Non-invasive Monitoring of Fracture-Healing \textit{in-vivo} in an MRI-compatible Animal Fracture Model

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\section*{INTRODUCTION}
Angiogenesis and vascularization are important steps in fracture healing and callus remodelling. Research regarding the biology and stimulation of fracture healing needs to evaluate neoangiogenesis and perfusion in the course of healing.

So far, noninvasive monitoring of these crucial events in the healing process is not available in vivo. In experimental studies, research regarding fracture healing is based on the evaluation of quantity and quality of vascularization and neoangiogenesis in callus formation. The gold-standard in animal studies are histological and immunohistochimiscal assays to observe the stages of fracture-healing evaluating common histological parameters, including callus formation, bone union, marrow changes and cortex remodelling in the course of time or antibody-staining of vessels. An appropriate technique for a non-invasive in vivo measurement of neoangiogenesis during fracture healing has not been established yet.

In this study, a MRI-compatible animal fracture model was developed to determine neovascularization with a special molecular contrast agent for a non-invasive \textit{in-vivo} analysis of bone healing.

\section*{METHODS}
A standardized femur shaft fracture of eight female Sprague Dawley Rats (20 weeks) was stabilized with an intramedullar PLLA (poly-L-lactideacrylate) nail to avoid electromagnetic interferences during MRI-recording with a micro-MRI (Fig1). Radiographs were taken immediately after surgery to examine the location of the fracture and the quality of fixation. The MR-Relaxometrie to evaluate neoangiogenesis is a noninvasive monitoring of microvessel neoangiogeneses was performed by dynamic MRI imaging using ultrasmall superparamagnetic iron oxide (USPIO, Supravist\textsuperscript{®}, Bayer Schering Pharma AG, Berlin, Germany, 80 \textmu mol Fe/kg BW) contrast agents. 7, 14, 21 and 28 days postoperational MR imaging was performed on a clinical 3 Tesla MR system (Intera; Philips, Best, the Netherlands) with a solenoid receive only coil of 70 mm diameter (Philips, Best, the Netherlands). USPIO-induced changes in tissue R2* (\(\Delta R2^{*}\)) were measured with multi echo MR-Relaxometry. Assuming that the SPIO has a steady-state intravascular distribution during MR measurement, the equation can be simplified to a linear relationship between the \(\Delta R2^{*}\) and the perfused blood volume fraction: \(\Delta R2^{*}(t) = k V(t)\) or \(V(t) = \frac{\Delta R2^{*}(t)}{k}\), where \(k\) includes the concentration of the contrast agent in the blood pool and is therefore dose dependent. Parametric \(\Delta R2^{*}\) maps were analyzed with respect to perfusion patterns.\textsuperscript{1} To underline our results, histological and immunohistochimiscal assays were performed. The size, structure and neovascularization of fracture callus is quantified and qualified also at day 7, 14, 21 and 28- each with 8 test animals. The microvessel density (MVD) is quantified with digital image analysis (Olympus BX51 Microscope, Olympus Optical Co., Tokyo, Japan and Image-Pro Plus 5.0 software, Media Cybernetics Inc., USA). Differences between MVD and \(\Delta R2^{*}\) in callus formation are analyzed and correlated using Mann-Whitney U test with significance \(p < 0.05\).

\section*{RESULTS}
The significant increase (\(p<0.05\)) of neoangiogenesis in callus formation with a peak after 14 days and subsidence at day 21\textsuperscript{st} recorded with the MRI is shown in Fig 2. Our data matched with results in literature and with our preliminary histological and immunohistochimical data, which show similar characteristics in vascularization and vessel formation in the course of time during fracture healing.\textsuperscript{2,3}

\section*{DISCUSSION}
Angiogenesis and vascularization during fracture healing can be determined and monitored using this special MRI technique. The MRI-evaluation of quantity and quality of vessel formation and angiogenesis in the fracture callus might be a new non-invasive method for measurement of results and monitoring of fracture-healing \textit{in-vivo} in experimental studies and maybe for clinical application in the future. Furthermore in experimental studies, the amount of rodents sacrificed for evaluation of callus-formation in fracture healing, can be reduced significantly with USPIO-enhanced steady-state MRI as a new tool compared to histology.

\section*{REFERENCES}
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