Altering Spacer Material Affects Bone Regeneration in the Masquelet Technique

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INTRODUCTION: The Masquelet or Induced Membrane technique is a two-phase surgical technique to treat segmental bone loss. Phase one requires that cement spacer (plastic poly methyl methacrylate, PMMA) is placed into the defect area. Over time the spacer is encapsulated with a foreign-body membrane which is utilized to protect and nurture a bone graft implanted during the second phase surgery. The membrane facilitates graft consolidation, with subsequent limb salvage, in situations that otherwise would fail due to graft resorption or necrosis (i.e. defects 6 to 20+ cm).

The technique has historically only been performed with a PMMA spacer. Decades of implant research has established that foreign-body membrane formation is highly dependent on an implant’s material. For example, titanium (Ti) generally evokes a thinner, more osteogenic membrane than plastics or stainless steel. Also, increasing the implant surface hydrophilicity, decreases membrane thickness. It is unclear what, if any, effect this could have on subsequent bone regeneration. The goal of this project was to determine if spacers made of Ti or polyvinyl alcohol sponge (PVA) would have any effect on membrane formation and bone regeneration. We hypothesize that Ti and PVA spacers, which are more hydrophilic, would alter membrane thickness and increase regenerative factors expression thus improving bone regeneration.

METHODS: All experiments were conducted with the approvals of our institutional IACUC board (protocol #2451). An externally stabilized, critical-sized defect (6mm) was created in the right femora of ten-week-old, male Sprague Dawley rats (Charles River). The defect was filled with a pre-fabricated cylindrical spacer made of either PMMA (PalacosR bone cement, Zimmer), medical grade Ti (McMaster-Carr), or PVA sponge (80um pore size, Shima). Spacers made of PMMA and Ti were roughened with 1200 grit sand paper to normalize surface roughness and increase hydrophilicity. Four weeks later, the animals were euthanized for tissue harvest or underwent a second surgery to implant freshly harvested allograft from an unoperated cohort animal. Limbs harvested at this time were fixed, scanned with microCT (Skyscan, 10um voxel size), and processed for cryosectioning and histology. MicroCT quantified the total volume of new bone growing over the spacer. Serial histological sections were picrosirius red stained to measure membrane thickness or processed for IHC of key regenerative factors typically analyzed for this technique (BMP2, IL6, TGFβ, and VEGF). To control for background fluorescence, the intensity of each sample’s negative control was subtracted from other IHC intensity values. Animals that underwent the second surgery were euthanized 10 weeks later. Both femora were harvested with surrounding tissues and stored at -20°C until the day before they were subjected to mechanical testing. Then the operated femora were scanned with microCT (10um voxel) to quantify bone quantity, morphology, and density in the defect. The microCT reconstructions and gentle manual manipulation were used to determine which samples could be stripped of soft tissues and undergo mechanical testing.}

RESULTS: After 4 weeks of spacer implantation, PMMA and Ti spacers created membranes of similar thickness (42.4±6.8um and 39.2±6.6um, respectively, n=4-5/group, Fig 1 A-B). In contrast PVA spacers did not create a distinct membrane. Matrix and cells infiltrated the spacer and muscle tissue appeared to directly appose the spacer (n=5, Fig 1C). All of the factors analyzed via IHC were elevated in PMMA membranes, but there are currently no statistically significant differences (n=2-3/group, Fig 1D). Both PMMA and Ti spacers had bone extending from the bone ends to partially cover the spacer while PVA spacers did not (n=4-6/group). In the PMMA and Ti groups significantly more extended from the proximal side than the distal (10mm vs 6mm, p<0.03, Fig 2A). After 10 weeks of graft implantation, PMMA animals had significantly higher BV/TV than PVA animals (PMMA: 0.40±0.05, Ti: 0.37±0.07, PVA: 0.32±0.09). There were no other significant differences between groups as measured by microCT (n=7-8/group). However, when the 3D reconstructions were evaluated there were marked differences in bone appearance. Six animals in the PMMA group had clearly remodeled the graft and new trabecular bone bridged the defect (Fig 2B). However, when the Ti and PVA groups only 2 and 1 animals, respectively, had healed similarly. In the others, some appeared to be filled with unremodeled graft (Fig 2C). In others the bone in the defect appeared globular and did not bridge the defect (Fig 2D). Gentle manual bending with the soft tissues intact proved that these latter two types of samples were insufficiently stiff for mechanical testing. So mechanical testing was not performed on any samples, and thus, the results were fixed for future histological analysis.

DISCUSSION: Contrary to our hypothesis, using a titanium spacer did not alter membrane morphology or new bone encapsulation over four weeks of implantation. This may be due to the relatively rough surface finish that was applied. These membranes were much thinner than previously reported so a minimal threshold may have been reached for both materials. On the other hand, PVA spacers, which are extremely hydrophilic, thinned membrane formation to a point that it was not distinct and did not permit bone extensions. Regenerative factor expression in the membrane is similar whether PMMA or Ti is used. Although there is a trend for increased factor expression with PMMA spacers which may reach significance as additional samples are processed. Despite the similarities between PMMA and Ti evoked membranes and differences in PVA evoked ones, PMMA was the only material that consistently repaired the bone defect in the distal end to partially cover the spacer while PVA spacers failed to adequately regenerate bone in the defect to bear even minor loads. Additional research is needed to identify the critical differences between membrane environments causing the divergent functional outcomes.

There could be differences in regenerative factors, barrier properties, or vasculature that were beyond the scope of these studies.

SIGNIFICANCE: The Masquelet or Induced Membrane technique is a surgical treatment to regenerate large amounts of bone and salvage limbs. It is currently unknown why the treatment is successful in very challenging healing environments. This research is a first step towards identifying critical membrane characteristics and elucidating the biological mechanisms.

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