The Masquelet Induced Membrane Technique with BMP and a Synthetic Scaffold Can Heal a Rat Femoral Critical Size Defect.

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Introduction: Large bone defects are difficult clinical problems. Smaller defects can often be managed by bone autograft. For larger defects, the induced membrane or Masquelet-technique is an option(1), a two stage procedure with a success rate of up to 90 %. An intramedullary nail is inserted and the bone defect is filled with a PMMA spacer. The spacer induces a surrounding vascularized membrane in which growth factors are expressed(2). Later, the spacer is removed and the membranous tube filled with autograft. Defects up to 20 cm have been reported to be bridged in a couple of months. Even though the method appears promising and meets unsolved clinical problems, alternatives to autograft would be attractive, to avoid autograft bone harvest but also allowing the operation to be performed as a single stage procedure.

Bone morphogenetic proteins (BMPs) are commercially available proteins that induce recruitment, differentiation and proliferation of osteoblasts. BMPs can heal critical size defects in rat femurs, but paradoxically also induce osteoclastic activation(3) through the RANK/RANKL pathway(4). BMP induced resorption can be controlled by a bisphosphonate. In a rat femoral osteotomy model, we have shown that a combination of allograft (osteocductive properties), BMP-7 (osteoinductive properties) and zoledronate (anti-catabolic properties) is a powerful replacement of autograft(5). We hypothesized that by controlling the BMP-induced early resorption, the combination of scaffold, BMP-7 and zoledronate, would be superior to BMP-7 alone, scaffold alone or the combination of the two in a rat Masquelet model.

Methods: Sprague Dawley rats (n=40) were randomized to four treatments; A) scaffold, B) BMP-7, C) BMP-7+scaffold and D) BMP-7+scaffold+systemic bisphosphonate. BMP-7 putty was prepared mixing 0.33 g of Osigraft (Stryker; =1 mg BMP-7), and 65 mg carboxymethyl cellulose (CMC). The animals in groups B, C and D received 25 µg BMP-7. The scaffold used was Bonesave (Stryker) in groups A, C and D. Zoledronate (Novartis, Basel, Switzerland) was administered as a single subcutaneous injection of 0.1 mg/kg two weeks after grafting. The RatNail XL (RISystem, Davos, Switzerland is a locked femoral nail with a length of 39 mm and a diameter of 1.6 mm. A saw guide was used to create diaphyseal defects of 6 mm. For spacer fabrication, a two- component epoxy filler (Plastic Padding Elastic, Henkel, Düsseldorf Germany) was cast and cut to 6 mm pieces. Each spacer was halved longitudinally to make implantation easier.

Each animal was operated twice, four weeks apart. A medial para-patellar incision was made, the medullary canal reamed, the nail introduced and distal locking performed. With a saw guide and a Gigli
wire saw, the femur was cut at two levels. The cut bone segment was replaced by two spacer halves, held together around the nail with a non-resorbable suture (Fig). The second operation was performed four weeks later. The membrane was incised longitudinally and the two spacer halves were carefully removed. The defect was filled/grafted according to the protocol and the membrane approximated.

11 weeks later the rats were killed and the bones explanted for radiography, manual assessment, micro-CT, histology and Fourier Transform Infrared spectroscopy (FTIR). The study was approved by the local animal ethics committee at Lund University

**Results:** Radiography and manual assessment; Isolated scaffold (group A), were all rubbery to palpation and failed to produce radiographic healing or rigid union in any of the rats. Isolated BMP-7-treatment (group B), led to radiographic healing and rigid union in 7/10 samples, two with visible fracture lines. BMP-7+scaffold (group C) induced radiographic healing and rigid union in 10/10 samples, nine with visible fracture lines. BMP-7+scaffold+systemic bisphosphonate treatment (group D) led to radiographic healing and rigid union in 9/10 samples, one with visible fracture line (Fig and Table).

**Micro-CT**

Both quantitative and qualitative differences were noted between the groups. The total callus volume (TVc) of the two groups receiving combined treatment with BMP-7+scaffold (groups C and D) was greater (p < 0.001) compared to groups A and B (Table 2). The volume of highly mineralized bone (BVhigh) in group D (BMP-7+scaffold+systemic bisphosphonate) was greater compared to groups A, B (p < 0.001) and C (p < 0.01). In group D (BMP-7+scaffold +systemic bisphosphonate) both callus volume and bone volume fraction increased.

**Histology:** In group A, no bony callus was seen. Group B displayed bone tissue formation throughout the previous defects with bridging bony callus. In group C, most of the scaffold was resorbed and there were signs of resorption with large vacuoles in the newly formed bone. In group D, the calluses appeared larger compared to the other groups.

**FTIR:** When comparing the molecular composition within each sample, the mineral to matrix ratio and the collagen maturity were significantly lower in the callus region than in cortex for all samples. The acidic phosphate content was significantly higher in the callus regions than in the cortex. Due to the small number of samples per group, no statistical comparison was conducted between groups.

**Discussion:** We present an animal model of the induced membrane technique in which we replace the autograft with a synthetic composite graft combining a bone substitute, BMP-7 and bisphosphonate. A synthetic graft would eliminate autograft supply issues and donor site morbidity. To our knowledge a murine Masquelet model with purely synthetic grafts has not previously been used to study healing of critical size defects.

In clinical practice the PMMA spacer is cast during surgery, which was not possible in our model and a premade spacer was used. An autograft group, the current standard for Masquelet procedures, lacked
since harvest of sufficient amount of cancellous autograft from rats was not possible. Technical
difficulties with the nail extraction, due to ingrowth around the nails and the locking bolts, precluded
mechanical testing.

BMP has previously been combined with synthetic bone substitutes. In a canine ulnar defect model,
rhBMP-2 mixed with biphasic calcium phosphate ceramic granules showed a higher histologic union
grade than rhBMP-2 mixed with allograft(6). Masquelet noticed resorption of the graft in follow up
radiographs and described cases with late deformity, after apparent radiographic consolidation. These
observations lead the Masquelet group to stop using BMP in conjunction with autograft. By speculation,
these adverse events can be explained by the previously mentioned paradoxical resorption caused by
the BMPs. The BMP mediated osteoclastic activation can be counteracted by bisphosphonates(3), which
delay remodeling.

**Significance:** This study utilized a novel rat Masquelet model and showed that a synthetic scaffold
together with BMP-7 and zoledronate can heal a critical size defect.

<table>
<thead>
<tr>
<th>Healing rate</th>
<th>Ascaffold</th>
<th>BBMP-7</th>
<th>CBMP-7+scaffold</th>
<th>DBMP-7+scaffold+BP</th>
</tr>
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<tbody>
<tr>
<td>radiographically totally healed</td>
<td>0/10</td>
<td>7/10</td>
<td>10/10</td>
<td>9/10</td>
</tr>
<tr>
<td>still visible radiographic lines</td>
<td>n/a</td>
<td>2/7</td>
<td>9/10</td>
<td>1/9</td>
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Fig. Radiographs from groups A) scaffold, without bridging callus; B) BMP-7, healed with bridging callus; C) BMP-7+scaffold, healed with bridging callus but visible fracture line proximally; D) BMP-7+scaffold+bisphosphonate, healed with bridging callus without visible fracture lines.