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A new technique for internal fixation of femoral fractures in mice: Impact of stability on fracture healing

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Abstract

Mouse models are of increasing interest to study the molecular aspects of fracture healing. Because biomechanical factors greatly influence the healing process, stable fixation of the fracture is of interest also in mouse models. Unlike in large animals, however, there is a lack of mouse models which provide stable osteosynthesis. The purpose of this study was therefore to develop a technique for a more stable fixation of femoral fractures in mice and to analyze the impact of stability on the process of fracture healing. The new technique introduced herein includes an intramedullary pin and an extramedullary metallic clip. Ex vivo biomechanical analysis revealed a significantly higher implant stiffness of our pin-clip technique when compared with previously described intramedullary fixation techniques. In vivo, we studied the course of healing after the more stable fixation with our pin-clip technique and compared the results with that observed after unstable fixation with the pin-clip technique after cutting the clip. After 2 and 5 weeks of fracture healing radiological analysis demonstrated that the more stable fixation with the pin-clip technique results in a significantly higher union rate compared to the unstable fixation. Torsional stiffness at 5 weeks was almost 3-fold of that measured after unstable fixation. Histomorphological analysis further showed that fractures stabilized with the pin-clip technique healed with a smaller periosteal callus area, an increased fraction of bone and a reduced amount of fibrous tissue. Of interest, the pin-clip fixation showed reliable union after 5 weeks, whereas the unstable pin fixation did not regularly achieve adequate fracture healing. In conclusion, we introduce a novel, easily applicable internal osteosynthesis technique in mice, which provides rotational stability after femoral fracture fixation. We further show that a more stable osteosynthesis significantly improves the process of fracture healing also in mice.

Keywords: Fracture healing; Fracture stabilization; Osteosynthesis; Fracture fixation; Mouse; Animal model; Mechano-biology; Biomechanics; Femur fracture

1. Introduction

Fracture healing is a complex, well-orchestrated process, and animal models are essential to enhance our understanding of the mechanisms involved. Whereas in the past large animals like sheep and dogs were chosen for fracture-healing studies, nowadays, smaller animals like mice and rats are used, especially to analyze the molecular aspects of fracture healing. Mouse models are cheap and easy to handle. The possibility of genetic targeting made them preferred animals in a wide field of medical research. Further, due to the great availability of distinct biomedical tools, such as monoclonal antibodies, mice may promise deeper insights into the physiology and pathophysiology of fracture healing. In recent years, mice also came into the focus of trauma research and several mouse fracture studies have been reported, most of them analyzing tibia or femur healing (Cheung et al., 2003; Gardner et al., 2006;

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Hiltunen et al., 1993a, b; Holstein et al., 2007; Isefuku et al., 2004; Manigrasso and O'Connor, 2004; Thompson et al., 2002).

Because mechanical stimuli are well known to play a pivotal role in the course of fracture healing (Augat et al., 2005), stable fixation techniques should also be considered in mice. Most mouse fracture studies used an unstable fixation technique or fractures were even left unstabilized. Some studies have described the use of external fixators for stable osteosynthesis in mice. However, there is a lack of stable internal fixation devices in mice, most probably due to the small size of the animal. The aim of this study was to develop an internal fixation system for femoral fractures in mice, which provides more stability than that provided by the techniques currently used. We further proved the hypothesis that the more stable fixation of femur fractures in mice improves the process of fracture healing when compared to that observed after unstable fixation.

2. Methods

2.1. Animals and specimens

The mice were fed a standard diet with water ad libitum. After fracture stabilization, the animals were killed after 2 and 5 weeks by cervical dislocation and fracture healing was evaluated by biomechanical, histomorphometric and radiological analysis. Each group consisted of 5 or 6 animals (Table 1). All experiments were performed in adherence to the National Institute of Health guidelines for the use of experimental animals and were approved by the German legislation on the protection of animals (permission code K110/180-07).

