HEALOS® MINERALIZED COLLAGEN MATRIX COMBINED WITH GDF – 5 REGENERATES BONE IN A CRITICAL-SIZED RABBIT RADIAL DEFECT MODEL

**+*Cook, A D ;  *Thompson, A Y;  **Godin, C S;  ***Shih, M S;  *Volence, F J;  *Kadiyala, S;  *Bruder, S P  
+DePuy Biologics, Raynham, MA  
acook@depuy.jnj.com**

**INTRODUCTION:** The use of bone morphogenetic proteins (BMPs) for bone grafting procedures in orthopaedic surgery requires an effective delivery system. HEALOS® mineralized collagen matrix (Matrix) is an osteoconductive bone grafting material that is currently used clinically in conjunction with autologous bone marrow aspirate. Growth/differentiation factor-5 (GDF-5) is a member of a divergent subgroup of the BMP family, with a primary role in the regulation of the development of the appendicular skeleton and joint formation. Recombinant human growth/differentiation factor-5 (rhGDF-5, also known as MP52), is being evaluated with HEALOS mineralized collagen matrix as an osteoinductive bone grafting material in human clinical studies for spinal fusion and tibial non-unions. The HEALOS matrix provides optimized biological and handling characteristics for the delivery of rhGDF-5, an osteoinductive protein. The combination product has demonstrated efficacy equivalent to autograft in preclinical animal models of cervical, posterolateral fusion in rabbits and sheep, and baboons, and in anterior interbody fusion in baboons. In addition, the delivery of rhGDF-5 from the HEALOS matrix has demonstrated bone healing efficacy in a rat cranial defect model, a baboon femoral gap model, and an avascular necrosis model in sheep. This study was designed to evaluate the ability of the HEALOS matrix combined with rhGDF-5 to stimulate bony repair in a critical-sized rabbit radial defect model. Three different concentrations of rhGDF-5 were tested.

**MATERIALS & METHODS:** The treatment group implants containing Matrix plus rhGDF-5 were fabricated in advance under aseptic conditions. Each strip of Matrix (1.7 cm x 0.5 cm x 0.5 cm; 0.425 cc in volume, DePuy Biologics, Raynham, MA) was saturated with rhGDF-5 (Biopharm GmbH, Heidelberg, Germany) in solution at 0.25, 0.5 or 1 mg/mL, lyophilized and stored frozen. Prior to implantation, the treatment group implants were thawed and hydrated with sterile saline. All procedures involving animals received IACUC approval (MPI Research, Inc.) prior to initiation of treatment and were conducted in accordance with USDA Animal Welfare guidelines. A unilateral, 1.7 cm segment of the midshaft region of the radius in male New Zealand white rabbits was excised. Implants were placed in the defects of six rabbits in each of three treatment groups, comprising Matrix + 0.25 mg/cc rhGDF-5, Matrix + 0.50 mg/cc rhGDF-5, and Matrix + 1.0 mg/cc rhGDF-5, and in two control groups, autogenous bone implant (ABI) and HEALOS mineralized collagen matrix without bone marrow aspirate (Matrix). At 12 weeks post-operative, all rabbits were euthanized, the defect sites were scored for union by manual palpation, and plain radiographs were taken. The radii were harvested and the defect sites were further analyzed by microCT, histomorphometry and subjective histological examination. A Dunnett’s analysis of the histomorphometric data was performed to determine statistical significance of each group vs. Matrix.

**RESULTS:** All doses of rhGDF-5 were found to have osteoinductive activity and stimulated the formation of new bone in a manner similar to ABI in contrast to the Matrix, which showed limited bone growth (0/6 unions). Radiographic evaluation at 12 weeks showed 3/6 unions for Matrix with 0.25 mg/cc rhGDF-5, 5/6 unions for Matrix with 0.50 mg/cc rhGDF-5, and 4/6 unions for Matrix with 1.0 mg/cc rhGDF-5. Radiographic union for ABI was 6/6. MicroCT and histomorphometric analyses (see Table 1) confirmed the results obtained from radiographs and suggested that more robust bone formation occurred at rhGDF-5 doses of 0.5 mg/cc and 1.0 mg/cc. Qualitatively, defects treated with rhGDF-5 or ABI showed a similar pattern of bony repair, with re-establishment of the bony cortex and remodeling of the central spongia in progress. No significant inflammation and no ectopic bone formation were observed in association with any treatment. Remnants of autograft bone were present in ABI-treated defects whereas only minute amounts of residual HEALOS matrix could be discerned.

**DISCUSSION:** The design of this combination product provides for a simple, off-the-shelf approach for osteoinductive bone grafting. The lyophilized matrix containing a pre-determined quantity of rhGDF-5 was very easy to handle during surgery. In this challenging, critical-sized defect model, all test groups and ABI control showed enhanced healing vs. Matrix alone, which would not be expected to produce bone. Clinically, HEALOS mineralized collagen matrix is used with bone marrow aspirate and regenerates significant amounts of new bone. This combination product of Matrix with rhGDF-5 provides an alternate method of inducing bone formation without the need for aspirating bone marrow, relying instead on the ability of the osteoprogenitor cells to migrate into the defect site.

Although treatment with ABI appeared to produce a higher rate of union than rhGDF-5 treatment, specific placement of the graft material, which may not have been in direct contact with the cut end of the bone, may account for this discrepancy. Despite sufficient bone growth throughout the mid-diaphysis, the one implant that did not qualify as a complete union at the 0.5 mg/cc dose failed to integrate at the host interface on only one end, resulting in a non-union. The other 11 of the 12 host-implant interfaces in the 0.5 mg/cc rhGDF-5 group were well integrated. Alternatively, this result may be a limitation of the model due to instability of the defect site. Nonetheless, the results of this study demonstrate the biocompatibility and efficacy of rhGDF-5 delivered from HEALOS mineralized collagen matrix to an orthotopic site. (When rhGDF-5 is placed in an ectopic site, there is minimal bone formation). This feature provides a margin of safety for the use of rhGDF-5, as bone would not be expected to regenerate in an undesirable location.) The mature nature of the bone that formed as a result of treatment with rhGDF-5, with evidence of solid cortices and reestablishment of the marrow canal, is further evidence of the effectiveness of rhGDF-5 in healing critical-sized, segmental bone defects.

**REFERENCES:**

**AFFILIATED INSTITUTIONS FOR CO-AUTHORS:**
** MPI Research, Inc., Mattawan, MI
*** SkeleTech, Bothell, WA

**Table 1. Histomorphometric Results**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N =</th>
<th>% New Bone</th>
<th>% Residual Implant</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI</td>
<td>6</td>
<td>36 ± 9</td>
<td>NA</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Matrix</td>
<td>6</td>
<td>10 ± 10</td>
<td>0.1 ± 0.2</td>
<td>NA</td>
</tr>
<tr>
<td>0.25 mg/cc</td>
<td>6</td>
<td>33 ± 20</td>
<td>0.1 ± 0.2</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>0.50 mg/cc</td>
<td>6</td>
<td>42 ± 19</td>
<td>0.2 ± 0.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>1.0 mg/cc</td>
<td>6</td>
<td>41 ± 16</td>
<td>0.2 ± 0.3</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Data are reported as AVE ± SD, NA = Not applicable