High-Throughput Osteoinductive Assessment of Demineralized Bone Fibers via Automated Digital Histo-Imaging and BMP Growth Factor Analysis

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BACKGROUND: Demineralized bone fibers (DBFs) provide an ideal architectural scaffold for more robust osteoinductivity (OI) and osteoconductivity (OC) [1]. DBFs can possesses prominent levels of OI growth factors (i.e., BMP-2 and BMP-7) demonstrating new bone formation via *in vivo* rodent models. However, current segmented histology imaging for OI (i.e., Edwards/ASTM scoring) [2, 3] may not assess the totality of OI potential in DBFs; whole slide imaging (WSI) can be analyzed under computational software (i.e., SlideViewer, etc.) to support OI lessening the subjectivity of segmented histology analysis. This study aims to corroborate OI potential of DBFs through automated WSI techniques of hematoxylin and eosin (H&E) pathology comparing to BMP-2 and BMP-7 ELISA concentrations.

HYPOTHESIS: Automated WSI can support the high-throughput assessment of DBF OI potential with support of empirical BMP-2/BMP-7 concentrations.

METHODS: All test article (TA) DBFs from their respective donors underwent the same *in vitro/in vivo* methods. Evoke™, a processed DBF of Pinnacle Transplant Technologies (PTT), is used.

1) *Whole Slide Automated OI Histo-Imaging*: DBF TAs were obtained from twelve (12) donors for testing in a 28-day *in vivo* athymic rat model following ASTM F2529-13 [2, 3]. After 28 days, extracted TAs were prepared for H&E and histopathology was assessed via the Edwards [3] scoring scale. Automated WSI was performed.

2) *BMP-2 & BMP-7 ELISA*: DBF TAs were obtained from three (3) donors. A GuHCl digestion was performed on the TAs and prepped for BMP-2/BMP-7 ELISA (per internal qualified methods).

3) *BMP-2 Spiked Control*: DBF TAs were loaded with 5 ug of rhBMP-2 for testing as a control for OI in a 28-day *in vivo* athymic rat model (ASTM F2529-13). The *in vivo* study involved implanting the TAs into an intermuscular pouch-bilateral dorsal area. Pathology confirmed via H&E by the Edwards score [3].

RESULTS: 1) Figure 1 demonstrates an automated digital WSI of a DBF TA, for which a SlideViewer imaging software is utilized to highlight magnified areas of OI bone forming elements. 2) Figure 2 illustrates BMP-2 and BMP-7 relative empirical concentrations over 3 donors for robust OI potential. 3) Figure 3 highlights new bone forming elements of a rhBMP-2 spiked DBF TA.

