Fabrication of TiO_2 Nano-fiber Meshes by Electrospinning and Evaluation of their Osteoblast Differentiation Potential

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Statement of Purpose: Ideal outcomes in the field of tissue engineering and regenerative medicine involve biomaterials that can enhance cell differentiation and tissue repair without the use of systemic drugs. Tissue engineering biomaterials include polymeric scaffolds that mimic the native 3-D structural environment of the final application, but that usually need to be functionalized with proteins or small peptides to improve their bioactivity [1]. Particularly for bone applications, titanium implants, or more appropriately the titanium dioxide (TiO_2) passive layer formed on their surface, have been shown to enhance osteoblast differentiation *in vitro* [2] and to promote osseointegration *in vivo* [3]. However, few studies have looked at the effect of pure TiO_2 porous biomaterials for tissue engineering. In this study, we evaluated the effect of pure TiO_2 nano-fiber meshes prepared by electrospinning on osteoblast differentiation.

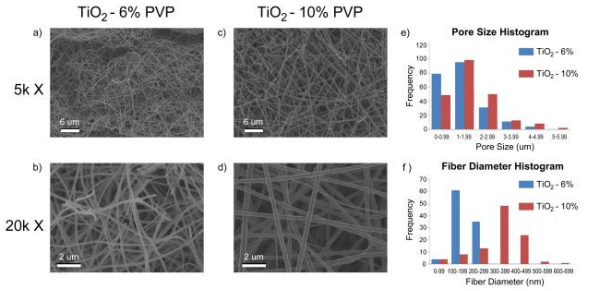
Methods:

Sample preparation and characterization: The TiO_2 nano-fiber meshes were prepared from a TiO_2 gel solution prepared by hydrolysis of titanium(IV) isopropoxide (TiP) in acetic acid/ethanol solution mixed with solutions of either 6% or 10% poly(vinyl pyrrolidone) (PVP, $M_w \approx 1\,300\,000$) in ethanol. The electrospun fibers (8 kV applied voltage) were collected on a bronze mesh covering a flat plate. Finally, the PVP was removed from these fibers by heating in air at 700 °C for 3 h. These as-prepared 6% PVP and 10% PVP TiO_2 meshes were characterized using SEM, EDX, XRD, and confocal laser microscopy.

Cell Culture Model: MG63 osteoblast-like cells were seeded on 12-well plate tissue culture polystyrene (TCPS) wells and the two different TiO_2 meshes: 6% PVP and 10% PVP. Prior to culture, the meshes were sterilized by UV over night. After confluence on TCPS, cells were treated with fresh media for 24 hours and cell number, alkaline phosphatase specific activity (ALP), osteocalcin (OCN), osteoprotegerin (OPG) and vascular-endothelial growth factor (VEGF) were analyzed (mean ± SEM for n=6 cultures/variable, analyzed by ANOVA)

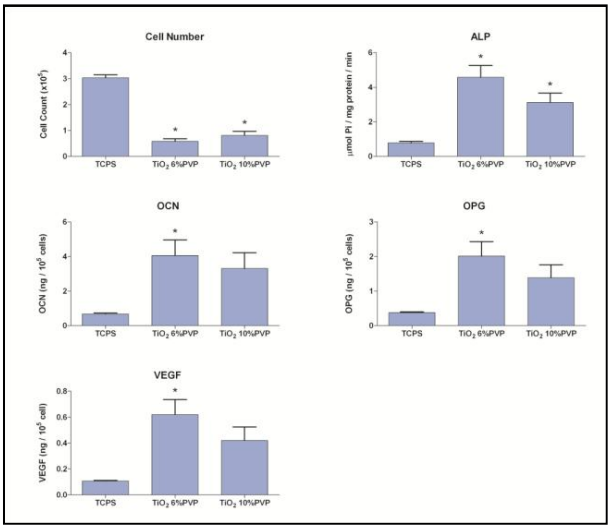
Results: The final products after calcination of the electrospun TiO_2 gel solution with 6% and 10% PVP were white and relatively thin fibrous meshes. By qualitative inspection, the 6% PVP meshes were brittle, while the 10% PVP meshes were more resistant to handling. SEM images and image analysis of the two different scaffolds revealed that the meshes were composed of non-woven nano-fibers with random orientations (Fig. 1a-d). The pore size of the 6% and 10% PVP samples was evaluated with image analysis of the lower magnification images, and were found to have similar values of 1.4 ± 0.1 µm and 1.8 ± 0.1 µm, respectively (Fig. 1e). However, fiber diameter evaluated on the higher magnification SEM images changed, with 6% PVP meshes having smaller average diameters (183.6 ± 2.8 nm) than 10% PVP (343.2 ± 23.3 nm) (Fig. 1f). Further material characterization showed that the TiO_2 meshes had similar chemical composition, containing mainly O and Ti, and had rutile crystal structure.

Figure 1. (a-d) SEM images and (e,f) image analysis of TiO2 nano-fiber meshes made with 6% and 10% PVP.



Cells attached throughout the entire meshes, formed multiple layers and exhibited elongated morphology. Cell numbers were lower and differentiation markers and local factors were higher on the TiO_2 meshes when compared to TCPS. Moreover, cell response was similar between 6% and 10% PVP samples.

Figure 2. MG63 cell response to TCPS and 6% or 10% PVP samples.



Conclusions: The results indicate that pure TiO_2 nano-fiber meshes can enhance osteoblast differentiation and local factor production. Furthermore, fiber diameters in the range of 180 to 350 nm do not seem to affect cell response. Additionally, these results suggest that TiO_2 porous materials may provide a good substrate for bone tissue engineering and osseointegration.

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