Performance of polymer + OCP composite scaffolds in the CSD rabbit calvaria model

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Statement of Purpose: Our approach to the treatment of osseous avulsions is focused on the development of synthetic, degradable bone grafts that perform equal to or better than commercially available predicate devices [1]. To this end, tyrosine-derived polycarbonate, E1001(1K), scaffolds containing calcium phosphate (CaP) were fabricated, characterized, and analyzed *in vivo*. We hypothesized that octacalcium phosphate (OCP), a possible precursor of bone apatite, would promote mesenchymal stem cell (MSC) differentiation into osteoblasts and thereby, enhance bone regeneration. The addition of OCP may serve as a possible alternative to bone grafts requiring bone morphogenetic protein-2 (BMP-2). In this study, we evaluated the *in vivo* performance of E1001(1K)+OCP scaffolds in the rabbit calvaria critical size defect (CSD) model.

Methods: Scaffolds made of E1001(1K) were fabricated using a previously published solvent casting process [2]. OCP was synthesized as described by LeGeros [3]. E1001(1K) scaffolds containing OCP were prepared in two ways: 1) OCP was mechanically added during the scaffold fabrication process ("composite" method), and 2) scaffolds were coated with OCP post-fabrication ("precipitated" method). E1001(1K) scaffolds containing dicalcium phosphate dihydrate (DCPD) were prepared as previously reported [2]. Scanning electron microcopy (SEM, Amray) and X-ray diffraction (XRD, Phillips) were used to characterize the scaffolds. The *in vivo* performance of the scaffolds was evaluated in the rabbit calvaria 15 mm critical size defect (CSD) model [2]. A commercially available, predicate device (PD) made of collagen and β-tricalcium phosphate (β-TCP) was also tested with and without 50 µg BMP-2. Scaffolds (n=5) were explanted at 6 weeks. Samples containing E1001(1K) scaffolds were stained with Trichrome, PDs were stained with Sanderson's rapid bone stain / van Gieson's. Microcomputed tomography (microCT) (ImageIQ), and histomorphometry was used to quantify bone formation within the defects. MicroCT data is reported as the trabecular bone volume/total volume x 100.

Results: SEM analysis confirmed a scaffold architecture that contained both macro (200-400 µm) and micro (10-20 µm) pores. Further, XRD confirmed the phase of calcium phosphate (CaP) incorporated into the scaffolds, such as OCP or DCPD. MicroCT was used to assess the amount of trabecular bone regenerated after 6 weeks (Fig.1). Trabecular bone volume within native bone was determined to be 23%. Defects treated with the PD controls without BMP-2 formed minimal amounts of bone (7%). However, defects treated with E1001(1K)+OCP composite produced, on average, more bone formation (>13%) compared to PD control. Further, bone formation was comparable to defects treated with PD+50ug BMP-2 (16%). Interestingly, the type of method used to incorporate the OCP (composite vs. precipitate) into the scaffold did not significantly affect average bone formation. The standard deviation of group 2 (Fig 1) was relatively high suggesting that the OCP precipitation process may require further optimization. However, defects treated with E1001(1K)+DCPD produced the lowest bone formation (3%).

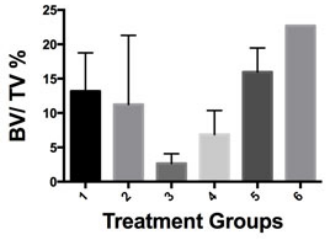

Fig 1: Effect of scaffold type on trabecular bone formation (BV/TV%) at 6 weeks in the rabbit calvaria CSD model. Defects were treated with:1) E1001(1K)+OCP composite; 2) E1001(1K)+OCP precipitate; 3) E1001(1K)+DCPD precipitate; 4) PD; 5) PD+50µg BMP-2; 6) Native rabbit calvaria bone. Data is presented as mean ± standard deviation.

Histological assessment showed a non-toxic, biocompatible response to all scaffold types (Fig. 2). Interfacial bone contact between the implant and native calvaria bone was qualitatively assessed and, in general, found to lack any inflammatory response. Further, osteoblasts were present throughout the defects treated with E1001(1K) + OCP scaffolds, suggesting that OCP enhanced osteogenesis. Osseo-integration at the native bone / defect interface was also evident. In the case of the PD implants, the sample without BMP-2, produced an abundance of fibrous tissue (Fig. 2e), whereas a significant increase in new bone area was noted only when the PD was treated with 50 µg BMP-2 (Fig. 2f).

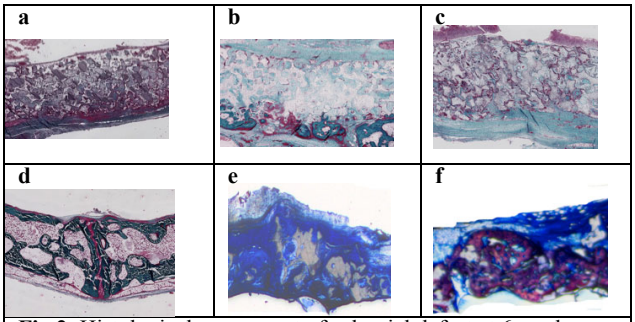

Fig 2: Histological assessment of calvarial defect at 6 weeks post-treatment. Defects were treated with (a) E1001(1K)+OCP composite; (b) E1001(1K)+OCP precipitated; (c) E1001(1K)+DCPD precipitated; (d) native bone; (e) PD; and (f) PD+50µg BMP-2. (a-d) were stained with Trichrome (green/blue indicates bone, red indicates osteoids), and (e-f) with Sanderson's rapid bone stain (blue indicates fibrous tissue) and van Gieson's picrofuchsin (red/pink indicates bone).

Conclusions: These results suggest that E1001(1K)+OCP scaffolds, without the addition of any biologics, are osteoconductive and promote bone regeneration within a rabbit calvaria CSD. In future studies, the performance of these scaffolds will be evaluated in a large animal calvaria CSD model.

References:

[1] M. Bohner, Materials Today, 2010, **3**, 24-30.
[2] M.H.R. Magno, et al., J. Mater. Chem., 2010, **20**, 8885-8893.
[3] R.Z. LeGeros, Monographs in Oral Sci. Vol.15, 1991, 11-15.