OSTEGENESIS AND VASCULARIZATION OF THE FRACTURE CALLUS ARE AFFECTED BY CONTINUOUS LOCAL APPLICATION OF GROWTH FACTORS IGF-1 AND TGF-BETA 1

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Introduction:
Osteogenesis and vascularization are important elements in fracture healing and bone remodelling. Growth factors are known to be important stimulants of fracture healing [1]. Several in vitro and in vivo studies have indicated a stimulatory effect of growth factors like Insulin like growth factor-I (IGF-I) and transforming growth factor-β1 (TGF-β1) on cell proliferation and differentiation. Osteoinductive and angiogenesis promoting properties have been described for these growth factors in vitro [1,2]. The mechanisms of these growth factors in vivo, however, remain unknown.

Therefore, the influence of IGF-I and TGF-β1 locally applied from poly(D,L-lactide) coated implants on osteogenesis and vascularisation during different phases of fracture healing was investigated in a rat model.

Materials and Methods:
A standardized closed fracture of the right tibia of 5-month-old female Sprague Dawley rats (n= 60) was performed. The fractures were intramedullary stabilized with coated versus uncoated titanium-K-wires.

The following groups were investigated:

G I: implant uncoated
G II: implant coated with PDLLA
G III: implant coated with PDLLA + rhIGF-I (5%) + rhTGF-β1 (1%)

After 5, 10, 15 and 28 days animals were sacrificed. After dissection of the right tibia, the bones were fixed in formalin for 48 h, decalcified and paraffin-embedded. Serial paraffin sections (5 µm) were performed in sagittal plane. Monoclonal mouse antibody against E11 [3], a cell surface marker of mature osteoblasts, and Smooth Muscle Actin (SMA), a marker of the contractile filaments in smooth muscle cells of the vessels, pericytes and myofibroblasts, were used to detect osteoblastic cells and blood vessels. The ABC detection system was coupled with alkaline phosphatase to perform immunohistochemical staining. Slices were counterstained with methyl green to detect cell nuclei and cartilage. Osteogenesis and vascularization of the callus was determined by localisation and distribution of E11- and SMA-immunoreactivity (IR) and judged by semiquantitative score.

Results:
Osteogenesis:
5 days after surgery we found single osteoblasts in the periost at a distance to the fracture gap in the uncoated group I. Only few E11-positive cells could be detected in group I at day 10 and 15, but strong immunoreactivity (IR) at day 28. In group III with the growth factors coated implants several islands of osteoblastic cells could be detected at day 5, especially in the soft callus tissue close to the fracture. The IR increased until day 15 and decreased at day 28.

After 5 days a strong IR of E11-positive cells could also be seen in group II with a continuous decrease from day 5 to day 28. After 28 days the fracture callus was still composed of many osteoblasts and woven bone in group I (Fig.1). Only occasional osteoblasts, less woven bone but narrow like structures were detectable in group II and III at this time point.

Vascularization:
The uncoated group showed an almost constant level of SMA-positive cells from day 5 up to day 28 with less vascularization compared to the PDLLA and growth factor group at all time points. After 5 days the fracture callus showed small vascularization in the uncoated group. High IR of SMA positive cells could be detected in the soft callus tissue close to the fracture gap in the growth factors group III (Fig.2).

Group II and III showed both a high amount of SMA positive cells in the soft callus tissue, and group III also in the woven bone at day 5. The signal decreased continuously in group II. In group III an increase of SMA positive signals was detectable until day 15. In group II, the signal decreased from day 5 to day 15, to rise strongly after that with high IR at day 28.

Discussion:
Different behaviour of osteogenesis and vascularization could be observed in the course of fracture healing among the different groups. Osteoblastic cells showed an earlier and stronger presence in group II and III. After 28 days only few osteoblasts were detectable in these two groups, but still many osteoblasts were detectable in group I. These results lead to the conclusion that osteogenesis is stimulated by local application of the growth factors but also by the PDLLA-coating. The callus of group II and III showed an accelerated and increased ingrowth of vessels compared to group I in the early phase of fracture healing. These effects must be explained by the influence of the growth factors and an yet unknown stimulatory effect of the PDLLA.

The results indicate that the local application of growth factors IGF-I and TGF-β1 from a biodegradable PDLLA-coating influences osteogenesis and vascularization in the early phase of fracture healing. These results are supported by biomechanical and histomorphological data of recent investigations [4]. Further studies should be performed to examine the stimulating properties of the PDLLA-coating and the activity of different growth factors during fracture healing.