Effect of Allograft Bone Processing on Structural Cortical Grafts: A Comparison of Three Proprietary Processing Methods

Michael G. Dunn, Ph.D.
Associate Professor, Department of Orthopaedic Surgery
Director, Orthopaedic Research Laboratories
UMDNJ-Robert Wood Johnson Medical School
1 Robert Wood Johnson Place, New Brunswick, NJ 08903

SUMMARY

The objective of this study was to determine the effect of three commercial bone cleaning processes on the bone remodeling characteristics of cortical bone. Cortical bone is useful for its load bearing properties. Cortical struts are typically used in applications where internal stabilization is desired. The cortical allografts serve as a stabilizing strut while undergoing bone remodeling by the mechanism of creeping substitution. The processing of cortical bone, intended to remove undesirable immunologically active elements, may also remove desirable elements (including endogenous growth factors) and affect the bone's ability to undergo creeping substitution.

The osteoinductive potential of cortical bone is utilized during creeping substitution. The bone morphogenic proteins (BMPs) and other growth factors are liberated from the bone mineral matrix by osteoclast action. This is the same basic mechanism of action that occurs with the use of demineralized bone.

Accordingly, we elected to evaluate the latent osteoinductive properties of cortical bone by making a demineralized bone matrix and measuring the osteoinductive properties. This allows a quantitative assessment of the effect of process cleaning methods on osteoinductive potential.

The study was conducted to determine the effect of the bone cleaning process on creeping substitution through quantification of osteoinductivity of the DBM samples prepared from tissues processed using MTF ATP™, Regeneration Technologies (RTI) BioCleanse®, and LifeNet Allowash®. It was our hypothesis that bone cleaning processing protocols have an impact on the inherent ability of the bone to reincorporate (as determined by measuring osteoinductive potential).

Osteoinductivity, or the ability to produce de novo heterotopic bone, was assessed histologically (on a scale of 0-4) following intramuscular implantation of eight DBM samples from each test group, as well as positive and negative controls, in an athymic mouse model.

Results of this study suggest that:

- DBM prepared from MTF ATP cortical tissue was consistently osteoinductive in this model; 7 out of 7 ranked samples were osteoinductive (1 sample could not be ranked due to histological artifacts). The average bone score for the 7 ranked samples was 2.17 ± 0.82.

- DBM prepared from RTI BioCleanse cortical tissue was not osteoinductive in this model; 7 out of 7 ranked samples were not osteoinductive (1 sample could not be ranked due to histological artifacts). The average bone score for the 7 ranked samples was 0.00 ± 0.00, exactly the same as the heatinactivated negative control. In addition, histological views showed fibrous tissue/inflammatory response.

- DBM prepared from LifeNet Allowash cortical tissue was partially osteoinductive in this model; 3 out of 7 ranked samples were osteoinductive (1 sample could not be ranked due to histological artifacts). The average bone score for the 7 ranked samples was 0.57 ± 0.74.

- Positive control DBX® putty had a score of 2.55 ± 0.51; heat-inactivated negative control DBX putty had a score of 0.00 ± 0.00.

- These results support the hypothesis that bone cleaning processes may have an effect on the rate of creeping substitution of cortical bone. This is suggested by the higher osteoinductivity of DBM prepared from ATP treated tissue compared to DBM prepared from BioCleanse and Allowash treated tissue.

INTRODUCTION AND BACKGROUND

The process of creeping substitution occurs when structural cortical allografts are incorporated into host bone, whereby osteoclasts reabsorb the mineral content of the allograft thus exposing BMPs and growth factors, which, in turn stimulates osteoblast differentiation. The osteoblasts then lay down new bone. This process occurs until the allograft tissue is remodeled into the patient's own bone (generally 1-2 years for full incorporation). The objective of this study was to determine the effect of three commercial bone cleaning processes on the bone remodeling characteristics of cortical bone by comparing the osteoinductivity of DBM prepared from MTF ATP cortical tissue, RTI BioCleanse cortical tissue, and LifeNet Allowash cortical tissue.

When implanted into normal animals, human DBM is xenogeneic, and is expected to provoke an immune response that may compromise the analysis of osteoinduction. To avoid this, we used the athymic mouse model. The athymic mouse lacks a thymus gland and therefore cannot mount a humoral immune response to the human DBM implants. Precedence of the use of an athymic mouse (*Nu/Nu*) model for studying the osteoinductive potential of demineralized bone allograft was noted in Schwartz *et al.*[1]

Samples of the test groups and controls were implanted bilaterally into the mouse hamstring muscle. The hamstring muscle group (*biceps femoris*

muscle) is a large, easily accessible muscle which is commonly used as an implant site to evaluate heterotopic bone formation. Histological evaluation of the control and test articles was conducted 28 days after implantation to assess osteoinduction.

