TIME COURSE OF UNSTIMULATED AND GROWTH FACTOR (IGF-I AND TGF-ß1) STIMULATED RAT TIBIAL HEALING

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Introduction
Locally applied growth factors have shown to stimulate fracture healing mainly in the early phases [1].
Purpose of the present study was to investigate the time course of healing processes during growth factor (GF) stimulated and unstimulated fracture healing in a closed tibial fracture model in rats in order to evaluate possible long term effects of the growth factors.
IGF-I and TGF-ß1 were locally applied using a thin polylactide (PDLLA) coating of intramedullary implants [2].

Material and Methods
A standardized closed fracture of the right tibia of five-month old Sprague Dawley rats (n=180) was performed with a guillotine-like fracture device. The fractures were intramedullary stabilized with uncoated versus coated titanium K-wires.

Following groups were examined:
Group I: uncoated
Group II coated with PDLLA
Group III: coated with PDLLA & IGF-I (50µg)+ TGF-ß1 (10µg)
n=60 each group, sacrifice: 28, 42, and 84 days after fracture
PDLLA: poly(D,L-lactide)

To evaluate the soft tissue trauma the compartment pressure was measured postoperatively in 12 animals using a Micro sensor (Codman® micro sensor, Johnson & Johnson). After fracture of the right tibia and throughout the experimental period x-ray examinations (p.a. and lat.) were performed. After sacrifice both tibiae were dissected for biomechanical torsional testing using a material testing machine (Zwick 1455, Ulm, Germany).
For histological and histomorphometric analyses the tibiae were fixed and embedded in methylmethacrylate. 5µm sections were cut and stained with Safranin O/light green and v. Kossa. The histomorphometry of the calluses was performed using an image analyzing system (Zeiss KS 400).

Results
The compartment pressure increased over a 12 hours observation period and showed a decrease after 24 without differences between the groups:
<table>
<thead>
<tr>
<th>Time [h]</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>[mmHg]</td>
<td>7.3</td>
<td>13.6</td>
<td>17.2</td>
<td>14.6</td>
</tr>
</tbody>
</table>
(physiological pressure of the anterior compartment: 7.4 ± 0.4 mmHg)

No differences in investigated systemic parameter such as body weight, temperature and blood counts were detectable between the groups.

•  Radiology
The animals revealed radiologically a transverse midshaft fracture of the tibia and a fracture of the fibula with a comparable soft tissue trauma.
The healing process showed differences in the radiology 28 and 42 days after fracture between the groups. The growth factor treated group revealed significantly more completely bridged fractures at these time points compared to the groups I and II. After 84 days no differences in consolidation of callus morphology were detectable radiologically.

Radiological score (**p<0.05 Chi²**)

<table>
<thead>
<tr>
<th></th>
<th>Uncoated</th>
<th>PDLLA</th>
<th>IGF-I + TGF-ß1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete bridging</td>
<td>1 3 10 3 5 10 5* 9* 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete bridging</td>
<td>2 2 0 3 3 0 4 1 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No bridging</td>
<td>7 5 0 4 2 0 1* 0* 0</td>
<td></td>
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</table>

•  Biomechanics
The biomechanical strength increased with healing time and showed the highest values after 12 weeks in group I and II.
The animals treated with growth factors revealed a significantly higher maximum load and torsional stiffness compared to the PDLLA and the uncoated groups 28 and 42 after fracture. 84 days after fracture no significant differences were detectable between the groups. The growth factor treated group already reached comparable torsional stability at the early time points compared to the unstimulated groups after 84 days.

Biomechanical testing

Discussion
The radiological, biomechanical and histological investigation revealed a significantly enhanced fracture healing in the growth factor treated group at days 28 and 42.
However, 84 days after fracture all groups showed almost identical parameters without any hypertrophic reactions or differences in systemic parameters. All invested groups were not completely remodelled three month after fracture. The growth factor treated group already reached comparable torsional stability with consolidated fractures and progressed callus remodeling at the early time points compared to the unstimulated groups after 84 days.
This study clearly confirms an enhanced fracture healing due to growth factor application in the early phases without alteration of the healing processes during time course compared to physiological fracture healing.

1 Linkhart et al. 1996 Bone 19S :1-12