Final results of a clinically relevant bone regeneration study in the goat calvaria model.

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Statement of Purpose:
Clinically used, synthetic bone void fillers (BVFs) usually consist of collagen or aliphatic polyesters, in combination with various calcium phosphate (CaP) formulations. None of these devices exhibit a strong regenerative response, thus requiring the use of biological additives (blood components, cells, or signaling molecules such as rh-BMP-2). The challenge of developing synthetic bone regeneration scaffolds that match the clinical performance of bone autograft has so far not been adequately addressed.

We have developed a new polymer composition, referred to as E1001(1k). E1001(1k) is a member of large combinatorial library of tyrosine-derived polycarbonates [1]. E1001(1k) has an appropriate resorption rate in vivo while also exhibiting exceptional biocompatibility and significant osteoconductivity. In combination with CaPs, porous E1010(1k) scaffolds strongly adsorb BMP and have healed critical sized defects in small animal models. Based on these results, a clinically relevant goat calvaria study was performed.

Materials and Methods: *Scaffold fabrication.* A combination of solvent casting and porogen leaching was used to prepare scaffolds [2]. A CaP coating was produced inside the pores by dipping the scaffolds in solutions containing the minerals, followed by drying. *Scaffold characterization.* Porosity and interconnectivity was assessed by microCT and scanning electron microsocopy. The CaP coating was identified using X-ray diffraction (Philips X'Pert). *Pre-clinical studies.* Critical size defects in goat calvaria (20 mm diameter) were created to assess the performance of scaffolds after 16 weeks in life. *Explant characterization.* microCT and histology/ histomorphometry were used to assess bone regeneration within the defects. Histology slides were stained with Stevenel's Blue and counterstained with van Gieson's picrofuchsin.

Results and Discussion: MicroCT and SEM confirmed high porosity (>80%) and presence of macro and micro pores. The performance of three treatment groups was tested in the goat calvaria CSD model: (1) ChronoOS, a commercially available BVF composed of PGLA and beta-TCP served as control; (2) E1001(1k) with a precipitated CaP coating of brushite (E1001(1k) + CaP)); (3) E1001(1k)+CaP+400 μg rh-BMP-2. After 16 weeks in life, explants were analyzed via microCT and histology/ histomorphometry (Fig. 3). The control group had minimal bone regeneration. With no rh-BMP-2, the E1001(1k) + CaP explants formed enough bone to bridge the defects. In the third treatment group, there was significant and full bridging with a relatively low dose of rh-BMP-2. Based on quantitative microCT measurements, the average bone volume regenerated by chronOS was 18% of the total defect volume, for E1001(1k) scaffolds with CP, the average bone volume was 25%, and for E1001(1k) scaffolds with CaP and BMP-2, the total bone volume was 30% of the defect volume. Another noteworthy observation was that for chonOS and E1001(1k)+CaP, bone seemed to grow into the scaffold from the sides, while for E1001(1k)+CaP+BMP, bone appeared to regenerate from the dura (bottom of the defect) to the top of the defect (Fig. 1).

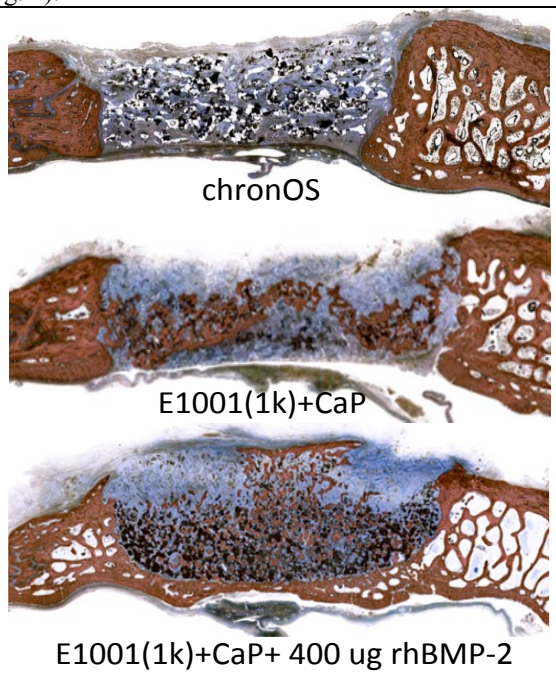

Figure 1. Histology images of the three treatment groups (bone tissue is stained red). The control group showed no bone; the groups with CaP with or without rh-BMP-2 showed bridging. The group with rh-BMP-2 showed essentially complete healing.

Conclusions: The E1001(1k) + CaP scaffolds are a viable option for the treatment of critical size defects, as has been confirmed in several studies using the critical size rabbit calvaria model. The current study in the clinically relevant goat model confirms the results obtained in the rabbit model and provides two additional insights: (i): E1001(1k)+CaP scaffolds resulted in almost complete bridging of the defect, even without the addition of rh-BMP-2; and (ii), the addition of a relatively low dose of only 400 μg of BMP-2 resulted in the close to complete regeneration of the entire critical sized defect area. We are now conducting a second study using a critical size defect in the sheep tibia model to evaluate the ability of E1001(1k) scaffolds to regenerate defects in long bones.

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References: [1] H.R. Magno *et al*, J Mater Chem 2010;20:8885–93. [**2**] J. Kim, H.R. Magno *et al***,** Regen Biomater. 2015 Mar; 2(1): 1–8.