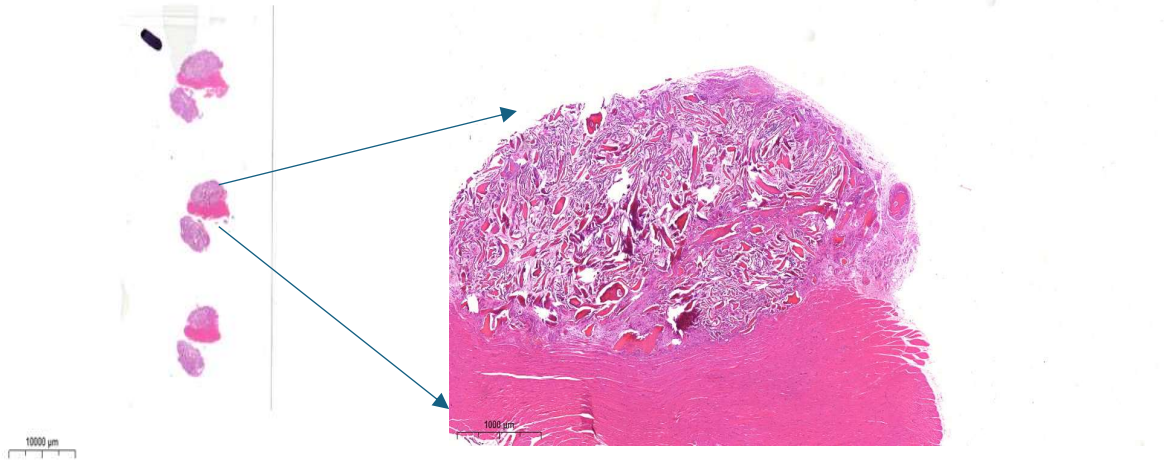

Figure 1. Left) H&E whole slide sectioned image of Evoke DBF TA; scale bar = 10000 µm Right) Magnified 1.5X SlideViewer image illustrating OI visualization of new bone and new cartilage (OI score ~1); scale bar = 1000 µm.

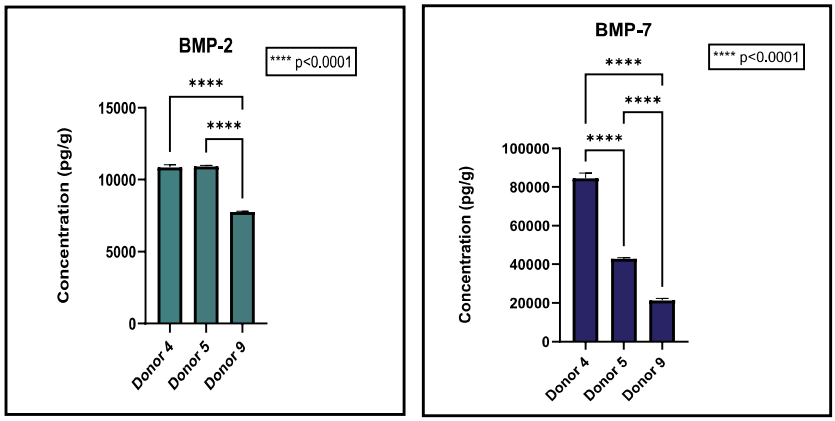

Figure 2. Left) BMP-2 & Right) BMP-7 concentrations across 3 different donors. Respectively donors 4, 5, & 9 age/sex are: 61F, 78M, 73M.

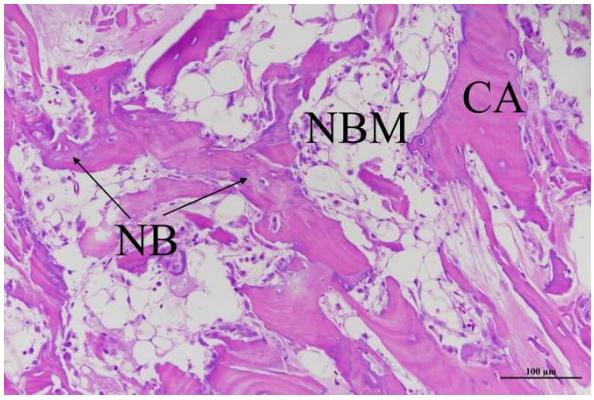

Figure 3. H&E image of implanted BMP-2 spiked DBF illustrating new bone (NB) and new bone marrow (NBM). [Implanted DBF TA (CA)]. 200X total mag., scale bar = 100 µm; (OI score ~ 3).

CONCLUSION: High-throughput characterization of OI in DBFs can be performed via automated WSI H&E histopathology (with SlideViewer) and supported with BMP-2 and BMP-7 concentrations. BMP-2 and BMP-7 concentrations in Figure 2 are within an empirical range per gram of Demineralized Bone Matrix tissue [4]. High-throughput digital imaging can confirm the totality of OI potential in DBFs that are spiked with 5 ug of rhBMP-2 for inducing higher OI scores. Thus, DBFs can be corroborated for the totality of OI under automated histology imaging highlighting new bone marrow, new bone, and even new cartilage. Further studies will be evaluated in OI WSI assessments with scanning software (i.e., SlideViewer, Image J, QuPath, etc.) for which also AI applications could be applied to further lessen the dependency on traditional OI segmented histology assessments.

