Bio-inspired Nanocomposite Fibrous Scaffolds for Hard Tissue Regenerative Medicine

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Statement of Purpose: Aging of population increases the need for human organ/tissue repairs. Autografts supply, however, has limitation, and allografts may cause the risk of disease transmission and anti-immunization response. One of the alternatives is nanofibrous biomaterials. Nanofibers are the fundamental building blocks of human tissues. Bone tissue is composed of mineralized collagen nanofibrils with carbonated hydroxyapatite [1]. This nanofibrous nature inspired us to design calcium phosphate (CaP)/biopolymer nanocomposite fibrous scaffolds for the reconstruction/regeneration of hard tissues. This study presents a novel design concept for the fabrication of the nanocomposites by electrospinning (ES) and biomimetic in-situ synthesis. The ES process produces a non-woven nanofibrous architecture that has 3D interconnected pores and high surface area. This design mimics the natural extracellular matrix of human tissues and is of great interest in biomedical applications [2]. The in-situ synthesis of CaP with biopolymer matrix for ES is shown to induce better dispersion and distribution of the CaP nanophase within the biopolymer nanofibers than a mechanical blending method. Two distinctive nanocomposite fibers systems explored in this research; i) poly(lactic acid) (PLA), a synthetic biodegradable polymer, with dicalcium phosphate anhydrate (DCPA) and ii) alginate, a natural biopolymer, with hydroxyapatite (HAp).

Methods: Calcium phosphate nitrate tetrahydrate (CNT) and sodium phosphate monobasic dihydrate (SPM) were used as precursors for the in-situ synthesis of DCPA in PLA solution (THF/DMF), which were electrospun into fibrous scaffolds. Aqueous Na-alginic acid/poly(ethylene oxide) solutions containing SPM were electrospun with the addition of Triton X-100. The scaffolds were cut into 1.5 x 1.5 in. pieces and agitated in aqueous CNT solution for cross-linking of the alginate and precipitation of HAp. The scaffolds were washed twice with de-ionized water and freeze-dried by lyophilizer. The nanocomposites were characterized by XRD, FT-IR, SEM, EDS, STEM, and TGA. Micro-tensile testing and in-vitro bioactivity, biodegradability, and cellular activity tests are under way.

Results: The in-situ synthesized DCPA/PLA solutions were electrospun into self-fused nanofibers, ranging broadly from 100 nm to 3.0 um in thickness, which contained intra-nanopores (Fig. 1 (c)). The homogeneous dispersion of DCPA nanocrystallites was confirmed by TEM and a selected area diffraction pattern (Fig. 1 (d)), and compared with the pure PLA nanofibers (Fig 1 (a, b)). STEM/EDS supported the observation of the uniform dispersion of the DCPA nanocrystallites by mapping the distribution of Ca, O, and P elements in the DCPA/PLA nanofiber. In contrast, the mechanically blended and electrospun DCPA/PLA nanofibers exhibited severely agglomerated DCPA crystal clusters of ~ 3.0 um in sizes. FT-IR spectra clearly indicated that the C=O bands of PLA shifted to lower wave numbers after the in-situ

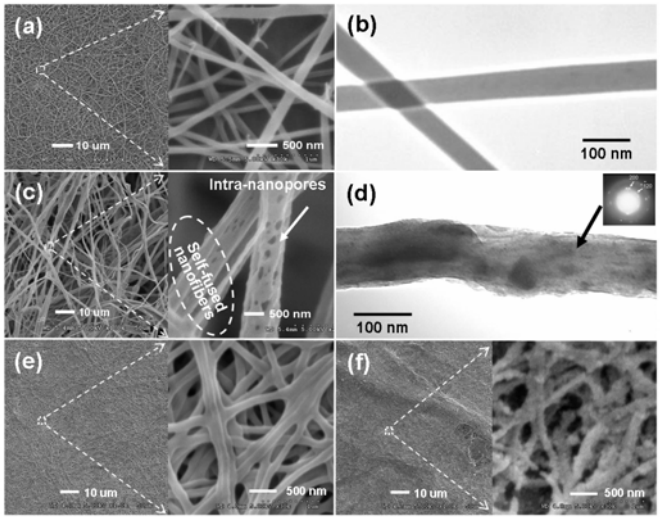

Figure 1. Morphology of (a, b) PLA, (c, d) DCPA/PLA, (e) alginate and (f) HAp/alginate fibrous scaffolds.

synthesis. It is well-known that Ca^{2+} ions form chelae bonds with the unpaired electrons of the oxygen in the PLA carbonyl groups [3]. The PLA/Ca^{2+} complexes may function as nucleation sites for DCPA mineralization, and the neighboring PLA molecular chains surrounding the nanocrystallites prevent agglomeration of the DCPA, inducing its uniform distribution in the PLA fiber host after ES. Fig. 1 (e) shows the electrospun pure alginate nanofibers after cross-linking. FT-IR spectrum confirmed with the double slit of the $PO_4(v_4)$ bands that the nanocrystals surrounding all along the nanofibers are HAp, even though XRD shows no characteristic peaks for HAp but amorphous calcium phosphate [3]. The homogeneous HAp coating on the nanofibers was achieved by the adjustment of PO_4^{3-} ion concentration and processing time. It appears that the formation of [–COO–]–Ca^{2+}–[–COO–] linkages in the "egg box" model [4] resulted from the interaction between the ionized carboxyl groups in sodium alginate and Ca^{2+} ions, serving as HAp nucleation sites in the alginate molecular chains and resulting in the shifts of the carboxyl bands in the FT-IR spectra.

Conclusions: The in-situ processes combined with ES successfully fabricated homogeneous calcium phosphate/biopolymer nanocomposite fibrous scaffolds, mimicking the mineralized collagen fibers of bone tissue. The unique fibrous topography promises to enhance biological performance and structural stability of the scaffolds for the applications in hard tissue regenerative medicine.

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