

MM IRCON EDICAL

www.mirconmedical.com



 **Exactech**[®]
DENTAL BIOLOGICS

Headquarters / Sede operativa **Mircon Medical**

C.P. 157 Mount Royal **North America**

H3P 3B9 Quebec, Canada

Tel: +1 514 384 3293 / +1 888 333 3044

Fax: +1 514 384 0331 / +1 888 333 3048

mtl2@mirconmedical.com



mirconmedical.com

Mircon Medical srl Headquarters / Sede operativa

Europe Via delle Robinie, 50 - 47842 S.Giovanni M. RN, Italia

Legal Office / Sede legale

Via Felice Casati, 20 - 20124 Milano, Italia

Tel: +39 0541 173 6512 / Fax: +39 0541 957 644

info@mirconmedical.com (Cod. Fisc. 06621590964 - P.Iva 06621590964)

Regenafil[®]

Allograft Paste

In Vivo determination of the optimal amount
of DBM in a DBM/carrier composite
required to optimize bone formation.

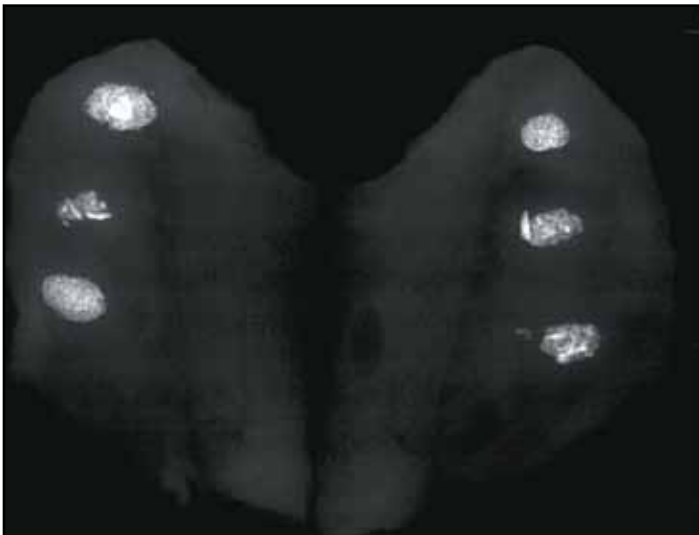
April 1999

INTRODUCTION

The intra-muscular rat model is the standard model for testing the osteoinductivity of demineralized bone and other osteoinductive factors. Urist, Strates¹ and others have nearly 30 years experience with this model. The purpose of this study was to determine the optimal percentage of DBM (Demineralized Bone Matrix) that must be present in a DBM/gelatin composite in order to maximize bone formation.

MATERIALS AND METHODS

Demineralized bone matrix was prepared from Sprague-Dawley rats and added to a gelatin-based carrier matrix. The amount of DBM added to the carrier matrix was 0, 15, 19, 24 and 33 percent by weight. The composite was blended thoroughly by hand mixing, and the implants were prepared by ejecting a thread of composite onto a petri dish, cut into short segments (pellets), frozen and subsequently thawed for implantation. Young Sprague-Dawley rats (200-410 g) were anesthetized, and a parallel to mid-line incision was made from the tip of the sternum to just above the groin. The lateral aspects of the rectus abdominus were accessed by blunt dissection to either side of the animal. Three short incisions were made in the muscle on each side and the implants inserted into the intramuscular pockets thus created. One positive (standard powdered DBM) or one negative (carrier only) control as well as two experimental compositions were inserted on each side.



Mammography film showing six ossicles that have formed in the rectus abdominus of a rat at 28 days post operative.

Implant locations were random except that each rat had one positive control (DBM only) on one side and one negative control (carrier only) on the contralateral side.

Animals were returned to their cages and provided ad-lib food and water. All animals of the study group were kept for four weeks except one animal (R1), which was sacrificed after two weeks for histology. After four weeks, animals were sacrificed, and the rectus abdominus was removed and x-rayed using mammography equipment.

EXPLANT ANALYSIS

Two of each variety of explant were removed from the muscle and fixed in 10 percent buffered formalin. Histological sections were taken, and consecutive sections were stained with H&E and Masson's trichrome stain.

The remaining explants were cut from the muscle tissue and ashed in a muffle furnace. Ash weight was determined and normalized to original implant weight. Calcium content was measured by atomic absorption spectroscopy. All analysis was conducted in a blinded manner with decoding done only after processing of the data was complete.

EXAMPLES OF HISTOLOGY OF REGENAFIL® ALLOGRAFT PASTE IN THE RECTUS ABDOMINUS OF A RAT AT 28 DAYS POST OP.

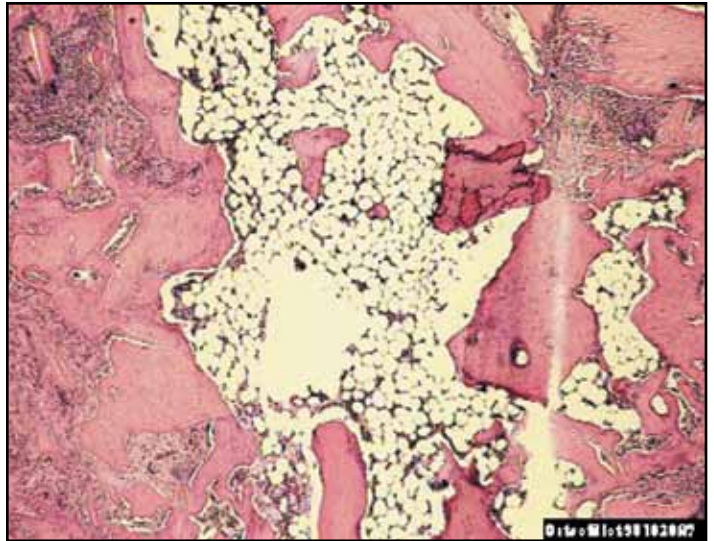


Figure 1: Low magnification image of a whole ossicle showing that most of the DBM is resorbed and replaced with new bone and marrow. The exterior of the explant has a neocortex and a newly formed periosteum. The interior of the explant resembles cancellous bone (H&E 40x).

