

The role of periosteal cells and mesenchymal stem cells in the physiological healing process of long bone defects. Potential regeneration acceleration by autologous cell transplantation.

K. Kaspar, G. Matziolis, G. Kasper, H.J. Bail, G.N. Duda

Research Laboratory, Center for Musculoskeletal Surgery, Charité- University Medicine Berlin.

Introduction: Reconstruction of the periosteum has received little attention in most fundamental research works on the healing of bone defects, partially due to the lack of phenotypic markers for periosteal cells. The exact course of periosteal reconstruction and its influence on fracture healing still remain unclear. Mesenchymal stem cells (MSCs) offer new options for research into the healing of bone defects and their transplantation may help to overcome some of the problems during the treatment of critical healing conditions. We hypothesise that: a) the integrity of the periosteum is essential for bone healing processes, beyond its function as a source of progenitor cells, and b) autologous MSCs have the potential to enhance bone healing in an impaired situation that is known to lead to an atrophic non-union.

Methods: 120 male Sprague Dawley rats (410-460g) (3 groups: sham, non-union, MSC) received an osteotomy of the left femur, which was stabilized with an external fixator. In the sham group, no further intervention was performed. In the non-union and MSC groups, the bone marrow was removed up to the inner K-wires and the periosteum was cauterized 2 mm proximally and distally of the osteotomy. 3 weeks prior to the osteotomy, bone marrow was harvested from the right tibia of each animal and MSCs were cultivated in vitro. Two days post-osteotomy $\sim 2 \times 10^6$ MPCs were injected percutaneously into the osteotomy gap of the MSC group. The non-union animals received an injection of culture medium. Animals were sacrificed 14 or 56 days post-op and evaluated by radiology and biomechanical testing, as well as by histological, histomorphometrical and immunohistochemical analysis.

Results: The mean torsional stiffness was significantly larger ($p < 0.001$) in the sham group than the non-union group ($136.2 \pm 34.5\%$ vs. $2.3 \pm 1.2\%$). No maximum torsional failure moment was measurable in the non-union group. The MSC group showed higher torsional stiffness, but no significant difference ($p = 0.141$) compared to the non-union group ($5.3 \pm 5.6\%$ vs. $2.3 \pm 1.2\%$).

At 56 days, the X-rays of the sham group showed a completely bridged osteotomy and evidence of cortical remodelling. The non-union group showed no callus at the osteotomy site and a partially widened osteotomy gap with rounded, hypodense cortical ends. The MSC group showed partial callus

with some degree of bridging and X-rays similar to the non-union group. Interestingly, most animals of the MSC group had mineralization of the intramedullary cavity at the osteotomy site (Fig. 1), as supported by the histology and histomorphometric data. Whilst the sham group showed a bony bridging of the osteotomy at 56 days, the non-union group displayed bone resorption at the cortical ends and no sign of bridging. The MSC group showed signs of bridging as well as of resorption (Fig. 2).

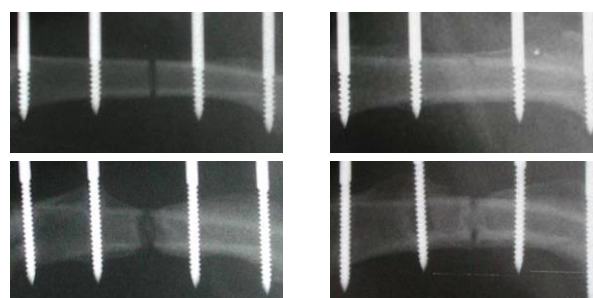


Fig. 1) X-rays of osteotomised femur at 56 days. Top: directly post OP (left) and sham (right); Bottom: atrophic non-union (left) and MSC treated (right).

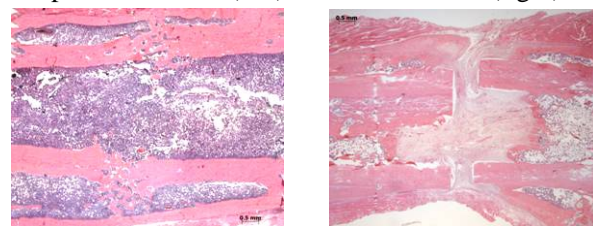


Fig. 2) Hematoxylin Eosin staining at 56 days. Top: sham (left) and atrophic non-union (right); Bottom: MSC treated.

Discussion & Conclusions:

The cauterisation of the periosteum and removal of the bone marrow, in combination with a high stiffness of the external fixator, may create an atrophic non-union under well defined biomechanical conditions and with minimised interaction between the healing zone and the implant. The diversity of the results strongly indicates that the regenerative potential of MSCs has not been exploited consistently enough. Nevertheless the results are promising and demand further investigations, especially during the initial phase after transplantation.

Acknowledgments: This work was supported by the AO Biotechnology Advisory Board, Davos.