2.2. Surgical procedure

CD-1 mice with a body weight of 34 ± 3 g were anesthetized by an intraperitoneal injection of 25 mg/kg xylazine and 75 mg/kg ketamine. Under sterile conditions a 4 mm medial parapatellar incision was performed at the right knee and the patella was dislocated laterally. After drilling a hole ($\varnothing = 0.5$ mm) into the intracondylar notch, a distally flattened 24 G needle was implanted intramedullary. Then, the femur was exposed through a lateral approach and an osteotomy was created in the middle of the femur, using a gigly-wire-saw. After reduction of the gap and reposition of the bone ends, a metallic clip, bridging the osteotomy, was implanted ventro-dorsally. This was done with the use of an operating microscope by first drilling a hole ($\varnothing = 0.5$ mm) 2 mm distally of the osteotomy through the lateral third of the femur. Then, a custom-made clip of 4 mm length, made of a 27 G needle ($\varnothing = 0.4$ mm), was implanted through the medullary cavity ($\varnothing = 1.0$–1.2 mm), passing the intramedullar pin ($\varnothing = 0.5$ mm) laterally. After drilling the proximal hole and implanting the clip, the dorsal ends were bent over to avoid secondary dislocation. The pin at the right knee was cut appropriately and wound closure completed the procedure (Fig. 1A). In a second group of animals, the same procedure was performed for osteotomy fixation. However, the functionality of the clip was eliminated, by cutting it in the middle after implantation (Fig. 1B and C).

Table 1
Experimental protocol including groups, time points and parameters of measurements

	2 weeks		5 weeks	
	Biomechanics	Histology	Biomechanics	Histology
Pin-clip	$n = 5$	$n = 5$	$n = 6$	$n = 5$
Unstable	$n = 6$	$n = 6$	$n = 5$	$n = 6$

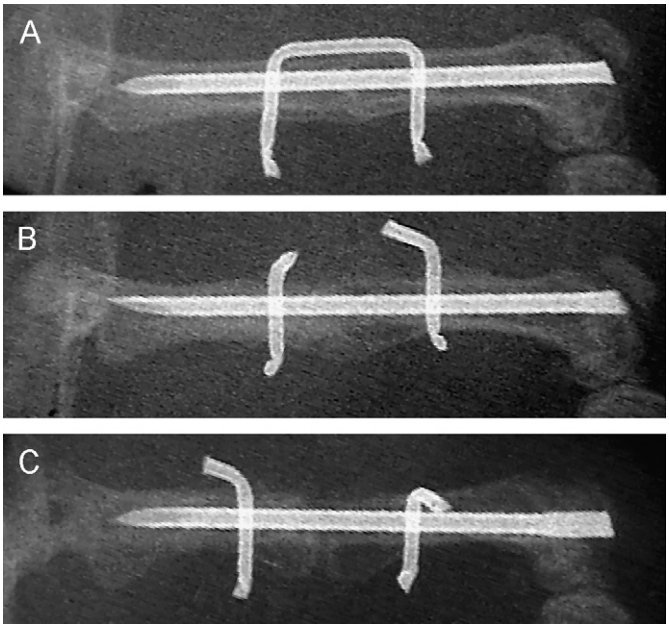

Fig. 1. Representative X-rays of right femora at 5 weeks after pin-clip (A) and unstable (B and C) fixation of the osteotomies. The more stable fixation regularly showed adequate radiological healing (Goldberg 2, A). Unstable fixation resulted frequently in only possible radiological union (Goldberg 1, B) or, occasionally, in radiological non-union (Goldberg 0, C).

2.3. Radiological analysis

At the end of the 2 and 5 weeks observation period, the animals were re-anesthetized and ventro-dorsal X-rays of the healed femora were performed. Fracture healing was analyzed according to the classification of Goldberg et al. (1985), with stage 0 indicating radiological non-union, stage 1 indicating possible union and stage 2 indicating radiological union.

2.4. Biomechanical analysis

For ex vivo analysis of implant stiffness, cadaveric femora of CD-1 mice with a body weight of 32 ± 3 g were stabilized with (i) a normal intramedullar pin ($n = 6$), (ii) a previously described locking femur nail with flattened ends (Holstein et al., 2007) ($n = 6$), (iii) the herein introduced metallic clip in combination with an intramedullar pin (pin-clip fixation) ($n = 6$) or (iv) with pin-clip fixation and a cut clip ($n = 6$). Unfractured femura ($n = 6$) served as controls. The locked pin was included in our study, because in mice, to our knowledge, this is the internal fixation device with the highest torsional stability.

For biomechanical analysis after in vivo healing, the right and the left femora were resected, carefully freed from soft tissue, the intramedullary pin was removed and the clip was cut in the middle. The proximal and distal ends of the femur were fixed in metallic tubes using PMMA with a working gauge length of 5 mm. The bones were then mounted on a computational-based torsional testing device (teststand FMT-400 and digital force gauge FMI-210B2, Alluris, Freiburg, Germany). Applying a gradually increasing angle (0.15°/s), peak torque at failure (N mm) and peak rotation angle at failure (deg) were measured and torsional stiffness (N mm/deg) was calculated. To account for differences in bone strength of the individual animals, the non-osteotomized left femora were also