METHODS AND MATERIALS

The tissue from each treatment group was made into a formulation of DBM (using acid demineralization based upon standard techniques[2]) and normal saline (0.9% Sodium Chloride, Irrigation, USP). Fifty implants were made, ten per group. Eight samples from each test group and 5 controls from each control group were implanted bilaterally in the hamstring muscles of athymic mice.

For each test group, 10 samples were packaged in individual syringes (8 for implantation; 2 extra). For each control group, 7 samples were packaged in individual syringes (5 for implantation; 2 extra).

Each sample weighed approximately 20-25 mg. The samples were randomized and implanted bilaterally in the hamstring muscles of athymic nude mice. Intramuscular implantation of active DBM is expected to induce cartilage and then bone formation within the implants, a process termed osteoinduction. Animals were sacrificed at 4 weeks post-implantation. Decalcified histology was then performed on the explanted samples; 5 histological slides with 2 sections per slide were prepared for each sample (10 sections total per sample). Slides were stained with hematoxylin and eosin and samples were evaluated for osteoinductivity. A semi-quantitative scoring system was utilized to assess osteoinduction.

The relative amount of osteoinduction was evaluated semiquantitatively by the study investigator using the scoring system described below; the observer was blinded to the identification of the implant. Osteoinductive scores were based on the degree to which new bone, bone cells, osteoid, calcified cartilage remnants, and marrow elements are present. To be consistent with proposed standards in the industry,[3] the following scoring system was utilized:

0	No evidence of new bone formation
1	1-25% of the section is covered by new bone
2	26%-50% of the section is covered by new bone
3	51%-75% of the section is covered by new bone
4	>75% of the section is covered by new bone

The overall score for each implant was obtained by averaging the highest 5 scores from the histological slides; scores for each experimental group were determined by pooling the overall scores of the individual implants. The results of semi-quantitative scoring are presented as a mean ± standard deviation. Images of histological slides from each test and control group were also captured and stored using a digital camera and computer system (*Image Pro Plus*™ imaging software).

RESULTS AND CONCLUSIONS

In developing the ATP process, MTF conducted extensive research to maintain the bone's biologic activity post ATP treatment.[4] DBM prepared from MTF ATP treated cortical tissue was consistently osteoinductive in this model; 7 out of 7 ranked samples were osteoinductive (1 sample could not be ranked due to histological artifacts). The average bone score for the 7 ranked samples was 2.17 ± 0.82 (Table 1). *Figure 1* shows the representative histological response to DBM prepared from MTF ATP cortical tissue, with robust new bone formation including bone marrow.

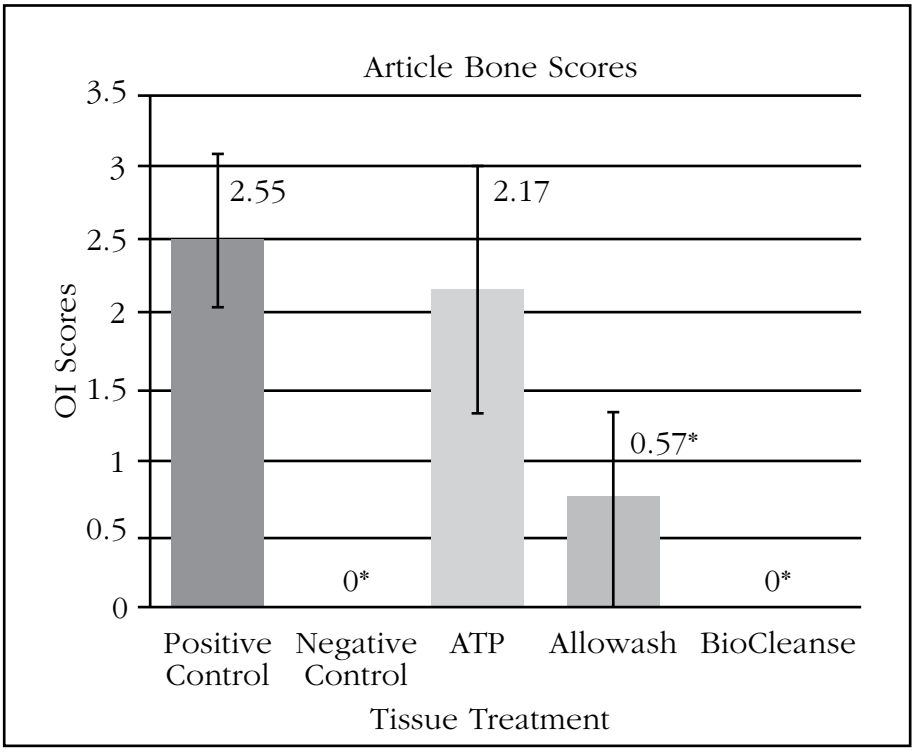
DBM prepared from RTI BioCleanse cortical tissue was not osteoinductive in this model; 7 out of 7 ranked samples were not osteoinductive (1 sample could not be ranked due to histological artifacts). The average bone score for the 7 ranked samples was 0.00 ± 0.00 (Table 1), exactly the same as the heat-inactivated negative control. *Figure 2* shows the representative histological response to DBM prepared from RTI BioCleanse cortical tissue, with a primarily fibrous tissue/ inflammatory response, with no new bone formation.

