

Is the Bioactivity of Induced Membranes Time-Dependent?

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Purpose: The Masquelet technique is a 2-stage surgical procedure for the reconstruction of segmental bone defects. Bone grafting is recommended at 4-6 weeks after cement-spacer implantation assuming the highest bioactivity of the cement spacer induced membranes at this point in time. In clinical practice, this narrow time interval is often not met due to ongoing soft-tissue healing (eg, after free flap surgery). Furthermore, successful treatment has also been reported if bone grafting was performed after several months or even years. Therefore, the aim of this study was the structural and cellular characterization of cement spacer induced membranes and the analysis of membrane bioactivity over time.

Methods: Membranes from 65 patients (35-82 years) were analyzed. Based on the time point of membrane biopsy, 4 groups were formed: group 1, 8-28 days; group 2, 29-49 days; group 3, 50-63 days; and group 4, 78- 113 days. The bioactivity of the membranes was histologically and immunohistologically studied by analysis of protein profiles of membrane lysates using protein microarrays. In addition, after co-culture of membranes with mesenchymal stem cells (MSCs) outgrowth and osteogenic differentiation of MSCs were measured. Osteogenic differentiation was assessed by calcification (alizarin red), alkaline phosphatase (ALP), C-terminal propeptide of procollagen I (CICP), osteoprotegerin (OPG), osteopontin (OPN), osteocalcin (OC), and bone-specific ALP (BAP).

Results: Microarray analyses revealed increased protein expression of angiogenesis factors, inflammatory mediators, and osteoinductive growth factors in Masquelet membrane lysates. The angiogenesis factors showed highest expression in the early time group (8-28 days). The expression of osteoinductive growth factors was comparable with slight variations at all time points. Histologically, increased vascularization occurred especially in the younger membranes (8-28 days). Immunohistochemical analyzes confirmed increased angiogenesis by the expression of angiogenin, CD31, CD34, CD90, CD105, and EMMPRIN. In the group of 29-49 days predominantly fibrotic tissue with parallel collagen fiber orientation was detected. In addition, the osteogenic markers (matrix metalloproteinase [MMP]-9, AP, OP, and OC) and osteoclasts could be detected by TRAP (tartrate-resistant acid phosphatase) staining. MSCs (CD105/CD73) were found at all time points.

Conclusion: The induced membranes showed specific time-dependent, but not significantly different, stages of development with increased angiogenesis and vascularization in the early phases and increased fibrosis in later phases. Comparable osteoinductive bioactivity and MSCs were observed at all time points. Thus, the previously postulated narrow time frame of 4-6 weeks until bone grafting can be questioned. However, further clinical trials are necessary to verify these in vitro results.