic nature of osteochondral defects. This serves to introduce the main caveat of ITE, where interfaces have shared characteristics of the tissues being connected but also contains regions of distinct composition and biological function [4]. As a result, the development of new methods, biomaterials, and techniques to manufacture biomimetic constructs linking two distinct tissues within certain biological and mechanical constraints presents considerable challenges. In addition, it is important to note that natural human osteochondral tissue ECM is nanometer in dimension composed of many nanostructured components (such as nanocrystalline hydroxyapatites, collagen and various other proteins) [5]. Thus, the objective of this study is to develop a biphasic biomimetic nanocomposite scaffold to provide sustained biological cues for enhanced human bone marrow-derived mesenchymal stem cell (hMSC) differentiation and new tissue formation.

In particular, one of the novel features of the biphasic osteochondral scaffold developed herein is the use of co-axial wet electrospray in the manufacture of growth factor-encapsulated core-shell nanospheres allowing for efficient encapsulation of tissue-specific growth factors within a wide range of biodegradable polymers. Traditional emulsion-based micro-/nanosphere fabrication techniques have exhibited positive results, but limitations regarding initial burst and uncontrolled release have inhibited their full clinical potential due to the disparity in particle size.

The co-axial wet electrospray technique developed herein has shown to produce nanospheres with good size distribution aiding in more controlled growth factor release.

In addition to core-shell nanosphere fabrication, a photocurable co-porogen system was used in creating the biomimetic biphasic nanocomposite construct as a means of physically and chemically attaching the two distinctly different biomaterials, (poly(caprolactone) (PCL) and poly(ethylene glycol) (PEG)). Therefore, the current work has developed an efficient wet electrospray growth factor nanosphere technique, as well as a novel biphasic osteochondral nanocomposite scaffold for directed and enhanced human MSC differentiation.

2 MATERIALS AND METHODS

2.1 Polymeric Nanosphere Preparation

Briefly, bone morphogenic protein-2 (BMP-2) and transforming growth factor-β1 (TGF-β1) lyophilized powders were resuspended per manufacturer's instructions and working concentrations of 10 ng/mL were used in all experiments. For BMP-2 encapsulated PDO nanospheres, a 2.5% (wt%) solution of PDO in 1,1,1,3,3,3-hexafluoropropanol (HFIP) was fed through the shell feed inlet (Figure 1b) at a flow rate of 4.0 mL/hr. BMP-2 was fed through the core feed inlet at the same flow rate. Voltage was adjusted during collection to prevent fiber formation and maintain adequate Taylor cone morphology. Similarly, TGF-β1 encapsulated PLGA nanospheres were fabricated using the same concentrations and flow rates wherein acetone was used as the solvent.

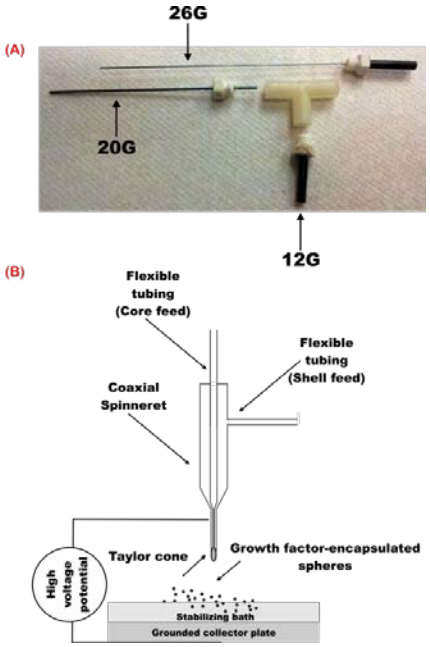

Figure 1: Co-axial electrospray system used in the manufacture of growth factor encapsulated polymeric nanospheres.

PDO nanospheres were collected in a chloroform stabilizing bath to assist in the prevention of agglomeration and replenished periodically during electrospraying. PLGA nanospheres were collected in an ultrapure water stabilizing bath. After collection, the baths were transferred to centrifuge tubes and ultrasonicated for 30 seconds. Emulsified samples were then immediately frozen and lyophilized for 24 hours to remove the stabilizing bath prior to use.

