Osteoinductivity of Puros® DBM Putty in Athymic Rat Model Following a One Year Shelf-Life Study

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Introduction:
The use of bone graft substitutes in orthopedic bone grafting procedures has increased dramatically in recent years. This is due in part to the wide range of materials, structures, and delivery systems that are available to be used. Additionally, bone graft substitutes often possess osteoconductivity and/or osteoinductivity. These characteristics make bone graft substitutes useful in augmenting the healing of bony defects caused by traumatic injury, tumor removal, abnormal skeletal development, cyst removal and prosthetic loosening.

One widely used bone graft substitute material is demineralized bone matrix (DBM) constituted with a carrier. The DBM consists of insoluble collagen and non-collagenous proteins and has inherent osteoinductive and osteoconductive properties (Mulliken et al., 1984). While growth factors in DBM provide an osteoinductive effect, the collagen structure provides the osteoconductive effect. The carrier's function is to facilitate handling characteristics and graft containment. The carrier keeps the DBM in place which allows the osteoinductive and osteoconductive nature of the DBM to facilitate bone regeneration to occur throughout a defect rather than simply at the edges (Mulliken et al., 1981). Although osteoinductive potential differentiates DBMs from synthetic bone graft substitutes, there are no known reports of osteoinductive potential over the entire shelf life period for commercially available DBMs.

Puros DBM Putty is a commercially available product that is 100% human derived, consisting of stage 1 DBM (DBM-1) and stage 2 DBM (DBM-2). DBM-1 is a demineralized bone matrix powder that preserves the osteoinductive potential of the bone and DBM-2 is a further processed demineralized bone which, when mixed with DBM1, gives the final product a "putty-like" consistency. Puros DBM Putty has demonstrated osteoinductive potential in the athymic rat model described by Urist (1965). To verify the osteoinductive potential of Puros DBM Putty throughout the product shelf-life, samples of the finished product were implanted into the ectopic muscle pouches of athymic rats after 11, 30, 60, 210, 270, and 365 days of real-time aging respectively. After 28 days in the rat, the implants were removed and histology was reviewed to assess the osteoinductive potential as well as the inflammatory response.

Materials and Methods:
All human tissue used in this study was derived from consented cadaveric donations. DBM from a total of nine different donors that previously scored positive for osteoinductivity and did not exhibit significant inflammation by the QC athymic rat assay, was selected for the current study. Each Puros DBM Putty sample was prepared from a single donor (9 donors total). All samples and controls (inactivated DBM as negative control and inactivated DB + BMP-2 as positive control) were gamma irradiated. Samples were stored at room temperature (15°-25°C) in RTI Biologics’ finished goods warehouse. At 11, 30, 60, 210, 270, and 365 day timepoints post-production (T0), individual samples were implanted into ectopic pouches of athymic rats.

Biological activity was assessed (qualitatively and quantitatively) using the Urist (1965) athymic nude rat model. Puros DBM Putty samples were placed into the muscle pouches created in the ventral abdominal muscles of anesthetized athymic homozygous nude (nu/nu) rats. Seven athymic rats were used at each time point. Each rat received 6 implants. Twenty seven of the 42 implant sites at each time point were composed of Puros DBM Putty (3 samples from each of the 9 donors). The remaining 15 implant sites were composed of control DBM material. Puros DBM Putty implants and control DBM implants were randomized to eliminate any erroneous conclusion due to animal response variations. The implants were removed after 28 days. Explants were formalin fixed, decalcified, paraffin embedded, and cut into five micron sections. At least five sections were obtained per explant. The sections were stained with Hematoxylin-Eosin. Osteoinductivity was analyzed using a semi-quantitative histological scoring system as described by Edwards, et al., (1998). In addition to the Edwards scoring system, bone maturity and the degree of inflammation for each section were also scored in accordance with Katz, et al., (2006).

Results:
Osteoinductivity and Bone Maturity:
Previous studies have demonstrated that the 100% human Puros DBM Putty is osteoinductive (Moore et al., 2008). Osteoinductivity (OI) and bone maturity scores for the Puros DBM Putty stored for up to 365 days from one representative donor are presented in Table 1. Puros DBM Putty from each of the nine donors was shown to have potential for inducing new bone growth at each of the time points throughout this study (data not shown). Although there appears to be a trend towards a loss of osteoinductivity over time, these differences were not statistically significant. In addition, there were no significant differences in the bone maturity of each sample throughout the study (Table 1).

