1928
doi:10.1017/S1431927610061854

Microsc. Microanal. 16 (Suppl 2), 2010
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Application of Various Imaging Techniques in the Study of Bone Tissue Formation Enabled by Biomimetic Nanomaterials

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Bone tissue engineering has been considered as a promising alternative to autografts with potential applications in orthopedic surgery [1]. In bone tissue engineering, scaffolds are used to provide a temporary substrate for cells to attach and grow and support new bone formation. To appropriately maintain the phenotype of osteoblasts and guide the functional bone tissue formation, it is desirable for the scaffolds to maximally recapitulate the major features of native bone extracellular matrix (ECM) on a multiscale and meanwhile provide the cells with a 3D microenvironment. In native bone, osteoblasts are embedded in between ECM fibers encased within hydroxyapatite (HAp) crystals [2]. In this regard, nanofibrous scaffolds containing HAp nanocrystals would be of benefit for bone tissue engineering. We have demonstrated that electrospun biomimetic nanofibers containing chitosan or chitosan/HAp can accelerate the osteogenic differentiation of murine preosteoblasts for bone formation [3]. The 3D environment formulated by biomimetic nanofibers allows the rapid deposition of bone minerals. While biochemical assays are used to evaluate cell proliferation and expression of bone markers, various microscopic techniques are extensively adopted to characterize the scaffolds, visualize the cell morphology and their distribution in the formed tissue.

Scanning electron microscopy (SEM) was used to examine the surface morphology of the electrospun nanofibers and the obtained images were used to measure the fiber diameter and the pore size of fiber meshes. As shown in Fig. 1, the PCL/chitosan nanofibers had smooth surface (Fig. 1A), however, the HAp nanoparticles directly blended with PCL/chitosan formed large aggregates (Fig. 1B), unfavorable to the spreading and growth of preosteoblasts. An effective solution to this is to in situ synthesize HAp nanocrystals into chitosan and then is electrospun into nanofibers. The transmission electron microscopy image clearly shows the presence of HAp in the nanofibers as nanosize crystals (Fig. 1C). The average fiber diameter of either PCL/chitosan or PCL/chitosan/HAp nanofibers is in the range of 300-600 nm. The preosteoblasts cultured on these nanofibers maintained their polygonal morphology (Fig. 2A) and formed a continuous layer after 4 days (Fig. 2B). Following the nanofiber enabled alternating cell assembly approach developed in our laboratory [4] osteoblasts can be assembled together with biomimetic nanofibers into 3D constructs. Further culture of these constructs can lead to a 3D tissue formation (Fig. 3). SEM examination of the transverse interface of cultured constructs reveals an integral piece without distinguishing cells, new ECM and nanofiber scaffold (Fig. 3A). To better visualize the cell distribution across the cultured constructs, thin cross-sections were stained with Hematoxylin and eosin (H&E) and examined under an optical microscope. Osteoblasts homogeneously distributed in the constructs with elongated morphology (Fig. 3B). The mineralization of cultured constructs was determined by von Kossa staining of the thin cross-sections. Optical image shows that dark brownish minerals deposit in the constructs after culturing for 14 days (Fig. 3C).

In conclusion, the biomimetic nanofibers favor the adhesion, proliferation and osteogenic differentiation of preosteoblasts. Culturing the assembled constructs with preosteoblasts and

https://doi.org/10.1017/S1431927610061854 Published online by Cambridge University Press

biomimetic nanofibers leads to the formation of 3D tissue with accelerated deposition of bone minerals and enhanced expression of alkaline phosphatase (ALP). Microscopy technology provides critical and effective tools to characterize the nanofiber scaffolds and newly formed tissues.

