

# Comparison of Osteoinductive Properties Between Optefil® Paste and DBX®

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## Background:

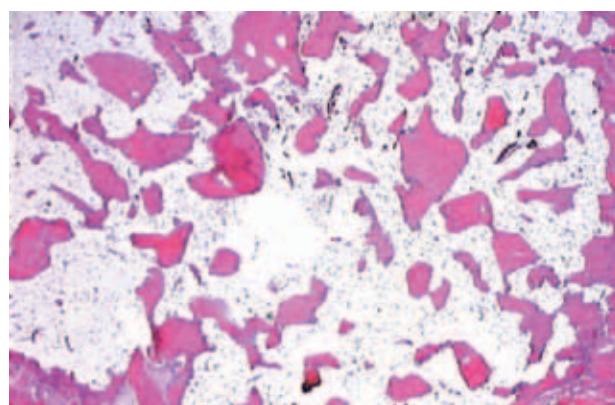
The biological effectiveness of any bone paste product is dependent on the osteoinductive potential of its constituents. The key inductive component of most commercially available bone paste products is demineralized bone matrix (DBM). Originally described in the 1960s, DBM is the end product of ground cortical bone, from which the mineral components are removed, exposing the collagenous matrix and its resident proteins. Resident non-collagenous proteins, such as TGF- $\beta$  and BMP's, are largely, but not entirely, responsible for the osteoinductive properties of DBM. Osteoinductivity of DBM can be measured by a variety of ways. This includes both *in vitro* and *in vivo* methods. The *in vitro* methods focus on the presence of growth factors<sup>1</sup> and the ability of DBM to support the growth<sup>2</sup> of osteoblast-like cells. However, they fail to recapitulate the complex chemical and biological processes that are involved in the new bone formation.<sup>3</sup> The *in vivo* athymic nude rat model, described by Urist<sup>4</sup>, offers the additional sensitivity of capturing the complete sequence of events. In this study, the osteopromotive ability of the DBM-based paste product, Optefil®, produced by RTI Biologics, Inc. (RTI), was compared with a similar product, DBX, prepared by Musculoskeletal Transplant Foundation (MTF). Comparable amounts (by weight) from 3 different lots of each paste product were subjected to a series of *in vitro* and *in vivo* osteoinductivity tests.

## Results:

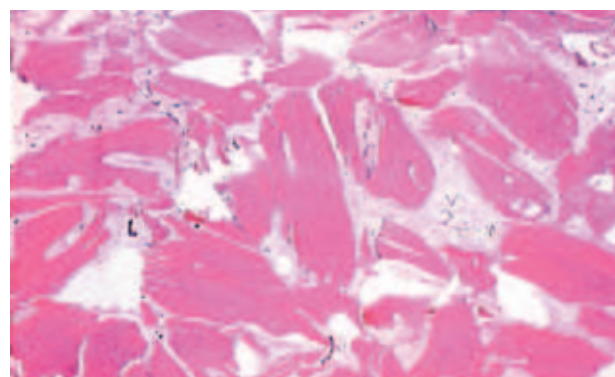
### *In vivo* Rat Model Results

Equivalent amounts of Optefil and DBX were implanted into abdominal muscle pouches of athymic nude rats. The volume of the implants and explants were measured to determine shrinkage. Inflammation and osteoinductivity were determined and scored via histology (H&E staining of decalcified explants) at 28 days post implantation.

*A sample representation of histological appearance of Optefil and DBX bone paste following 28 days post implantation in rats. Note active remodeling, new bone and bone marrow formation in the Optefil as compared to minimal activity (inactive DBM particles) in the DBX sample.*