Synthesized core-shell growth factor encapsulated nanosphere morphologies were characterized by transmission electron microscope (TEM). Particle size analysis of nanospheres was conducted with ImageJ. Briefly, a calibrated TEM micrograph was imported in to the software and converted to a binary image with adjusted threshold to remove noise and background. Particle analysis of the binary image was conducted and particle diameters were extrapolated from calculated areas.

2.2 Biphasic Scaffold Fabrication

Biphasic osteochondral scaffolds were fabricated via a novel photocrosslinking/co-porogen leaching method for integration of two disparate polymeric materials as illustrated in Figure 2. Scaffolds were prepared as a proof of concept and each layer was evaluated separately for respective hMSC differentiation.

2.2.1 Bone Layer Preparation

PCL was employed as the base bone layer material in the current system. It was dissolved in an excess of chloroform to allow for efficient mixture of all composite materials with PCL resultantly constituting 38% of the total mass of the bone layer. nHA (20 wt%) was added to the dissolved PCL. Separately, a 60:40 mixture of Poly(ethylene glycol)):Poly(ethylene glycol)-diacrylate (PEG-Da, Mn=700) was prepared. A photoinitiator, Bis(2,4,6-trimethylbenzoyl)-phenylphosphineoxide (BAPO), with excitation in the ultraviolet (UV) range was added to the PEG:PEG-Da mixture at 0.5 wt% of PEG-Da and allowed to rest overnight for adequate dissolution. Several samples were prepared and used for further hMSC differentiation studies. PDO nanospheres were added to the dissolved PCL/nHA mixture. Upon complete dissolution of the photoinitiator, both PCL and PEG solutions were mixed and mechanically stirred. For hMSC differentiation experiments, complete PCL/PEG mixture was cast in to a glass petri dish allowed to rest for 10 minutes and cured for 30 seconds under UV light. 5 mm samples of crosslinked samples were collected with a biopsy punch and leached in ultrapure water for 3 days with periodic exchange of fresh ultrapure water.

2.2.2 Cartilage Layer Preparation

For more efficient layer integration, the same 60:40 PEG:PEG-Da mixture served as the base material for all cartilage layer samples. As previously described, 0.5 wt% BAPO was added to the PEG:PEG-Da mixture and allowed

to rest overnight. Lyophilized TGF-β1 encapsulated PLGA nanospheres were subsequently added and mixed for adequate dispersion within the hydrogel matrix. The hydrogel mixture was then cast in to a 9 cm glass petri dish and UV cured for 15 seconds. 5mm samples of crosslinked samples were collected with a biopsy punch and used for differentiation studies.

2.2.3 Fabrication of Osteochondral Scaffold

Biphasc biomimetic osteochondral scaffolds were prepared as depicted in Figure 2. The complete PCL/PEG-DA one layer misture was cast into a 9 cm glass petri dish and allowed to rest for 5 minutes prior to partial (15 second) UV curing. The solvent (chloroform) was allowed to partially evaporate prior to casting of the PEG-DA cartilage layer mixutre. The PEG-DA cartilage layer was cast directly on top of the partially cured PCL bone layer and UV cured for 1 additional minute. The biphasic scaffolds were imaged under scanning electron microscopy. (SEM)

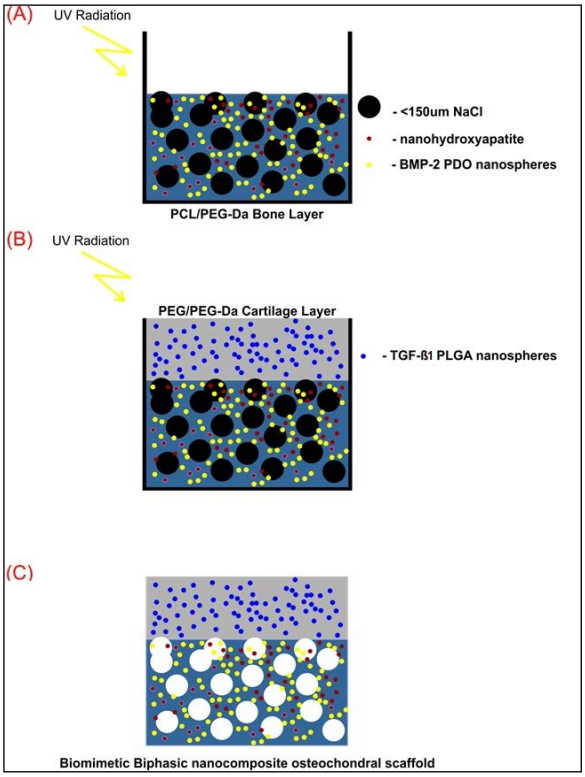

Figure 2: Photocrosslinking/Co-porogen leaching method for the fabrication of biphasic biomimetic osteochondral scaffolds.

