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Synthesis, characterization and osteoblastic activity of polycaprolactone nanofibers coated with biomimetic calcium phosphate

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Abstract

Immersion of electrospun polycaprolactone (PCL) nanofiber mats in calcium phosphate solutions similar to simulated body fluid resulted in deposition of biomimetic calcium phosphate layer on the nanofibers and thus a highly bioactive novel scaffold has been developed for bone tissue engineering. Coatings with adequate integrity, favorable chemistry and morphology were achieved in less than 6 h of immersion. In the coating solutions, use of lower concentrations of phosphate sources with respect to the literature values (i.e., 3.62 vs. 10 mM) was substantiated by a thermodynamic modeling approach. Recipe concentration combinations that were away from the calculated dicalcium phosphate phase stability region resulted in micron-sized calcium phosphates with native nanostructures. While the nano/microstructure formed by the deposited calcium phosphate layer is controlled by increasing the solution pH to above 6.5 and increasing the duration of immersion experimentally, the nanostructure imposed by the dimensions of the fibers was controlled by the polymer concentration (12% w/v), applied voltage (25 kV) and capillary tip to collector distance (35 cm). The deposited coating increased quantitatively by extending the soak up to 6 h. On the other hand, the porosity values attained in the scaffolds were around 87% and the biomimetic coatings did not alter the nanofiber mat porosities negatively since the deposition continued along the fibers after the first 2 h. Upon confirming the non-toxic nature of the electrospun PCL nanofiber mats, the effects of different nano/microstructures formed were evaluated by the osteoblastic activity. The levels of both alkaline phosphatase activity and osteocalcin were found to be higher in the coated PCL nanofibers than in the uncoated PCL nanofibers, indicating that biomimetic calcium phosphate on PCL nanofibers supports osteoblastic differentiation.

Keywords: Polycaprolactone; Nanofiber; Calcium phosphate coating; Nanocomposite; Bone tissue engineering

1. Introduction

Controlling and regulating the potential of natural tissue regeneration mechanisms and use of mimics of the natural extracellular matrix (ECM) for defect repair or even organ regeneration is the challenge in tissue engineering. A tissue-engineering scaffold would be considered successful if it has biocompatibility, biodegradability, reproducibility, high porosity with interconnected pores, and no potential for serious immunological or foreign body reactions. The scaffold's ability to promote ECM secretion and carry biomolecular signals are additional factors to consider. The principal components of ECM are collagen biopolymers, which are mainly in the form of fibers and fibrils. Recently, due to the resemblance of their physical nanofeatures to natural ECM, biocomposites composed of nanocrystals and nanofibers have been popularized in supporting cell proliferation and organization [1].

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Electrospinning has been widely used to produce nanoscale fiber mats of several biocompatible and biodegradable polymers, including synthetic–natural polymer blends for tissue engineering purposes [2–4]. Polycaprolactone (PCL), which is among the most popular scaffold polymers approved by the US Food and Drug Administration, has also been electrospun successfully [3,5–8]. Mimicking of the structural dimensions of the ECM by small-sized fibers has been shown to promote the adhesion and proliferation of cells [6,8,9]. A slow biodegradation rate (over 24 months) and hydrophobicity are the major drawbacks of this otherwise promising biopolymer [1].

Hybrid nanocomposite hard tissue scaffolds fabricated with approaches involving the electrostatic co-spinning of a natural or a synthetic polymer along with nanoparticles of hydroxyapatite (nHA) [10,11], tricalcium phosphate (TCP) or $CaCO_3$ (CC) [12] have also been shown to improve the cell attachment kinetics [2]. Such studies include those with PCL–nHA and PCL–CC nanocomposites [9,12–14]. In the other co-spinning approach, rather than using nHA, precursors of HA were blended with appropriate polymers before electrospinning [15,16]. However, the fact that biodegradation rate of the polymer matrix can limit the extent of availability of the inorganic component has raised some concerns, and the coating of polymer fibers has been offered as an alternative to the straightforward blending approaches mentioned above. The coating of polymers with a calcium phosphate layer has been claimed to provide the obtained structures with adequate bone-bonding or osteoconductive properties, and help in tailoring the degradation and resorption of the polymer matrix and improving cell adhesion, proliferation and differentiation [17]. Biomimetic coatings are promising candidates for this hybridization approach.