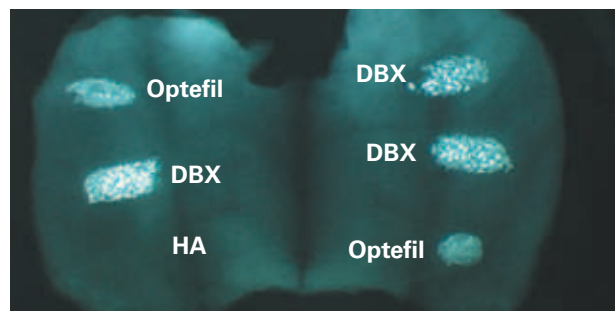


*Optefil histology H&E stain 28 days post implantation.*



*DBX histology H&E stain 28 days post implantation.*

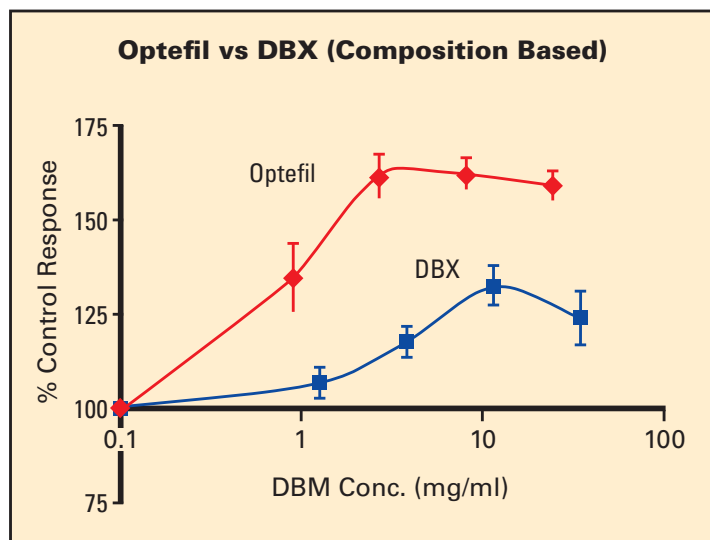
Qualities Measured	Optefil	DBX	P value (t-test)
Initial Density	1.17 (n=10)	1.12 (n=10)	NA
% Decrease in Volume (Average for group)	6%±21% (n=10)	7%±11% (n=10)	.818
% Osteoinductive Implants	100% (n=45)	58.8% (n=34)	0.01
Histology Score (Avg. observed OI)	2.3±1.1 (n=45)	0.76±0.7 (n=34)	.001
% DBM base OI RTI=60%, DBX=33%	3.07	0.76	NA
Average Inflammatory Score	1.1±0.3 (n=45)	1.1±0.3 (n=34)	1



*A sample representation of radiographic appearance of muscle flaps implanted with Optefil or DBX bone paste samples. Note organized radio-dense appearance of all Optefil implants.*

### *In vitro* Osteoinductivity Results

For *in vitro* assessment of inductive potential, equal amounts of Optefil and DBX paste were weighed and dispersed in serum free tissue culture medium. Graded doses (n=6) of each preparation were added to wells of 96-well-tissue culture plates containing SAOS cells<sup>2</sup> (a human osteoblast cell line). The ability of each preparation to support/promote osteoblast growth was measured colorimetrically following 96 hours of culture in a humidified incubator (37°C, 5% CO<sub>2</sub>). Results are expressed as a percent of the control response, where control represents values obtained from SAOS cells cultured without the paste products. Data expressed in the following graph depicts osteoblast growth in the Y-axis, in response to the DBM concentration in the paste product that was tested, depicted in the X-axis. Average values for each paste product (pooled donor lots) are shown in the graph.



### Growth Factor ELISA Results

Levels of key growth factors implicated in the osteoinductive potential of DBM, namely BMP 2/4 and TGF-β1 were assessed in both products. For this analysis a commercial enzyme linked immuno-assay (ELISA) kit<sup>1</sup> that included antibodies specific for human growth factors mentioned above was employed. Comparable amounts of each paste preparation were subjected to collagenase digestion, to release the growth factors from the matrix, concentrated, and tested. Data presented below represents values obtained from replicate samples following collagenase extract of each paste preparation. As observed in an earlier report<sup>1</sup>, wide variations in the levels of these growth factors were seen, presumably due to differences between the donors used to process DBM.

	Optefil	DBX
BMP 2/4 range (pg/g DBM)	180 – 1295	1.2 – 21.3
TGF-β1 range (pg/g DBM)	97100 - 115800	23341 – 98032

### Discussion:

In a variety of tests employed to address the osteoinductive potential of any given bone paste product, Optefil performed considerably better than DBX in all the tests. All the product lots used in this series of experiments (both DBX and Optefil) were stored under manufacturer recommended conditions, and used well within the indicated expiration dates. In the more sensitive, and AATB approved<sup>3</sup>, animal model to measure inductivity, not only was the overall score of DBX lower than the Optefil (p<0.001, t-test), but a

significant number of the DBX implants (42%) failed to demonstrate any measurable new bone formation. The radiographs further supported this observation, where the Optefil implants exhibited a honeycomb structure with x-ray dense granules, indicative of new bone formation rather than mineralization, while the DBX implants exhibited diffuse x-ray dense material without organization. Further, in the *in vitro* model, which operates under less stringent requirements than the animal model, the DBX lots demonstrated diminished ability to promote osteoblast growth. The difference was significant (p<0.001, t-test) at all DBM concentrations that were tested. Since success in the *in vitro* assay is almost entirely dependent on growth factor levels, an ELISA was performed to measure the same. Once again, the DBX lots that were tested demonstrated virtually no BMP 2/4, and significantly lower levels of TGF-β1 in comparison to Optefil. While wide variations have been observed in the levels of these two growth factors even within DBM lots that are known to be inductive from the rat assay<sup>1</sup>, the levels displayed by the DBX lots tested were particularly low. Taken together, the data suggested that the lower osteoinductive properties of DBX might be attributable to the quality of the DBM in these lots.

### Conclusion:

The osteoinductive ability of a bone paste product is determined by both the quality of the DBM and the carrier used to deliver the DBM in the finished product. Since most carriers used in commercial bone pastes are by themselves non-inductive, the quality of the DBM becomes a major determinant of inductive ability of the end product. All of the DBM used to prepare Optefil paste are subjected to an *in vivo* testing prior to use in preparing the end product. Employing such a rigorous quality control measure that necessitates the use of DBM meeting minimum osteoinductivity requirements appears to be the reason for better performance by Optefil.

### References

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