IN VIVO MONITORING OF FRACTURE HEALING IN RATS
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Introduction
The healing process can be divided into at least three phases, the early, inflammatory phase, the repair phase and the remodelling phase. Because of the strong correlation between the callus stiffness and the phases of healing, the callus stiffness is a very important parameter for evaluating the fracture healing process. However, in most animals studies, the callus stiffness is only determined at one time point via ex vivo mechanical testing. Therefore, differences of the begin and duration of the repair phase cannot be determined. The aims of this study were (a) to developed a method which allows to continuously determine the callus stiffness in vivo over the whole healing time in rats under a more rigid and a more flexible fixation, and (b) to determine the time to heal, and the duration of the repair phase of the fracture healing process for both fixator configurations.

Methods
The right femurs of sixteen wistar rats were osteotomized and stabilized with either a more rigid or a more flexible external fixation device. The fixator body was instrumented with a stain gauge to measure the deformation of the fixation device. Gait analysis was performed once per week in a gait wheel equipped with a ground reaction force measuring device. Curves was fitted against the in vivo measured strains to determine the course of the callus stiffness over the healing time for the animals by using the curve Fitting Toolbox™ of Matlab (MathWorks, Ismaning, Germany):

\[ \varepsilon = \lambda \cdot \frac{K_F}{K_C + K_F} \quad \text{with} \quad K_C = a \left( 1 + e^{\frac{-t}{b}} \right) \]

\( \lambda \) is a fixation specific factor describing the maximum strain during gait when the callus stiffness is negligible small, \( K_F \) is the Fixation stiffness, \( a \) is the callus stiffness after 12 weeks of healing, which was determined by ex vivo mechanical testing The parameters \( t_0 \) and \( b \) were subjected to the fitting process. For each group, the time to heal (timepoint, when the callus stiffness reached 95% of the stiffness of the intact bones) and the duration of the repair phase (healing time in the range of the callus stiffness between 5 and 95% of the intact bone stiffness) were determined.

Results
There was a statistical significant difference in the healing time (30 days for the rigid and 64 days for the flexible fixated animals) and an increased duration of the repair phase for the flexible fixated animals (Fig. 1), which was 51 days vs. 19 days for the rigid fixated animals.

Discussion
Beside the reliable detection of differences of the fracture healing time between experimental animal groups, this method also allows to identify the begin and duration of the repair phase of the healing process. This provides information about the healing process itself, e.g. an elongated repair phase under flexible fixation. As long as the confidence intervals of the two curves are not overlapping a significant difference on a 5% level between both groups can be expected (in this experiment between 14 and 38 days). The largest difference in stiffness (\( \Delta K_{\text{callus}} > 60\% \)) can be expected between day 24 and day 35.