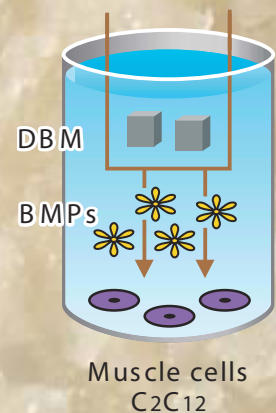


# VALIDATED ASSAY FOR OSTEOINDUCTIVITY

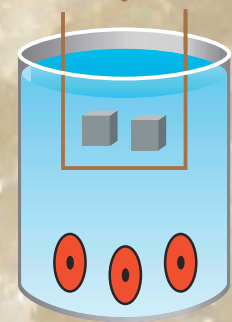
A validated in vitro assay is used to quantify the osteoinductivity of each lot of human demineralized bone matrix (DBM) prior to its use in IsoTis' bioimplants. Cultured C<sub>2</sub>C<sub>12</sub> mouse muscle cells are incubated with the DBM for a specified period of time to determine the conversion of the muscle cell line to bone-forming cells. The assay measures the production of alkaline phosphatase, which is an accurate indicator of osteoblastic activity.

Bone regeneration and healing by surgical intervention may be facilitated by osteoinductive and osteoconductive bioimplants. As an osteoinductive material, DBM induces the differentiation of mesenchymal (stem) cells into osteoblasts (bone-forming cells), and is used in a variety of orthopedic surgical applications.

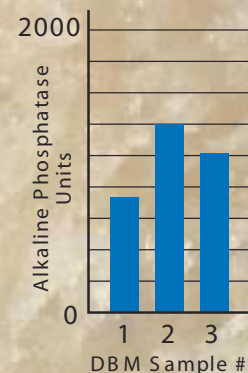
DBM contains a variety of biologically active bone morphogenetic proteins (BMPs), such as BMP-2, BMP-4, and BMP-7, which are among the osteoinductive components of bone. However, the osteoinductive potential of DBM is highly variable depending on the individual donor and the demineralization process. Therefore, it is essential to measure the osteoinductivity of each lot of DBM to ensure the bone-forming potential of the final product.



Days



Extract cells



**Figure 1** Mouse muscle cells growing in culture are converted to bone-forming cells (osteoblasts) in the presence of osteoinductive DBM. The number of active osteoblasts is quantified as units of alkaline phosphatase.

# VALIDATED ASSAY FOR OSTEOINDUCTIVITY

## QUANTITATIVE ASSAY MEASURES OSTEOINDUCTIVITY VALIDATION AGAINST IN VIVO MODEL

To determine the osteoinductivity of DBM, IsoTis employs a quantitative in vitro assay that is both rapid and sensitive. In the presence of an osteoinductive substance, the assay measures the conversion of a skeletal muscle cell (myoblast) line to osteoblasts via assessment of alkaline phosphatase production. As such, it is an appropriate extension of Dr. Urist's in vivo osteoinductivity test in which DBM is implanted into a skeletal muscle.

In choosing a cell line suitable for assaying the osteoinductivity of DBM, it is vital that the cells do not, under normal culturing conditions, spontaneously differentiate into osteoblasts or produce any bone-specific proteins (e.g., osteocalcin) or bone-selective proteins (e.g., alkaline phosphatase). The assay utilizes the skeletal muscle cell line C<sub>2</sub>C<sub>12</sub>, which fits these criteria as shown by validation of a negative control.

In the presence of BMPs, the mouse muscle cell line has been shown to convert its differentiation pathway from myoblast to osteoblast. The DBM and myoblasts are incubated together for sufficient time (up to 15 days) to permit differentiation into osteoblasts (Figure 1). Deactivated DBM serves as the negative control, while rhBMP-4 is used as the positive control. The number of active osteoblasts is quantified as units of alkaline phosphatase, a sensitive and reliable measure of osteoblastic activity.

The in vitro assay has been validated against an in vivo osteoinductivity model at an independent laboratory. All lots of DBM induced proportional amounts of ectopic bone formation and alkaline phosphatase production in vivo. There was a positive correlation between in vivo DBM osteoinductivity, measured as percent new bone or alkaline phosphatase production, and in vitro osteoinductivity, measured as alkaline phosphatase production. Based on these results, it was determined that the in vitro assay confidently predicts the osteoinductive potential of DBM.

### SUMMARY

Each production lot of DBM is tested in the in vitro assay before it can be used in IsoTis' bioimplants, and is electron beam sterilized at the standard dose prior to testing. IsoTis' products are released if the DBM component increases the production of alkaline phosphatase to a value significantly greater than the established baseline. DBM lots that are not shown to be highly osteoinductive are rejected and not used in the production of our bioimplants. The use of the validated assay thus helps to ensure successful clinical outcomes when implanting IsoTis' products.

1. Shigemasa Y, D'Errico JA, Stone R, et al. Commercially-prepared allograft material has biological activity in vitro. *J Periodontol* 1995;66:478-87.
2. Jortikka L, Laitinen M, Wilkund J, et al. Use of myoblasts in assaying the osteoinductivity of bone morphogenetic proteins. *Life Sciences* 1998;62:2359-68.
3. Urist MR. Bone: formation by autoinduction. *Science* 1965;150:893-9.
4. Katagiri T, Yamaguchi A, Komaki M, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C<sub>2</sub>C<sub>12</sub> myoblasts into the osteoblast lineage. *J Cell Biol* 1994;127:1755-66.



ISOTIS ORTHOBIOLOGICS, INC.

2 Goodyear • Irvine, California 92618 • Phone 800.550.7155 / 949.595.8710 • Fax 800.471.3248 / 949.595.8711  
18-20, Avenue de Sévelin • 1004 Lausanne, Switzerland • Phone +41 21 620 6020 • Fax +41 21 620 6021

[www.isotis.com](http://www.isotis.com)