Evaluating Controlled Sequential Delivery of Two Growth Factors To Enhance Osteoinduction In Young Mice

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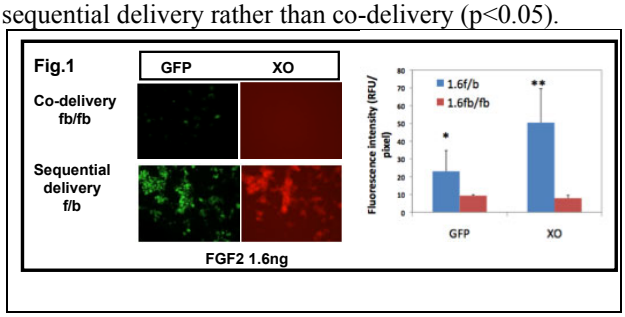
Statement of purpose: The primary goal of this study was to evaluate whether the controlled sequential delivery of fibroblast growth factor-2 (FGF2) followed by a low dose of bone morphogenetic protein-2 (BMP2) could enhance osteoinduction as compared to either growth factor alone in young mice. A previous study applied 5ug BMP2 co-delivered with FGF2 to enhance bone formation in a mouse and saw a modest effect [1]. It has been shown that FGF-2 expands osteoblast progenitors in tissue culture thus there is a rationale to deliver it first, prior to the application of the differentiation factor BMP2 [2]. We hypothesized that sequential, rather than co-delivery of FGF2 followed by BMP2 could increase the new bone induction at even lower doses of BMP2.

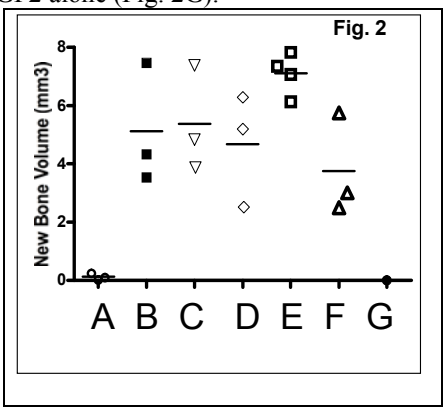
Methods: *In vitro osteogenesis experiments.* Neonatal mouse osteoblast progenitors were obtained from 5 day-age Col2.3-GFP pups after calvarial digestion with collagenase [3]. The osteoblast progenitors were seeded at a density of 1.5 x 10^4 cell/cm^2 and treated for the first three days with either co-delivery of both factors or FGF2 alone (FGF2 0.16ng/ml or 1.6ng/ml, BMP2 50ng/ml) in DMEM with 10% FBS and 1% P/S. After 3 days in the proliferation/growth medium, the cells were cultured in the osteogenic medium (α-MEM with 10%FBS, 4 mM β-glycerophosphate, 50 ug/ml ascorbic acid, 1%P/S) with either continued administration of both growth factors as before or BMP2 only. Fluorescent images were taken on day 14 after addition of xylenol orange to stain the mineral and both GFP expression and xylenol orange staining was quantitated by Image J (NIH).

In vivo study. The controlled sequential release scaffolds were prepared by a novel combination of Healos® collagen/hydroxyapatite sponges (DePuy Spine Inc, Raynham, MA) over-coated with a polyethylene glycol (PEG) hydrogel (Straumann, Switzerland). The scaffold size was 4.0 mm x 1 mm. 2ug of rhBMP2 in 0.04% HAc was absorbed on Healos® and dried overnight. A few minutes before placement in the animal, 5ul of PEG-hydrogel solution with 1ng, 5ng or 50ng of FGF2 was added to coat the Healos®. In one group BMP-2 was added in the PEG phase only with no FGF-2. A 4.5mm of defect was made in the right parietal bone of the calvaria. The scaffold was placed into the defect. Seven groups were tested in this study: (A) Healos® -PEG, (B) Healos® /PEG+BMP2, (C) Healos® /BMP2-PEG, (D) Healos® /BMP2-PEG/FGF2 (FGF2 1ng), (E) Healos® /BMP2-PEGh/FGF2 (FGF2 5ng), and (F) Healos® /BMP2-PEG/FGF2 (50ng), (G) Healos® -PEG/FGF2 (50ng). The mice were euthanised at day 28 and the new bone volume (BV) was measured using microCT and assessed histologically. One-way ANOVA was used to compare the difference between different groups.

Results: *In vitro* osteogenesis experiment results are shown in Fig. 1. The 1.6ng/ml FGF-2 combined with BMP-2 resulted in more rapid differentiation (more GFP expression) and mineral deposition than the lower dose. Statistically there was more mineral deposited after sequential delivery rather than co-delivery ($p<0.05$).



In vivo study. The new bone volume in the BMP2 alone and FGF2/BMP2 treatment groups were significantly different than the scaffold alone ($p<0.05$). There was less bone formed in the scaffold alone (Healos® -PEG) group. 5ng of FGF2 with BMP2 formed more bone than 1ng of FGF2 with BMP2 ($p<0.05$). There was no significant difference between either BMP2 alone group and both growth factors combined; however, we found that 5ng of FGF2 with BMP2 showed less individual variation (Fig. 2E) as compared to the other treatments which is encouraging. No new bone was induced by 50 ng of FGF2 alone (Fig. 2G).



Conclusions: Our preliminary data demonstrates that the sequential release of FGF2 followed by BMP-2 could enhance osteoinduction both *in vitro* and *in vivo*. Healos® -PEG has potential as a delivery system for sequential release of two growth factors with more rapid release of the first factor (FGF2) from the PEG phase, followed by the second factor (BMP2) adsorbed to the hydroxyapatite phase of Healos®. The mouse calvarial defect model is a good model to test the osteoinduction of both growth factors. Ongoing studies are being conducted in aged mice that might benefit more greatly from pre-stimulation of osteoprogenitors by FGF2, prior to differentiation by BMP2.

References:

1. Nakamura YK, et al. Bone. 2005; 36: 399-407.
2. Hurley MM, et al. Academic Press. 1996:627-645.
3. Kalajzic I, et al. J Bone Miner Res. 2002; 17:15-25.