DBM prepared from LifeNet Allowash cortical tissue was partially osteoinductive in this model; 3 out of 7 ranked samples were osteoinductive (1 sample could not be ranked due to histological artifacts). The average bone score for the 7 ranked samples was 0.57 ± 0.74 (Table 1). *Figure 3* shows the representative histological response to DBM prepared from LifeNet Allowash cortical tissue, with moderate new bone formation.

Positive control DBX putty had a score of 2.55 ± 0.51 (Table 1). *Figure 4* shows the representative histological response to positive control DBX putty, with robust new bone formation including bone marrow.

Article	Treatment	Group	Group Article	Std Dev
Test Article 1	ATP Treated DBM Putty	G1	2.17	0.82
Test Article 2	BioCleanse Treated DBM Putty	G2	0.00	0.00
Test Article 3	Allowash Treated DBM Putty	G3	0.57	0.74
Positive Control	DBX Putty	G4	2.55	0.51
Negative Control	Heat-Inactivated DBM Putty	G5	0.00	0.00

Table 1. Osteoinduction scores for DBM made from MTF ATP cortical tissue, RTI BioCleanse cortical tissue, and LifeNet Allowash cortical tissue, as well as positive and negative control DBX Putty.



Results in Table 1 shown in graph form.
*Statistically different to ATP & Pos. Control

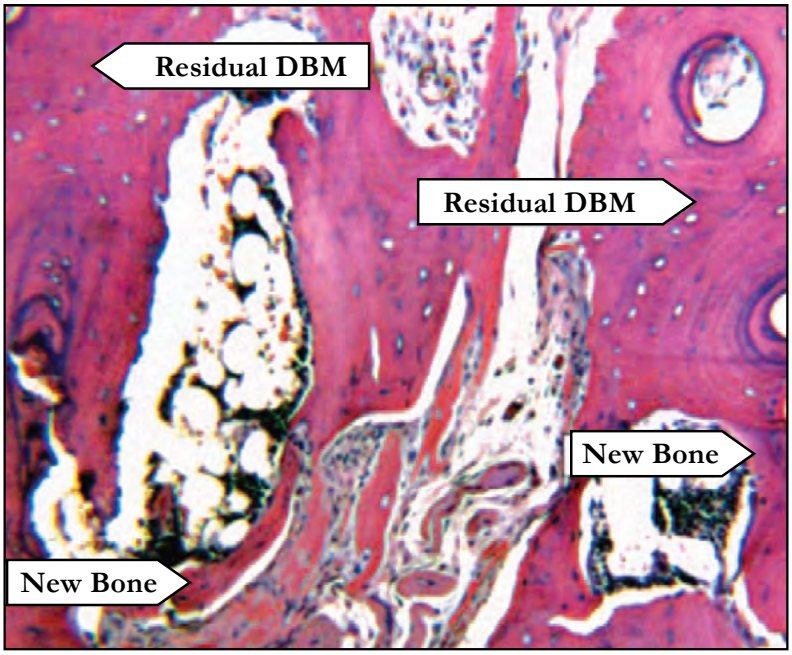


Figure 1. DBM prepared from MTF ATP cortical tissue, GROUP G1. H&E stain; 100X magnification; BAR = 100 MICRONS.

