Journal of Biomechanics xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

# Masquelet technique: The effect of altering implant material and topography on membrane matrix composition, mechanical and barrier properties in a rat defect model

Natalie Gaio [1], Alice Martino [1], Zacharie Toth, J. Tracy Watson, Daemeon Nicolaou, Sarah McBride-Gagyi *

*Department of Orthopaedic Surgery, Saint Louis University School of Medicine, 1402 S. Grand Blvd, Schwitalla Hall M176, St. Louis, MO 63132, USA*

ARTICLE INFO

*Article history:*
Accepted 18 February 2018
Available online xxxx

*Keywords:*
Masquelet technique
Induced membrane
Animal model
Membrane mechanics
Membrane barrier properties

ABSTRACT

The Masquelet technique is a surgical procedure to regenerate segmental bone defects. The two-phase treatment relies on the production of a vascularized foreign-body membrane to support bone grafts over three times larger than the traditional maximum. Historically, the procedure has always utilized a bone cement spacer to evoke membrane production. However, membrane formation can easily be effected by implant surface properties such as material and topology. This study sought to determine if the membrane's mechanical or barrier properties are affected by changing the spacer material to titanium or roughening the surface finish. Ten-week-old, male Sprague Dawley rats were given an externally stabilized, 6 mm femur defect which was filled with a pre-made spacer of bone cement (PMMA) or titanium (TI) with a smooth (~1 μm) or roughened (~8 μm) finish. After 4 weeks of implantation, the membranes were harvested, and the matrix composition, tensile mechanics, shrinkage, and barrier function was assessed. Roughening the spacers resulted in significantly more compliant membranes. TI spacers created membranes that inhibited solute transport more. There were no differences between groups in collagen or elastin distribution. This suggests that different membrane characteristics can be created by altering the spacer surface properties. Surgeons may unknowingly effecting membrane formation via bone cement preparation techniques.



## 1. Introduction

The Masquelet or Membrane Directed Bone Formation technique (MDBF) is a newer two-step procedure to address segmental bone defect reconstruction (Aurégan and Bégué, 2014; Chadayammuri et al., 2015; Giannoudis et al., 2011; Masquelet and Begue, 2010; Taylor et al., 2012). The procedure has shown promise in addressing a wider clinical need while also providing a less arduous treatment regime than distraction osteogenesis (Aurégan and Bégué, 2014; Giannoudis et al., 2011; Gouron, 2016; Taylor et al., 2012). During the first phase, a bone cement (polymethyl methacrylate, PMMA) spacer is implanted where bone regeneration is desired. Over the following weeks to months a foreign-body or 'induced' membrane encapsulates the spacer (Aho et al., 2013; Bosemark et al., 2015; Christou et al., 2014; Cuthbert et al., 2013; Fischer et al., 2016; Gouron et al., 2014; Gruber et al., 2016, 2013, 2012; Henrich et al., 2013; Klaue et al., 2009; Liu et al., 2013; Luangphakdy et al., 2017; Nau et al., 2016; Shah et al., 2017; Viateau et al., 2006; Wang et al., 2015). Then a second surgery is performed to remove the spacer leaving the membrane in place. The membrane compartment is filled with morselized bone graft material which mineralizes over the following months independent of defect size (Karger et al., 2012; Masquelet and Begue, 2010).

There are three main theories for the MDBF technique's success: (i) the membrane's pre-established vascular network helps revascularize the graft quickly preventing necrosis, (ii) the membrane secretes factors to modulate cell behavior and promote regeneration, (iii) the membrane serves a barrier to prevent soft tissue invasion and resorption (Giannoudis et al., 2011; Taylor et al., 2012). However, none of these hypotheses have been tested nor have the effects of procedural alterations been thoroughly assessed.

Decades of previous implant research has shown that implant surface properties impact foreign-body membrane development (Franz et al., 2011; Kenneth Ward, 2008; Richards, 2007). Different spacer materials and topologies adsorb different proteins thus changing the original matrix formed around the spacer and thus the cells it attracts (Richards, 2007). All objects implanted in the

* Corresponding author.
*E-mail address:* Sara.McBrideGagyi@health.slu.edu (S. McBride-Gagyi).
[1] Co-first authors.

https://doi.org/10.1016/j.jbiomech.2018.02.026
0021-9290/

body that cannot be degraded will eventually be enveloped in a foreign-body membrane to effectively wall it off from the body (Franz et al., 2011; Kenneth Ward, 2008).

Titanium (TI) implants have become the most favorable biomaterial used for orthopaedic implants because TI induces a relatively thin membrane and promotes osteogenic factor expression and enhances osseointegration (AOTrauma, 2013; Geetha et al., 2009; Goriainov et al., 2014; Nuss and von Rechenberg, 2008). Plastics, like the PMMA used for the MDBF technique, have been shown to produce thicker membranes when implanted into bone (Nuss and von Rechenberg, 2008). Perhaps equally important as spacer material in influencing foreign body membrane formation is spacer topography (Franz et al., 2011; Goriainov et al., 2014; Nuss and von Rechenberg, 2008; Richards, 2007). Roughened implants have been shown to provide more traction, decreasing tissue motion and resulting in the formation of a thinner membrane (Nuss and von Rechenberg, 2008). Thin membranes are advantageous in the context of orthopaedic implants because they allow better implant integration into surrounding bony tissue, preventing surrounding bone necrosis, resorption, or fracture (Geetha et al., 2009). However, in the context of MDBF, membrane properties that may be advantageous to bone regeneration and healing have not been identified. It is possible that altered spacer surface properties could positively or negatively affect membrane formation and ultimate healing outcomes.

