Bone Regeneration using Bone Morphogenetic Protein-2 Loaded Tyrosine Polycarbonate Scaffolds

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Statement of Purpose: There is a significant need for effective delivery of growth factors from suitable osteoconductive substrates to heal large bony defects. In this study, E1001(1k), an inherently osteoconductive polymer composition chosen from the library of tyrosine-derived polycarbonates (TyrPC) was used to create porous scaffolds[1]. These were evaluated as carriers for bone morphogenetic protein-2 (BMP2) to function as synthetic bone grafts in a critical sized defect (CSD) in the rabbit calvaria. The CSD rabbit calvarial model[2] is widely used to evaluate the handling of bone void fillers as a safety and efficacy benchmark in translational development. It is essential that comprehensive analysis of regeneration be carried out; for screening of the graft material to identify optimal composition, and also with a broader outlook to ensure ability to assess performance in future clinical trials if the material meets the requisite demands. While groups have tested a variety of grafts[3,4,5] in the rabbit CSD, few have used all the characterization tools available to evaluate in vivo regeneration.

Methods: E1001(1k) scaffolds loaded with various dosages of BMP-2 (0, 10, 25 and 50 µg) were assessed after 16 weeks of *in vivo* implantation. Micro computed tomography (µCT) analysis was used to quantify the volume, density and spatial distribution of the regenerated bone. Histological and morphometric analysis was performed post euthanasia as the traditional gold standard of bone regeneration and to evaluate the biology of the bone defect environment. For comparison to clinical protocols, 64-slice CT scans (CT) were carried out at 4 & 16 weeks to confirm ability to monitor temporal bone healing patterns. Additionally, traditional radiography (X-ray) was used to assess defect bridging and opacity.

Results: A clear dose based response of increasing regenerated bone volume with rhBMP-2 loaded on the graft was observed in the E1001(1k) scaffolds at 16 wks. Significantly more bone formation was observed at the highest dose of rhBMP-2 (50 µg), which is 25 – 50% of the recommended dose (100-200 µg) for this defect size. A significant difference in regenerated bone volume was observed between the lowest (10 µg) and highest (50 µg) doses using radiographs (% defect that was opaque) ($p<0.001$), µCT (bone volume in mm^3) ($p=0.002$) and CT (bone volume in mm^3) ($p<0.001$) and histology (bone area/ slice in mm^2) ($p<0.001$) as shown in Table 1.

Table 1. Mineral volume measurements by technique. (** is significantly > than all, * is > than 0 µg dose, $p<0.05$).

BMP	64 slice	XRay	µCT	Histology
0 µg	196±17	26 ± 3	179±9	0.63±0.1
10 µg	215±44	35 ± 8	177±20	0.70±0.1
25 µg	307±22*	32 ± 4	226±13	0.81±0.1*
50 µg	407±19**	79 ± 5**	254±12**	0.86±0.2*
	mm^3	**% fill**	**mm^3**	**mm^2**

In addition, a high correlation between the mineralization quantification methods existed (µCT vs CT R^2= 0.86 being highest, CT vs XRay R^2= 0.67, µCT vs XRay R^2= 0.60, histology vs CT R^2=0.59, histology vs µCT R^2= 0.51 and histology vs XRay R^2= 0.36 being lowest).

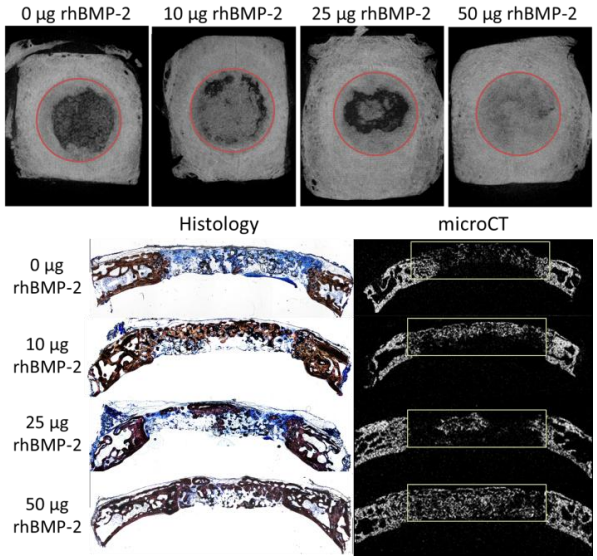

Figure 1. Representative radiographs, histological slides and microCT images showing bone regeneration in the defect space after 16 weeks at each dose of rhBMP-2.

MicroCT based evaluation of mineral density of bone indicated no significant differences without or at any higher dose of BMP2. Radiography analysis however indicated a significant increase at 50 µg compared to 0 µg, probably due to the increased mineral thickness.

Discussion: Multiple techniques were used to evaluate bone regeneration in a critical sized rabbit calvarial model. E1001(1k) scaffolds showed increased bone regeneration with rhBMP-2 dose, effectively healing a critical size defect at a fraction of the recommended dose. Radiography analysis provided quantifiable % defect coverage and radio-opacity, µCT provided spatial volumetric and bone density measures, histomorphometry provided biological confirmation and CT allowed for establishing translational guidelines. All techniques showed high quantitative correlation. To the best of our knowledge this is one of the first studies in which all 4 techniques have been used and validated against one another. These methodologies allow for a standardized and comprehensive description of bone regeneration with clinical translational implications.

References:

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