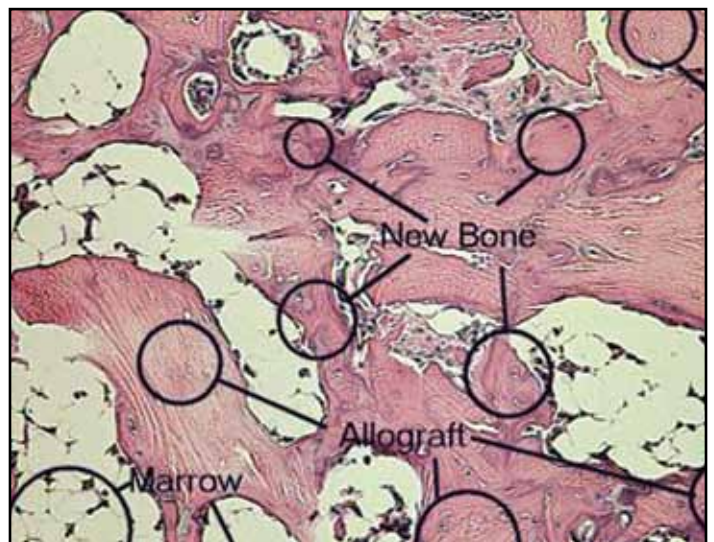


Figure 2: Higher magnification image of an explanted ossicle showing de novo bone forming around the original allograft particles. The areas between the particles have filled in with hematopoietic and fatty marrow. New woven bone generally forms in areas of higher mechanical stress (between allograft particles) and marrow forms in areas of lower stress (H & E 100x).

HIGH MAGNIFICATION IMAGES

Figures 3, 4 and 5: High magnification images of the active remodeling and bone formation on the surface and between allograft particles. All slides show the formation of new osteoid and significant osteoclastic and osteoblastic activity (H & E 400x).

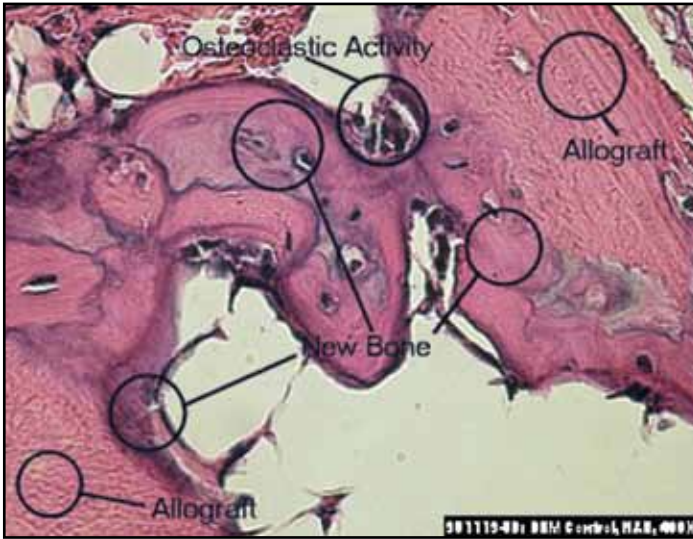


Figure 3



Figure 4



Figure 5

HISTOLOGIC REVIEW

Implants of DBM and the 15, 19, 24 and 33% compositions all showed bone growth. It is difficult to measure bone growth quantitatively, so histological analysis was instead used to confirm that the ash and calcium observed in the explants was due to bone formation and not ectopic calcification unrelated to osteogenesis.

Figures 1-5 show explant histology of Regenafile® Allograft Paste.

RESULTS

The ash analysis yielded results listed in the table below. In this table, % ash corresponds to % calcium, which correlates directly with the amount of bone formed.^{1,2}

Composition (%DBM)	% Yield Ash/cc Implant	% Yield Ash/g Implant
0 {(-) control}	10	10
15	7	6
19	14	12
24	42	35
33	36	30
DBM Powder	6	32

The amount of bone formed in the 24 & 33 percent compositions was significantly higher than that seen in the 0,15 and 19 percent compositions. ($p < 0.01$, Student's-T). There was no statistically significant difference between the amount of bone formed with the 24 & 33 percent compositions ($p > 0.05$).

REFERENCES:

1. Urist, M., and B. Strates. "Bone formation in implants of partially and wholly demineralized bone matrix. Including observations on acetone-fixed intra and extra cellular proteins." *Clinical Orthopaedics and Related Research* 71:271-8. 1970.
2. Wironen, J. "In Vivo Determination of Composition and Activity of a DBM/Gelatin Composite." *University of Florida Tissue Bank Study*. 1997.

Regenafi® is processed by RTI Biologics, Inc. and distributed by Exactech.

Exactech®

2320 NW 66th Court
Gainesville, Florida 32653
1-866-284-9690
www.exac.com

717-02-80 Rev. B
Regenafil Rat Summary 0310