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INTRODUCTION

Demineralized bone fibers (DBFs) provide an ideal architectural scaffold of elongated fiber lengths and widths for more robust osteoinductivity (OI) and osteoconductivity (OC).[1] While OC of DBFs can be more acutely assessed under scanning electron microscopy (SEM) imaging, OI evaluation of such DBFs need histology imaging for gauging new bone (NB), new bone marrow (NBM), and/or new cartilage (NC) (Fig.1). High-throughput OI screening of DBFs can be performed under automated whole slide imaging (WSI) histology. However, to corroborate such high-throughput OI screening, BMP growth factor measurements (i.e., BMP-2 and BMP-7) of DBFs should be evaluated. The quantitation of BMP-2 and BMP-7 in DBFs are important indicators of OI [2], which can bolster automated WSI histology. Current non-automated/manual segmented histology imaging for OI (i.e., Edwards/ASTM scoring)[3,4] may not properly assess the totality of OI potential in DBFs; automated WSI can be analyzed under computational software (i.e., SlideViewer, etc.) to support comprehensive OI, lessening the subjectivity of non-automated segmented histology analysis.[5] This study aims to corroborate OI potential of DBFs through automated WSI techniques of hematoxylin and eosin (H&E) pathology comparing to BMP-2 and BMP-7 ELISA concentrations.

Figure 1. Left) Conglomerate of cut DBFs. Middle) Assembly of DBFs into Evoke™ DBF PTT product. Right) SEM images of sectioned image of Evoke DBF test article (TA) assessing OC properties (from top to bottom) at 195X, scale bar = 300 µm ; 1000X, scale bar = 80 µm ; 5000X, scale bar = 10 µm respectively & H&E whole slide sectioned image of Evoke DBF test article (TA) assessing OI potential visualization of new bone (NB) and new bone marrow (NBM) [OI Score ~3] ; scale bar = 5000 µm.

HYPOTHESIS/OBJECTIVE: Automated WSI can support the high-throughput assessment of DBF OI potential with the augmentation of empirical BMP-2/BMP-7 concentrations.

MATERIALS AND METHODS

All test article (TA) DBFs from their respective donors underwent the same *in vitro/in vivo* methods. Evoke™, a processed DBF of Pinnacle Transplant Technologies (PTT), is used in the methods below.

1) *Whole Slide Automated OI Histo-Imaging*: DBF TAs were obtained from twelve (12) donors for testing in a 28-day *in vivo* athymic rat model following ASTM F2529-13.[3,4] After 28 days, extracted TAs were prepared for H&E and histopathology was assessed via the Edwards[3] scoring scale. Automated WSI was performed.
2) *BMP-2 & BMP-7 ELISA*: DBF TAs were obtained from three (3) donors. A GuHCl digestion was performed on the TAs and prepped for BMP-2/BMP-7 ELISA (per internal qualified methods).
3) *BMP-2 Spiked Control*: DBF TAs were loaded with 5 µg of rhBMP-2 for testing as a control for OI in a 28-day *in vivo* athymic rat model (ASTM F2529-13). The *in vivo* study involved implanting the TAs into an intermuscular pouch-bilateral dorsal area. Pathology confirmed via H&E by the Edwards score.[3]

RESULTS

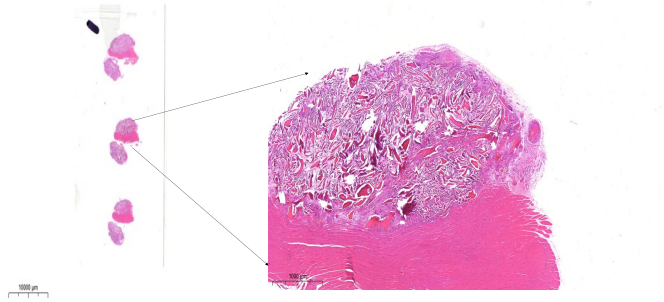

Figure 2. Left) H&E whole slide sectioned image of Evoke DBF TA; scale bar = 10000 µm Right) Magnified 1.5X SlideViewer image illustrating OI visualization of new bone and new cartilage (OI score ~1); scale bar = 1000 µm.

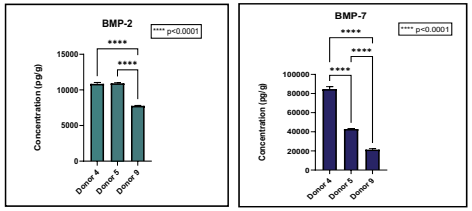

Figure 3. Left) BMP-2 & Right) BMP-7 concentrations across 3 different donors. Respectively donors 4, 5, & 9 age/sex are: 61F, 78M, 73M.

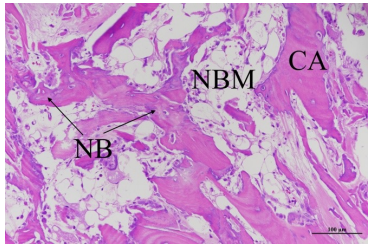

Figure 4. H&E image of implanted BMP-2 spiked DBF illustrating new bone (NB) and new bone marrow (NBM). [Implanted DBF TA (CA)]. 200X total mag., scale bar = 100 µm; (OI score ~ 3).

DISCUSSION

The results demonstrate that high-throughput characterization OI in DBFs can be performed via semi-automated WSI H&E histopathology (with SlideViewer) and supported with BMP-2 and/or BMP-7 concentrations. Traditional non-automated manual segmentation histology provides high variability in assessing OI totality in DBFs; an automated WSI approach along with OI growth factor (i.e., BMP-2 & BMP-7) assessment can provide a more dependable and accurate holistic OI potential depiction of DBFs.

Figure 2 demonstrates a whole slide semi-automated digital image of a DBF TA, for which a SlideViewer imaging software is utilized to highlight magnified areas of OI bone forming elements. A more comprehensive view of the whole tissue section allows better understanding/visualization of OI than traditional manual segmentation. This sectioned TA has an Edwards semi-quantitative OI score ~1.

Figure 3 illustrates BMP-2 and BMP-7 relative empirical concentrations over 3 DBF donors for robust OI potential. The concentrations and ratio of BMP-7:BMP-2 are within general range of demineralized bone matrix (DBM) tissue that historically have exhibited OI potential.[2]

Figure 4 highlights new bone forming elements (i.e., NB and NBM) of a rhBMP-2 spiked (~5 µg) DBF TA under H&E histopathology. This non-automated manually segmented OI scored histology image (OI score ~3) corroborates that BMP-2 is an important driver for NBM and thus needs to be considered when assessing high-throughput OI screening of DBFs.

Histology imaged sections in Figures 2 & 4 depict visual *in vivo* assessments of actual new bone forming elements that are crucial to confirm OI potential under ASTM F2529-13/Edwards methods. However, this is also supported by understanding the relative pertinent BMP factors in DBFs for OI potential. High-throughput OI assessment of DBFs can be achieved through both a more automated WSI approach along with the respective understanding of the OI biological cues (i.e., BMPs) that DBFs possess. These results give a great blueprint into fully autonomizing high-throughput OI screening of DBFs.

CONCLUSION

In all, OI potential of DBFs should be assessed through both imaging modalities and the support of BMP growth factors (i.e., BMP-2 & BMP-7). Traditional non-automated/manual segmentation histology imaging analysis can provide variability in the totality of OI evaluation, and thus to achieve robust accurate high-throughput screening a more automated WSI approach needs to be established. DBFs can be corroborated for the totality of OI under automated histology imaging highlighting NBM, NB, and even NC. Further studies will be evaluated in OI WSI with scanning software (i.e., SlideViewer) for which AI applications could be applied for fully autonomous high-throughput OI screening via deep learning artificial neural networks.

CONTACT and REFERENCES

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