A Comparison of the Host Inflammatory Response between Peptide Amphiphile-Based Scaffolds and Recombinant BMP-2 in a Rat Model

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INTRODUCTION: Recombinant human bone morphogenetic protein-2 (rhBMP-2) is currently used in clinical settings to stimulate bone formation during spinal fusion surgery. Although rhBMP-2 is effective, its use is associated with a broad range of side effects, most notably inflammation. While a controlled inflammatory response is essential to initiate bone formation, excessive inflammation can lead to complications. AMFX-100 is a novel bone graft substitute developed to overcome these limitations. It is composed of peptide amphiphile molecules, polyethylene glycol, and tri-calcium phosphate granules, which together create a biomimetic scaffold that supports osteogenesis while potentially minimizing inflammatory side effects.

METHODS: 44 female rats undergoing posterolateral spinal fusion (PLF) were divided into 4 treatment groups: ACS alone, ACS+100 µg BMP2 (bone morphogenic protein-2), ACS+10 µg BMP2, and AMFX-100. Female rats were used in this study due to its exploratory nature. Future research will include both sexes to assess potential sex-based differences. Scaffold materials were implanted between the L4-L5 transverse processes to elicit bone formation (Fig. 3). At postoperative days (POD) 4, 7, and 21, inflammation and fusion were assessed via MRI and microCT imaging. Blood was collected for ELISA-based quantification of cytokines. Spines and major organs were collected for histology. Cells at the fusion bed were also collected for flow cytometry to assess macrophage infiltration and polarization.

RESULTS SECTION: Preliminary findings from 22 animals showed that high-dose rhBMP-2 significantly increased TNF-α (~4-fold, $p<0.05$) and IFN-γ (~7-fold, $p<0.001$) relative to ACS alone (Fig. 1). AMFX-100 did not induce TNF-α relative to the control but showed a slight elevation of IFN-γ ($p<0.05$). Cells harvested from implants from 2 rats at POD4 and POD7 in the AMFX-100 group and 2 rats from POD7 in the ACS+10 µg BMP2 showed no statistical differences in M2 polarization (Fig. 2).

DISCUSSION: Preliminary data suggest that AMFX-100 demonstrates a limited inflammatory response compared to recombinant BMP-2 and no significant shift in macrophage polarization. These findings support the biosafety of AMFX-100 and its potential as a bone graft substitute with an improved safety profile relative to rhBMP-2 + ACS. The study is limited by its reliance on a single rat model, which restricts the generalizability of the findings to human spine fusion. Additionally, the relatively small sample size may limit the statistical power of the analyses. Further study with longer follow-up and assessments are necessary.

SIGNIFICANCE/CLINICAL RELEVANCE: rhBMP-2 is effective for spinal fusion but causes dose-dependent inflammation that can lead to complications and is often used at supraphysiological doses clinically. This study evaluates AMFX-100, a novel biomimetic scaffold designed to promote bone formation while reducing inflammatory side effects. Preliminary data show lower cytokine levels with AMFX-100 compared to rhBMP-2, supporting its potential as a safer, clinically relevant alternative for spinal fusion.

REFERENCES:

1. Hsu WK, Polavarapu M, Riaz R, Larson AC, Diegmueller JJ, Ghodasra JH, Hsu EL. Characterizing the host response to rhBMP-2 in a rat spinal arthrodesis model. Spine (Phila Pa 1976). 2013 May 20;38(12):E691-8. doi: 10.1097/BRS.0b013e31828cb977. PMID: 23429681.
2. Yamaguchi JT, Weiner JA, Minardi S, Greene AC, Ellenbogen DJ, Hallman MJ, Shah VP, Weisz KM, Jeong S, Nandurkar T, Yun C, Hsu WK, Hsu EL. Characterizing the host response to rhPDGF-BB in a rat spinal arthrodesis model. JOR Spine. 2021 Oct 7;4(4):e1173. doi: 10.1002/jsp2.1173. PMID: 35005440; PMCID: PMC8717117.
3. Hsu WK, Polavarapu M, Riaz R, Roc GC, Stock SR, Glicksman ZS, Ghodasra JH, Hsu EL. Nanocomposite therapy as a more efficacious and less inflammatory alternative to bone morphogenetic protein-2 in a rodent arthrodesis model. J Orthop Res. 2011 Dec;29(12):1812-9. doi: 10.1002/jor.21454. Epub 2011 May 16. PMID: 21590717.

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IMAGES AND TABLES:

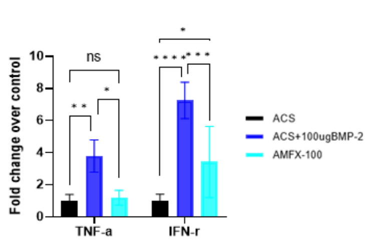

Figure 1. Quantification of TNF–α and IFN-γ by scaffolds. Serum TNF-α and IFN-γ were quantified with commercial ELISA kits. Data are presented as the fold change relative to ACS control.

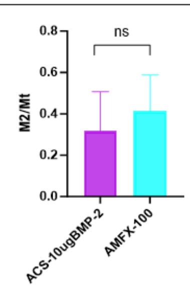

Figure 2. Polarization of macrophages present in the scaffold at fusion bed. Total macrophages (Mt) and polarized macrophages (M2) were measured by flow cytometry. Polarization of macrophages was reflected by the ratio of M2/Mt.

Figure 3. Intraoperative images depicting exposure of bilateral transverse processes (TP) of L4 and L5 (A) and subsequent posterolateral spinal fusion with implants (B)