Osteoinductivity of DBM is Independent of Donor Bisphosphonate Use

INTRODUCTION
Many clinical applications require additional bone graft, including orthopaedic applications such as spinal fusions and fracture repair and dental applications. Autologous bone graft may be used, but this procedure can be painful and may require a second surgery. Demineralized bone matrix (DBM), a human allograft material, is commonly used as a bone graft substitute because of its osteoinductive properties, either alone or to supplement an osteoconductive material. The aging population has lead to an increase in the number of prospective DBM donors who have taken bisphosphonates for conditions such as osteoporosis, because this class of drugs prevents osteoclast-mediated bone resorption. However, it is not known if the bisphosphonates affect the osteoinductivity of donor DBM. The aim of this study was to determine whether oral bisphosphonate usage affects the osteoinductive capacity of DBM in a mouse implant model.

METHODS
DBM was produced at four tissue banks from allograft bone. Samples were provided from 3-4 sex-matched and age-matched pairs of samples. Donors had a mean age of 68.07±2.04 for the DBM without bisphosphonate group and 69.07±2.48 for DBM with bisphosphonates. Each pair of donors included one donor known to take bisphosphonates and the other not known to have taken bisphosphonates. DBM confirmed as osteoinductive in the nude mouse gastrocnemius implant model was used as a positive control and heat-inactivated DBM was used as a negative control. As a final control, DBM that had been incubated with 1 ml PBS containing 0, 0.002, 2 or 2,000 ng/ml alendronate was tested. Samples were processed by the tissue banks and provided in sterile packaging. Gelatin capsules were loaded with 15 mg DBM and bilaterally implanted in the gastrocnemius muscle of male mice (n=8 implants per group). Mice were harvested 35 days post-implantation and the implant site was processed for decalcified histology. Sections were made at three points through the implant site and the section that contained the largest implant area was selected for analysis. Thus, all samples were biased for positive scoring. Each section was scored by two blinded independent examiners. Osteoinduction evaluated by qualitative score (1=DBM only; 2=one ossicle; 3=2 or more ossicles) and histomorphometric measurements of new bone formation, ossicle formation, and remnant DBM. Statistical significance was determined using Bonferroni’s modification of Student’s t-test.

RESULTS
Heat inactivated DBM did not support new bone formation. At harvest, only the originally implanted DBM was present at the treatment site. Ten of 15 samples from bisphosphonate donors were osteoinductive and nine of 15 samples from patients believed not to have taken bisphosphonates were osteoinductive. There was no difference in the qualitative score for the two treatment groups (1.71±0.40 without; 1.85±0.66 with bisphosphonates, Fig. 1, left panel). The osteoinductive DBMs produced ossicles of comparable size, whether they were from donors that had a known history of taking bisphosphonates or from donors believed not to have taken them (Fig. 1, right panel). Histomorphometric measurements of the area of new bone formation and residual DBM were also comparable (data not shown). Addition of alendronate to control DBM also did not affect its osteoinductivity, whether the qualitative scoring system (Fig. 2, left panel) or quantitative histomorphometric measurements were used (Fig. 2, right panel; ossicle size, area of new bone formation and residual DBM data are not shown).

DISCUSSION
The results of this study show that DBM from patients with and without bisphosphonates have the same ability to induce bone formation. In fact, adding alendronate to samples of DBM known to be osteoinductive had no effect on osteoinduction. However, there were some limitations in the study design. Small sample sizes were used from each of the four tissue banks, and each bank has different processing methods. Donors that were known to have used bisphosphonates were assigned to that treatment group, but the history of their bisphosphonate medication was not known for all donors, including dose and duration of treatment. The donors selected for the non-user group may have used bisphosphonates at some time, but were not using them at the time of death. Despite these limitations, the results suggest that bone formation via DBM was not affected by oral bisphosphonates or the addition of bisphosphonates to DBM. However, remodeling of the new bone was not studied, so it is possible that bisphosphonates do not affect bone formation but have longer-term effects on the bone created.