In Vitro and In Vivo Studies Support Osteoinductivity for VelvetDBF

Derek C. Dashti, MS, PhD, MBA; Candice Chen, MS; Chad J. Ronholdt, MBA; and Nathaniel T. Remlinger, PhD

Introduction:

Demineralized bone fibers (DBFs) provide a more ideal architectural scaffold for robust osteoconductivity (OC) and osteoinductivity (OI) compared to traditional demineralized bone matrix (DBM) particulates [1]. These DBF products possess elongated fibers, which provide a cellular migration conduit to facilitate enhanced OI. Demineralized bone fibers also contain a larger surface area as compared to DBM particulates, enhancing higher binding capacity for host progenitor cells and leading to improved bone healing [2]. VelvetDBF, consists of long cut and demineralized fibers from human donor cortical bone tissue. The fibers are cut by computer numerical control (CNC) - machining of human cortical bone enabling optimal surface geometry of fiber-to-fiber self-adhesion [3]. This process results in a malleable and fibrous bone matrix that preserves osteoinductive properties without relying on a viscous carrier [4].

PTT has performed *in vitro* and *in vivo* studies to illustrate osteoconductive and osteoinductive properties of VelvetDBF. SEM imaging, BMP-2 and BMP-7 ELISA quantification, Alkaline Phosphatase cellular data, and *in vivo* OI pathology analysis studies from an athymic nude rat model support VelvetDBF's capabilities to promote osteogenesis. These studies demonstrate that VelvetDBF 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 generating favorable OI responses in both *in vitro* and *in vivo* testing. Ultimately, new bone tissue formation is confirmed via implantation of VelvetDBF in an athymic nude rat model post 28 days, supporting its intrinsic OI properties.

Materials & Methods:

All VelvetDBF test articles (TA) from their respective consented cadaveric donors underwent the same *in vitro* and *in vivo* methods outlined below. All TAs underwent CNC-machining along with PTT's proprietary tissue processing methods.

1) *Scanning Electron Microscopy (SEM) Imaging*: VelvetDBF TAs 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:* VelvetDBF 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 [5] was used as a tissue reference control (TC). Following the Blum et al. methods [6], TAs were prepped for BMP-2/BMP-7 ELISA assays per manufacturer instructions (Quantikine ELISA, R&D Systems, Minneapolis, MN).

3) *Alkaline Phosphatase (ALP):* VelvetDBF 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*: VelvetDBF 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. [5, 7]. 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 semi- quantitative scoring scale [7].

Results:

1) Figure 1 illustrates physical properties of VelvetDBF. SEM images show malleable elongated interwoven fibers that are crucial for establishing cellular migration highways. The SEM images also demonstrate pore sizes of 100 µm+ which are essential for cellular integration into the VelvetDBF scaffold [3]. Furthermore, the zoomed SEM images at 1000X and 5000X depict a rugged topography for which cellular adhesion can occur to initiate osteoinductivity [8].

2) Figure 2 demonstrates statistical significance of BMP-2 and BMP-7 levels higher than positive test controls (TC) across a wide variety of donors.

3) The average ALP results (1.46) indicate that the DBF samples scored statistically above the assay control (0.2) and at levels that are considered to have positive osteoinductive properties. Figure 3 illustrates the statistically significant scores of ALP above 0.2 across 5 donors.

4) Figure 4 highlights the OI potential of VelvetDBF compared to a positive control (DBF-PC) and negative control (NC). H&E analysis reveals that the implantation of VelvetDBF results in new bone (NB), new cartilage (CG), and new bone marrow (BM) formation from an *in vivo* athymic nude rat model with an OI score of ~2.

