In Vitro And In Vivo Characterization Of A Unique Human Cortical Bone Allograft

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Disclosures:
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Introduction: Finding a suitable alternative to autograft to help achieve a successful posterolateral spine fusion continues to be a clinical challenge in many cases. The ideal matrix would have compression resistant properties, yet be flexible enough to conform to patient anatomy, bridging across the transverse processes. Numerous demineralized bone matrix (DBM) formulations are available today for clinical use as an alternative to autograft. In most cases, the DBM powder or fibers are combined with a carrier to improve the handling characteristics. However, the carrier dilutes the concentration of active DBM. This study evaluates a unique machined allograft consisting of 100% demineralized human cortical bone with no added carrier. The demineralized graft allows for flexible handling characteristics, while retaining the osteoinductive potential of the cortical bone.

Methods: Human cortical bone was harvested from the shafts of long bones and machined into a cross-hatched shape, 14mm x 50mm x 5mm (Figure 1). Grafts were demineralized in HCl, rinsed in PBS, and lyophilized. Osteoinductivity Assay: Tissue samples from three unique donors were evaluated for osteoinductive potential in the C2C12 cell culture assay as described previously.1 Briefly, tissue samples were added to a culture of C2C12 myoblast cells. After culture alkaline phosphatase (ALP) activity levels were quantified and compared to those obtained from both a positive control lot of DBM powder previously shown to be osteoinductive in athymic rat ectopic implantations, and values from control wells with BMP-2 added. Growth Factor Content: Grafts were ground and growth factors were extracted using a ratio of 0.1g of tissue to 2ml of 4M guanidine hydrochloride (GuHCl)/0.05M Tris-HCl at 4°C for a total of 21 hours, followed by a 20 hour dialysis in a 20KDa molecular weight cutoff membrane at 4°C. Commercially available kits of human BMP-2, BMP-7, TGF-ß1, VEGF, IGF-1, PDGF, FGF-basic (R&D Systems, Minneapolis, MN) and BMP-4 (Abcam Cambridge, MA) were used to assay the amount of growth factors present using the ELISA (enzyme-linked immunosorbent assay) method with optical densities measured at 450nm on a microplate reader (ELx800, Biotek Instruments, Winooski, VT) with Gen5 Analysis Software. Sample concentrations from the average of triplicate measurements were determined using standard curve analysis. 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 Briefly, following the administration of injection anesthesia, the paraspinous muscles were deflected to access the transverse processes, which were then decorticated to bleeding bone. Tissue was rehydrated with either saline or human bone marrow aspirate, 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, decalcified, paraffin embedded, sectioned, and stained for histological analysis.

Results: Samples from each donor were determined to have on average an osteoinductivity level twice that of the positive control lot and 80% of the BMP-2 control, as determined by measurement of ALP activity levels in the C2C12 in vitro assay. Growth factor concentrations as determined by ELISA are reported in Table 1 in units of (ng) of growth factor per (g) of freeze-dried tissue. Levels of BMP-2, BMP-4, BMP-7, TGF-ß, VEGF, and IGF-1 were all detected in each sample. Trace amounts of FGF-basic were detected in one sample. PDGF was not detected in any sample. Samples for the rat implantation studies were easily rehydrated with either saline or bone marrow aspirate. At sacrifice, both microCT scans and radiographs (Figures 2a and 2b, respectively) displayed dense areas 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 (Figure 2c). Good integration between the host bone (transverse process) and implanted graft was seen. Additionally, evidence of the formation of marrow cavities was also present. A comparison between the grafts rehydrated with bone marrow aspirate and the grafts rehydrated with saline indicated a trend towards a higher average new bone formation score by histology for the grafts rehydrated with bone marrow aspirate; however the difference was not significant for the sample size evaluated.

Discussion: Achieving a successful posterolateral spine fusion is often challenging, and typically requires a large amount of graft material. Since the volume of autograft available may be limited, the use of a bone graft substitute material is often required. This study evaluates the in vitro and in vivo characteristics of a human tissue allograft machined from a single piece of cortical bone designed to span two transverse processes clinically. Due to the unique geometry of this construct, upon demineralization, the graft becomes flexible and bendable. Furthermore, unlike other DBM formulations, it retains its integrity and is resistant to irritation. However, finding the balance between the desired flexible handling properties and the osteoinductive potential can...
be challenging. The osteoinductive potential of this graft was characterized thru three different accepted methods. The in vitro C2C12 alkaline phosphatase assay is a sensitive method for evaluating the level of osteoinductive potential of various graft materials. Samples from each of three donors were shown to be osteoinductive in the C2C12 assay. Further analysis using growth factor quantification by ELISA confirmed the results of the C2C12 assay by reporting the presence of osteoinductive growth factors at levels consistent with those reported in the literature for osteoinductive DBM powder. Finally, in vivo evaluation in the athymic rat further demonstrated the osteoinductive nature of these grafts in a challenging posterolateral spine fusion model designed to mimic potential clinical use. Histological analysis indicated the ability of this graft to induce new bone formation with good integration with the host bone.

**Significance:** This study was a preliminary characterization of a potential autograft alternative consisting of 100% human cortical bone with unique handling properties and demonstrated osteoinductive potential for use in posterolateral spinal fusion applications.

**Acknowledgments:**

**References:**
2. Bae et al. JBJS 2010;92:427-35

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Figure 1: Machined Demineralized Cortical Allograft

Figure 2: Athymic Rat Posterolateral Spine Fusion

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