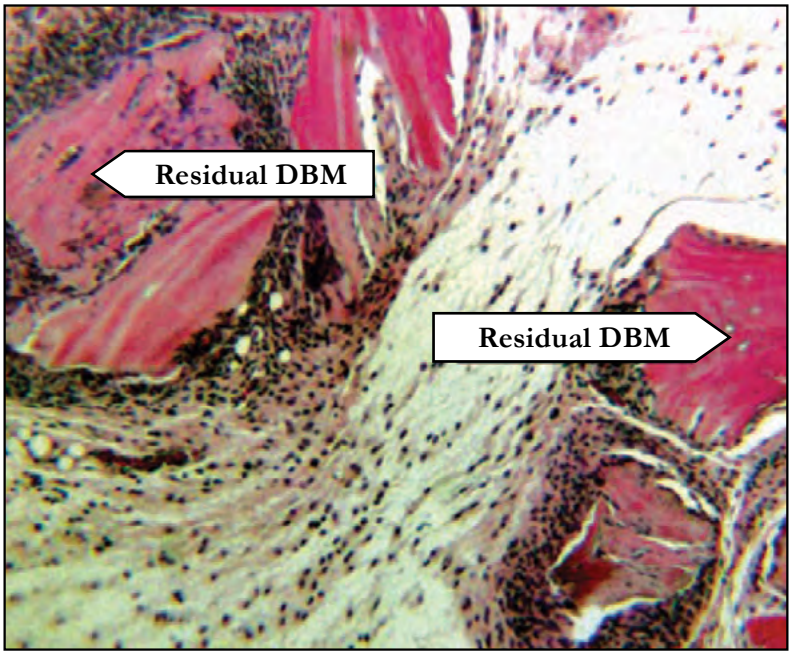


Figure 2. DBM prepared from RTI BioCleanse cortical tissue, GROUP G2. H&E stain; 100X magnification; BAR = 100 MICRONS

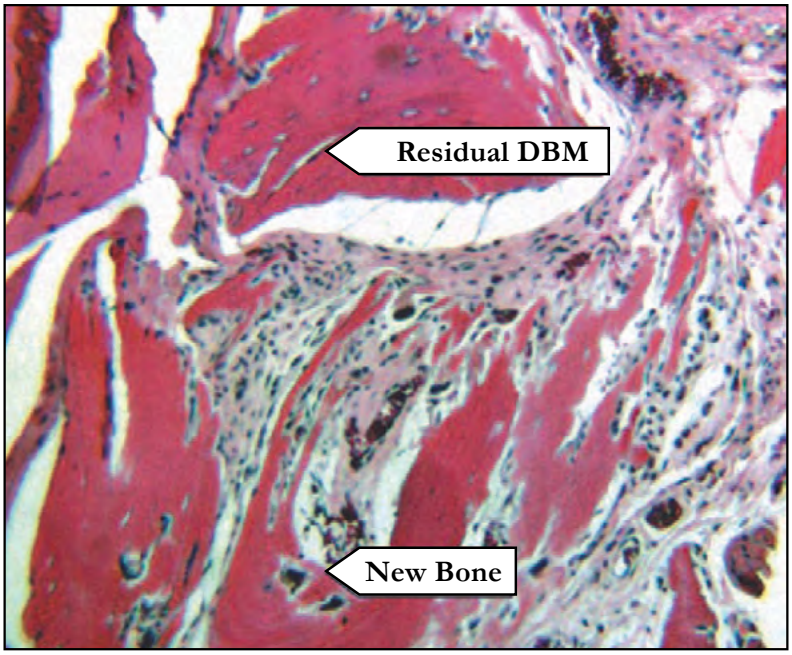


Figure 3. DBM prepared from LifeNet Allowash cortical tissue, GROUP G3. H&E stain; 100X magnification; BAR = 100 MICRONS.

