**Evaluation of a Freeze-Dried DBM Formulation as an Autograft Extender in a Posterolateral Lumbar Fusion Model**

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**INTRODUCTION:** Iliac crest autograft is considered the gold standard graft material despite limitations in the quantity available and potential complications associated with harvesting procedure. These disadvantages have motivated clinicians and investigators to seek alternative graft materials to extend, enhance and/or substitute for autograft. Examples of such alternatives include: allografts, demineralized bone matrix (DBM), synthetic materials and recombinant human bone morphogenetic proteins (BMPs).

In this study, a freeze-dried DBM formulation was created from DBM mixed with a gelatin carrier derived from the same donor. This formulation can be rehydrated with various biologic solutions at the point-of-care to allow the surgeon flexibility in determining the carrier for the DBM. The purpose of this study is to evaluate this DBM formulation for osteoinductivity (Study I) and determine if it is a suitable bone graft extender for posterolateral spine fusion (Study II).

**METHODS:**

**Study I:** In order to confirm the bioactivity of the freeze-dried DBM formulation, osteoinductivity was evaluated in an athymic rat muscle model. Human DBM powder was used to create a resorbable, freeze-dried DBM formation comprised of DBM powder and gelatin derived from the same donor (Bonus® DBM, Biomet Biologics, Warsaw, IN). Three donors of the DBM formulation were rehydrated with saline or human platelet-rich plasma (PRP) (GPS®III, Biomet Biologics, Warsaw, IN). In vivo osteoinductivity (OI) of the DBM formulation was determined in both the abdominal and thigh intramuscular implants in Rh-μn drummer rats. Following a 28-day implantation, explants were removed and OI was determined by both alkaline phosphatase activity and histological evaluation (1).

**Study II:** Ability of the DBM formulation to perform as an autograft extender in a posterolateral spine fusion was evaluated in rabbits. Freeze-dried DBM formulations were manufactured from rabbit DBM in order to avoid immune rejection in a rabbit animal model. Implantation groups included autograft (n=6), Bonus® DBM rehydrated with saline and mixed with bone marrow aspirate (50:50 ratio) (n=8), and InterGro® Plus (DBM in a lechitin carrier with resorbable ceramic granules, n=8). For the surgical procedure, the dorsal cortices of the transverse processes of L5 and L6 vertebrae were removed to provide a bleeding vascular bed for placement of the graft. Approximately 1.5-3.0cc of corticocancellous bone graft from the iliac crest (ICBG) was obtained bilaterally. The morselized ICBG was mixed with the Bonus® DBM to form a homogeneous graft and then placed bilaterally in the paraspinal bed from the cranial to caudal transverse processes. The lateral two thirds of the transverse processes were covered with the graft.

Following 12 weeks, fusion was evaluated by radiographs, manual palpation, and histological scoring of hard tissue-processed slides. The histology slides were evaluated for new bone formation (scale 0-3), residual graft (scale 0-3), and fusion (scale 1-10) (2).

Data are presented as mean ± standard deviation. Differences between groups were determined by a Student’s t-test (p<0.05).

**RESULTS:**

**Study I:** Bonus® DBM was osteoinductive in the athymic abdominal muscle pouch model (Fig. 1), though donor-to-donor variability was seen. Rehydration with a biologic solution such as PRP did not hinder the OI of the DBM formulation (p>0.05).

**Study II:** Digital plain films (ventral/dorsal) were used to assess graft placement and fusion. The ICBG had a radiographic fusion rate of 67%, InterGro® Plus 75%, and the Bonus® DBM+ICBG 87%. Fusion was assessed with manual palpation of the grafted level by 3 blinded observers. Final result was based on agreement of 2 of the 3 observers. Four of six (67%) ICBG grafted rabbits were graded to have no motion present (fused), while 4 out of 8 (50%) of InterGro® Plus were fused, and 7 out of 8 (87%) Bonus® DBM+ICBG were fused. The manual palpation results correlated with radiographic interpretation in the ICBG and Bonus® DBM+ICBG groups, but there were two false positive radiographic findings in the InterGro® Plus group.

Histological evaluation of new bone formation within the grafted site was assessed and new woven bone was present in all groups (Table 1). There were no adverse inflammatory responses seen in any of the treatment groups. Residual graft was most apparent in the InterGro® Plus group and fusion scores were highest in the Bonus® DBM+ICBG group (Fig. 2).

**DISCUSSION:** The purpose of this study was to evaluate the potential of Biomet Bonus® DBM + autograft as a graft extender in a rabbit posterolateral lumbar fusion model. InterGro® Plus and iliac crest autogenous bone were used as positive controls. The grafted motion segments were judged as fused by radiographs, manual palpation, and calcified histology.

Bonus® DBM was osteoinductive in the athymic rat muscle model. In the OI study, Bonus DBM was successfully rehydrated with PRP to make a carrier. Previous studies have demonstrated that the addition of PRP to DBM powder can increase OI in this model (3). Both the Intergro® Plus and Bonus® DBM were easily molded and retained shape after placement in the paraspinal bed. The addition of ICBG with the Bonus® DBM formed a homogeneous graft with even distribution of the ICBG and Bonus® DBM. The residual blood within the ICBG added a binding effect to the test article/graft.

Using the most widely published and validated method of assessing fusion in this model (manual palpation), the Bonus® DBM+ICBG had the highest fusion rate (87%) followed by ICBG (67%) and Intergro® Plus (50%). Regardless of treatment group, motion segments deemed not fused often had a solid fusion mass attached to either the cranial or caudal transverse process, but not both.

Histological assessment of the limited number of fusion masses within each group showed the two groups that included ICBG had the highest fusion scores and new bone formation. This also correlated with radiographic and manual palpation assessment. Residual graft (i.e. ceramic granules) in the Intergro® Plus treated rabbits was evident and had the highest score when compared to the other two groups.

In this commonly used rabbit spine fusion model, Bonus® DBM + ICBG produced increased radiographic, mechanical, and histologic fusion results when compared to ICBG and Intergro® Plus.

**REFERENCES:**