SCIENTIFIC ARTICLE

# IN-VIVO AND IN-VITRO OSTEOINDUCTIVE POTENTIAL OF OPTECURE

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## SUMMARY

Optecure bone graft is composed of allogenic DBM and a synthetic carrier. In this study, we evaluated the in-vivo osteoinductivity potential of the graft and the BMP-2 content of 36 random lots of DBM from two different tissue banks in 2010. All the lots tested were osteoinductive based on the in-vivo assay with an average score of 2.3 ± 0.08 (Mean ± SEM). The average BMP-2 content is 20.5 ± 2.6 (Mean ± SEM), with a range of 93 ng of BMP-2/ g of DBM. Our results are consistent with previously published data with BMP-2 content of 21.4 ± 2.7 ng/g (Mean ± SEM) (Pietrzak, Woodell-May, & McDonald, 2006). **There was a positive correlation between the BMP-2 content and the in-vivo OI score. All of the lots tested contained BMP-2 and passed the in-vivo OI acceptance criteria of 2 or better out of 4.**


## BACKGROUND

Allogenic demineralized bone matrix (DBM) is ¬¬-used as the osteoinductive component in most bone graft substitute products. Marshall Urist demonstrated that DBM induces endochondral bone formation when implanted at ectopic sites. Bone morphogenetic proteins (BMPs) are the growth factors responsible for the osteoinductive potential of DBM.

Osteoinductivity (OI) of DBM can be measured both via in-vitro and in-vivo assays. The "gold standard" for determining OI is the in-vivo assay. As an alternative to in-vivo testing, in-vitro assays for determining OI include ELISA measurements of growth factors and the ability of DBM to induce a dose-dependent increase in alkaline phosphatase activity in C2C12 cell line.

In this study we measured the osteoinductive potential of Optecure via an in-vivo assay. In addition, quantitative ELISA measurements on BMP-2 content of the DBM used in the in-vivo assay was determined.

## RESULTS

Optecure putty samples were implanted over each biceps femoris muscle of athymic nude mice. There were two implants per sample and two mice per sample for a total of 4 implantations per sample. The explants were collected 35-days post implantation and OI determined histologically. The extent of ectopic bone formation within the implants was described by using the following semi-quantitative scale: 0 (no implant present), 1 (no evidence of new bone formation), 2 (1-25% new bone formation), 3 (26-50% new bone formation), and 4 (51-100% new bone formation) based on a 100x magnification field of view (Figure 1-2). The final score of each lot is based on the mean of these 4 implantation site. Implants displaying a maximum score greater than 2 are considered osteoinductive.

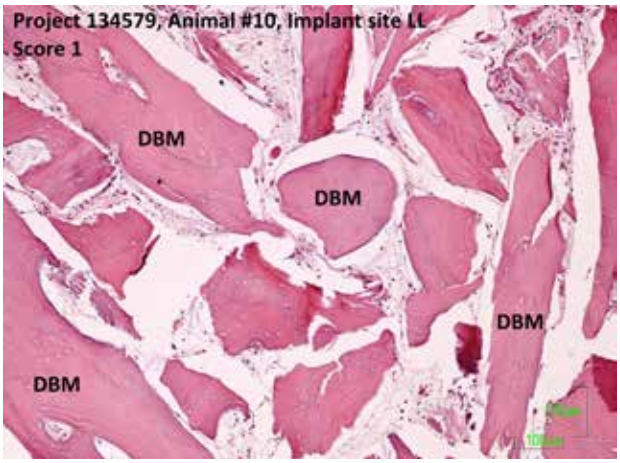


**Figure 1:** A representative H&E histology section of a non-osteoinductive sample with a score of 1 following 35 days post implantation.

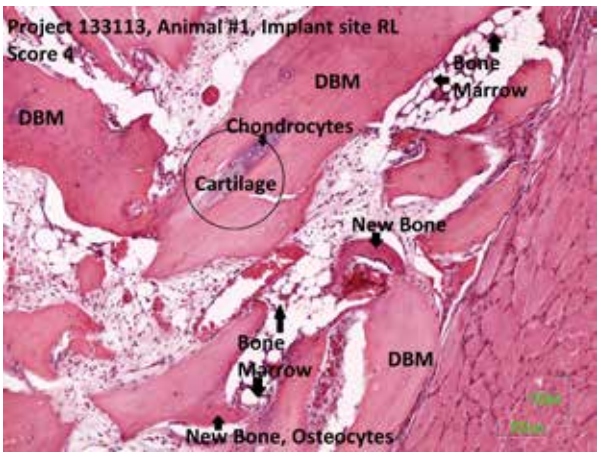


**Figure 2:** A representative H&E histology section of an osteoinductive sample with a score of 4 following 35 days post implantation.

All DBM lots tested via the in-vivo assay were also tested for BMP-2 content. BMP-2 has been demonstrated to be the best single predictor of osteogenic potency (Murray, et al., 2007). Protein extractions of DBM in guanidine hydrochloride were performed at 4°C overnight with constant shaking. The extracts were dialyzed against water at 4°C for 24 hours. BMP-2 content in the extracts was quantified using enzyme-linked immunosorbent assay (ELISA) (R&D Systems). The analysis was performed according to the instructions of the manufacturer. The BMP-2 data were normalized to the DBM weight used during the extraction.

The average BMP-2 content is 20.5 ± 2.6 ng/g of DBM with an average OI score of 2.3 ± 0.1 (Table 1). The minimum content was 5.0 ng/g of DBM with an OI score of 1.5. The maximum content was 98.3 ng/g of DBM with an OI score of 3.8. Our results are consistent with previously published data with BMP-2 contents of 21.4 ± 2.7 ng/g (Pietrzak, Woodell-May, & McDonald, 2006).

**Table 1:** Content of BMP-2 found in guanidine hydrochloride extracted DBM and the in-vivo osteoinductivity score

| BMP-2 (ng/g DBM) | In-Vivo Score |
|---|---|
| 20.5 ± 2.6 | 2.3 ±0.1 |

*Data shown as mean ± SEM, n=36*

## DISCUSSION

The OI potential of each lot of DBM used in Optecure is tested via an in-vivo assay. In this study, 36 DBM lots were also analyzed for BMP-2 content. Correlation analysis shows a statistically significant positive correlation ($r=0.54$, $p<0.05$) which indicates that when the BMP-2 content increases, the OI score tends to increase as well.



## REFERENCES

1. **Murray, S. S., Brochmann, E. J., Harker, J. 0., King,E., Lollis, R. J., & Khaliq, S. A.** (2007). A Statistical Model to Allow the Phasing Out of the Animal Testing of Demineralised Bone Matrix Products. Alternatives to Laboratory Animals, 405-409.
2. **Pietrzak, W., Woodell-May, J ., & McDonald, N.** (2006). Assay of Bone Morphogenetic Protein-2, -4,and - 7 in Human Demineralized Bone Matrix. The Journal of Craniofacial Surgery, 84-90.