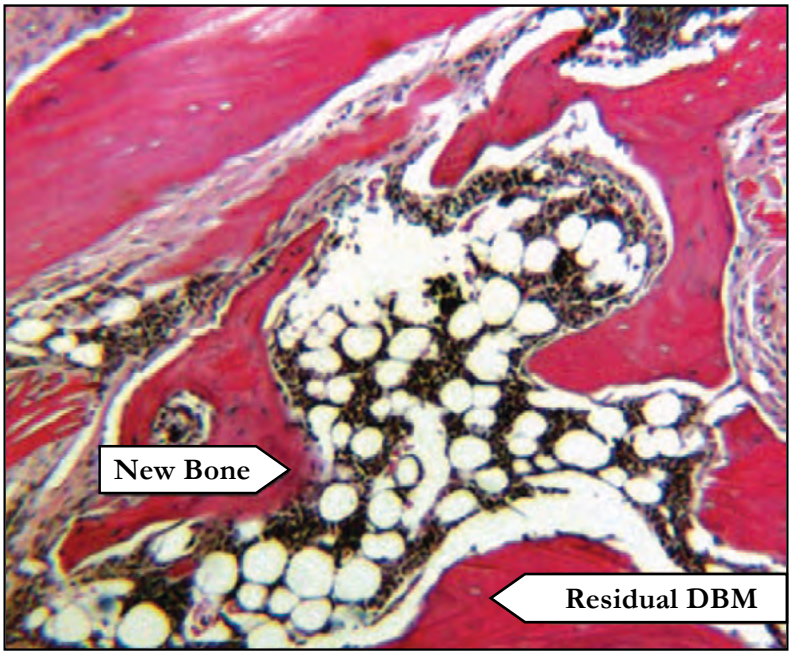


Figure 4. Positive control DBX putty, GROUP G4. H&E stain; 100X magnification; 0BAR = 100 MICRONS.

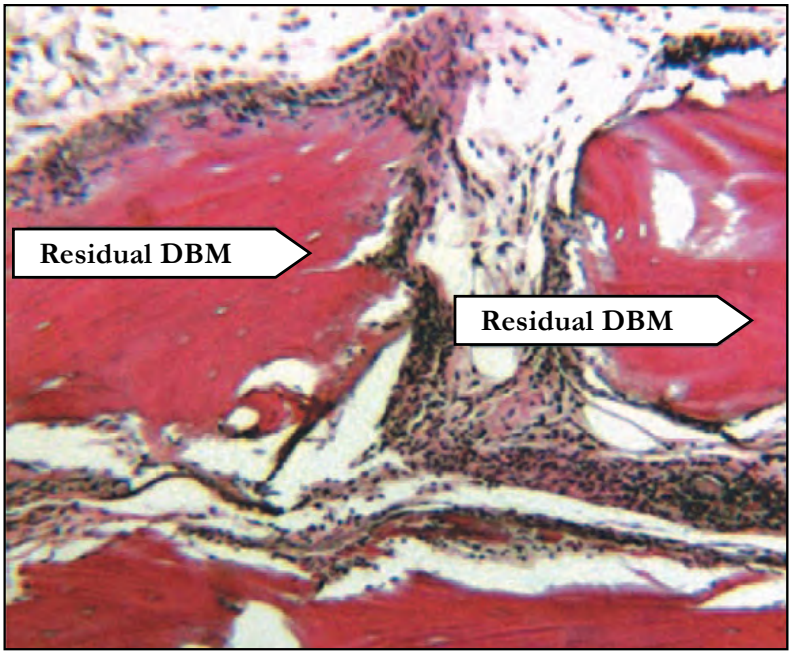


Figure 5. Heat-inactivated negative control DBX putty, GROUP G5. H&E stain; 100X magnification; BAR = 100 MICRONS

Group	p-Value
ATP vs. Positive Control	0.409
ATP vs. BioCleanse	0.000
ATP vs. Allowash	0.002

Table 2. Statistical Data

Heat-inactivated negative control DBX putty was not osteoinductive (Table 1), as expected, with a primarily fibrous tissue/inflammatory response, and no new bone formation (*Figure 5*). This is an important result that validates the utility and sensitivity of the animal model.

A statistical analysis was conducted using the average bone score values from each sample group. A t-test for two samples was used with a 0.95 confidence

level and a null hypothesis mean of zero. A p-value <=0.05 is considered statistically significant (Table 2). The ATP group when compared to both the Allowash and Biocleanse group was statistically different. The analysis also showed that ATP is not statistically different to the positive control (DBX).

In summary, the higher osteoinductivity of DBM prepared from ATP treated tissue compared to DBM prepared from BioCleanse and Allowash treated tissue may result in improved incorporation of allografts treated with the ATP process. These results support the hypothesis that bone cleaning processes may have an impact on the inherent ability of the cortical bone to reincorporate.

REFERENCES:

1. Schwartz, et al., J. *Periodontol Surg*. 69: 470 – 478, 1998.

2. Urist, *Science*. 150: 893-899, 1965.

3. Draft Standard: Standard Guide for the Assessment of Bone Inductive Materials, ASTM F04.4 Division, Draft by Barbara Boyan, University of Texas Health Science Center at San Antonio, downloaded from ASTM website 5-8-2000.

4. DePaula C.A., Truncale K.G., Gertzman A.A., Sunwoo M.H. and Dunn M.G. 2005. Effects of hydrogen peroxide cleaning procedures on bone graft Osteoinductivity and mechanical properties. Cell and Tissue Banking. 6:287-298.

MTF Musculoskeletal Transplant Foundation
the better approach

125 May Street ■ Edison, NJ 08837 ■ 800-433-6576 ■ mtf.org