CHARACTERIZATION OF THE OSTEOINDUCTIVE POTENTIAL OF DEMINERALIZED CANCELLOUS ALLOGRAFT

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Introduction: Although the growth factor content and resultant osteoinductive potential for demineralized cortical bone powder (DBM) has been characterized in the literature, little information is available concerning the osteoinductive potential of demineralized cancellous bone. This study evaluates the *in vitro* and *in vivo* osteoinductive potential of human demineralized cancellous allograft.

Methods: Human cancellous bone was harvested from the metaphyseal ends of long bones from 20 unique donors and machined into either 14mm cubes or strips (50 x 20 x 5mm). Grafts were demineralized in 1N HCl, rinsed in PBS, and lyophilized. *Osteoinductivity Assay:* Demineralized cancellous grafts were evaluated for osteoinductive potential in the C2C12 cell culture alkaline phosphatase (ALP) assay as described previously.[1] *Growth Factor Content:* Demineralized cancellous grafts were ground into powder and growth factors were extracted using 4M guanidine hydrochloride (GuHCl)/0.05M Tris-HCl. Commercially available kits of human BMP-2, BMP-7, TGF-β1, VEGF, IGF-1, PDGF, FGF-basic (FGF-2) (R&D Systems, Minneapolis, MN), BMP-4 and BMP-6 (Abcam Cambridge, MA) were used to quantify the amounts of growth factors present using the ELISA (enzyme-linked immunosorbent assay) method. *Rat Posterolateral Spine Fusion:* Grafts were implanted into athymic rats in a posterolateral spine fusion model to assess fusion and new bone formation, using methods previously described.[2] Tissue was rehydrated with either saline or human bone marrow aspirate (BMA), cut to approximately 15 x 5 x 5mm, and implanted between the transverse processes of L4-L5, bilaterally. After 8 weeks *in vivo*, animals were euthanized. Faxitron radiographs were taken, and selected explants were chosen for microCT analysis. Fusion segments were removed, fixed in formalin, and processed for decalcified histology.

Results: The osteoinductive potential as determined by measurement of ALP activity levels in the C2C12 *in vitro* assay although variable from donor to donor, ranging from 32% to 146% that of the DBM powder positive control lot (average: 64% ± 34%) were all positive for OI. Growth factor concentrations determined by ELISA are reported in Table 1 (ng of growth factor per g of freeze-dried tissue). Samples for the posterolateral fusion studies were easily rehydrated, and became compressible upon rehydration. At sacrifice, despite the presence of localized new bone formation observed with all the grafts, the fusion rates were 66%. MicroCT scans and radiographs of fused implants displayed regions of bone bridging from one transverse process to another, indicative of new bone formation. Histological analysis confirmed the presence of new bone formation along the surface of the implanted graft. Good integration between the host bone (transverse process) and implanted graft was also seen. Non-fused implants had evidence of localized new bone formation histologically, but bone was not seen bridging the transverse processes. In the small sample size evaluated, there was no difference between samples hydrated with saline vs. BMA.

Conclusions: Demineralized cancellous allograft has been shown in this study to contain a broad panel of growth factors including BMP-2, -4, and -7, and has demonstrated osteoinductive potential both in the *in vitro* C2C12 assay as well as in the rat posterolateral spine fusion model. While these grafts consistently demonstrated new bone formation in the rat model, the fusion outcomes varied from animal to animal. Visually, grafts that obtained fusion were different than those that only showed localized new bone formation. Grafts that did not proceed to a fusion were initially substantially more porous with less cancellous structure on to which bone could grow. When these more porous grafts were excluded from the study, fusion rates increased. It may be possible that the more porous human cancellous graft made bone bridging difficult in the smaller rat spine and that larger animal models may be better suited to evaluate the osteoinductive potential and fusion capabilities of these grafts *in vivo*. Despite the varied fusion outcomes, the consistent localized new bone formation observed in this study confirmed the ability of these grafts to support and promote bone formation. Given the donor to donor variability observed with human allograft, as with DBM powder processed from cortical bone, lot-to-lot testing of demineralized cancellous allograft is warranted, to ensure that the processing conditions have been optimized for the unique geometry of cancellous grafts and that the osteoinductive potential of each donor lot has been retained. Through careful verification testing and QC screening to identify highly porous grafts which may be less desirable, an osteoinductive human allograft with unique handling properties to meet specific surgical needs can be obtained.

Table 1: Growth factor content by ELISA method (n=20)

	Avg (ng/g)	SEM
BMP-2	61.99	7.80
BMP-4	22.98	3.03
BMP-6	0.97‡	0.57‡
BMP-7	353.20	48.28
TGF-ß1	109.51	11.22
FGF-2	7.96	3.92
VEGF	4.15	1.10
IGF-I	2118.69	76.99
PDGF	1.04	0.22

‡Values are displayed as pg/g

References:

1. Han et al. J Orthop Res 2003; 21(4):648-5
2. Bae et al. JBJS 2010;92:427-35