## Osteogenic, Stem Cell, and Molecular Characterization of the Human Biomembrane ("Induced Membrane") from Trauma Patients

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Purpose: The biomembrane (induced membrane) formed around polymethylmethacrylate (PMMA) spacers has great value as reflected in its clinical application in the Masquelet technique. Few studies, however, have evaluated cellular, molecular, or stem-cell features of the human biomembrane. The objective of this study is to evaluate and characterize the human biomembrane in terms of its osteogenic, stem cell, morphologic, and molecular characteristics. We hypothesize that a better understanding of its biologic properties will lead to development of methods that can optimize long-term functional outcomes for traumatic limb salvage/military amputee patients.

Methods: Following IRB approval, biomembrane specimens were obtained from 12 surgeries (11 patients) with complex fractures (mean age 42.7 ± 13.2 years; 3 females, 8 males). Biomembranes from 8 tibias and 2 femurs were processed for routine morphology and molecular analysis or
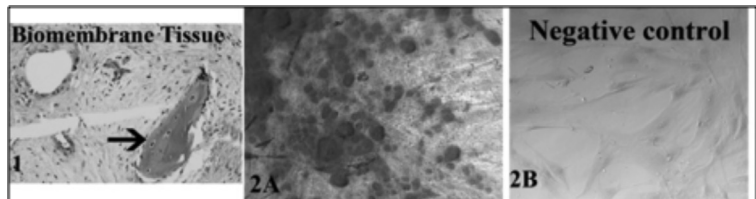
Table 1. Stem Cell Potency of Human Biomembrane Cells			
	Osteogenic differentiation	Chondrogenic differentiation	Adipogenic differentiation
% (#/total)	70% (7/10)	90.9% (10/11)	90% (9/10)

minced and utilized for monolayer cell culture to determination of the presence of stem cell populations. Cells were tested for their ability to differentiate into osteoblasts, chondroblasts and adipose cells using accepted differentiation criteria employing differentiation media (Lonza) and alizarin-red staining of calcified nodules formed by osteoblasts, micromass formation by chondroblasts, and adipocyte formation. The GCOS Affymetrix GeneChip Operating System was used to determine gene expression. Data were normalized and GeneSifterTM web-based software used to analyze microarray data. Statistical significance was determined using the Student t-test (two-tailed, unpaired, $P \leq 0.05$ as the significance level).

Results: Average duration of the PMMA spacer in vivo was 13.5 weeks (range, 6-21). Trabecular bone was present in 33.3% of the biomembrane specimens (Fig. 1, arrow). Biomembrane morphology showed high vascularity and collagen content; all specimens showed positive immunologic presence of bone morphogenetic protein 2 (BMP2) and RUNX2 in the biomembrane stroma. Differentiation of stem cells is shown in Table 1 and osteogenesis (alizarin-red staining of calcified nodules) in Figure 2A. Positive osteogenesis was found in cells from patients with PMMA present for 6-17 weeks (mean, 13.4 weeks). Molecular analyses compared 3 older (mean age, 56.7 years) versus 3 younger patients (mean age, 33.6 years). Biomembranes from older patients showed significant upregulation of aldehyde oxidase

1 (a producer of hydrogen peroxide/superoxide, $P = 0.03$) and type I collagen ($P = 0.008$), and significant downregulation of matrix metalloproteinase 13 ($P = 0.03$) and tenascin XB (an extracellular matrix protein, $P = 0.01$).

Conclusion: Stem cell differentiation data showed greater variability in pluripotency for osteogenic potential (70%) versus chondrogenic or adipogenic potentials (90.9 and 90%, respectively). Due to the importance and increased use of the Masquelet technique in complicated large bone defects, analysis of data such as these is valuable because it leads to improved understanding of the human biomembrane's osteogenic potential.