Inflammation:
Previous studies have shown that Puros DBM Putty compared favorably to DBM-1 alone with respect to generating bone growth and inflammatory response. Puros DBM Putty from a single donor, at the T0 + 30 time point only, had an unacceptable inflammation score. All other inflammation scores, including those of the DBM-1 from the deviating donor, were within the acceptable range (inflammation ≤ 2). The inflammatory response is scored on an increasing inflammation range of 1 to 4; a score of 2 is the highest acceptable inflammatory score.
Histological Analyses:

Histological analyses of each sample yielded comparable remodeling features that are associated with new bone formation at all time points that were measured. Only Puros DBM Putty derived from Donor 8 showed no features of new bone formation at the T0 + 270 time point; however, at all previous time points and the T0 + 365 time point, this donor material did show evidence of new bone formation (data not shown). All other donor Puros DBM Putty samples demonstrated features of new bone formation. There were visible areas of marrow stromal matrix as well as pockets of osteoblasts and osteocytes. Previous studies have shown that the athymic rat model is capable of responding to DBM implants from allogeneic and xenogeneic sources. This model distinguishes between active and inactive DBM implants. Recent studies have demonstrated that this model is the most dependable in terms of accurately determining the osteoinductive potential and inflammatory properties of bone paste products (Katz et al., 2008).

The data presented clearly supports that Puros DBM Putty maintains its osteoinductive properties for at least one year. Puros DBM Putty Samples from nine different donors were stored as final product and were tested at the given time points. Puros DBM Putty derived from each of the nine donors maintained its osteoinductive potential without eliciting a significant inflammatory response.

Results Summary

Puros DBM Putty samples, with a range of osteoinductive scores representing the final product, were used in this study to establish their osteoinductive potential and inflammatory responses over a 1 year period. The results showed the samples maintained osteoinductive potential with only a slight decrease in osteoinductivity as the year progressed. With the exception noted previously, all the donor materials induced limited inflammatory responses (≤2). The results show that Puros DBM Putty has the potential for inducing new bone growth for the duration of the 1 year shelf-life.

Conclusion:

Ideal bone graft substitutes should possess osteoinductive and osteoconductive properties, be biocompatible, and breakdown in concert with bony replacement. The osseous integration of a bone graft substitute depends on the activity of the surrounding bone cells and their precursors. Puros DBM Putty was shown to have osteoinductive potential in the athymic rat ectopic pouch model. The new bone formation was characterized by marrow stromal matrix and pockets of osteoblasts and osteocytes. Previous studies have shown that the athymic rat model is capable of responding to DBM implants from allogeneic and xenogeneic sources. This model distinguishes between active and inactive DBM implants. Recent studies have demonstrated that this model is the most dependable in terms of accurately determining the osteoinductive potential and inflammatory properties of bone paste products (Katz et al., 2008).

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<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Test</th>
<th>11 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>210 Days</th>
<th>270 Days</th>
<th>365 Days</th>
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<tbody>
<tr>
<td>Donor 3</td>
<td>Puros DBM</td>
<td>OI ± SD</td>
<td>3.0 ± 0.00</td>
<td>3.0 ± 0.00</td>
<td>3.0 ± 0.00</td>
<td>2.0 ± 0.00</td>
<td>1.7 ± 0.58</td>
<td>2.7 ± 0.58</td>
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<td></td>
<td>Putty</td>
<td>Mat. ± SD</td>
<td>8.7 ± 0.58</td>
<td>9.0 ± 0.00</td>
<td>8.7 ± 0.58</td>
<td>7.7 ± 0.58</td>
<td>8.0 ± 0.00</td>
<td>7.7 ± 0.58</td>
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<tr>
<td></td>
<td></td>
<td>Inf. ± SD</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
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<tr>
<td>Negative Control</td>
<td>Inactivated</td>
<td>OI ± SD</td>
<td>1.1 ± 1.95</td>
<td>1.0 ± 1.53</td>
<td>1.0 ± 1.73</td>
<td>1.1 ± 1.95</td>
<td>1.1 ± 1.68</td>
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<td>DBM-1</td>
<td>Mat. ± SD</td>
<td>2.5 ± 4.32</td>
<td>3.7 ± 4.64</td>
<td>2.6 ± 4.39</td>
<td>2.5 ± 4.39</td>
<td>3.6 ± 4.50</td>
<td>2.6 ± 4.39</td>
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<td></td>
<td></td>
<td>Inf. ± SD</td>
<td>2.5 ± 0.69</td>
<td>2.0 ± 0.58</td>
<td>1.6 ± 0.98</td>
<td>1.9 ± 0.69</td>
<td>2.0 ± 0.82</td>
<td>2.3 ± 0.95</td>
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<td>Positive Control</td>
<td>Inactivated</td>
<td>OI ± SD</td>
<td>4.0 ± 0.00</td>
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<td>DBM-1 + BMP2</td>
<td>Mat. ± SD</td>
<td>9.0 ± 0.00</td>
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<tr>
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<td></td>
<td>Inf. ± SD</td>
<td>1.0 ± 0.00</td>
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Table 1. Mean ± Standard Deviation of Osteoinductivity (OI), Bone Maturity (Mat), and Inflammation (Inf) Scores
Figure 1: Histology sections at each study time point for Purus DBM derived from a single donor.
References Cited:


Moore ST, Cobb RR. Osteoinductivity of Puros® DBM Putty in Athymic Rat Model. RTI Biologics Control Doc # 5330 (whitepaper), 2008.
