An external fixation method and device to study fracture healing in rats

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ABSTRACT We wished to establish a reproducible model for fracture fixation to be used in fracture healing research and therefore developed an external fixation construct and surgical procedure adapted to Sprague-Dawley rats. We evaluated the mechanical properties of the construct in brass rods and rat bone, in an Instron test machine with axial and transverse loading, and the in vivo performance. We found that the mechanical properties of the construct in brass rods were predictable and could be repeated in rat femora. In all tests, the axial load was about 10 times the transverse for the same degree of deformation. The stiffness among fixators was uniform. 1 mm pins caused about 50% less stiffness than 1.2 mm pins in axial loading of rat bone ($p < 0.001$) and brass rods ($p < 0.001$) as well as in transverse loading of brass rods ($p < 0.001$). Loosening of 1 or 2 screws that lock the pins to the fixator reduced stiffness by about 50% in axial loading of rat bone ($p = 0.009$) and brass rods ($p = 0.05$). A change in the distance between the bone surface and the fixator was linearly related to the stiffness in axial loading of rat bone ($p < 0.001$) and brass rods ($p < 0.001$) and in transverse loading of brass rods ($p < 0.001$). If the bone ends touched each other, the axial stiffness of the construct increased almost 10 times (265 N/mm), as compared to a fracture gap size of 2 mm (31 N/mm). In vivo experiments had a complication rate of less than 10% when we used 1.2 mm pins, 6 mm offset and rats weighing 350–450 g.

Our method and device for experimental external fixation of rat femora are reliable and the findings are reproducible. These can be used in bone repair and fracture healing research.

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Several animal models have improved our understanding of the biological mechanisms involved in bone tissue repair (Einhorn et al. 1984, Feighan et al. 1995, Hunt et al. 1996, Jazrawi et al. 1998, Probst et al. 1999, Richards et al. 1999). An important problem in experimental models is reproducibility. However, data on compliance, stiffness, torsional rigidity and intrafragmentary movements are frequently not described in detail about existing models for fracture fixation (Chao et al. 1989).

In accordance with principles used in humans, experimental fracture treatment can be divided into internal or external fixation. Intramedullary fixation of a standard closed fracture in rats is a widely-used model known, by Bonnarens and Einhorn (1984). The reproducibility for production of the fractures is well described but the stiffness, torsional rigidity and interfragmentary movement of the fracture fixation are not. Probst et al. (1999) reported a reproducible intramedullary fixation method of standard closed fractures which permits two stiffnesses of fixation. Utvåg et al. (1999) described another method of intramedullary fixation, using transverse locking pins, which provides torsional stability and dynamizing the fracture fixation. However, no data are given about the reproducibility of the fixation principle.

Plate fixation is another principle of internal fixation, mainly used for fixation of segmental bone defects (Feighan et al. 1995, Hunt et al. 1996). However, hardly any information is available concerning the reproducibility of this type of internal fixation.

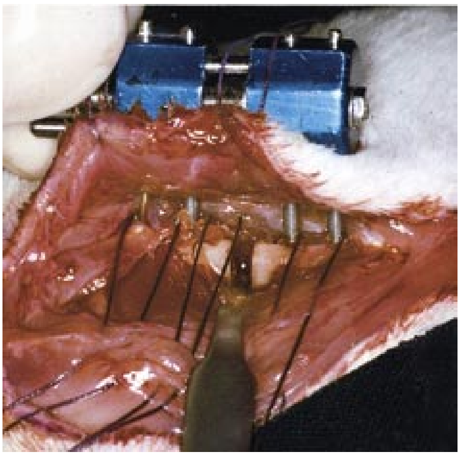

Figure 1. An osteotomized rat femur with a fixator fastened to pins cannulated through the skin flap and pre-set sutures through the muscle fascia.

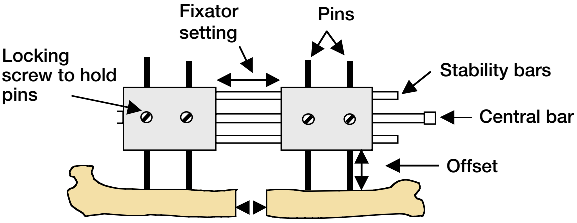

Figure 2. Schematic drawing of the fixator.

