In-vitro Bioactivity and Osteoblast Responses on 3D PCL/BG Hybrid Fibrous Scaffolds

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Introduction

Three-dimensional (3D) bioactive organic-inorganic (O/I) hybrid fibrous scaffolds are attractive extracellular matrix (ECM) surrogates for bone tissue engineering. In this study, a novel biodegradable and bioactive O/I hybrid fibrous scaffolds were designed to mimic the morphological functions of ECM and to provide favorable microenvironment for cells to adhere, migrate, proliferate, and differentiate. The application of the PCL/BG hybrids for bone regeneration requires porous 3D scaffold architectures to mimic the trabecular bone structure of the native tissue. Therefore, a 3D poly (ε-caprolactone) and tertiary Bioactive glass (PCL/BG) hybrid constructs were prepared by a sol-gel/electrospinning process. Some of the critical scaffold properties for bone regeneration applications, including wettability, pore-size distribution, porosity, mechanical properties and bioactivity were evaluated. In addition, the role of PCL/BG hybrid fibrous scaffolds on osteoblast cell proliferation and osteoblast phenotype markers gene expression were evaluated *in vitro*. Furthermore, the effect of fiber diameter on the physical and biological properties of the PCL/BG hybrid fibrous scaffolds was demonstrated.

Materials and Methods

The electrospinning of sol-gel derived PCL/BG hybrids was carried out as described previously [1]; except that instead of a stationary collector, we have utilized a custom designed rotating mandrel collector to produce larger mat size and to control mat morphology. The electrospinning parameters were optimized to produce two distinct fiber diameters (260 nm (HF) and 600 nm (HC)). For fabricating PCL control nanofiber scaffolds, 10 wt. % of the polymer solution in 4:1 chloroform/ dimethyl sulfoxide ratio was prepared and electrospun. Scaffold properties including morphology, porosity and pore-size, wettability, mechanical properties, and in *vitro* bioactivities were characterized. *In vitro* cellular studies were conducted to evaluate osteoblast cell attachment, proliferation and differentiation on PCL/BG fibrous scaffold surfaces. In order to evaluate osteoblast differentiation, ALP activity and the gene expression level of osteoblast phenotype markers, RT-PCR analysis and ALP activity assay were used.

Results and Discussion

3D PCL/BG fibrous scaffolds with two different fiber diameter were successfully fabricated by a combined sol-gel and electrospinning process. The fibrous scaffolds characterization results indicated that the PCL/BG hybrid scaffolds exhibited high porosity, greatly improved hydrophillcity, significantly enhanced tensile mechanical properties, and *in vitro* bone-like apatite formation ability as compared to the PCL fibrous scaffolds. The PCL/BG fibrous scaffolds supported the attachment and proliferation of osteoblast cells. It was observed that rat cavarial osteoblast cells cultured on the

electrospun PCL/BG hybrid fibrous scaffolds maintained their phenotypic expression and was characterized by an increased alkaline phosphatase (ALP) activity, and enhanced expressions of bone-associated markers as compared to the pure PCL fibrous scaffold over the period of the culture time. In general, results of this study demonstrated that the PCL/BG fibrous scaffolds showed superior physical and biological scaffold properties versus PCL fibrous scaffolds for promoting bone regeneration.

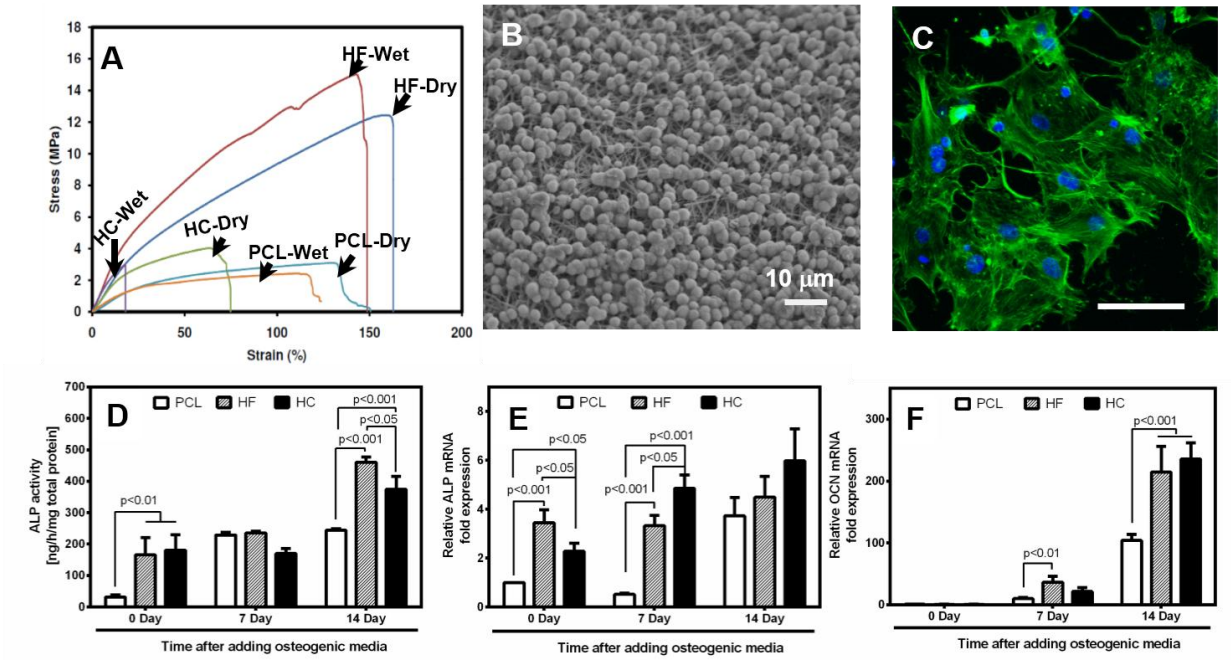

Figure 1: (A) Representative Stress-Strain Curves; (B) SEM micropgraphs of PCL/BG hybrid fiber after soaking in SBF for 24 h; (C)Confocal images MC3T3-E1 cells after 4 days ; (D) Quantifications of ALP activity and; (E,F) Temporal expressions of ALP and Osteocalcin (OCN) mRNAs by qRT-PCR.

Reference

[1] Allo BA, Rizkalla AS, Mequanint K. Synthesis and electrospinning of epsilon-polycaprolactone-bioactive glass hybrid biomaterials via a sol-gel process. Langmuir 2010;26:18340-8.