Relevance to Musculoskeletal Conditions: Although the clinical importance of the vascular integrity of musculoskeletal soft tissue in providing beneficial influence on fracture healing has been established (1), the microvascular and cellular mechanisms accounting for this phenomenon are very poorly understood. Several microcirculatory studies of skeletal muscle and cortical bone blood flow following fracture have been performed, in which diminished cortical blood flow and impaired fracture healing have been linked to skeletal muscle and periostial injury (2). However, these indirect studies, did not directly visualize fracture-induced microcirculatory disturbances and have not demonstrated leukocyte-endothelial cell interactions in skeletal muscle and periostium. To advance our understanding of the early microcirculatory and inflammatory events that mediate the fracture-induced disturbances in nutritive blood flow we have explored the microvascular consequences of closed fracture in periostium and skeletal muscle. We hypothesized that closed tibial fracture adversely affects microcirculation in skeletal muscle and periostium. To test this hypothesis, we aimed to quantitatively assess immediate microcirculatory changes in skeletal muscle and periostium following standardized closed fracture in vivo.

Material and Methods: Standardized closed fracture of left tibia (AO type A 2 & A 3; no significant closed soft tissue damage) in isoflurane-anesthesized (isoflurane 1.5 vol%, N 2O 0.5 l/min and O 2 0.3 l/min) SD-rats (n=14) was induced using a modified weight-drop-technique (2) and intramedullary stabilization by manual insertion of a 1.0 mm k-wire. All procedures were performed with the ethical and according to the NIH guidelines. For hemodynamic monitoring of mean arterial blood pressure (MABP); heart rate (HR) and fluid administration the left carotid artery and right jugular vein were cannulated with PE-catheters. Following fracture the left extensor digitorum longus (EDL) muscle (n=7) and the meta-/diaphyseal tibial periostium (n=7) were surgically exposed for in vivo fluorescence microscopy (IVM) (4,5). Non-fractured rats ( sham; n=14) served as controls, i.e. periostium (n=7) and EDL-muscle (n=7). For IVM of either EDL-muscle or periostium (15 minutes after preparation, i.e. 1 hour after fracture) each tissue was scanned (2mm steps, 8 observation areas. For contrast enhancement of the vascular network and for in vivo staining of leukocytes FITC-dextran and rhodamine 6G was injected intravenously prior to each observation. Microhemodynamic analysis included the determination of microvessel diameters (D in µm), functional capillary density (FCD: length of perfused capillaries per observation area, cm⁻¹), leukocyte-endothelial cell interactions (leukocyte rolling fraction: number of rolling leukocytes as % of the total leukocyte flux; number of sticking leukocytes per mm³ of endothelial surface), microvascular permeability (macromolecular leakage) and red blood cell velocity (Vbac). Volumetric capillary and venular blood flow (VBF, picoliter/sec) was calculated from Vbac and D for each vessel as: VBF=π/4 x D² x Vbac. After sacrificing the rats, the left and right EDL-muscles were harvested for measurement of the wet to dry weight ratio and calculation of the EDL-muscle water content and edema index (EI = exp./contralat. limb).

Results: MABP; HR remained stable with no significant difference between groups.

EDL-Muscle: In non-fractured rats, capillaries were arranged in parallel and straightened in the longitudinal axis of the muscle fibers with no significant microvascular thrombosis, macromolecular leakage or leukocyte adherence. Following closed tibial fracture, a heterogeneous perfusion pattern with severe microvascular dysfunction, including stasis and collapse of capillaries, increase in intercapillary distance, microvascular thrombosis and direct disruption of microvessels with subsequent hemorrhage was found. FCD and Vbac of EDL-muscle following fracture were found to be significantly reduced compared to controls. Fracture-induced microvascular deteriorations were further characterized by a significant hypoperfusion as demonstrated by a marked decrease in capillary VBF. Fracture-induced changes in microvascular diameters displayed a simultaneous vasodilation of capillaries and postcapillary venules. In addition, a significant increase in microvascular leakage) was found, reflecting substantial endothelial disintegration in response to fracture. Analysis of leukocyte-endothelial cell interaction revealed a two-fold increased leukocyte rolling and adherence, mostly restricted to the endothelium of postcapillary venules when compared to non-fractured animals.

Periostium: In non-fractured rats, a homogeneous periostal microcirculation with no capillary dysfunction or leukocyte adhesion was found. Whereas the metabolism a densely meshed capillary network with many intercapillary connections was observed, the arrangements of diaphyseal capillaries, showed a parallel alignment to the tibia axis. Microvascular response of periostium to fracture demonstrated a total microvascular perfusion failure and significantly increased microvascular permeability at the diaphyseal fracture site. Metaphyseal areas remote from the fracture site displayed heterogeneous and severely impaired perfusion with decreased Vbac and scattered capillary thrombosis, increased microvascular leakage and leukocyte activation. Again, endothelial rolling and adherence of leukocytes were increased by two-fold when compared to controls, most pronounced in post-capillary periostial venules. Closed tibial fracture caused a significant increase in EDL-muscle water content and EI, demonstrating fracture-induced skeletal muscle edema formation.

Discussion: The present model permits for the first time direct in vivo visualization and quantification of capillary-function-induced microhemodynamic changes within the surrounding periostium and skeletal muscle. It could be demonstrated that a simple fracture leads to profound microvascular injury, endothelial dysfunction, leukocyte-endothelial cell interaction and tissue edema in skeletal muscle and periostium, also remote from the diaphyseal fracture site. Therefore, we conclude that these findings provide an useful approach for the pathophysiological analysis of tissue-confined microcirculatory disturbances and their local interaction. In conclusion, the present study may have therapeutic consequences in view of developing novel treatment strategies to improve fracture healing by counteracting fracture-induced microvascular dysfunction.


**Dept. Trauma & Reconstructive Surgery, University of Rostock, Rostock, Germany.**