Scaffold-Mediated Delivery of Inducible BMP2-Lentivirus Promotes Atrophic Nonunion Fracture Healing without Excessive Ossification

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INTRODUCTION: Fracture nonunions occur in 5-10% of patients, and current treatments are expensive and have variable success. Controllable and localized delivery of BMP2 using porous, degradable scaffolds may be a promising alternative to existing treatments to rescue nonunion fracture. Herein, we designed tube-shaped cryogel scaffolds as a BMP2-Lentivirus (BMP2-LV) delivery vehicle. Previous results showed that adipose-derived stem cell (ASC)-seeded scaffolds, in addition to BMP2-LV, promoted endochondral ossification at the atrophic nonunion fracture site, but also stimulated excessive bone formation. Therefore, in this study we tested whether an acellular approach based on scaffold-mediated gene delivery of doxycycline (dox) inducible BMP2-LV could promote atrophic nonunion fracture healing in mice without excessive ossification.

METHODS: In this study, we tested two cryogel scaffolds: **chitosan-gelatin** (**CG**), which has a smaller average pore size (30μm); and **chitosan-agarose-gelatin** (**CAG**), which has a larger average pore size (60 μm). First, we assessed *Bmp2* expression and osteogenesis using an *in vivo* subcutaneous implantation model in three groups: 1) cellularized scaffolds seeded with BMP2-transduced ASCs (**CG/CAG BMP2-ASC**), 2) acellular scaffolds with BMP2-LV (**CG/CAG BMP2-LV**), and 3) plain scaffolds (**scaffold only**). All mice (C57BL/6J; n=3/group, both sexes) were administered with 625 mg/kg dox chow to activate BMP2-LV. X-rays were performed weekly to track longitudinal mineralization. Scaffolds were harvested either 2 weeks post implantation for qPCR or 4 weeks post implantation for μCT. Next, to evaluate scaffold potential to enhance nonunion fracture healing, we implanted scaffolds at the site of an established atrophic nonunion at 4-weeks post fracture (Col1-TK mice; n=8-12/group/timepoint, both sexes). Weekly x-rays were performed and scored by the mRUST method to assess radiographic healing. At 2- or 8-week post implantation, both fractured and intact femurs were dissected for qPCR, μCT and histology. All mouse studies were approved by IACUC.

RESULTS: Two weeks following subcutaneous implantation, mineralization was evident by x-ray only in the BMP2-ASC group, while qPCR showed higher *Bmp2* expression in both BMP2-LV and BMP2-ASC groups compared to the scaffold-only group (data not shown). Four weeks after subcutaneous implantation, μCT showed there was some bone formed in BMP2-ASC groups, whereas we did not observe bone in samples from the BMP2-LV or scaffold-only groups (data not shown). In the atrophic nonunion model, less than 50% of the nonunion fractures reached an mRUST score of 6 (two cortices bridged) at 8-week post implantation in CG/CAG scaffold-only groups, whereas 100% of the fractures reached a score of 6 in CG/CAG BMP2-LV and BMP2-ASC groups at the same timepoint (Figure 1A, B). At 2-weeks post implantation, as determined by μCT, we observed a significantly higher callus bone volume (BV) and callus tissue volume (TV) in BMP2-LV and BMP2-ASC groups than the scaffold-only group, while BV/TV, BMD and TMD were similar among groups (Figure 2A, B). In addition, most of the BMP2-ASC samples had visible excessive ossification, with a significantly higher excessive BV in the CG BMP-ASC group than the CAG BMP2-ASC group, whereas the scaffold-only and BMP2-LV groups had negligible excessive bone formation (Figure 2C). Histology images showed that scaffold-only groups have more cartilage and fibrous tissues while BMP2-LV and BMP2-ASC groups had more woven bone (Figure 3A). At 8-weeks post implantation, histology showed full bridging in all samples from BMP2-LV (both CG and CAG) and CAG BMP2-ASC groups. In contrast, only 50% CG and 57% CAG scaffold-only samples showed fully bridged cortices. 75% CG BMP2-ASC samples had excessive bone formation, and only 50% showed full bridging. Furthermore, fully bridged cortices without a visible fracture line were seen in 33% CG and 30% CAG BMP2-LV samples, as well as 12.5% CG and 33% CAG BMP2-ASC (Figure 3B), indicating full healing with remodeling of the fracture site.

