Evaluation of a Next Generation DBM Putty in a Posterolateral Spinal Fusion Model

INTRODUCTION
Successful spinal fusion outcomes are generally achieved through the promotion of osteobiologic processes including osteoinduction and osteoconduction. Although autogenous bone graft (autograft) is widely considered the gold standard of bone graft materials, this procedure has numerous drawbacks including increased blood loss, risk of harvest site infection and long term morbidity [1]. Demineralized bone matrix (DBM) based bone grafts have gained popularity as an alternative to autograft to promote bone formation by processing ground bone in a manner that attempts to expose natural bioactive growth factors contained within bone matrix. The standard method for demineralizing ground cortical bone, however, gives rise to DBM particles having an inherently dense matrix structure with limited surface area exposure to promote osteogenesis. Advanced methods have recently been developed to further process DBM particles to generate a dispersed form in order to increase the surface area thereby improving exposure of host tissue to native cell adhesion proteins and growth factors contained within the donor bone matrix. We hypothesize that utilizing this improved processing method will result in bone matrix-derived constituents that can more effectively drive osteoconductive and osteoinductive processes necessary for promoting the formation of bone.

This study examined if the use of a formulation of DBM consisting of a combination of particulate-DBM and dispersed-DBM in a poloxamer-based reverse phase medium (RPM) carrier could result in spinal fusion outcomes equivalent to autograft in a rabbit posterolateral lumbar fusion model.

METHODS
The DBM formulation (Accell Evo3™) was prepared using rabbit bone and consisted of particulate and dispersed forms of DBM. Particulate-DBM was produced from ground bone by exposure to hydrochloric acid (0.5N) to remove the mineral component. Dispersed-DBM was generated by further exposure of a portion of DBM particles to citric acid (3M) and agitation as a means of gently disrupting the dense organization of the bone matrix. The resulting dispersion was neutralized by diafiltration and lyophilized prior to recombining with the particulate-DBM and adding the RPM carrier. To further characterize these components, an ELISA kit (R&D Systems) was used to measure, over time, soluble BMP-2 from samples of human derived particulate and dispersed DBM incubated in HEPES buffer and collagenase (20U/ml) [2].

The animal portion of this study was performed by an independent research laboratory, the Surgical & Orthopaedic Laboratory (SORL) of the University of New South Wales (Randwick, Australia). The single level (L5-L6) posterolateral fusion model in 6 month old New Zealand white rabbits was used [3]. Treatment groups for the in vivo evaluation consisted of Accell Evo3™ versus autograft harvested from the iliac crest of the rabbit. For each of the treatment groups, 1.2 cc of material was grafted between the decorticated transverse processes on the left and right side of the spine (n = 8). Decorticated non-treated (empty) defects served as negative controls (n = 3). Rabbits were euthanized at 12 weeks and evaluated using radiography (Faxitron), computed tomography (CT), 3D model visualization, biomechanical testing, and paraffin-embedded histology. Statistical analysis was performed using 1-way ANOVA and significance was set at p < 0.05.

RESULTS
In vitro characterization of the DBM components demonstrated that soluble BMP-2 was detected in dispersed-DBM samples at an earlier time (peak at 4hrs) compared to particulate-DBM (peak at 48 hrs; Figure 1). In vivo radiographic analysis assessed bilaterally for each animal treated with Accell Evo3™ at 12 weeks revealed a continuous fusion mass in 15 of 16 sites (94%) compared to 13 of 16 sites (81%) treated with autograft (Table 1). Empty controls did not fuse, evidenced by the absence of fusion mass at 0 of 6 sites (0%). CT scans revealed mature peripheral cortices formed in both Accell Evo3™ and autograft treatments. Biomechanical testing of intact spines demonstrated equivalent load to failure measurements for both Accell Evo3™ and autograft treatments (p = 0.81) while empty controls were statistically inferior to both treatment groups (p < 0.05, Figure 2). Histology confirmed the osteogenic properties of both Accell Evo3™ and autograft as robust new bone formation was apparent throughout the treated graft sites (H&E and tetrachrome stained sections). Bone formation and remodeling of the fusion masses were normal in appearance with no adverse cellular reactions observed in either treatment groups. Ex vivo analysis of intact sites containing Accell Evo3™ at 12 weeks revealed that resorption of dispersed-DBM appeared complete and that of particulate-DBM was nearly complete. The Accell Evo3™ treatment resulted in fusion with substantial bone formation consistently observed at the central areas of the fusion masses.

DISCUSSION
Based on in vitro ELISA data, dispersed-DBM appears to result in earlier exposure of soluble BMP-2 and possibly other bioactive factors contained within the processed bone matrix, whereas exposure to similar factors from particulate-DBM appears to occur at later time points. The results of the in vivo data demonstrate that Accell Evo3™ alone was as effective as autograft in achieving bilateral fusion in a rabbit posterolateral lumbar model. Overall, these data suggest there may be an advantage to the combination of a high surface area dispersed-DBM with high density particulate-DBM in a single formulation as a means to promote bone formation, and may represent an effective alternative to autograft for achieving bilateral arthrodesis in posterolateral spinal fusions.

REFERENCES