The principle of external fixation has been used in fixation of segmental bone defects and in experimental models of distraction osteogenesis (Einhorn et al. 1984, Jazrawi et al. 1998, Richards et al. 1999). Likewise, little information is available on reproducibility of experimental external fracture fixation in rats (Probst et al. 1999).

We wished to develop a reproducible experimental model for fixation of femoral fractures in rats. This should include: 1) the design of a new external fixation construct; 2) evaluation of the mechanical properties of the construct in brass rods; 3) development of a reproducible surgical procedure intended to compare the mechanical properties of the construct in brass rods and excised rat femora; and 4) evaluation of the in vivo performance.

Material and methods

Design of the fixator

The fixator was designed for unilateral external fixation, especially in the rat femur (Figures 1 and 2). The fixator consists of two octagon-shaped blocks (9.0 × 10.0 mm) made of titanium. The other components are made of stainless steel. The two blocks are connected with two sliding bars, diameter 2.0 mm and length 30.0 mm, which run parallel through the two blocks, and to the longitudinal axis of the blocks. The latter can be moved in relation to each other along the two sliding bars. This is done by turning a 3 mm screw with a length of 35.0 mm, pitch 0.5 mm, parallel to the two sliding bars. Consequently, distraction and compression in a bone defect are controlled with this device. The two blocks are anchored into the two femur fragments with four pins, each conically threaded in one end. The four pins are fitted into corresponding holes in the fixator's two blocks—two pins in each block—and fastened with locking screws, with the two blocks' center axis estimated as parallel to the bone surface and fixed at a distance, pre-set for each study (offset). Depending on the aim of the study, different pins can be used. These are 25.0 mm long and, in this study, their diameter was 1.0 mm or 1.2 mm and they were conically threaded over 7.0 mm in one end. The total weight of the device is 6.0 g, including four 1.0-mm wide pins.

Mechanical testing

The aims were to evaluate the stiffness of the external fixation construct regarding the device and its performance in bone. Therefore, we did two sets of mechanical tests: 1) assessment of construct stiffness using brass rods, 2) assessment of construct stiffness using excised rat femora.

In the first test set-up, different pins and external fixators of identical design were fixed to two cylindrical brass rods, 5 mm in diameter and 15 mm in length, corresponding to femora fragments of rats weighing 350–450 g. The pins were screwed into tapped holes in the brass rods. Measurements were made in two ways: with the load cell in the test machine applied in an axial or transverse direction to the brass construct (Figure 3).

In the second test set-up, different pins and external fixators of the same design as in the first test set-up were applied to osteotomized femora in 13 rats, in accordance with the surgical procedure.

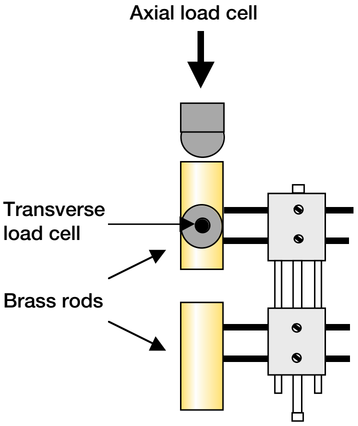

Figure 3. Axial and transverse load-testing set-up.

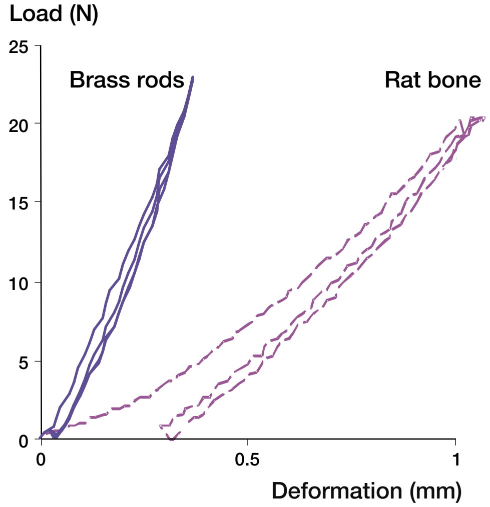

Figure 4. Load-deformation curves from brass rods and rat bones. Representative examples of loading and unloading sequences from axial tests.

Immediately after surgery, the bones were disarticulated and for testing purposes, the epiphyses were cut to flat and parallel surfaces. Before testing, the bones were stored deep-frozen, wrapped in gauze saturated with physiological saline. The bones were thawed at room temperature and care was taken to avoid loss of water. When defrosted and at room temperature (about 20 °C), they were tested immediately. In the second test, measurements were made only when the axial load was applied to the osteotomized bone.

