Introduction
More than 100,000 bone grafting procedures are treated with human demineralized bone matrix (DBM) annually in the US. There are significant differences amongst commercial preparations of DBM and their ability to induce bone in laboratory animals. Many materials, including synthetic and natural polymers, are used as DBM carriers to improve handling properties and delivery. These materials include hydrous and anhydrous bases. The effect of moisture in the carrier material on DBM activity is unknown. In this study, we investigated the effects of moisture and temperature on the preservation and expression of osteoinductive activity of DBM. Such study provides information for DBM product stability, storage and shelf life.

Methods:
Heat treatment on DBM: A single human DBM batch, whose osteoinductivity was confirmed both in vitro and in vivo assay previously [1] was used in this study. Particle sizes ranged from 200-500 micrometers. All procedures were aseptic. Each 100mg DBM particles were weighted and divided into 2 groups for incubations. In the 1st group, samples were incubated directly. In the 2nd group, 100 ul of PBS was added to DBM aliquots and sealed before incubation. Both groups were incubated at 21°C, 37°C, 45°C, and 65°C respectively for different time intervals (7d, 14 d and 35d).

In vitro and in vivo osteoinductivity assay: At the end of the incubation period, the osteoinductive activities of DBM particles were assayed using a C2C12 cell culture method [1]. 5x10^3 cells of C2C12 (ATCC) were plated in each well of 24-well plates in a medium of 10% FBS/DMEM. After 5-hour attachment, the medium was changed into DBM assay medium containing 1% FBS/DMEM. Five milligrams of DBM were added into the assay well. After 48-hour of incubation, cells were washed with cold PBS three times prior to alkaline phosphotase and total protein assays.

Nude rat model: Twenty-two male athymic nude rats, weighing 100-120 g each (NCI) were used in this study. Muscle pouches were created in abdominal muscles bilaterally at 8 sites and subsequently packed with DBM loaded gelatin capsules. Implants were retrieved after 28 days postop and underwent Faxitron radiography, histological staining and ALP assay. Paraffin-embedded sections were cut with a microtome (5mm) and stained with H&E for examination by light microscopy.

Results:
Heat treatment under anhydrous conditions: At temperatures below 65°C, DBM activity was well preserved under anhydrous condition. At 65°C, DBM activity decreased. 7-day heat-treated DBM preserved the activity better compared with 14-day treated groups (Fig 1, anhydrous part). If temperature lower than 65°C, 35-day treated DBM were still active compared with negative control (guanidine inactivated DBM) (data not shown).

Heat treatment under hydrous condition: 65°C-treated DBM completely lost its capability to induce C2C12 cell ALP activity (Fig 1, hydrous part). After 7- or 14-day incubation at 37°C and 45°C, the detectable osteoinductive proteins by C2C12 cells was significantly increased as compared with anhydrous DBM. 14-day incubation released more osteoinductive factors from DBM than 7 days’ incubation.

In vivo osteoinductivity of heat and moisture treated DBM: The osteoinductivity of DBM heat treated for 35 days was tested in a nude rat intramuscular implantation model. Histological results showed that anhydrous DBM heat-treated up to 65°C showed osteoinduction with new bone formation, but the new bone volume was significantly lower than those treated with lower temperature (data not shown). There is no difference on new bone formation between room temperature and 45°C treated samples. On the other hand, when DBM incubated with trace amount of PBS (5%) at different temperature for 5 weeks, the in vivo osteoinductivity decreased with the increase in temperature. The osteoinductivity was completed impaired at 65°C (Fig 2).

Conclusion and Discussion:
Stability and accessibility of DBMs determines the DBM product’s activity and shelf life. In DBM serves as growth factors control release device, by releasing growth factors, to continually stimulate surrounding cells to proliferate and differentiate. With the increase of temperature under the hydrous environment, collagen molecules packed with BMPs start to unwind and could release out the active factors prematurally. The in vitro measured ALP activity increases while the in vivo bone induction capacity decreases. When temperature higher than collagen transition temperature (which is around 60°C), the DBM activity is completely lost. This study indicates that DBM products with water-based carrier should be carefully evaluated for activity when stored at room temperature. The scenario for low temperature storage is under study.

Reference:

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