Bone mineral has a defective structure. In contrast to the perfectly stoichiometric compound calcium hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$), it is considered to be doped with mono- or divalent cations (BM: $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$). Due to its defective chemistry, BM is associated with enhanced bioresorbability [18]. Simulated or synthetic body fluids (SBF) have the ability of forming apatitic calcium phosphates on the immersed osteoinductive (metals, ceramics or polymers) materials within a few days to 2 weeks. Due to the resemblance of their defect chemistry to BM, SBF-induced calcium phosphates can be considered as bone-like mineral (BLM). Biomimetic coating of implant surfaces with BLM in SBF recipes has been offered as a practical and robust way of increasing the bone-bonding ability of the implants or testing their bioactivity [19–21]. The supersaturation levels in the SBF recipes commonly experimented with do not allow for rapid deposition rates. However, the use of concentrated (i.e., *n*SBF, where *n* denotes the multiples of concentrations of ions found in human plasma such as $n = 1.5$, 2, 5, 10) recipes permits similar thicknesses of coatings in as short a time as 2–6 h [18,20,22]. Although *n*SBF solutions seem like straightforward extensions of classical SBF (c-SBF) solutions, the advantages brought by their use are not limited to increased coating rates. In the application of c-SBF solutions, a buffering agent like Tris or HEPES is used to keep the solution pH at 7.4 throughout the coating process. Elimination of the buffering agents from *n*SBF solutions not only facilitates the preparation steps, but also promotes the formation of calcium phosphate phases with different resorption rates [20]. The nature and crystallinity of the phases forming depends on various parameters, including concentrations of phosphate/carbonate sources (i.e., pH), the ionic strength of the *n*SBF solution (i.e., activity coefficients of the ions and aqueous species), and the kinetics of the nucleation and growth processes (affected by the available surface area for deposition, exposition to free-flowing or closed atmosphere conditions, the presence and concentration of Mg^{2+} ions, the extent of Posner's clusters formation in the stock solutions, etc.) [20,23–28]. Nevertheless, compared to perfectly stoichiometric HA, any of these possible phases (i.e., calcium-deficient hydroxyapatite (CDHA), dahllite, amorphous calcium phosphate (ACP), octacalcium phosphate (OCP), dicalcium phosphate dihydrate (DCPD–brushite), dicalcium phosphate (DCPA–monetite, etc.) would have a higher dissolution tendency [23,29]. Although HA is osteoconductive, it does not directly take part in the bone remodeling process. On the other hand, with their higher resorbability, phases like CDHA, ACP, OCP, DCPD and DCPA do take part in the bone remodeling and biomineralization process with greater efficiency [30]. While the use of c-SBF generally leads to only carbonated CDHA (BLM), the use of *n*SBF solutions (with $n > 5$) leads to the deposition of a combination of the highly resorbable phases mentioned, along with BLM. Therefore, besides its obvious advantage of increasing the deposition rate, the use of *n*SBF could also adjust the resorption rate of a coating. This would be important in providing a flexible control parameter to the variability of the calcium and phosphate ionic concentrations in the cell microenvironment at different time scales; a concept that could help solve several "time-dependent cell viability" issues mentioned in recent related work [17]. In addition, the stringent constraint on the solution temperature, which needs to be set to 37 °C in c-SBF, could be abated by the use of 10SBF [18].

Besides metallic implants like titanium alloy, bulk polymers [31,32], including PCL [21,33–42], used in bone tissue engineering have also been treated with various SBF recipes to test or increase their bioactivity. While the c-SBF recipe has been employed to increase bioactivity of a few electrospun polymer nanofiber systems [43,44], Ngiam and co-workers recently reported the employment of c-SBF on PCL nanofibers with modified surfaces [45]. However, they did not provide an account of the produced composite's bioactivity. In a concurrent work, Araujo and co-workers [17] successfully employed 1.5SBF to obtain biomimetic calcium phosphates (BCP) on PCL nanofibers. However, prior to the final soak in 1.5SBF,

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