Mechanically Competent Polyphosphazene Nano-structured Biomimetic Scaffolds: Accelerated Osteoblast Differentiation

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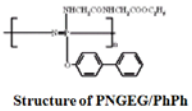
Statement of Purpose: Successful *in vivo* scaffold-based tissue regeneration greatly depends on the scaffold material biocompatibility, mechanical stability, and scaffold architecture to promote tissue in-growth. The long term goal of this work is to develop mechanically competent bioresorbable nano-structured three-dimensional (3D) biomimetic scaffolds for bone tissue engineering. A polymer blend system composed of poly[(glycine ethyl glycinato)$_1$(phenylphenoxy)$_1$phosphazene] (PNGEG/PhPh) and poly(lactide-*co*-glycolide) (PLAGA) was chosen to fabricate nanofibers in the range of 50-500 nm to mimic dimensions of collagen fibrils in native bone due to its superior mechanical stability, *in vitro* osteoblast performance, and *in vivo* biocompatibility[1]. Further a mechanically competent 3D scaffold mimicking the bone marrow cavity, as well as, the lamellar structure was created by orienting electrospun blend nanofibers in a concentric manner with an open central cavity.



Structure of PNGEG/PhPh

Methods: PLAGA 50:50 ($\overline{Mw}$ 34 kDa) was purchased from Boehringer Ingelheim KG. PNGEG/PhPh was synthesized via a two-step polymerization route[2]. The BLEND was prepared with a PNGEG/PhPh:PLAGA weight ratio of 25:75[1]. Nonwoven polymer nanofibers were fabricated at an optimized condition using an electrospinning set-up[3]. The osteocompatibility of the electrospun nanofibers was evaluated using a previously reported protocol[2,3]. Biomimetic 3D scaffolds were created by rolling-up the blend nanofiber sheets in a concentric manner with an open central cavity. The potential of this scaffold for bone repair was investigated by monitoring the cellular activity and mechanical performance over time using primary rat osteoblast cells. Compressive testing was conducted using an Instron machine. Cell morphology was visualized by SEM. Secretion of extracellular matrix proteins was characterized by immunohistochemical staining.

Results: The BLEND fibers obtained from an optimized electrospinning condition were found to be uniform and bead-free with an average diameter of 342.8±87.6 nm (Fig. 1A, B). It was confirmed by EDX that PPHOS component was presented within the BLEND nanofibers (Fig. 1C). Furthermore, a single thermal transition at 41ºC was found for BLEND fibers, indicating its suitable use for tissue engineering (Fig. 1D). As shown in Fig. 2A, cell numbers on BLEND nanofibers were increasing during the *in vitro* culture and were found to be comparable to PLAGA nanofibers at all time points, which indicated good osteocompatibility. However, ALP activity of the cells on BLEND nanofibers was significantly higher than on PLAGA nanofibers at day 3 (Fig. 2B), which indicated that the phenotypic expression of osteoblasts was enhanced due to the presence of polyphosphazene[2]. Furthermore, the nano-structured 3D scaffolds shown in Fig. 3A exhibited a similar mechanical behavior to bone under compression and possessed an average compressive modulus of 236.6±29.5 MPa (Fig. 3B), which was within the range of 20–900 MPa for human trabecular bone. As seen from Fig. 3C, the surface of biomimetic scaffolds was covered by osteoblast cell layers after 28 days. Robust osteoblast matrix deposition was found to bridge the gap space between the fiber layers, indicating that the cells were functioning actively (Fig. 3D). In addition, osteopontin (OPN), an osteoblast extracellular matrix protein, was secreted robustly inside the center cavity and the gap space between layers (Fig. 3E, F). The matrix mineralization quantified by alizarin red staining confirmed the progression of calcium deposition during the cell culture.

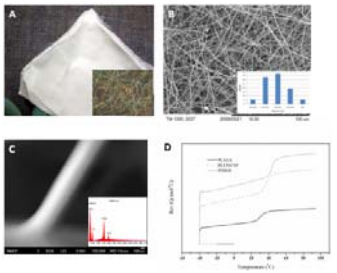


Fig. 1. (A) Light micrographs of BLEND nanofibers; (B) SEM micrographs of BLEND nanofibers; (C) SEM micrographs showing the morphology of single BLEND nanofiber (inset, EDX patterns of the BLEND nanofiber); (D) DSC thermograms.

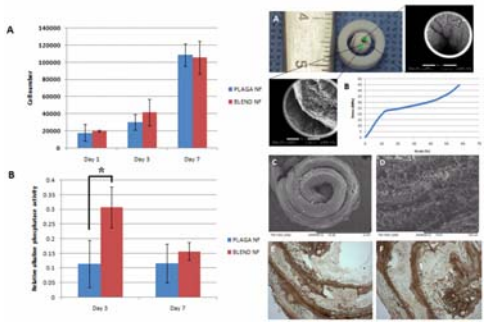


Fig. 2. (left) (A) Cell proliferation by MTS assay; and (B) ALP activity, (*) denotes significant difference ($p<0.05$); **Fig. 3. (right)** (A) Light micrographs of the 3D biomimetic scaffolds and SEM micrographs showing the detailed morphology of central cavity and fiber lamella structures; (B) Representative stress-strain curves of the 3D biomimetic scaffolds under compression; (C) SEM micrographs of the cell-seeded 3D biomimetic scaffolds after 28 days of culture; (D) SEM micrographs showing osteoblast extracellular matrix deposition bridging the fiber layers; (E) OPN staining for the lower portion of 3D biomimetic scaffolds; and (F) OPN staining for the upper and center portion of the 3D biomimetic scaffolds.

Conclusions: When combined with the desirable polymer blend properties, the concentric open macrostructures of nanofibers that structurally mimic native bone can be a potential scaffold design for accelerated bone healing.

References: 1. Deng et al., 2008 WBC [2383] 2. Deng et al., Biomaterials 29(3);337 (2008); 3. Kumbar et al., Biomaterials 29(30);4100 (2008)

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