Collagen Films on Hydroxyapatite Scaffolds Enhance rhBMP-2 Based Regeneration in Large Bone Defects

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STATEMENT OF PURPOSE:

INFUSE (Medtronic), which is a combination of recombinant human bone morphogenetic protein 2 (BMP2) and an acellular collagen sponge (ACS) is currently the clinical standard for large open bone defects in the extremities[1]. Apart from high cost of therapy, there is great concern regarding the side effects of delivering supra-physiological doses of rhBMP-2 such as transient bone resorption, cyst-like bone void formation and heterotopic bone formation[2]. We previously demonstrated that pairing the HA scaffold with a collagen wrap aids bone regeneration over HA alone by providing a protected environment and a directed periosteal scaffold[3]. In this study, in an attempt to reduce BMP2 dose, we focus on delivery of BMP2 from the ACS with a porous structural hydroxyapatite (HA) scaffold occupying the defect volume in a critical sized defect in the rabbit radius. Since HA being brittle is a concern in load bearing applications, we develop a collagen film coated HA to improve mechanical properties. We additionally investigate the benefit of bone mesenchymal stem cell (MSC) seeding to increase bone regeneration. The primary objective of this study was to determine whether the total BMP2 required to heal critical sized defects could be reduced using a combination of osteoconductive and osteogenic factors.

METHODS:

HA scaffolds (15 mm length and 3 x 5 mm oval cross section) with high porosity (~80%) and interconnectivity were prepared as previously described[3]. Collagen films were deposited by twice coating the HA scaffolds with 4% collagen in 0.05M acetic acid solution. Changes in porosity, mechanical strength and in vitro MSC osteogenesis were measured. The ability to regenerate bone *in vivo* was evaluated using a critical sized 15 mm rabbit radius mid diaphyseal defect model. 5 groups were evaluated: ACS+ 76 µg BMP2 (INFUSE), HA+76 µg BMP2, HA+ 15 µg BMP2, HA/Col+ 15 µg BMP2 and HA/Col+ 15 µg BMP2+ BMSCs. In the INFUSE group, the ACS was used to fill the defect while in the HA groups, ACS loaded with BMP2 was wrapped around the defect extending 2 mm over either interface. BMP2 dose was based off the recommended 430µg/ml dose[1] for this defect size. 10 rabbits were used in each group and euthanized 8 weeks after surgery. Study was approved by the IACUC at the US Army Institute of Surgical Research. All samples were examined using microCT (Fig 1A) and quantified to evaluate regenerated bone quantity (Fig 1B). 3 per group were further evaluated by histology and 7 per group by torsional mechanical testing.

RESULTS AND DISCUSSION:

Scaffold development: The collagen coating reduced the porosity of the scaffold by 3.7% but increased compressive strength by 188% and toughness by 536%. Additionally, catastrophic strut collapse was not observed during mechanical testing with scaffolds maintaining their shape post failure. No differences in osteogenic properties of MSCs were observed between the HA and HA/Col scaffolds over 14 days *in vitro* in ALP production while cell number was greater in the HA/Col group over the first 9 days.

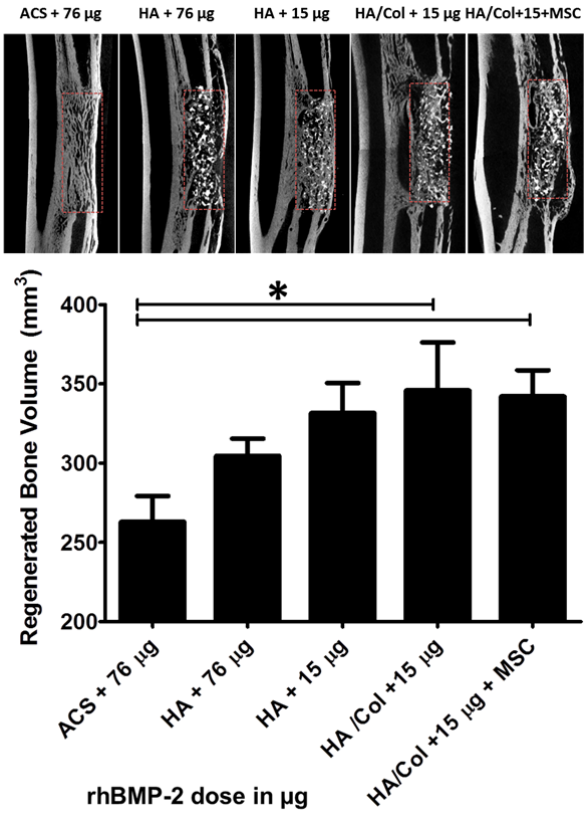

Figure 1. (A) *Longitudinal microCT sections of the defect site after 8 weeks in vivo* **(B)** *Regenerated bone volume within the defect after 8 weeks (significant differences shown by *, $p<0.05$).*

Robust bone healing and complete bridging of the critical size defect was observed in all groups indicating the strong osteoinductive nature of BMP2 (Fig 1A). No statistical difference was found between the low and high doses of BMP2 with the HA scaffold alone and INFUSE® in terms of bone regeneration. However, the use of the collagen coated scaffold significantly increased bone regeneration in the critical sized defect at 20% of the recommended dose (Fig 1B). We have previously reported that without an osteo-inductive signal, the addition of MSCs did not increase bone regeneration in the rabbit radius[4]. In the current study it was similarly observed that even with BMP2 delivery, no benefit of additional MSC seeding on HA/Col was seen in terms of bone volume regenerated (Fig 1B).

CONCLUSION:

Using a structural collagen coated HA scaffold, rhBMP2 dose needed to achieve complete healing of a critical size bone defect could be reduced to 20% of recommended dose with significantly greater bone volume regenerated.

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