2.2.4 Cell Study Preparation

hMSCs were seeded at a density of 10^5 cells/scaffold for osteogenic differentiation evaluation. Cell seeded bone layer scaffolds were cultured in complete media supplemented with osteogenic factors (10 nM Dexamethasone, 20 mM β-glycerophosphate, 50 µM *L*-Ascorbic acid) for 1 and 2 weeks, respectively. Total collagen content was measured spectrophotometrically.

3 RESULTS

TEM imaging was employed to evaluate the morphology of the PDO/PLGA nanospheres. Particle size ranged between 75 and 250 nm, with an average particle size of 150 nm. (Figure 3)

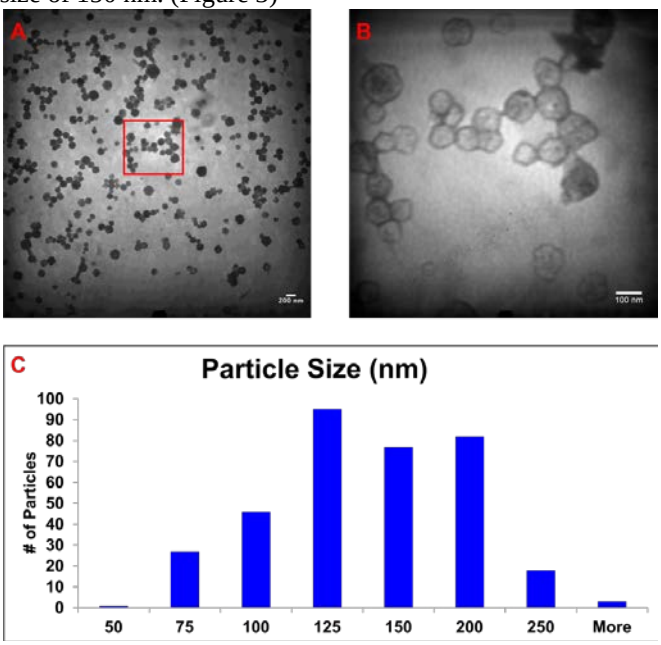

Figure 3: TEM images of BMP-2 encapsulated PDO nanospheres (A&B) (B) depicts the highlighted region in (A) at a higher magnification. (C) depicts PDO particle size distribution. PLGA spheres exhibited similar results.

Optical and scanning electron micrographs (Figure 4) of fabricated osteochondral scaffolds revealed excellent integration between the PEG-Da (cartilage) and PCL/PEG-Da (bone) layer (Figure 3C). Figure 3C illustrates the UV crosslinked interface between the respective layers with higher magnification images (Figure 3D-E) further illustrating good integration. An interesting feature noted in the osteochondral scaffold is the formation of a fibrous nanostructured network formed orthogonally to the cured surface. In addition, due to the presence of the nHA nanoparticles within the bone layer, a more biomimetic scaffold has been developed with respect to morphology and composition. The interface serves to not only integrate the two respective tissues, but also serves to aid in transferring shear stress from the articulating surface to compressive stress in the deep cartilage and subchondral region. The presence of the "nano-struts" may aid in enhancing the compressive strength of the overall scaffold thus further adding a mechanical component to the overall enhancements of the osteochondral scaffold developed herein.