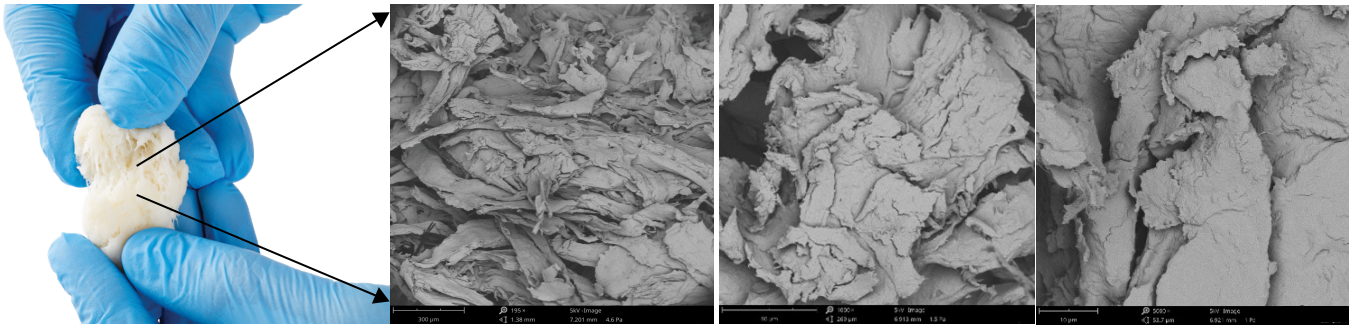

Figure 1. Left) Phase image of VelvetDBF. Right) SEM images (from left to right) at 195X, scale bar = 300 µm; 1000X, scale bar = 80 µm; 5000X, scale bar = 10 µm respectively.

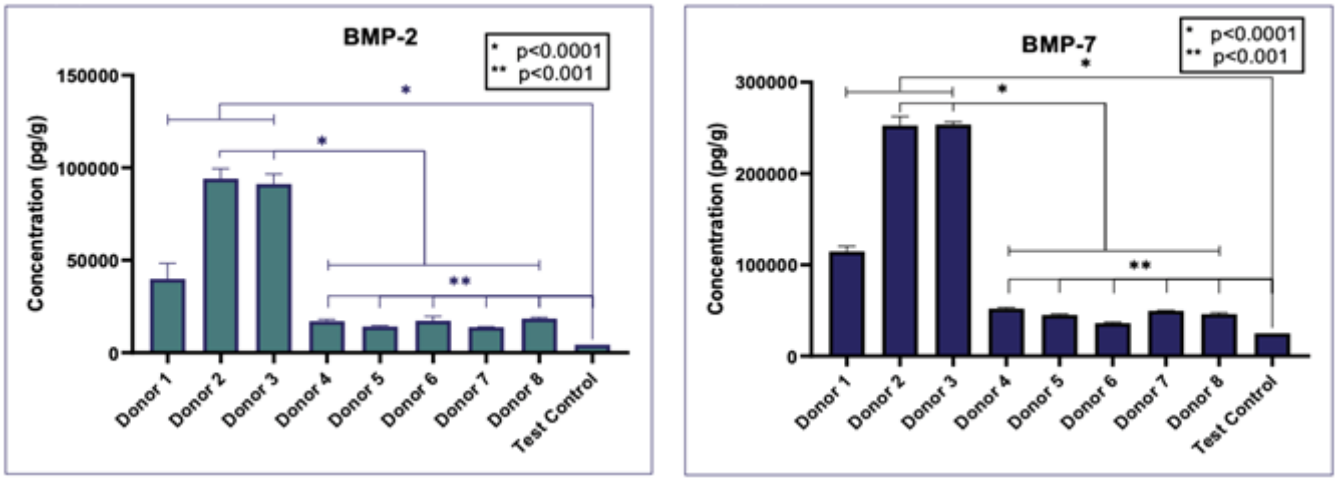

Figure 2. Left) BMP-2 & Right) BMP-7 concentrations across 8 different donors. Respectively donors 1-8 age/sex are: 31M, 85M, 61F, 61F, 78M, 70M, 81M, 81M.

