Bone wax spacers increase induced membrane thickness and vessel more than PMMA spacer in Masquelet technique

Takushi Nakatani[1], Hajime Mishima[1], Sho Tostuka[1], Ryunosuke Watanabe[1], Norihito Arai[1], Yohei Tomaru[1]
Hisashi Sugaya[2], Tomofumi Nishino[1], Masashi Yamazaki[1]
[1]University of Tsukuba, Ibarki, Japan [2]Tsukuba University of Technology
Nk-taxi-@hotmail.co.jp

Disclosures: Takushi Nakatani(N), Hajime Mishima(N), Sho Tostuka(N), Ryunosuke Watanabe(N), Norihito Arai(N), Yohei Tomaru(N), Hisashi Sugaya(N), Tomofumi Nishino(N), Masashi Yamazaki(N)

Abstract body using the following format: abstract information must not appear on or outside the margins. Abstracts must be created in a Word document formatted to 8.5" x 11" (21.59cm x 27.94cm) with the following margin settings: Top & Bottom 0.75 inch (1.91cm); Left & Right 0.75 inch (1.91cm). Font should be 10 points for the Title and 8 points for all authors, institutions, disclosures, and body sections. Times New Roman is the only font approved for use. (If noting a brand name of a product you used, please do not list the city, state or country.)

INTRODUCTION: The treatment of critical bone defects is a challenging condition. Masquelet reported a new treatment for critical bone defects in 2000. The Masquelet procedure is a two-stage treatment in which the bone defect is filled with polymethylmethacrylate (PMMA) bone cement in the first stage. After 4-6 weeks, the PMMA spacer is removed and an autologous cancellous bone graft is placed around the PMMA spacer, forming an induced membrane due to the foreign body reaction. Induced membrane promotes remodeling of grafting bone and lead to bone union. So far, PMMA spacers have been used in the Masquelet technique, but it is not known whether there are more useful spacers. Bone wax is widely used as a hemostatic material for bone. On the other hand, inflammation induced by foreign body reaction and fibrous granuloma formation have been frequently reported as complications as side effects of bone wax. Fibrous granulomas and induced membranes occur by the same mechanism by a foreign body reaction. We hypothesized that in the first stage of the Masquelet procedure, the induced membrane formed with bone wax as a spacer would have a different membrane morphology compared to the induced membrane formed with a PMMA spacer.

METHODS: Individuals were randomly assigned so that each group consisted of six rats. A 6-hole 1.5 mm titanium plate (Synthes, Dübendorf, Switzerland, VA-LCP hand plate) was placed on the right femur. Two cortical screws were used to fix the plate proximally and two distally. A 10 mm critical size bone defect was created with a bone saw and a spacer was inserted. Fascia and skin were sutured with 3-0 absorbable suture. Animals were euthanized at 3 and 6 weeks after surgery, induced membrane were harvested. Induced membrane was fixed with 4% paraformaldehyde. After fixation, the tissues were embedded in paraffin.Paraffin-fixed induced membranes were thinned to 3 μm and stained with hematoxylin and eosin (HE). A computerized imaging system (BZ-X710, KEYENCE, Osaka, Japan) was used to image the tissues: the entire tissue was observed at 4× magnification, six fields of view were determined for tissue evaluation, shifted by 60°, and images were taken at 10× magnification. Evaluation included thickness of each inner and outer layer at three locations in an image. Vascular endothelial cells were immunostained with CD31 antibodies to assess the number of vessels in the induced membrane. The area of the vessel lumen was measured and the ratio of vessel area to membrane area was calculated.

RESULTS SECTION: The median thickness of the inner layer removed at 3 weeks postoperatively was 93 μm for bone wax and 26 μm for PMMA, a significant difference ($p<0.01$). The thickness of the inner layer at 6 weeks was also 58.5 μm for bone wax and 23 μm for PMMA, with significantly thicker inner layers for bone wax ($p<0.01$). The outer layer was significantly thicker with bone wax 232 μm compared to PMMA 50 μm at 3 weeks ($p<0.01$). When the same spacers were compared over time, bone wax showed significant thinning over time in both the inner and outer layers (3 weeks; $p<0.01$, 6 weeks; $p<0.01$), while PMMA showed thinning over time in the inner layer ($p<0.01$).The vessel area ratio in the membrane was 3.4 % after 3 weeks of bone wax, 2.8 % after 3 weeks of PMMA, 1.9 % after 6 weeks of bone wax and 1.7 % after 6 weeks of PMMA. Statistically significant differences were found between bone wax 3 and 6 weeks ($p<0.02$) and between bone wax 3 weeks and PMMA 3 weeks ($p<0.04$), but not between the other groups.

DISCUSSION: The Masquelet technique is a new treatment modality in the treatment of critical bone defects, but the pathophysiology remains unclear: induced membranes are formed as a result of an inflammatory response due to a foreign body reaction to the PMMA spacer. The composition of the induced membrane formed differs depending on the type of spacer. A possible reason for this is that macrophages, which play an important role in the foreign body response, may experience different degrees of inflammation depending on the roughness of the material surface they recognize. This is because macrophages have an approximate diameter of 10-20 μm and larger surface features are perceived as flat by the cells. Bone wax has also been reported to induce inflammation and fibrous granuloma formation due to foreign body reactions. Immunostaining revealed multinucleated giant cells, foamy macrophages, small numbers of lymphocytes and eosinophilic leukaemic cells, and mast cells. Although foreign body reactions also occur in bone cement, it is known that foreign body reactions such as giant cell and lymphocyte aggregation to bulk cement are more limited than those to particulate cement fragments. The cement spacer in the Masquelet technique is often implanted as a block, which may have reduced inflammatory responses due to foreign body reactions compared to bone wax.

SIGNIFICANCE/CLINICAL RELEVANCE: Bone wax spacers increase induced membrane thickness and vessel more than PMMA spacer after transplantation. Which may further improve the outcome of the Masquelet technique.

REFERENCES: Include references here. (References are Optional)

ACKNOWLEDGEMENTS: Include acknowledgements here. (Acknowledgements are Optional)

IMAGES AND TABLES:

Spacer	Period	Inner layer (μm)	Outer layer (μm)	Vessel area ratio(%)
Bone wax	3wks	93 (53.3-166.5)	232 (151-387)	3.4 (2.4-4.7)
	6wks	58.5 (38-103)	63.5 (34.3-94.5)	1.9 (1.5-3.6)
PMMA	3wks	26 (13.8-53.5)	50 (29-110)	2.8 (1.6-3.3)
	6wks	23 (16.8-31.8)	54 (34-96)	1.7 (1.1-2.3)