DISCUSSION: First, we showed that only ASC-seeded cryogel scaffolds coated with BMP2 lentivirus (BMP2-ASC) had evidence of bone formation when implanted subcutaneously, whereas acellular BMP2-LV scaffolds did not form bone. This finding indicates that BMP2 lentivirus alone is not sufficient to induce cell infiltration and subsequent bone formation at this non-skeletal site. However, in an atrophic nonunion fracture model, we showed that both acellular BMP2-LV and cellular BMP2-ASC groups had improved healing at the fracture site compared to plain scaffold. The positive result with acellular BMP2-LV suggests that cells initially recruited at the fracture site after scaffold implantation are transduced by BMP2-LV which promotes the healing process. One of the limitations of cellular BMP2-ASC approach is excessive ossification, which we also observed in a previous study using CG scaffolds. Excessive Ossification has been associated with pain and may also impede the healing process, as only half of the CG BMP2-ASC showed fully bridging in histology at 8-week post implantation. In summary, these results indicate that acellular BMP2-LV cryogel scaffold delivery may promote atrophic nonunion fracture healing without excessive bone formation. In the future, we will further analyze bone formation at 8-weeks post implantation and determine the compositional changes in callus at 2-week and 8-week post implantation to compare the efficiency of rescuing atrophic nonunion fractures through cellular or acellular BMP2 delivery methods.

SIGNIFICANCE: Controllable and localized delivery of lentiviral BMP2 using degradable scaffolds may be a promising treatment approach to rescue atrophic nonunion fracture.

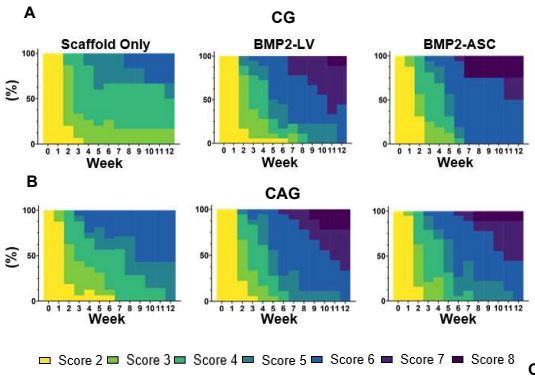

Figure 1. mRUST scoring of A) CG and B) CAG scaffold groups indicate faster healing in BMP2-LV and BMP2-ASC compared to scaffold-only group in atrophic nonunion model. Scaffolds were implanted at 4-wk post fracture.

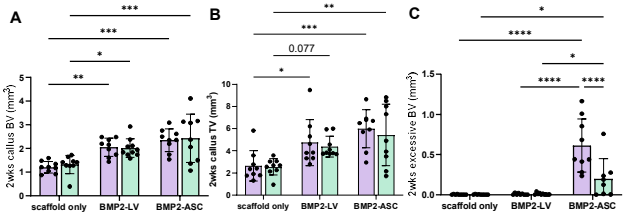

Figure 2. μCT showed that 2-wk post implantation, A) callus BV and B) callus TV are significantly higher in BMP2-LV and BMP2-ASC. C) excessive BV is significantly higher in BMP2-ASC.

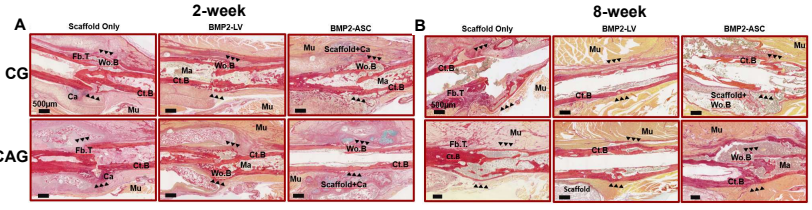

Figure 3. A) Histological images of 2-wk post implantation samples show more cartilage and fibrous tissues in scaffold-only and more woven bone in BMP2 groups. B) At 8-wk, the BMP2-LV groups had cortical bridging in all samples (6/6 CG, 10/10 CAG), as did the CAG BMP2-ASC group (9/9). Ct.B: Cortical Bone; Wo.B: Woven Bone; Ca: Cartilage; Mu: Muscle; Fb.T: Fibrous Tissue; Ma: Marrow.