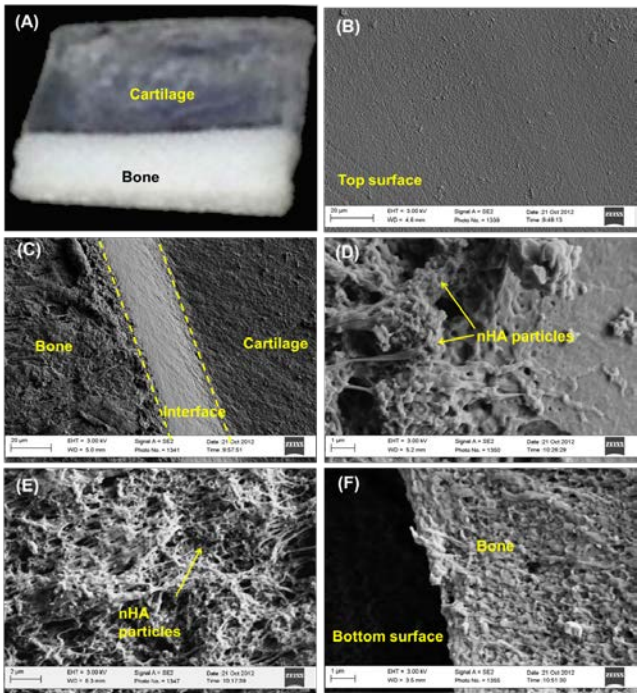

Figure 4: Optical(A) and SEM (B-F) images of biphasic biomimetic osteochondral scaffold

Total collagen synthesis (Figure 5) increased in all nanostructured bone layer samples with respect to control after 2 weeks of culture. Although no distinguishable difference was observed after 1 week, after 2 weeks all nHA/BMP-2 PCL samples performed better than PCL control samples, as well as showed a statistically significant increase in collagen production when compared to week 1. Moreover, our results show that BMP-2 encapsulated PDO nanospheres can achieve the highest collagen synthesis when compared to BMP-2 blended and all other samples after 2 weeks.

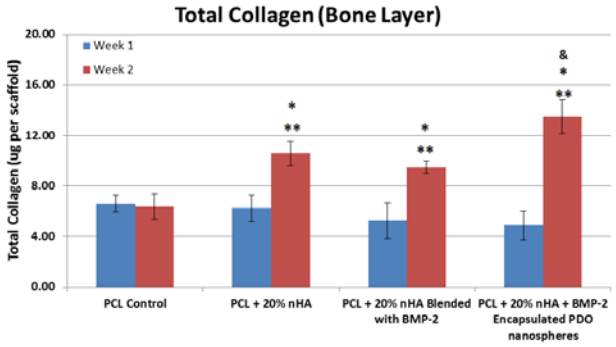

Figure 5: Significantly improved total collagen content in nanocomposite bone layer scaffolds after 2 week of culture. Data are mean ± SEM, n=6; *p<0.01 when compared to PCL control after 2 week and **p<0.05 when compared to respective scaffolds in week 1.

4 Conclusions

The work presented herein served to illustrate the feasibility of manufacturing a novel biphasic biomimetic osteochondral nanocomposite scaffold with controlled growth factor release. Novel aspects of the current study include the development of an efficient wet electrospray technique to manufacture growth factor encapsulated core-shell nanospheres in addition to co-porogen UV crosslinking of two disparate polymeric materials. hMSC adhesion and differentiation were enhanced through the incorporation of tissue-specific nanomaterials including nHA, BMP-2-loaded PDO and TGF-β1-loaded PLGA nanospheres. Due to the nature of the model, additional growth factor encapsulated spheres can be readily incorporated and evaluated for neovascularization. The current biphasic osteochondral model and nanosphere fabrication method hold great potential for orthopedic tissue engineering applications.

REFERENCES

1. Hootman, J.M. and C.G. Helmick, *Projections of US prevalence of arthritis and associated activity limitations.* Arthritis Rheum, 2006. **54**(1): p. 226-9.
2. Castro, N., S. Hacking, and L. Zhang, *Recent Progress in Interfacial Tissue Engineering Approaches for Osteochondral Defects.* Annals of Biomedical Engineering, 2012. **40**(8): p. 1628-1640.
3. Langer, R. and J.P. Vacanti, *Tissue engineering.* Science, 1993. **260**(5110): p. 920-6.
4. Temenoff, J.S. and P.J. Yang, *Engineering Orthopedic Tissue Interfaces.* Tissue Engineering Part B-Reviews, 2009. **15**(2): p. 127-141.
5. Zhang, L., J.Y. Li, and J.D. Lee, *Nanotechnology for Cartilage and Bone Regeneration*, in *Nanomedicine: technologies and applications*, T.J. Webster, Editor 2012, Woodhead Publishing Limited. p. 571-598.