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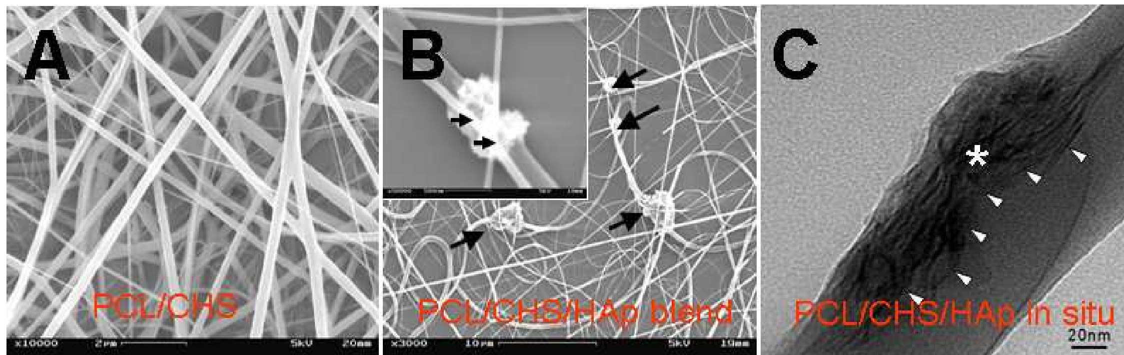

FIG. 1. Electron microscopy images of electrospun nanofibers of PCL/chitosan (A), PCL/chitosan with blended HAp (B), and PCL/chitosan with in situ synthesized HAp (C). Arrows indicate the HAp aggregates in (B) and nanocrystals in (C).

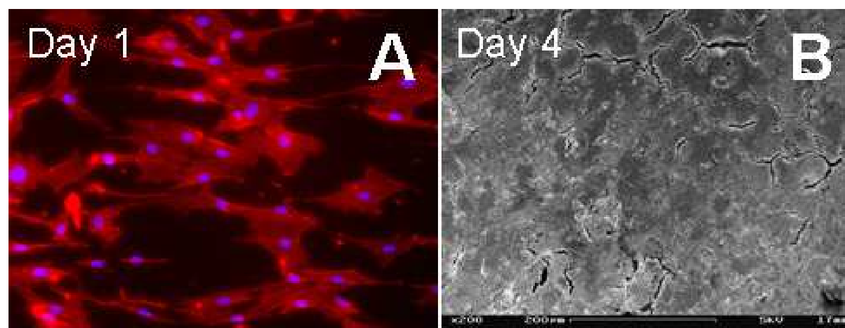

FIG. 2. Osteoblasts cultured on PCL/chitosan/HAp nanofibers. Fluorescence microscopy image of osteoblasts stained with TRITC conjugated phalloidin (red=actin) and DAPI (blue=nucleus) (A) and SEM image of the confluence layer of cells (B).

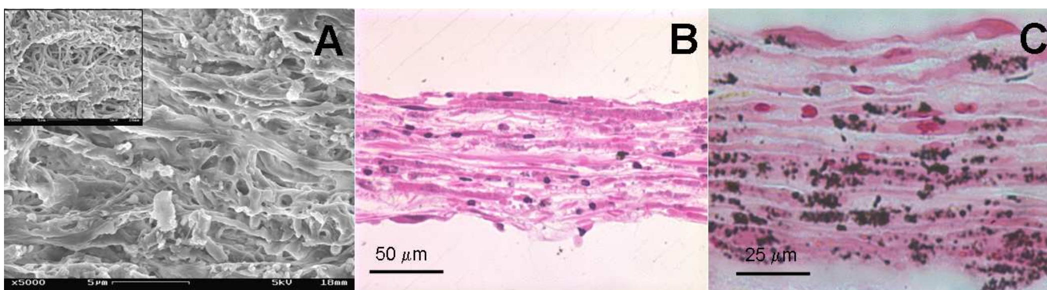

FIG. 3. 3D tissue formation by culturing the alternating assembled osteoblasts and PCL/chitosan nanofibers for 14 days [3]. (A) SEM image of the transverse section. (B) Optical image of the cross sections stained with H&E. (C) Optical image of the von Kossa stained cross sections.

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