Histological analysis of the processes underlying non-healing of a segmental bone defect in a rat model

INTRODUCTION:
Non-union and delayed healing are still clinical problems, despite continued technological advances in the field of orthopedic research and surgery. A better understanding of the failures in biological mechanisms that occur during defect healing is crucial for the development of therapeutics to avoid or treat non-unions and thus decrease patients’ suffering. There are many in vivo experimental models of delayed/non-union that rely on large segmental bone defects. However, most of these models have been used to evaluate different kinds of treatment strategies rather than to characterize and describe the biological changes that occur during defect healing. Our study performed basic research of the pathophysiology in a rat non-union model by various histological staining methods, in comparison to an uneventful healing situation. We hypothesized that bone healing processes during defect repair would be similar to those occurring during uneventful healing, even though a subsequent non-union developed.

METHODS:
A femoral middiaphyseal osteotomy (1mm vs. 5mm gap size) was performed in two groups of female Sprague Dawley rats (weight 250-300g, 12-weeks-old), stabilized with a unilateral external fixator with four titanium threaded 1.2mm diameter pins. The study was approved by the local legal representative (Berlin LAGeSo: G0071/07). Tissue samples (n=8 per group and time point) were fixed with formalin, decalcified in EDTA, embedded in paraffin, and cut into 4µm-thick sections. The temporal and spatial tissue distributions in the calluses were examined at 2, 3, 4 and 6 weeks postoperation by histology, immunohistochemistry and histomorphometry. Collagen fiber content and orientation were studied by the Picrosirius red-polarization method. Statistical comparison between both osteotomy groups for each time point was performed using the Mann-Whitney U-test and the Bonferroni Holm test procedure. Significance was set at the p<0.05 level.

RESULTS:
In the 1mm group at week 6, 5/8 specimen showed a partial bone consolidation with endosteal bony bridging and periosteal cartilage residues (Fig. 1B), and 3/8 specimen an incomplete bony bridging. Cartilage formation increased initially, followed by a slight decrease during the weeks 3 to 6. Furthermore, the healing outcome was characterized by a decrease of fibrous tissue (Figs. 1A+B, 3). Histological analysis of the 5mm group revealed a lack of bony consolidation, resorption of the cortical ends, minimal cartilage formation at week 2, gap filling with fibrous tissue and prolapsed muscle tissue, and bony sealing of the medullary canal covered by a layer of collagen fibers (Figs. 1C+D). Immunohistochemical analysis of this ‘soft sealing’ revealed the presence of type III collagen (Fig. 2C). A remodeling of the bony sealing (woven to lamellar bone) started mainly at week 4, combined with an increased density of transversally aligned collagen fibers with an altered morphology (Fig. 2B). The new bone formation was significantly lower (p<0.008) at week 4 and 6 compared to the 1mm group. Significantly less (p<0.038) cartilage formation was detectable at all investigated time points in comparison to the 1mm group (Fig. 3).

DISCUSSION:
This study demonstrates that during bone defect repair there was a failure in endochondral bone formation, which may have led to a subsequent failure in bone consolidation, and gap filling with fibrous and muscle tissue. Interestingly, the medullary canal was sealed by remodeled lamellar bone. This indicates that despite the lack of bone consolidation, the remodeling process from woven to lamellar bone occurred in the defect repair. But why does remodeling occur even though woven bone could sufficiently seal the medullary canal? This may be explained if the remodeling process of woven to lamellar bone would be an automatic cascade that proceeded during defect progression, despite the lack of general healing processes. The sealing of the medullary canal has been reported in non-healing defects in sheep models as well as in human patients. It may act as a “compensatory stabilization” of the system by means of tissue re-closure. Furthermore, the histomorphometric data have shown that initially, repair processes including cartilage and new bone formation occurred in and around the osteotomy gap. But, cartilage formation declined and endochondral bone formation failed, while the new bone formation proceeded to a minimal extent. We conclude that at an early time point the biological capability for repair was given, but declined over time. Thus, the hypothesis was supported with the repair processes having a similar onset, but a different repair course. Initially (2 weeks or earlier) the same repair processes were present, although less pronounced in the 5mm model than in the uneventful healing model. Over the time course of the investigation, a divergence in “healing” between the osteotomy models was seen.

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