Favored Osteoblast Interactions with Aerosol Printed 3D Nano-to-Macro Hierarchical Architectures: The Promise of Nanocomposites as Orthopedic Prostheses

Huinan Liu* and Thomas J. Webster**

Division of Engineering, Brown University, 182 Hope Street, Providence, RI 02912, USA,
*Huinan_Liu@brown.edu, **Thomas_Webster@brown.edu

ABSTRACT

Ceramic/polymer nanocomposites simulate bone in terms of its nanostructure and associated properties, thus, offering a promising new opportunity for bone regeneration in a natural way. This study focused on further mimicking bone by building 3D structures from ceramic/polymer nanocomposites using a novel aerosol based 3D printing technique. The 3D printed nanophase titania/PLGA composites demonstrated well-ordered 3D structures and the surfaces of such nanocomposite scaffolds demonstrated uniform dispersion of titania nanoparticles after 3D printing. In vitro osteoblast (bone-forming cell) results provided the first evidence that these 3D scaffolds further promoted cell infiltration into porous structures compared to previous cast-molded nanostructured surfaces. In conclusion, so far, these results have evaluated a promising new orthopedic nanocomposite and a means of fabricating a macro structure from such nanomaterials for more effective orthopedic applications.

Keywords: nanocomposite, ceramics, polymers, orthopedics, 3D fabrication, rapid prototyping.

INTRODUCTION

Currently, the main reason for bone substitute failure in orthopedics lies in a lack of osseointegration, that is, insufficient juxtaposed bone growth on material surfaces as a result of deficient osteoblast (bone forming cell) functions. Special properties (nano-scale surface topography, surface area and roughness) of nanophase ceramics enhance osseogenesis and new bone regeneration [1]. Specifically, greater osteoblast adhesion and long term functions have been observed on nanophase ceramics compared to conventional ceramics [1]. Moreover, the adsorption of vitronectin (a protein known to mediate osteoblast adhesion) has been reported to be much greater on nanophase ceramics compared to conventional ceramics [2]. However, single phase ceramics are inherently brittle and are difficult to be fabricated into complex structures with acceptable mechanical properties for orthopedic applications. For this reason, ceramic/polymer nanocomposites have received more attention.

The experimental focus of this research was to study in vitro osteoblast functions on ceramic/polymer nanocomposite scaffolds and to gain a deeper understanding of cell interactions with 2D and 3D scaffolds. Previous studies demonstrated that well-dispersed nanophase titania in PLGA (poly-lactide-co-glycolide) composites promoted bone cell adhesion and calcium deposition [3]. However, to date, relatively few advantages of nanocomposites have been incorporated into the orthopedic clinics due to challenges integrating nano-scale structures or components into macro architectures while preserving their nano-features. Traditional 3D fabrication methods (such as solvent-casting/porogen-leaching and phase separation) have difficulties in the precise control of 3D internal and external nano architecture of scaffolds, especially when a second phase (ceramic nanoparticles) is involved. Therefore, a novel aerosol based 3D printing technique ($M^3D^®$ system) developed by OPTOMEC® was used for the first time in this study to fabricate nanocomposite scaffolds for bone tissue engineering applications. This is because natural bone, similarly, builds its 3D macro hierarchical structures from constituent nano-components. The objective of this study was to test the effectiveness of this 3D printing technique for nanocomposite fabrication as well as osteoblast (bone-forming cells) adhesion and infiltration into these 3D printed nanocomposite scaffolds.

MATERIALS AND METHODS

PLGA (50/50 wt.% poly(DL-lactide/glycolide)) and nanophase titania were used as model materials in this study and were purchased from Polysciences, Inc. and Nanophase Technologies, Corp., respectively. The titania particle size was 32 nm according to BET adsorption measurements and the particle morphology was nearly spherical according to TEM images (Figure 1) [4]. PLGA was dissolved in chloroform and nanophase titania was then added to the PLGA solution to give a 30/70 ceramic/polymer weight ratio. The composite mixture was sonicated and processed in a $M^3D^®$ system, where it was aerosolized in an atomizer to create a dense aerosol of tiny droplets; the aerosol was carried by a gas to the deposition head and focused by a second gas flow within the deposition head; and finally the resulting high velocity

stream was "sprayed" onto the substrate layer by layer according to pre-designed CAD (computer-aided design) models. The 3D printed nanocomposite scaffolds were dried in air at room temperature for 24 hours and dried in an air vacuum chamber at room temperature for 48 hours. The final composite scaffolds were 1 cm × 1 cm squares with a thickness of 0.3 mm. These scaffolds were sterilized by soaking in 70% ethanol for 30 minutes and were dried completely before performing experiments with cells.

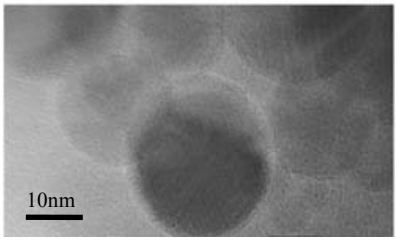

Figure 1: TEM image of nanophase titania powder. Magnification bar is 10 nm [4].

