Tyrosine-Derived Polycarbonates to Treat a Rabbit Critical-Sized Segmental Bone Defect

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Statement of Purpose: Autografts harvested from patients' iliac crest are the gold standard for the treatment of bone defects as a result of trauma, fracture, or tumor resection.[1,2] Due to the limited availability of autograft tissue and donor-site morbidity, tissue engineering-based products such as bone graft substitutes in combination with rhBMPs have been developed and some of those have been approved by the US FDA (*e.g.*, INFUSE, Medtronics) as an alternative to autografts. Tyrosine-derived polycarbonates (TyrPCs) represent a library of over 10,000 polymers. Several of these polymers have been developed as therapeutics for bone regeneration. The **purpose** of this study was to determine the biocompatibility of one particular TyrPC composition, referred to as E1001(1k), and to explore the effect of enhancing the polymer scaffold with calcium phosphate (CP) and rhBMP-2. The study was conducted in a rabbit critical-size defect (CSD) radius model.

Methods: E1001(1k) scaffolds with CP coatings were prepared as described previously.[3] In vivo performance of the scaffolds was determined using a rabbit radius critical-size defect (CSD). The different doses rhBMP-2 (0, 17, 35 μg/defect site) were incorporated into porous E1001(1k)+CP scaffolds. A unilateral segmental defect (15 mm long) was created in the radial diaphysis. The scaffolds were implanted into the rabbit radius CSD. Quantitative bone regeneration in the defects was determined at 4 and 8 weeks post-implantation using micro-computed tomography (μCT), histology and histomorphometry.

Results: The polymer, E1001(1k), was synthesized and its chemical structure **(Fig. 1)** was confirmed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The architecture of porous scaffolds was assessed by scanning electron microscopy (SEM). The in vivo data revealed that the E1001(1k)+CP scaffolds did not produce any adverse cellular and tissue responses and the incorporation of rhBMP-2 into the scaffolds (as low as 17 μg/defect site) significantly promoted bone regeneration in a rabbit radius CSD model at 8 weeks compared to E1001(1k)+CP without rhBMP-2. There were no significant differences in bone regeneration between different doses (17 μg vs. 35 μg rhBMP-2/defect site). Histological assessment of *in vivo* biocompatibility of the scaffolds revealed that there were no adverse tissue responses such as reactive inflammatory reactions (e.g., macrophages or foreign body giant cells) or osteolysis (bone resorption) in the radius defects (**Fig. 2**).

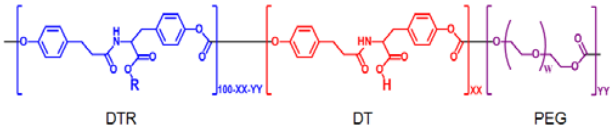

Figure 1. Chemical structure of E1001(1k), where xx = 10 mol% and yy = 1 mol%.

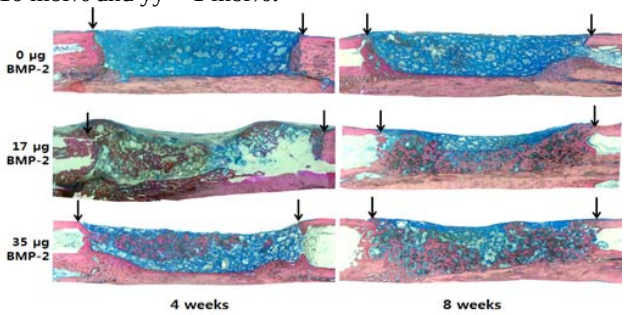

Figure 2. Representative histological images (1.5x) of the rabbit radius CSDs treated with different doses of rhBMP-2 at 4 weeks and 8 weeks post-implantation.

The images also suggested that without rhBMP-2, new bone formation was marginal and can be seen along the host bone margins at both time periods (4 and 8 weeks). However, good interfacial integration into the defects treated with E1001(1k)+CP scaffolds was evident, regardless of rhBMP-2 incorporation. When rhBMP-2 was present at 17 μg or 35 μg, substantial bone formation can be observed throughout the E1001(1k)+CP scaffolds. There appeared to be more bone regeneration at 8 weeks as compared to 4 weeks.

Conclusions: The μCT, histology, and histomorphometry data showed that the synthetic E1001(1k)+CP scaffolds were biocompatible, biodegradable and osteoconductive in the rabbit radius CSD model. The incorporation of a minimal dose of rhBMP-2 (17 μg) into the scaffolds significantly promoted new bone formation as compared to treating the defect with a E1010(1k)+CP scaffold alone. This trend was evident both at 4 weeks and 8 weeks post-implantation. Our data illustrate (1) the effectiveness of adding bioactive factors into synthetic scaffolds, (2) the potential therapeutic value of E1001(1k)+CP scaffolds when used in combination with a minimal effective dose of rhBMP-2, and (3) the ability of a synthetic, bioactive scaffold to regenerate bone and heal clinically challenging segmental defects.

References:

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