A COMPARATIVE EVALUATION OF THE OSTEOINDUCTIVITY OF TWO FORMULATIONS OF HUMAN DEMINERALIZED BONE MATRIX

*Takikawa, S (A-Osteotech); ++Bauer, T W. (A-Osteotech); *Kambic, H (A-Osteotech)
++The Cleveland Clinic Foundation, Cleveland, Ohio. The Cleveland Clinic Foundation, Dept. of Anatomic Pathology / L25, 9500 Euclid Av. Cleveland, OH, 44195 USA, 216-444-6830, Fax: 216-445-6967, osteoclast@aol.com

Introduction: Among the various preparations of bone allograft available for human use, demineralized bone matrix (DBM) provides an osteoconductive matrix and, if properly processed, also is osteoinductive. DBM is considered a transplantable tissue, not a “device”, so the Food and Drug Administration currently exerts relatively little control over performance “claims.” Nevertheless, we would expect that different tissue processing methods would yield DBM preparations with different osteoinductive properties. The purpose of this study is to use an established athymic rat model to compare the osteoinductive properties of two commercially available human DBM preparations with different methods of preparation but essentially identical product claims.

Material and Methods: Two putty-like formations of human DBM were assessed for osteoinductivity in a study approved by our Institutional Review Board. The first, Grafton Putty (Osteotech, Eatontown, NJ) is a combination of DBM fibers with a glycerol carrier (OT). The second, Osteofil (Regeneration Technologies, Aluchua, FL) is DBM particulate in a collagen carrier (RT). Both materials are commercially available for human use in the US. Two lots of Grafton material (OT-1 and OT-2) were purchased from a hospital and provided by Osteotech. Two lots of Osteofil (RT-1 and RT-2) were purchased from our hospital inventory. Both DBM preparations were handled as specified by the respective manufacturer. A positive control (rat DBM powder prepared using the same procedures as used in the preparation of human DBM) was provided by Osteotech. Sixteen female athymic homozygous rnu/rnu (nude) rats were used. The rats were handled using sterile technique and housed in sterile, microisolator cages. Aseptic surgical procedures were carried out in a laminar air-hood. Equal volumes (0.2cc) of OT (250mg) and RT (180mg) were aseptically implanted in the hind limbs of each animal. Rehydrated control rat DBM (40 mg) was implanted into the left pectoralis musculature of each animal. Animals were euthanized with CO2 after 28 days, and tissue in the implant site was retrieved. Ossicles of bone were fixed in 70% ethanol. Approximately half of each ossicle was embedded in plastic and processed without decalcification; the remaining half was decalcified and embedded in paraffin. Microscope slides were reviewed and a semiquantitative scoring method was used to measure osteoinduction (1). Criteria for new bone formation included any evidence of endochondral bone formation including the presence of chondrocytes, osteoblasts, osteoid, newly formed and mineralized bone, and/or marrow and associated fat cells. Differences between groups were analyzed using analysis of variance (ANOVA) with a Kruskal-Wallis multiple comparison test at the p<0.05 significance level.

Results: All animals recovered quickly after the surgical procedures. In general the sites of OT and rat DBM insertion contained hard, ossified nodules after 4 weeks, while the OT insertion site contained soft, friable material. The ossicles of rat DBM contained relatively mature cancellous bone and bone marrow, with little evidence of residual demineralized bone graft material visible. Most of the OT ossicles contained mineralized bone and bone marrow with a few areas of proliferating chondrocytes, while the RT ossicles contained very little evidence of bone formation. Residual fragments of demineralized graft material were evident in both OT and RT groups. The osteoinductive scores for each group are shown in Figure 1. There were no significant differences in bone formation between the two lots from each source, but there were significant differences between products. The osteoinductivity of the positive control rat DBM was significantly greater than that of OT (p<0.001), and that of the OT was significantly greater than RT (p<0.001).

Discussion: Several factors influence the osteoinductive properties of a DBM, including the concentration of osteoinductive proteins in the bone matrix of the individual donor, the methods of graft processing (demineralization), the carrier materials included in the final product, and the nature of the host response and implantation site. It is difficult to test each of these variables. In an effort to compare the influence of demineralization or carrier materials on osteoinductivity, we tested two different lots of commercially available DBM from different manufacturers in intermuscular sites in nude rats. There are a number of limitations to our study. First, the amount of DBM inserted was matched by volume, not by weight, as volume seems more clinically relevant. The preparations had different weights, reflecting different proportions of bone matrix and carrier materials. These differences might be important, but were not controlled in this study. Second, only two lots from each manufacturer were tested; a larger study testing more lots would be desirable. Finally, our results quantify the amount of bone at 4 weeks. Different results might be observed at different time intervals. Nevertheless, our results suggest that methods of graft processing may represent a greater source of variability than differences among individual donors. DBM from both lots of the Osteotech material showed significantly more osteoinductive potential in this rat model than either lot of the Regeneration Technologies material. Whether these differences relate to methods of demineralization or some other factor (such as the nature of the carrier) remains to be determined. Interestingly the histologic appearance of residual DBM suggests that many of the fragments in the RT material had not been completely demineralized (Fig. 2).