EFFECT OF GROWTH FACTORS RELEASED FROM A POLYLACTIDE COATING ON FOREIGN BODY REACTION DURING FRACTURE HEALING

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Introduction
Fracture healing can be stimulated by exogenous application of growth factors. Using porcine and rat models the efficacy of locally delivered IGF-I and TGF-β1 from an implant coating has been demonstrated (1,2). A thin and biomechanically stable biodegradable poly(D,L-lactide) was used to coat implants and serve as a drug carrier. Due to some reports of foreign body reactions caused by polymer screws or membranes, this study investigated the biocompatibility of the implant coating and the locally released growth factors during the time course of rat tibial fracture healing (days 5, 10, 15, and 28 after fracture). Rat monocytes/macrophages cells were detected using an monoclonal antibody against ED1 (compared to CD68 in mice and human).

Material and Method
A standardized tibia fracture of 5 month old Sprague Dawley rats (n=60) was performed and intramedullary stabilized with titanium Kirschner wires, uncoated or coated. Four different time points were investigated (days 5, 10, 15 and 28 after fracture).

Group I: uncoated titanium wire
Group II: PDLLA coated titanium wire
Group III: PDLLA + 50 μg IGF-I & 10 μg TGF-β1 coated wire
n=5 each group and time point

For immunohistochemistry the tibiae were fixed for 2 days in 10% formaldehyde. After decalcification with EDTA the tibiae were embedded in paraffin and 5 µm sections were prepared. Sections were incubated with monoclonal antibody against ED1 (analogue to human CD68) (BM4000, 1:100, DPC Biermann, Germany) followed by a biotinylated rat absorbed horse anti-goat IgG secondary antibody (Vector Laboratory, USA) and Avidin-Biotin-Complex-detection system (ABC-method; Vector Laboratories, USA) coupled with alkaline phosphatase. The slices were counterstained with Methyl Green to detect cell nuclei and cartilage. Incubation of slices without the primary antibody served as negative control.

The slices were analyzed using an image analysis system (Zeiss KS 400, Germany). The total callus area and the stained area was measured in defined ROI.

Statistic: ANOVA, Bonferroni correction (SPSS 10).

Results
At day 5 after fracture monocytes and polynucleated cells stained positive. The cells were predominately located in the medullary cavity (the space between the wire and the endost) and in the newly formed soft callus. There was no difference detectable between the groups.

10 and 15 days after fracture the amount of CD68 positive cells increased in all three groups. Both, in the medullary cavity and the callus a high amount of monocytes and macrophages were detectable. Also, polynucleated cells adjacent to the bone and in resorption lacunae were positive, indicating that CD68 is also expressed by osteoclasts (Fig. 1). At the latest investigated time point, day 28, the number of CD68 positive cells decreased in all three groups.

The quantitative analyses revealed a comparable time courses in the amount of stained cells between the investigated groups at days 5-15. Interestingly, 28 days after fracture significantly more positive cells were measured in the control group in the periosteal callus (Fig. 2a).

Discussion
During the last decades biodegradable polymers become more important in clinical use (3, 4). The general results reveal a good biocompatibility of the used materials and a satisfactory clinical outcome. However, due to reports of sever foreign body reactions resulting in the worst case in osteolysis (5, 6) biocompatibility tests are necessary before clinical use of newly developed polymer applications. This study investigated the biocompatibility of a biodegradable poly(D,L-lactide) coating of orthopaedic implants for local controlled drug delivery in vivo. Previous in vivo studies demonstrated the beneficial effect of the drug carrier analyzing the biomechanical stability, histomorphometry and the early proliferation pattern (1,2,7). Biocompatibility test revealed no differences in the amount and distribution of monocytes/macrophages and osteoclasts in the early phases (d5-15) of fracture healing between the investigated groups. Changes are only detectable between the investigated time points. The pure titanium implant served as control and neither the PDLLA coating nor the incorporated growth factors evoked an increase in the macrophages. Surprisingly, 28 days after fracture the titanium group showed significantly more CD68 positive cells compared to the PDLLA or the PDLLA+ growth factor group.