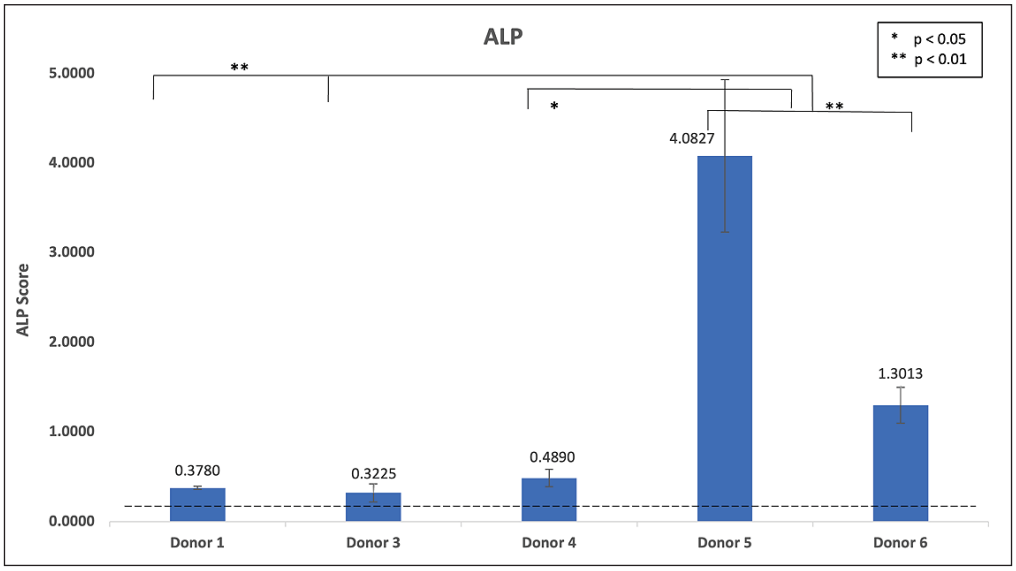

Figure 3. ALP scores among 5 different donors (all above the assay threshold control of 0.2). Donors 1, 3, 4, 5, 6 age/sex are: 77M, 85M, 61F, 61F, 78M. Dashed line denotes the 0.2 threshold assay control

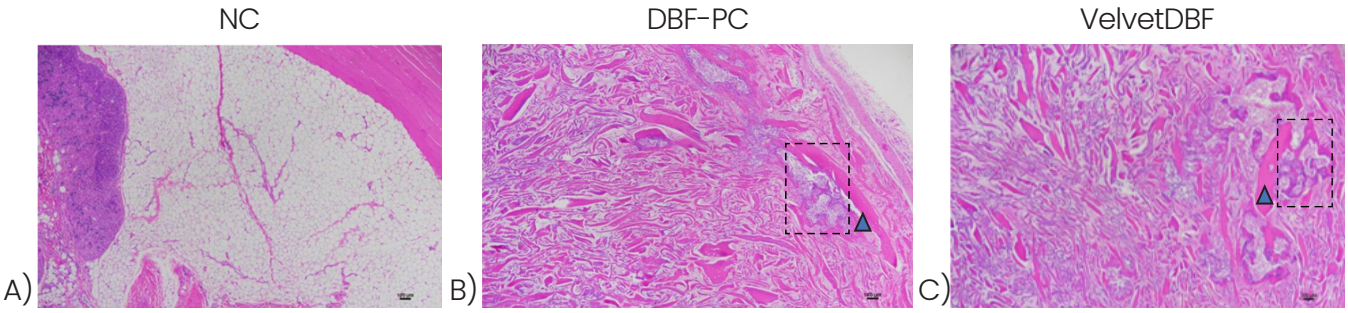

Figure 4. H&E images (40X total mag., scale bar = 100 µm.) of implanted TA illustrating A) Demineralized bone Negative Control-autoclaved cancellous tissue. No NB, CG, or BM is present. B) DBF Positive Control. NB & CG are present with an OI score of ~2. C) VelvetDBF. NB, CG, & BM are present with an OI score of ~2. *[*The blue triangle indicates TA allograft bone remnant and the dashed rectangle next to the blue triangle highlights NB, CG, and/or BM formation.] (NB – New Bone formation, CG – New Cartilage formation, BM – New Bone Marrow formation).*

Discussion:

The results demonstrate that VelvetDBF demonstrates favorable OI potential within multiple different assays and that the implantation of VelvetDBF tissue ultimately supports new bone formation in vivo. SEM imaging illustrates long fiber lengths greater than 500 µm with pore sizes greater than 100 µm and a rugged surface topography. 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 VelvetDBF 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 VelvetDBF produced new bone, new cartilage, and new bone marrow. This formation of new bone tissue (Fig. 4) confirms VelvetDBF has OI potential for eventual osteogenesis.

