

## **Introduction:**

Reconstruction of cranial defects is challenging and can require multiple operations. Previous research has implicated the BMP and TGF-Beta signaling cascades to be significant in calvarial osteogenesis. We hypothesized that locally silencing SMAD7, a negative regulator of both pathways, with small interfering RNA (siRNA) would enhance osteogenesis. Moreover, we aimed to develop a simple therapy that could be delivered percutaneously.

## **Methods:**

Critical-sized 3mm calvarial defects were trephined into the right parietal bones of C57 wild-type mice (n=24). Animals were divided into 3 groups (n=8/group): Group 1 received no further treatment; Group 2 received an agarose matrix containing nonsense siRNA placed into the cranial defects; Group 3 received an agarose matrix with SMAD7 siRNA placed in the cranial defects. Percutaneous injection of either nonsense siRNA or SMAD7 siRNA into the cranial defects continued on a weekly basis after trephination. At 12 weeks, microcomputed tomography was used to assess for bony ingrowth. PCR of drilled and non-drilled bony specimens was done to quantify differences in RNA expression of SMAD7, SMAD4, SMAD5, RunX2, Tak1, TGFB-R2, and Collagen Type 1. Gomori Trichome staining was used to assess for bony architecture. Significance was determined by One-Way ANOVA.

## **Results:**

Micro-CT revealed that bony ingrowth was significantly greater in the SMAD7 siRNA treatment group ( $91.2 \pm 5.9\%$ ) compared to the control ( $33.8 \pm 1.3\%$ ) and nonsense siRNA ( $32.1 \pm 1.0\%$ ) groups,  $p < 0.001$  and  $p < 0.001$ , respectively. Bony ingrowth in the nonsense group was not significantly different from that of the control ( $p = X$ ). Compared to uninjured parietal bone, SMAD7 was increased 2.6 fold in untreated trephined bone. In contrast, the SMAD7 siRNA treated group had 68% SMAD7 suppression as well as a 3.8 fold increase in SMAD4, 5.7 fold increase in SMAD5, 5.9 fold increase in TAK1, 7.1 fold increase in RunX2, 2.6 fold increase in TGFB-R2, and a 4.6 fold increase in Collagen Type 1. Histology demonstrated substantial bone formation in SMAD7 treated trephination defects.

## **Conclusions:**

We present a novel percutaneous drug delivery method to enhance calvarial osteogenesis. Local inhibition of SMAD7 via small interfering RNA allows for increased BMP and TGF-Beta signaling which, in turn, yields remarkable calvarial osteogenesis. This relatively noninvasive and efficacious means to bony ingrowth may have applicability to human cranioplasty.