Human osteoblasts (ATCC) were seeded at a concentration of 2500 cells/cm^2 onto the scaffolds of interest in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (P/S; Hyclone) and then incubated under standard cell culture conditions for 4 hours. After that time period, non-adherent cells were removed by rinsing with PBS and adherent cells were then stained with DAPI nucleic acid stain (Invitrogen); the cell nuclei were thus visualized and counted under a confocal laser scanning microscope (Leica TCS SP2 AOBS spectral confocal microscope, excitation wavelength 358 nm and emission wavelength 461 nm). Two detection channels were used in this study: one was for imaging fluorescence from stained cells and another was for collecting bright field images of scaffolds. Leica's confocal software, LCS version 2.5, was used for 3D-scanning image acquisition and 3D reconstruction. Cell counts were expressed as the average number of cells adherent around the pores and adherent on the surfaces away from the pores determined in twenty fields of view. All experiments were run in triplicate. Numerical data were analyzed using Student *t* test; statistical significance was considered at $p<0.05$. Osteoblasts morphologies on the composite scaffolds were observed using a Scanning Electron Microscope.

RESULTS AND DISCUSSION

The 3D printed nanophase titania/PLGA composite scaffolds had well-ordered 3D structures (Figure 2). The pores had a cubic shape and pore sizes were controlled at 100 µm. The porosity was 32%. The pore size, shape and distribution can be precisely controlled by the pre-designed CAD model using this 3D printing technique. Moreover, the surfaces of such nanocomposite scaffolds demonstrated uniform dispersion of titania nanoparticles after 3D printing (Figure 3). It was previously reported that well dispersed titania nanoparticles in PLGA promoted initial osteoblast adhesion and long-term functions [3].

The in vitro osteoblast adhesion results here demonstrated that these 3D scaffolds further promoted osteoblast infiltration into porous structures compared to previous nanostructured surfaces. The SEM image in Figure 4 shows a well-spread osteoblast attached on the nanocomposite surface. The confocal image in Figure 5 shows enhanced osteoblast adhesion around novel pore structures of such 3D printed nanocomposite scaffolds. Quantitative results of cell counts demonstrated that osteoblast infiltration into the pore structures was 4.2 times greater than osteoblast adhesion onto the scaffold surfaces (Figure 6).

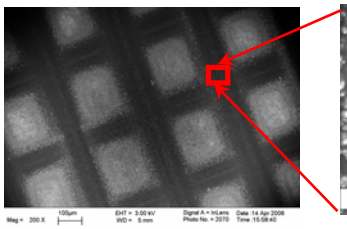

Figure 2: SEM image of 3D printed scaffolds. Bar=100 µm.

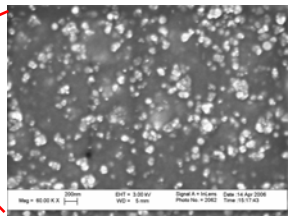

Figure 3: SEM image of a magnified region of the surface. Bar=200 nm.

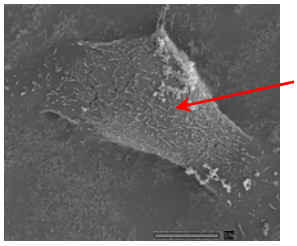

Figure 4: SEM image of an osteoblast adhering on the nanocomposite surface. Bar=10 µm.

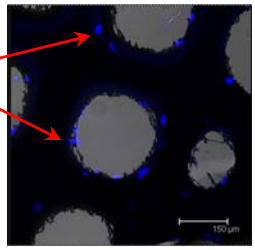

Figure 5: Confocal image of osteoblasts adhering around pore structures. Bar=150 µm.

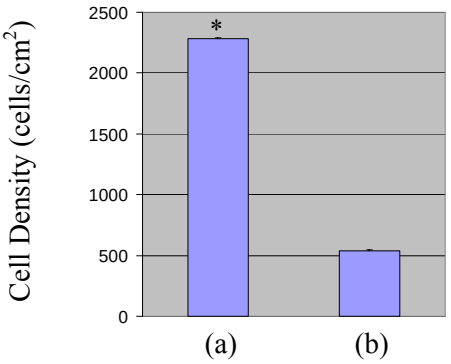

Figure 6: (a) The average number of osteoblasts adherent to pore structures. (b) The average number of osteoblasts adherent to the surfaces away from pores. Values are mean ± SD; n = 3; $*p < 0.05$ compared to (b).

CONCLUSIONS

Increased osteoblast infiltration into 3D porous structures is a crucial prerequisite for enhancing subsequent new bone ingrowth. These results have, thus, provided a promising new orthopedic nanocomposite and a means of fabricating a hierarchical macro-structure from such nanomaterials that can mimic properties of natural bone. Future work is needed to focus on understanding the mechanisms of osteoblast interactions with various nanostructured 3D patterns.

ACKNOWLEDGEMENTS

The authors would like to thank the NSF for a Nanoscale Exploration Research (NER) grant and OPTOMEC® for the assistance with the M3D® printing system.

REFERENCES

[1] Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R, 2000, "Enhanced functions of osteoblasts on nanophase ceramics", *Biomaterials*, 21(17): 1803-10.
[2] Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R, 2000, "Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics", *J Biomed Mater Res*, 51(3): 475-83.
[3] Liu H, Slamovich EB, Webster TJ, 2006, "Increased osteoblast functions among nanophase titania/poly(lactide-co-glycolide) composites of the highest nanometer surface roughness", *J Biomed Mater Res*, 78A: 798-807.
[4] Liu H, Slamovich EB, Webster TJ. 2005, "Increased osteoblast functions on poly-lactic-co-glycolic-acid with highly dispersed nanophase titania", *J Biomed Nanotechnol*, 1: 83-9.