Bone Morphogenetic Protein 2 (BMP-2) Levels are Predictive of the Osteoinductive Potential of Demineralized Bone Matrix

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INTRODUCTION:

Demineralized bone matrix (DBM) is rich in growth factors that influence the bone repair process. Among the growth factors associated with DBM, bone morphogenetic proteins (BMPs) are the most potent contributors of bone development and fracture healing[1].

Ectopic bone formation in athymic mice has been the "gold standard" measure of the osteoinductive potential of DBM tissue forms. This in vivo assay is time-consuming and costly and results in the sacrifice of numerous animals. In an effort to reduce the cost, time, and number of animals necessary for verification of the effectiveness of DBM, in vitro assays have been developed including extraction and quantification of BMPs or other fracture healing-related growth factors endogenous in DBM. Although there are many growth factors involved in the osteoinductive process, BMP-2 has been shown to be the best single predictor of osteogenic potential based on statistical analysis of the correlation between in vitro levels of various growth factors and in vivo osteoinductivity (OI) in athymic rodents [2].

The objectives of this study were to (1) determine the range of BMP-2 levels in multiple batches of DBM and (2) test the hypothesis that BMP-2 levels are predictive of the osteoinductivity of DBM and DBX Putty formulation (DBM in sodium hyaluronate).

METHODS:

Demineralized bone matrix (DBM): The DBM used for this study was prepared from cortical segments of human allograft long bones at the Musculoskeletal Transplant Foundation (MTF, Edison NJ).

Extraction and quantification of BMP: BMP-2 levels were measured in 91 lots of DBM and 18 lots of DBX Putty using a 4M guanidine hydrochloride solution in 0.05M TrisHCl as the extraction agent by incubating for 24h at 2-8°C under agitation. For the DBX Putty samples, the Hy carrier was removed with 4 consecutive water rinses under C until analysis. The levels of BMP-2 were determined using commercially available Quantikine ELISA (Enzyme-Linked ImmunoSorbent Assay) kits from R&D systems.

In vivo osteoinductivity (ectopic bone formation model): For the in vivo OI experiments, DBM was prepared as either DBM in saline or DBX Putty formulation by mixing 31% (by weight) with 69% normal saline or sodium hyaluronate putty respectively.

For each test group (9 lots of DBM; 8 lots of DBX Putty), 8 samples were randomized and implanted bilaterally in the hamstring muscles of athymic Nu/Nu male mice. The protocol for this study was approved by the University of Medicine and Dentistry of New Jersey Institutional Animal Care and Use Committee (IACUC). Animals were sacrificed at 4 weeks post-implantation. Decalcified histology was then performed on the explanted samples. Slides were stained with hematoxylin and eosin and samples were evaluated for osteoinductivity. Osteoinductive scores were based on the degree to which new bone, bone cells, osteoid, calcified cartilage remnants, and marrow elements are present. The following scoring system was utilized for evaluation of the in vivo osteoinductive potential: "0" when there is no evidence of new bone formation, “1” when 1-25% of the section is covered by new bone, “2” when 26-50% of the section is covered by new bone, “3” when 51-75% of the section is covered by new bone and “4” when more than 75% of the section is covered by new bone.

RESULTS:

In vitro BMP-2 analysis in DBM and DBX Putty (final formulation in Hy): Each of the 91 lots of DBM had measurable BMP-2 levels that ranged from 6,100 to 24,032 pg / g DBM, with an average value of 12,100pg / g DBM (Figure 1a). BMP-2 was also quantified in the final formulation of DBX Putty (n=18) and was found to be of equivalent level as in the respective DBM powder. The good correlation between BMP-2 levels in the final formulation and in DBM powder (n=18, Figure 1b), suggests equivalence in measuring BMP-2 in DBM and DBX Putty, and supports that the manufacturing processes do not affect the growth factor levels in the final formulation.

![Figure 1](image1.png)

**Figure 1:** (a) Histogram of BMP-2 levels in DBM (n=91 lots, average BMP-2 value: 12,100 pg/g DBM); (b) Correlation of BMP-2 levels in DBM powder and DBX putty formulation of the same lots (n=18, R²=0.82).

In vivo osteoinductivity using the athymic mouse model and correlation analysis with BMP-2 levels: The osteoinductive potential varied in different lots of DBM due to donor-to-donor differences, but all lots showed evidence of osteoinductivity. The levels of BMP-2 in DBM were shown to correlate well with in vivo osteoinductivity of both DBM (Figure 2a, R²=0.78) and DBX Putty final product (Figure 2b, R²=0.72) using the intramuscular athymic mouse model. The good correlation, irrespective of whether DBM was in formulation or not, indicates that there are no significant changes in the BMP-2 levels or in the osteoinductive potential of DBM after mixing with the carrier into final product.

![Figure 2](image2.png)

**Figure 2:** Correlation of in vitro BMP-2 levels in DBM with (a) in vivo OI of DBM in saline (R² of 0.78) and (b) DBX putty (R² of 0.72).

DISCUSSION:

The results support our hypothesis that BMP-2 is a good predictor of the osteoinductive potential of DBM in the form of a powder or combined with sodium hyaluronate carrier. The levels of BMP-2 in DBM quantified by ELISA were shown to have a linear correlation with in vivo osteoinductivity of both DBM (R-squared = 0.78) and DBX Putty final product (R-squared = 0.72) using the intramuscular athymic mouse model. By using processing techniques designed to preserve DBM quality and endogenous growth factors, BMP-2 levels in the DBM in the final formulation (after mixing with the carrier) remained the same as for the raw DBM.

REFERENCES: