Osteoinductivity of Puros® DBM Putty in Athymic Rat Model

By Steven T. Moore and Ronald R. Cobb

Commercially available bone paste products consist of formulations that combine demineralized bone matrix (DBM) with a carrier material. RTI Biologics, Inc. (RTI) currently markets a series of allograft DBM paste products that are produced using single donors and a porcine-derived carrier. Recent efforts have focused on generating a 100% human-derived DBM paste product made from a mixture of two types of DBM from different stages in the manufacturing process. Stage 1 DBM (DBM-1) is a demineralized bone matrix powder that preserves the osteoinductive potential of the bone. Stage 2 DBM (DBM-2) is a completely demineralized bone matrix that when mixed with DBM-1 gives the final product a "putty-like" consistency. This ready-to-use product is stored hydrated at room temperature in a one stage delivery system and is marketed as Puros DBM Putty. Preliminary in vivo studies, using the rat ectopic pouch model, have shown that this new DBM product retains its osteoinductive properties.

The athymic nude rat model described by Urist (1965) has been used to evaluate the osteoinductive potential and inflammatory response of DBM-based products for many years. To verify the osteoinductive potential of Puros DBM Putty (DBM-1 + DBM-2), samples of the finished product and DBM-1 alone, as a control, were implanted into the ectopic muscle pouches of athymic nude rats. After 28 days, the implants were removed and histology was reviewed to assess the osteoinductive potential as well as the inflammatory response of Puros DBM Putty (DBM-1 + DBM-2) and DBM-1 alone from the same donor.

Materials and Methods:

All human tissue used in this study was derived from consented cadaveric donations. DBM from a total of four different donors was selected for the current study, all had previously scored positive for osteoinductivity and did not exhibit significant inflammation by the QC athymic nude rat assay. The DBM-1 and DBM-2 for Puros DBM Putty were prepared from a single donor. All samples of Puros DBM Putty (DBM-1 + DBM-2) and DBM-1 controls were irradiated at a dosage of 25-31 kGy.

Biological activity was assessed (qualitatively and quantitatively) using the Urist (1965) athymic nude rat model. This in vivo model has been utilized by RTI to identify DBM with acceptable osteoinductivity for use in all paste products. After irradiation, each sample was implanted in triplicate in three separate rats with six samples per rat. The implants were extracted after 28 days and the samples were sent for histological preparation. The histological slides were scored for osteoinductivity and inflammatory responses in accordance with the scoring system of Edwards et al. (1998). In addition, bone maturity was scored in accordance with Katz et al. (2006).

Results:

Osteoinductivity and Bone Maturity:

Osteoinductivity (OI) and bone maturity scores for Puros DBM Putty (DBM-1 + DBM-2), DBM-1 alone, and control treatment groups are presented in Table 1 below (also see Figures 1-5 for histological slides). As shown in Figures 1-5, addition of the carrier did not significantly affect the OI results of the Puros DBM Putty (DBM-1 + DBM-2) compared to the DBM-1 alone. In addition, there was no significant effect on the bone maturity scores in the Puros DBM Putty (DBM-1 + DBM-2) relative to the DBM-1 alone. Although the sample size was small (n = 4 donors), this data suggests that there is no significant effect of the Puros DBM carrier (DBM-2) on the

osteoinductivity and bone maturity scores of DBM-1 alone. As expected, the OI scores for the negative control (deactivated DBM) samples were zero, as were the bone maturity scores. The average OI score for the positive control was 3.3 (± 0.58) with a bone maturity score of 9.0 (±0.00).

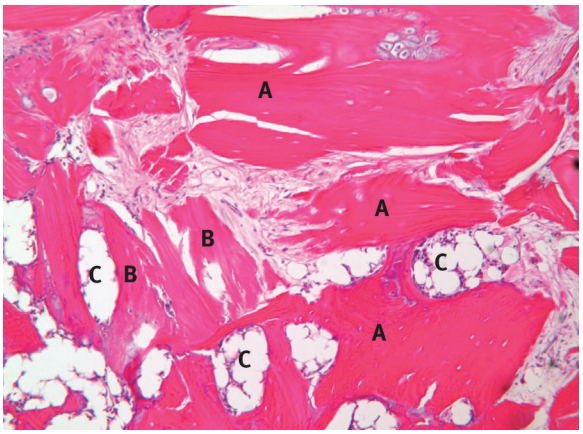

Figure 1: Donor 1, Puros DBM Putty (DBM-1 + DBM-2)

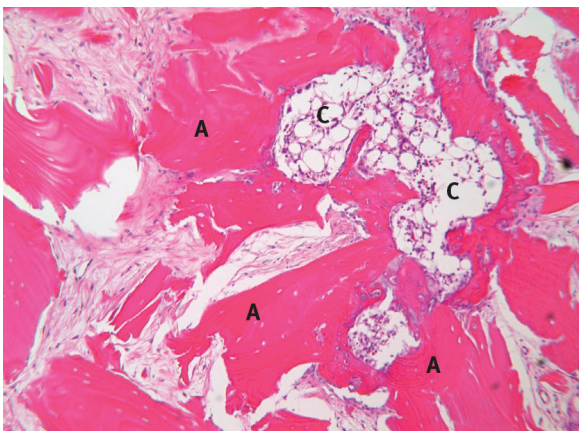

Figure 2: Donor 1, DBM-1 Alone

Inflammation:
As shown in Table 2 below, Puros DBM Putty (DBM-1 + DBM-2) compared favorably to the DBM-1 alone with respect to inflammation scores. The addition of the DBM carrier (DBM-2) to the DBM-1 alone yielded inflammation scores that were in the acceptable range (inflammatory scores < 2). The deactivated DBM negative control generated an inflammatory score that would have resulted in rejection of the product (inflammatory scores > 2), a result which is consistent with results of past in vivo tests.

Histological Analyses:

Histological analysis of the test implants failed to reveal qualitative differences between samples mixed with the DBM-2. Comparable remodeling features that are associated with new bone formation (cells associated with bone and marrow formation) were observed. Additionally there were no signs of inflammation associated with the Puros DBM Putty (DBM-1 + DBM-2) in the explants. Representative images of explants from matched donors with DBM-1 alone or Puros DBM Putty (DBM-1 + DBM-2) are shown in Figures 1-5.

Table 1. Mean ± Standard Deviation of Osteoinductivity (OI) and Bone Maturity Scores

Tissue	Treatment	OI ± SD	Maturity ± SD
Donor 1	*Puros* DBM Putty	2.3 ± 0.58	7.7 ± 0.58
	DBM-1 Alone	2.0 ± 0.00	7.7 ± 0.58
Donor 2	*Puros* DBM Putty	2.3 ± 0.58	7.7 ± 0.58
	DBM-1 Alone	2.3 ± 0.58	8.3 ± 0.58
Donor 3	*Puros* DBM Putty	2.7 ± 0.58	8.7 ± 0.58
	DBM-1 Alone	3.3 ± 0.58	9.0 ± 0.00
Donor 4	*Puros* DBM Putty	1.0 ± 0.00	6.3 ± 1.20
	DBM-1 Alone	1.7 ± 0.58	7.7 ± 0.58
Controls	Negative Control	0.0 ± 0.00	0.0 ± 0.00
	Positive Control	3.3 ± 0.58	9.0 ± 0.00

Conclusion:

A variety of qualitative features such as DBM particle size, calcium content, matrix integrity and the composition of the carrier are known to influence the osteoinductivity of bone paste products (Zhang et al., 1997; Mathukumaran et al., 1988; Sampath and Reddi, 1984a; Sampath and Reddi, 1984b). In the present study, the osteoinductivity of a bone paste product derived from 100% human material, Puros DBM Putty, was investigated in an in vivo rat ectopic pouch model. Recent findings demonstrate that this form of testing is the most reliable in terms of accurately determining the osteoinductive and inflammatory properties of bone paste products (Katz et al., submitted). The data clearly indicate that the DBM-2 (DBM carrier) has no significant dilutive effect on the osteoinductivity of DBM-1 alone as measured using the athymic nude rat

Table 2. Mean ± Standard Deviation of Inflammation Scores

Tissue	Treatment	Inf. ± SD
Donor 1	*Puros* DBM Putty	1.0 ± 0.00
	DBM-1 Alone	1.3 ± 0.58
Donor 2	*Puros* DBM Putty	1.0 ± 0.00
	DBM-1 Alone	1.3 ± 0.58
Donor 3	*Puros* DBM Putty	2.0 ± 0.00
	DBM-1 Alone	1.3 ± 0.58
Donor 4	*Puros* DBM Putty	1.0 ± 0.00
	DBM Alone	1.0 ± 0.00
Controls	Negative Control	2.3 ± 0.58
	Positive Control	1.0 ± 0.00

model. Histological evaluation of the test samples also demonstrated that Puros DBM Putty (DBM-1 + DBM-2) had essentially the same remodeling features associated with new bone formation as DBM-1 alone. In addition, this new DBM product did not cause an increase in inflammation.

The results of this study clearly reveal that Puros DBM Putty (DBM-1 + DBM-2) has similar osteoinductivity and inflammatory characteristics to the DBM-1 alone.

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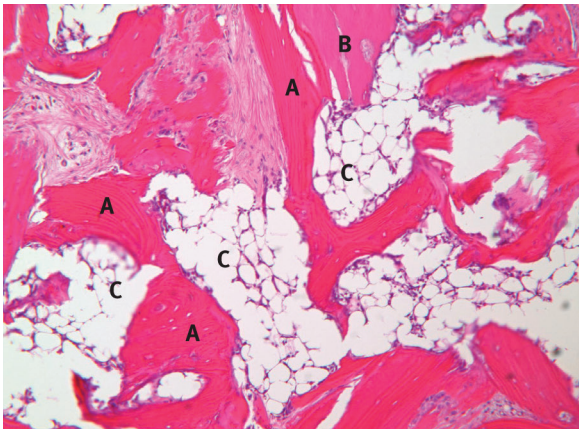

Figure 3: Donor 3, Puros DBM Putty (DBM-1 + DBM-2)

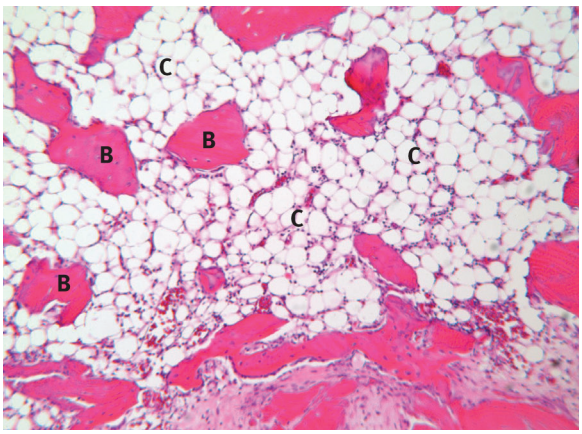

Figure 4: Donor 3, DBM-1 Alone

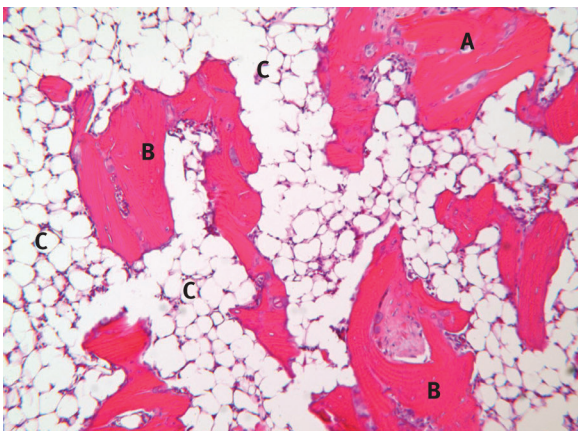

Figure 5: Positive Control

A= New Bone; B= DBM; C= Bone Marrow

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