

## LECITHIN ENHANCES THE OSTEOINDUCTIVITY OF DBM

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### INTRODUCTION:

Demineralized bone matrix (DBM), an osteoinductive bone graft material, is widely used for a variety of bone grafting applications. It is used alone or mixed with carriers to form gels, putties and sheets [1,2]. Lecithin is a phospholipid present in cell membranes, and found in significant amounts in the bone calcification front [3]. This study examined the importance of lipids in the osteoinductive phenomenon. It also assessed the osteoinductive potential of a putty-like grafting material consisting of lecithin and DBM. In order to separate the osteoinductivity from any osteoconductive effects, the test materials were implanted ectopically (subcutaneously and intramuscularly), as well adjacent to the cranial bone of rats.

### MATERIALS AND METHODS:

**Material and reagents:** Lecithin (Phospholipon 90G, American Lecithin Company, Oxford, Connecticut) is a purified phosphatidylcholine obtained from soybean.

**Preparation of DBM/lecithin composite:** Fresh bones were procured from 180-220 g Fisher 344 rats. The cortical shafts were cleaned with several rinses of PBS, and soaked in ethanol to partially remove lipids and cellular debris. After freeze-drying, they were further ground into particle sizes of 100-500  $\mu$ m, and decalcified with 0.6 N HCl/1% Triton X-100. For complete de-lipidation, particles were further soaked in 1:1 chloroform-methanol at room temperature for 12 hours. The DBM generated by such a process was designated as dDBM. Human DBM (Allosource, Denver, CO), was also tested, however it was subjected to this de-lipidation process. Rat or human DBM were blended with lecithin to generate pastes of various concentrations (DBM concentration from 20% to 80%). Composites were packed into gelatin capsules (No.3, Eli Lilly, IN) for implantation.

**Animal implantation:** 27 Fisher 344 rats and 12 athymic homozygous *nu/nu* (nude) rats were anesthetized and implants placed subcutaneously (SQ), intramuscularly, and subcutaneously adjacent to their cranial bones. Each animal received either four subcutaneous implants or two intramuscular implants. Implants were recovered 28 days postoperatively, fixed, decalcified, embedded and stained with H&E and Alcian Blue.

**Alkaline phosphatase assay:** alkaline phosphatase activity was determined as previously described [4].

### RESULTS AND DISCUSSION:

#### Properties of the DBM/Lecithin composite:

Lecithin mixed with the DBM particles gave rise to a composite with ointment-like consistency. It improved the handling properties of DBM which could be easily molded and fitted into irregularly shaped defects. The composite was insoluble in water and blood. When incubated at 37°C, the lecithin containing formulations maintained a solid state and did not liquify. The lecithin appeared to resorb within 7-14 days.

#### Endochondral and intramembranous bone formation using human DBM/lecithin composite:

Endochondral bone formation was observed in the DBM/lecithin implants placed in the anterior abdominal wall musculature or subcutaneously for 28-day. New bone formation, characterized by bone matrix with osteocytes, is shown bridging DBM particles together in the implants (Fig 1). The bridging of bone particles together generated tighter spaces within the implants that became hematopoietic marrow spaces. The devitalized DBM particles were clearly identified as amorphous stained material with empty osteocytic lacunae. Above the cranial bone there was a marked stimulation of new bone formation beyond the confines of the cranial structure

#### Lecithin enhances bone formation:

De-lipidizing with chloroform/methanol decreased bone formation compared to standard DBM, but when phospholipids in the form of lecithin were added back, bone formation rates were significantly enhanced, above the levels stimulated by DBM alone. The histological results were always confirmed by alkaline phosphatase analysis of the explants (Fig 2). Implantation of the composite suprapariosteally in the cranial region caused further marked bone deposition.

### DISCUSSION:

Lecithin is a relatively stable phospholipid present in significant amounts in the calcification front. It does not readily dissolve in water, disperse or emulsify. Composites containing DBM and lecithin appear to be practical for filling osseous defects particularly when there is concern of implant washout or migration. Recently, Urist [5] found that endogenous lipids are closely associated with BMP facilitated heterotopic bone formation. If completely delipidized with chloroform methanol during the process of preparation, the rate of ectopic bone formation by demineralized bone matrix was decreased by 80%. In our study, when lecithin was added back to de-lipidized-DBM, obtained from rats or from a human bone bank (which includes a partial de-lipidizing step), bone formation and the closely correlated alkaline phosphatase activities increased.

In conclusion, lecithin blended with DBM, not only provides better handling properties, but also has the ability to enhance the osteoinductivity of DBM.

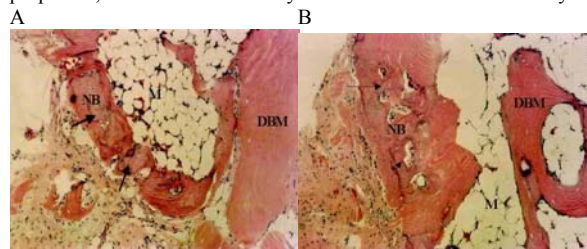
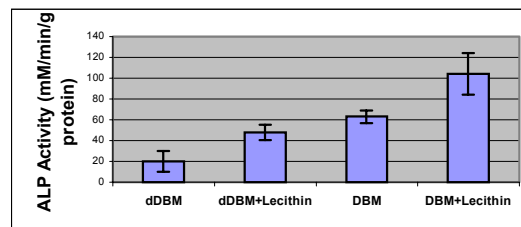


Figure1. New bone formation in the DBM/Lecithin composite after 28 days implantation. (a) subcutaneously; (b) Intramuscularly



Figures 2. Lecithin increases the alkaline phosphatase activities when composite with DBM. ALP activities in 28-day postoperatively explants.

### Reference:

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