Biological Activity of Human Induced Membranes: Temporal Differences Between Femoral and Tibial Sites

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What was the question?
Human induced membranes formed in association with femoral defects clinically appear to be more robust than those from tibial defects. The anatomical origin of an induced membrane may be a more important influence than the time interval between stages. This study examined the biological activity of human induced membranes with respect to both their anatomical site and the length of the interval between stages. Specific attention was directed towards identifying temporal changes in the gene expression pattern, tissue morphology, and osteogenic and angiogenic protein localization, considering the differences between tibial and femoral specimens.

How did you answer the question?
Membranes were harvested from 16 clinical cases of bone defects which were managed using the Masquelet technique, returning for the second stage between 4 and 20 weeks. Biopsies of induced membranes (n=16) and control samples (normal fascia; n=16) were collected from femoral and tibial defects. Samples (10x10mm) were morselized, and then stored at -80oC prior to gene expression analysis of relevant growth factors for bone repair using qRT-PCR. Different samples (20x10 mm) were used for histology, and stained with haematoxylin and eosin (H&E). Immunohistochemistry (IHC) was used to localize proteins with osteogenic and angiogenic potential. Comparisons were made between femur and tibia, and corrected for time differences between stages.

What are the results?
Bone-like tissue was observed on the outer layer of the induced membranes with H&E. CD68, a marker of macrophage lineage, was homogenously expressed within the membranes, while in the fascia it was mostly absent. VEGF, a potent angiogenic factor, was consistently expressed in blood vessels of both fascia and membrane. Quantitative analysis revealed the number of active cell nuclei and the average cell density in induced membranes (2.77 x 105 cells/mm3) was more than double when compared to fascia (1.14 x 105 cells/mm3; $p < 0.05$). Gene expression analyses revealed that the growth factors relevant to bone repair were significantly up-regulated in membranes as compared to fascia. Membranes revealed significantly up-regulated cell proliferation, cell-cell and cell-matrix adhesion, chemokines, interleukins, and platelet activation genes in femurs, as compared to tibias, which had more down-regulated genes. Growth factors specific to bone mineral metabolism and skeletal development were similarly expressed in both groups. Femoral membranes were more cellular and vascular at earlier time points, compared to tibial specimens. The optimal gene expression appeared to be between 8-12 weeks for femoral specimens, with maximal expression at 10 weeks. Tibial specimens appeared very similar in many respects, with a 4-week delay that may be related to less soft tissue cover locally. The optimal gene expression appeared to be between 12-16 weeks for femoral specimens, with maximal expression at 14 weeks. Most importantly, membranes from both sites demonstrated significant expression of growth factors relevant to bone healing for a prolonged period, and human induced membranes appear to be highly biologically active for many weeks.

What are your conclusions?

The Masquelet technique has been heralded by some as a revolution in the management of skeletal defects, although others continue to report mixed clinical outcomes. The preferred timing to return for the second stage has been an ongoing topic of considerable debate. Preliminary results from this study suggest the anatomical location of origin of a human induced membrane might be a more important factor in regards to influencing the biological response than the time interval between stages. However, this difference is most apparent in the differential gene expression, as it evolves with time following implantation of the PMMA spacer. Specimens from both femoral and tibial defects exhibited significant biological activity for a prolonged period post-operatively, exceeding previous expectations established from basic science research in small animal models.