Δ Biological Activity of Human-Induced Membranes: Differences Between Anatomical Sites are More Important than Time Interval Between Stages

Vaida Glatt, PhD*; Anna Woloszyk, PhD; Kevin Tetsworth, MD*
University of Texas Health SA, San Antonio, Texas, UNITED STATES

Purpose: Despite reports documenting the broad clinical appeal and widespread use of the Masquelet induced-membrane technique, key questions remain unanswered regarding the best time for second-stage surgery, and to what extent the anatomical location influences the biological response. The goal of this study was to examine the biological activity of human-induced membranes with respect to both their anatomical site and the length of the interval between stages.

Methods: Membranes were harvested from clinical cases of bone defects managed using the Masquelet technique, returning for the second-stage surgery between 4 and 20 weeks (as dictated by clinical considerations). Three groups were defined: 'Early' (≤ 6 weeks), 'Intermediate' (6-12 weeks), and 'Late' (≥12 weeks). Biopsies of induced membranes and control samples (normal fascia and periosteum) were collected from femoral and tibial defects (n = 5-8/per group). Samples were assessed using RNA sequencing. Histology is in progress. Comparisons were made between femur and tibia, and corrected for time differences between stages.

Results: Significant differences were observed between membranes and fascia within all of the groups tested, however, there were no differences identified between membranes and periosteum. Transcriptional regulators involved in osteogenic differentiation (TGFB1 [transforming growth factor β1], BMP2 [bone morphogenetic protein 2], WNT4, RUNX2, TNF [tumor necrosis factor], ERK, GLI1, GLI2, GLI3, IL10 [interleukin 10], IL13) were specifically activated at all stages in femoral membranes (FMs), whereas only ERK, TGFB1, and TNF were activated at the late stage in tibial membranes (TMs). The top 10 upregulated genes were related to activation and migration of bone marrow mesenchymal stem cells (fibroblast activation protein), promotion of osteogenesis (ADAM12, CDH11, MIR21), and formation of osteoblasts (SULF2). The highest expression of these particular genes was observed in the Early group of FMs, whereas in TMs the highest expression was identified in the Intermediate group. The top 10 downregulated genes were principally related to osteoclast function, and they had their lowest expressions in all of the FM groups compared to the TM groups.

Conclusion: Preliminary analysis has confirmed human induced membranes formed around the polymethylmethacrylate (PMMA) spacer closely resembled the biological activity of periosteum, and there was no significant difference identified between them at any of the stages. More importantly, this study indicates the anatomical location appears to have greater influence on the biological response than the time interval between stages. Biological activity increased earlier in FMs compared to TMs. This is not surprising, considering the femur is extremely well vascularized and enveloped circumferentially in thick muscles, a potential rich source of undifferentiated mesenchymal stem cells.

Δ OTA Grant