Controlling for variables such as implant material and topography may be important, as these factors both affect the initial protein matrix formed around the implant, which in turn affects cellular adhesion and matrix formation (Nuss and von Rechenberg, 2008; Richards, 2007). The matrix composition affects tissue mechanics which could in turn mediate cellular behavior on both the cell and tissue length-scales (Green et al., 2014). At the cell length-scale, the cellular matrix's elastic properties impact stem cell lineage differentiation and phenotypic expression (Discher et al., 2005; Engler et al., 2006; Sharma and Snedeker, 2010; Shin et al., 2013). At the tissue length-scale, exogenous mechanical forces have been shown to modulate cellular behavior (Califano and Reinhart-King, 2010; Chan et al., 2010). This is especially true for cells related to chondro- and osteogenic processes (Bonewald and Johnson, 2008; Chan et al., 2010; Galli et al., 2010; Glatt et al., 2016; McBride et al., 2008; McBride and Silva, 2012). Changes in membrane compliance could alter how whole bone forces are transduced to individual residing cells and indirectly modulate bone repair.

The induced membrane is also theorized to affect the cells within the defect by providing a barrier between the graft and surrounding soft tissues (Dimitriou et al., 2012; Giannoudis et al., 2011; Taylor et al., 2012). Thus, by altering the implant material and surface topography, diffusion could be varied. Thinner or less collagenous membranes may decrease diffusion time and allow movement of more/larger particles. This would impact both factor influx to the graft from surrounding soft tissues as well as factor efflux from the tissue compartment. Thus, local concentrations of positive and negative biochemical regulators could differ during the second treatment phase based on the membrane environment established during the first treatment phase.

Based on the understanding that implant material and topography can alter membrane morphology, we hypothesized that altering spacer material and topography will alter the matrix composition of the membrane in the MDBF milieu. In turn, the altered matrix composition will likely impact the mechanical properties of the induced membrane, including tensile and shrinkage properties, as well as barrier properties. Since it has been shown that TI and roughened implants produce thinner membranes, we expect these membranes to be inferior in matrix composition, mechanical properties, and barrier function.

## 2. Materials & methods

### 2.1. Animal model

10-week-old, male Sprague Dawley rats (Charles River, Wilmington, MA) were used for all experiments. Our Institutional Animal Care and Use Committee approved all procedures (protocol #2451). Euthanasia via carbon dioxide asphyxiation was performed following American Veterinary Medical Association 2013 guidelines (20–30% gradual replacement).

### 2.2. Surgical procedure

Phase one of the Masquelet technique (implantation of external fixator and spacer) was performed on all animals (Fig. 1, N = 120 for all studies). After installing an external fixation device in the right femur, a 6 mm long defect was created at approximately the bone mid-shaft. Animals were then randomly assigned to one of 4 spacer groups (PMMA Smooth, PMMA Rough, TI Smooth, or TI Rough) (For spacer fabrication and surgical details see supplemental section). Smooth spacers had surface roughness of approximately 1 μm while rough spacers had an estimated 8 μm surface roughness. These values were chosen based on previous studies (Goriainov et al., 2014) and the relative size of osteoblasts and macrophages (10–20 μm) (Krombach et al., 1997). If the larger texture is too big, the cells perceive it as a flat surface, and thus may not behave differently.

### 2.3. Immunohistochemistry (IHC)

In order to better understand the matrix protein composition, semi-quantitative IHC assays for collagen Type 1 and elastin (n = 7–11/group, N = 36) were performed (details in supplemental section). Four weeks after implantation, the operated limb was harvested, fixed, and processed for cryosectioning. Two serial sections per animal were processed for IHC for collagen type 1 (ab34710, Abcam, Cambridge, MA) or elastin (ab21610, Abcam). A third section served as a no primary antibody negative control. A fourth section was stained with picrosirius red and alcian blue and imaged under polarized light to assist in distinction of each membrane layer.

IHC sections were imaged under fluorescent light (Leica DMI4000B, Leica Microsystems, Buffalo Grove, IL). ImageJ (NIH, Bethesda, MD) was used to segment the non-birefringent and birefringent layers, and a MATLAB code was used to find the average green fluorescent intensity in each region. Then, the average green fluorescent intensity of the corresponding region in each animal's negative control was subtracted from the experimental sections' values to control for tissue auto-fluorescence and non-specific binding.

### 2.4. Tensile testing

Four weeks post-operatively, membranes were harvested for mechanical tensile testing (n = 7–9/group, N = 34, details in supplemental section). Briefly, as much overlying muscle as possible was removed and the membrane was incised longitudinally to create a flat sheet approximately 6 mm tall (axial direction) and 10 mm wide (circumferential direction) (Fig. 2A-B). Each membrane was split into two pieces (Fig. 2B). One piece was stretched in the axial direction while the other was stretched in the circumferential direction (1 mm/min, MTS Criterion 42 with 100 lb load cell, 10 Hz, Fig. 2C). Each sample's initial dimensions in the testing grips were measured by foil gauge (thickness) or calibrated images (minimum width and gauge length).

Please cite this article in press as: Gaio, N., et al. Masquelet technique: The effect of altering implant material and topography on membrane matrix composition, mechanical and barrier properties in a rat defect model. J. Biomech. (2018), https://doi.org/10.1016/j.jbiomech.2018.02.026