A new reliable model of atrophic non-union in mice
Patric Garcia1,2, Jörg Holstein1,2, Tina Histing1, Tim Pohlemann1, Michael D. Menger2
1Department of Trauma-, Hand- and Reconstructive Surgery, University of Saarland, Homburg/Saar, Germany; 2Institute for Clinical & Experimental Surgery, University of Saarland, Homburg/Saar, Germany

Introduction: Despite our growing knowledge on the mechanisms of fracture healing, non-unions remain a clinical problem. Murine fracture models are of increasing interest in fracture research to study molecular mechanisms, but until now it hasn’t been possible to reproducibly create non-unions in this animal. Thus, the aim of this study was to develop a reproducible murine model to study atrophic non-unions.

Materials and Methods: To prevent fracture healing we created segmental defects of 0.25 mm, 0.8 mm and 1.8 mm in the mouse femur and stabilization was done with an intramedullar pin. To preserve gap width and to achieve rotational stability, additionally an extramedullar metallic clip was implanted bicortically using an operating microscope (pin-clip fixation). Furthermore, the influence of periosteal resection on the healing process was studied in gaps of 0.8 mm and 1.8 mm. Radiological and histological healing was analyzed 5, 10 and 15 weeks after surgery. Radiological and histological healing were defined by bone bridging of both cortices on x-rays or histological sections. Each group consisted of 4-6 animals.

Results: In all animals it was possible to perform a stable pin-clip fixation and to create a gap size of 0.25 mm, 0.8 mm and 1.8 mm, respectively (Fig. 1). After 5 weeks all groups showed predominantly atrophic non-unions. After 10 weeks, 6 out of 6 animals with a gap of 0.25 mm showed radiological and histological union. However, animals with a gap of 0.8 mm or 1.8 mm still showed poor healing, with predominantly atrophic non-unions. Histologically, 2 out of 6 animals in the group with a gap of 0.8 mm and intact periosteum showed bone bridging of the fracture gap. When the periosteum was stripped, 1 out of 4 animals showed bone bridging of the fracture gap. In the group with 1.8 mm gap size and intact periosteum, also 1 out of 4 animals showed histological healing after 10 weeks. Only a gap size of 1.8 mm with periosteal resection resulted in 100% atrophic non-union after this time point (n=5). Non-union formation was confirmed after 15 weeks in animals with gap size of 1.8 mm and periosteal resection, with no signs of progressive repair (n=5). The non-unions observed appeared atrophic, with characteristic histological and radiological features: no fracture bridging with abundant fibrous tissue in the gap, absent callus formation, rounded bone ends, no signs of progressive repair. Of interest, the non-unions were not associated with failure of vascularization. In contrast, the fibrous tissue within the interfragmentary gap showed newly formed microvessels, which were lined by CD31-positive endothelial cells.

Discussion: Previously described fracture studies in mice, mostly reported on healing times between 3 and 5 weeks. However, our pin-clip model with a gap size of 0.25 mm showed delayed fracture healing with bone bridging after 10 weeks. Gap sizes of 0.8 mm represented a critical size defect, with predominantly atrophic non-unions, but were found healed in some animals, irrespective of periosteal resection. Only a gap size of 1.8 mm with periosteal resection resulted in reliable non-union formation, with no signs of progressive repair until the 15th week. Although our pin-clip fixation was able to preserve the gap width over time and to achieve rotational stability, our technique doesn’t result in a stable osteosynthesis, comparable to an osteosynthesis in humans. Thus, a certain amount interfragmentary motion, additionally to the introduced bone defects, could account for the impaired healing process in our pin-clip model. The interfragmentary biological and biomechanical environment is crucial to develop and maintain the healing process. The underlying molecular mechanisms, however, are still unknown. With the use of the herein presented murine non-union model, these mechanisms may be analyzed in future studies. Additionally, the present model could be helpful to evaluate and compare the plenitude of different therapeutic strategies in the treatment of atrophic non-unions. A murine model not only holds the possibility for molecular analyses, but also bears the advantage, compared to large animal models, that a great amount of animals can be studied in a short time period.

Acknowledgements: We gratefully acknowledge the excellent technical assistance of Janine Becker.