Biomimetic Calcium Phosphate-Polycarbonate Composite Scaffolds for Bone Tissue Engineering

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Statement of Purpose:

Large bone defects resulting from trauma, tumor resection, congenital abnormalities or reconstructive surgery are challenging clinical problems that are usually treated with autografts and allografts. However, donor site morbidity and limited supply of autograft and the limited bioactivity of allograft have stimulated the development of bone graft substitutes [1]. Initial results with bone regeneration scaffolds composed of tyrosine-derived polycarbonates such as E1001(1k) (Fig 1) have been promising, due to their desirable osteoconductivity, and excellent *in vivo* bone biocompatibility [2]. To further enhance the performance of these scaffolds *in vivo*, we developed a process that deposits a coating of calcium phosphate within the pores of the scaffold and confirmed a significant improvement of the *in vivo* performance of the coated scaffolds [3]. In this study, we further optimized the coating process and were able to define the type of calcium mineral (either hydroxyapatite (HA) or dicalcium phosphate dihydrate (DCPD)) formed within the pores of the E1001(1k) scaffolds. We studied in *vitro* cell viability, attachment, proliferation and osteogenic differentiation of human mesenchymal stem cells (hMSC). .

Methods:

3D porous scaffolds were fabricated from E1001(1k) (Fig. 1) using a combination of porogen leaching and freezing drying [2]. The polymeric scaffolds were coated with calcium phosphate using a modified alternate soaking method in basic and acidic phosphoric solution. Scaffold pore size and morphology were investigated using Scanning Electron Microscopy (SEM). The identity of calcium phosphates was determined by X-ray diffraction (XRD). The *in vitro* calcium phosphate dissolution and scaffold degradation were studied by incubating scaffolds in PBS at 37°C up to 28 days. The *in vitro* cell viability, attachment, proliferation and osteogenic differentiation on E1001(1k), E1001(1k)+HA, and E1001(1k)+DCPD scaffolds were investigated using hMSC.

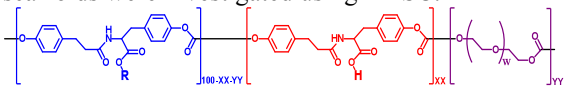

Fig. 1. General structure of Tyr-PC polycarbonate terpolymers where XX and YY are %mole fractions of DT and PEG respectively. The polymer used here has XX = 10 and YY = 01 and is referred to as E1001(1k).

Results:

E1001(1k) scaffolds are highly porous with interconnected pores and a characteristic bimodal pore size distribution of macropores (200-400µm) and micropores (<20µm) (Fig. 2). By controlling the pH used in the alternate soaking process, E1001(1k)scaffolds were coated with either HA or DCPD (Fig. 2). In basic reagent solution, a layer of fluffy-like aggregates of fine HA grains were found covering the scaffold pore wall. In acidic reagent solution, large plate-like DCPD crystals were formed throughout the scaffold. The DCPD crystals clustered together and formed 100µm flower-like structures. When conditioned in PBS, E1001(1k)+DCPD scaffolds generated a 5-6 times higher equilibrium calcium ion concentration as compared to that of E1001(1k)+HA scaffolds. In addition, DCPD transformed to bone-like apatite in 14 days in PBS as demonstrated by SEM and XRD. All tested scaffolds supported cell attachment and proliferation as shown by SEM and Alamar Blue Assay. DCPD and HA significantly enhanced the ALP activity of hMSC on those scaffolds (Figure 3).

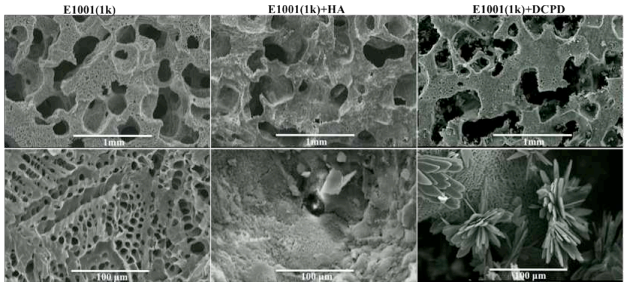

Fig. 2. SEM micrograph of representative E1001(1K), E1001(1K)+HA, and E1001(1K)+DCPD scaffolds

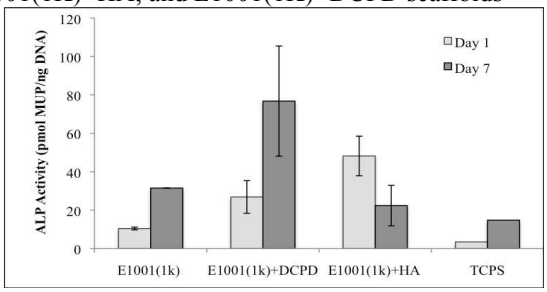

Fig. 3. Normalized ALP activity of hMSC cultured on different scaffolds substrates and on tissue culture polystyrene plates (TCPS).

Conclusions:

In this report, E1001(1k) scaffolds were coated with either HA and DCPD. The use of DCPD as a coating on polymeric scaffolds is reported here for the first time. E1001(1k) scaffolds coated with HA or DCPD are bioactive and support hMSC attachment, proliferation and differentiation into osteoblasts. hMSC on E1001(1k)+DCPD scaffolds showed their highest ALP activity at day 7, probably attributed to the higher calcium dissolution rate and ability to form bone-like apatite. E1001(1k)+DCPD scaffolds are a promising composition for bone tissue engineering and will be further investigated.

References:

1. DeLong, W. J Bone Joint Surg Am. 2007; 89: 649-58.
2. Magno, H. J. of Mat Chem. 2010; 20: 8885-8893.
3. Kim, J. Tissue Eng Part A. 2012; 18:1132-1139.