Significance-Clinical Relevance:

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

References:

1. Russell, N. *et al.*, In-vivo Performance of Seven Commercially Available Demineralized Bone Matrix Fiber and Putty Products in a Rat Posterolateral Fusion Model. *Front. Surg.* **7** (10), 1-11 (2020).
2. Verboket, D. *et al.*, Fibrous Demineralized Bone Matrix (DBM) Improves Bone Marrow Mononuclear Cell (BMC)-Supported Bone Healing in Large Femoral Bone Defects in Rats. *cells* **10** (1249), https://doi.org/10.3390/cells10051249 (2021).
3. Rodriguez, R. U. *et al.*, Demineralized bone matrix fibers formable as general and custom 3D printed mold-based implants for promoting bone regeneration. *Biofabrication* **8**, 1-16 (2016).
4. Heegaard, M., Bari, T. J., Dahl, B., Hansen, L. V. & Gehrchen, M., Demineralized cortical fibers are associated with a low pseudarthrosis rate in patients undergoing surgery for adult spinal deformity without three-column osteotomy. *Brain and Spine* **3**, https://doi.org/10.1016/j.bas.2023.101751 (2023).
5. ASTM F2529-13, Standard Guide for in vivo Evaluation of Osteoinductive Potential for Materials Containing Demineralized Bone (DBM) (2013).
6. Blum, B., Moseley, J., Miller, L., Richelsoph, K. & Haggard, W., Measurement of Bone Morphogenetic Proteins and Other Growth Factors in Demineralized Bone Matrix. *Orthopedics* **27** (1), s161 - s165 (2004).
7. Edwards, J. T., Diegmann, M. H. & Scarborough, N. L., Osteoinduction of Human Demineralized Bone: Characterization in a Rat Model. *Clinical Orthopaedics and Related Research* **357**, 219-228 (1998).
8. Kazimierczak, P. & Przekora, A., Osteoconductive and Osteoinductive Surface Modifications of Biomaterials for Bone Regeneration: A Concise Review. *Coatings* **10** (971), doi:10.3390/coatings 10100971 (2020).

Pinnacle Transplant Technologies™
Improving today. Advancing tomorrow.

OEM Marketing Materials Review & Approval Form

Please complete the below form and submit the completed form, along with copies of all marketing materials to marketing@pinnacletransplant.com for review.

Customer Information

Customer Name:	Cervos Medical	**Date Initiated:**	10/22/2025
Primary Contact:		**Email:**	

Marketing Materials Information

Document Name(s):	Cervos Medical VelvetDBF White Paper		
Document Number(s):	MKT10000	**Version(s):**	1

☒ US Distribution ☐ Global Distribution

☒ New Collateral ☐ Revised Collateral

For Revised Marketing Materials Only

Description of Changes:
(Or attach a redlined version of the marketing materials showing all changes)

☒ N/A

Internal Use Only

Reviewed and Routed for approval by:

Marketing Representative:	Signature	Senior Marketing Communications Manager Title	10/22/2025 | 23:10 MST Date
Head of Marketing:	Signature	Director of Marketing & NPD Title	10/23/2025 | 07:17 MST Date
Head of Quality & Regulatory:	Signature	Executive Vice President Title	10/23/2025 | 08:30 MST Date
Customer:	Signature	Brand Manager Title	10/27/2025 | 20:11 PDT Date

Signed by: Lindsey Gordon
Signer Name: Lindsey Gordon
Signing Reason: I approve this document
Signing Time: 10/22/2025 | 23:10 MST
76FC7F89AAD4E9E95828D7CF1FC906C

Signed by: Cristina Slade
Signer Name: Cristina Slade
Signing Reason: I approve this document
Signing Time: 10/23/2025 | 07:16 MST
4AE8112F89AB44D0800B0C2C5973BAAC

Signed by: Beverly Bliss
Signer Name: Beverly Bliss
Signing Reason: I approve this document
Signing Time: 10/23/2025 | 08:30 MST
477C459F726A433F8C9E44427D2D6E4D

Signed by: Signature
Signer Name: Sydney Kirstein
Signing Reason: I approve this document
Signing Time: 10/27/2025 | 20:10 PDT
DE3A986EF8B4AD32AA0017152709455DA