Enhanced Osteoblast Function and Infiltration into Nanostructured Titania/Poly(lactide-co-glycolide) Aerosol 3D Printed Orthopedic Tissue Engineering Scaffolds

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Statement of Purpose: Previous studies demonstrated that well-dispersed nanophase titania in PLGA (poly-lactide-co-glycolide) composites promoted bone cell adhesion and calcium deposition [1]. 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 purchased from Polysciences 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 [2]. 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. Human osteoblasts (bone-forming cells; ATCC) were seeded at a concentration of 2500 cells/cm^2 onto the scaffolds of interest in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin 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). Osteoblasts morphologies on the composite scaffolds were observed using a Scanning Electron Microscope.

Results: The 3D printed nanophase titania/PLGA composite scaffolds had well-ordered 3D structures (Figure 1). 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 2). It was previously reported that well dispersed titania nanoparticles in PLGA promoted initial osteoblast adhesion and long-term functions [1]. 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 3 shows a well-spread osteoblast attached on the nanocomposite surface. The confocal image in Figure 4 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.

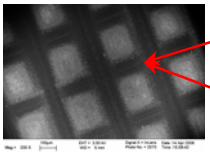

Figure 1: SEM image of 3D printed scaffolds. Bar=100 µm.

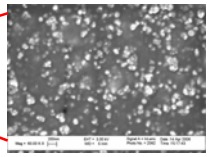

Figure 2: SEM image of a magnified region of the surface. Bar=200 nm.

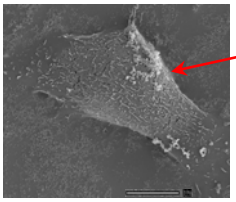

Figure 3: SEM image of an osteoblast adhering on the nanocomposite surface. Bar=10 µm.

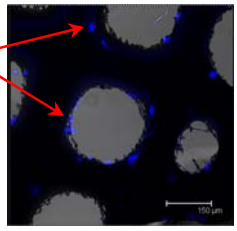

Figure 4: Confocal image of osteoblasts adhering around pore structures. Bar=150 µm.

Conclusions: Increased osteoblast infiltration into 3D porous structures is a crucial prerequisite for enhancing subsequent new bone ingrowth. The results provide a promising means of fabricating a macro structure from nanocomposites for more effective orthopedic applications. Future work is needed to focus on understanding the mechanism of cell interactions with various nanostructured 3D patterns.

References:

[1] Liu H et al. J Biomed Mater Res. 2006; 78A: 798-807.
[2] Liu H et al. J Biomed Nanotechnol. 2005; 1: 83-89.