Evoke™ Demineralized Bone Fibers Demonstrate Osteoinductive Properties

Derek C. Dashti[1], PhD, MBA; Candice Chen[1], MS; Chad J. Ronholdt[2], MBA; Nathaniel T. Remlinger[1], PhD
1 - Pinnacle Transplant Technologies LLC (PTT), Phoenix, AZ ; 2 – New Vision BioLOGIC INC., Dallas, TX
Email: ddashti@pinnacletransplant.com

Disclosures: Derek C. Dashti (3A - PTT), Candice Chen (3A - PTT), Chad J. Ronholdt (3B - PTT), Nathaniel T. Remlinger (3A - PTT)

INTRODUCTION: Demineralized bone fibers (DBFs) provide an ideal architectural scaffold for more robust osteoconductivity (OC) and osteoinductivity (OI) compared to traditional demineralized bone matrix (DBM) particulates.[1] These DBF products provide a cellular conduit for which consistent and enhanced OI is achieved. Evoke DBF consists of long cut and demineralized fibers from human donor cortical bone tissue. This study illustrates potent osteoconductive and osteoinductive properties of Evoke DBF through SEM imaging, BMP-2 and BMP-7 ELISA quantification, Alkaline Phosphatase cellular data, and *in vivo* OI pathology analysis. Evoke DBF possesses interwoven elongated fiber layers to create an osteoconductive scaffold of optimal porosity for cellular engraftment, while the tissue processing preserves high levels of osteoinductive growth factors of BMP-2 and BMP-7 and generates favorable OI responses in both *in vitro* and *in vivo* testing.

METHODS: All test article (TA) DBFs from their respective donors underwent the same *in vitro* and *in vivo* methods outlined below.

1) *Scanning Electron Microscopy (SEM) Imaging*: Test articles (TAs) of DBFs were obtained from a minimum of three (3) donors for SEM imaging. The samples were prepped and imaged using standard operating procedures (Nanofiber Solutions, Dublin, OH).

2) *BMP-2 & BMP-7 in vitro ELISA*: DBF TAs were obtained from eight (8) donors in addition to a negative control (NC) of heat inactivated cancellous. A DBF product with marketed OI claims based on an *in vivo* athymic rodent model[2] was used as a tissue reference control (TC). Following the Blum et al.[3] methods, TAs were prepped for BMP-2/BMP-7 ELISA assays per manufacturer instructions (Quantikine ELISA, R&D Systems, Minneapolis, MN).

3) *Alkaline Phosphatase (ALP)*: DBF TAs were obtained from five (5) donors for ALP testing. This *in vitro* cell-culture assay quantifies expression of the enzyme ALP as a cellular response to a demineralized biomaterial. The samples were terminally sterilized and sent to the University of Southern California Tissue Engineering Lab for processing.

4) *Osteoinductive (OI) Pathology Analysis*: DBF TAs were obtained from twelve (12) donors for testing in a 28 day *in vivo* athymic nude rat model following ASTM F2529-13 and Edwards et al.[2, 4] The *in vivo* study involved implanting the TAs into a muscle pouch in each rear limb. After 28 days, extracted TAs were prepared for hematoxylin and eosin (H&E) and histopathology was assessed via the Edwards[4] semi-quantitative scoring scale.

RESULTS: 1) Figure 1 illustrates that Evoke DBF possesses elongated osteoconductive fibers that are crucial for establishing cellular migration highways. The SEM image also demonstrates pore sizes of 100 µm+. 2) Figure 2 demonstrates statistical significance of BMP-2 and BMP-7 levels higher than compared to a positive test control (TC) across a wide variety of donors. 3) The average ALP results (1.46) indicate that the DBF samples scored well above the assay control (0.2) and at levels that are considered to have positive osteoinductive properties. 4) Figure 3 highlights the consistent OI potential of Evoke DBF in which H&E analysis reveals new bone, new cartilage, and new bone marrow formation from an *in vivo* athymic nude rat model (OI scores of 1 to 2).

DISCUSSION: The results demonstrate that Evoke DBF has favorable and consistent OI potential within multiple different assays that support new bone formation *in vivo*. SEM imaging illustrates long fiber lengths greater than 500 µm with pore sizes greater than 100 µm. These properties are critical for innate osteoblasts and other progenitor cells to attach, migrate through the ECM fiber network, and deposit new bone. Furthermore, all variable biological batches of Evoke DBF demonstrate statistically significant higher measured values of BMP-2, BMP-7, and ALP compared to assay controls. Higher levels of ALP, BMP-2, and BMP-7 are strong indicators of improved OI performance as confirmed with *in vivo* histopathology analysis where Evoke DBF produced new bone, new cartilage, and new bone marrow. This formation of new bone tissue (Fig. 3) confirms Evoke DBF has OI potential for eventual osteogenesis.

SIGNIFICANCE/CLINICAL RELEVANCE: In all, the results demonstrate that Evoke DBF has strong osteoinductive potential across multiple osteoinductivity characterization assays. The properties of Evoke DBF bone graft substitute indicate favorable suitability for clinical applications to repair, replace, or reconstruct osseous defects.

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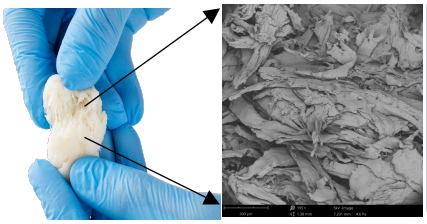

Figure 1. Left) Phase image of Evoke DBF. Right) SEM image at 195X, scale bar = 300 µm.

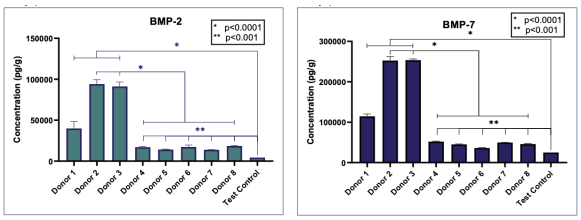

Figure 2. Left) BMP-2 & Right) BMP-7 concentrations across 8 different donors. Respectively donors 1 – 8 age/sex are: 31M, 85M, 61F, 61F, 78M, 70M, 81M, 81M.

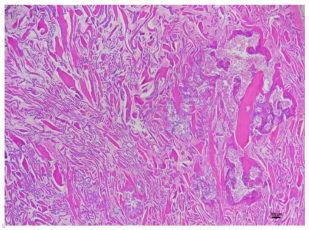

Figure 3. H&E image of implanted TA illustrating new bone, new cartilage, and new bone marrow. 40X total mag., scale bar = 100 µm.