In both test set-ups, we evaluated the uniformity of the mechanical properties among the various individual external fixators and pins. The effects of different parameters of the construct were also evaluated—i.e.: 1) a difference in the diameter of the external fixator pins (1.0 mm and 1.2 mm), 2) a change in the distance between the bone surface and the fixator blocks (offset); 3) a change in the distance between the fixator blocks, fixator setting, corresponding to the width of the fracture gap; and 4) loosening of one or two screws locking the pins into the fixator blocks. One parameter was changed in each test set-up.

The mechanical tests were done with a computer-controlled Instron test machine (Instron Corporation, Canton, MA, USA), model 4202, served by LABPAC software (Dennis Bergström Trading AB, Stockholm, Sweden). Two load cells were used because of accessibility with a maximum capacity of 100 N and 10 kN. The accuracy of the load is ± 4% and the accuracy of position is ± 0.1 mm. The load range was pre-set, 20 N in axial loading, 5 N in transverse loading and the deformation speed was set to 1 mm/min. Each measurement was made with a particular sequence of loading and unloading. The stiffness was calculated from the load-deformation curve, recorded by the computerized test machine, and using the equation Stiffness = $\Delta F/\Delta X$, where F = axial or transverse force, X = deformation and the values are given in N/mm (Figure 4).

Surgical procedure

We obtained approval from the Research Ethics Committee before using the animals. The male Sprague-Dawley rats weighed between 350 and 450 g. Surgery was performed under sterile conditions and with the animal anesthetized. Before surgery, clindamycin (4 mg per kilo body weight) was given i.m. and after surgery, the analgesic buprenorphine (0.01–0.05 mg per kilo body weight) s.c. The entire hind portion of the rat was shaved, including both legs. The anesthetized rat was washed with soap, water and chlorhexidine alcohol and draped in sterile towels, leaving one of the hind legs free. A curved incision was made through the skin, running from the base of the tail to the knee, with the convexity anteriorly. A skin flap could therefore be dissected from the underlying fascia. Via a “mail box” approach through the fascia, the shaft of the femur was exposed by gentle dissection between the quadriceps and hamstring muscles. The femur was exposed from the greater trochanter to the supracondylar region of the knee. To prevent bleeding, the small vessel crossing laterally and proximally to the knee joint was identified and avoided. A tunnel was dissected in the muscles around the mid-portion of the femur and care was taken to prevent injury to the periosteum. A self-locking nylon strap (2 mm in width, Stastraps, Panduit Corp, Tinby Park, Illinois, USA) was pulled through the tunnel for temporary fixation of a drill guide on the lateral aspect of the mid-shaft of the femur. A groove in the drill guide was placed on the greater trochanter of the femur for reproducible positioning. 4 holes were drilled with a diameter of 0.7 or 0.9 mm, corresponding to the pin diameters of 1.0 mm and 1.2 mm; these passed close to the central axis of the bone. During drilling, irrigation was done to prevent heat damage to the bone tissue. The drill holes (0.7 mm or 0.9 mm) through the near cortex were tapped. A pin was inserted into each drill hole and screwed through the bone until its tip reached the outer surface of the far cortex. The 4 pins were cannulated through the skin flap and the fixator was fastened to the pins at a pre-set distance from the bone surface (offset). Between the 2 middle pins, we made an osteotomy with a reciprocating saw under irrigation. Using the distraction/compression screw, the bone fragments were moved to a pre-set fixator setting—i.e., width of the fracture gap (Figures 1 and 2). The fixator settings from 0 mm to 5 mm were evaluated. The wound was closed in layers. On the first 2 postoperative days, the rats were given the same broad-spectrum antibiotic that they had received before surgery.

In vivo performance

In a first set of experiments, we evaluated the fatigue properties of the pins in terms of frequency of broken pins over a 6-week period. The external fixation construct was applied to the right femora of 20 rats, using a 1.0 mm pin diameter and 6 mm offset.

Because of the high frequency of broken 1.0 mm pins, new pins with a diameter of 1.2 mm were made. In a second set of experiments, the external fixation construct was applied to the right femora of 15 rats, using these pins and 6 mm offset. The frequency of broken pins was again evaluated during a 6-week period of healing.

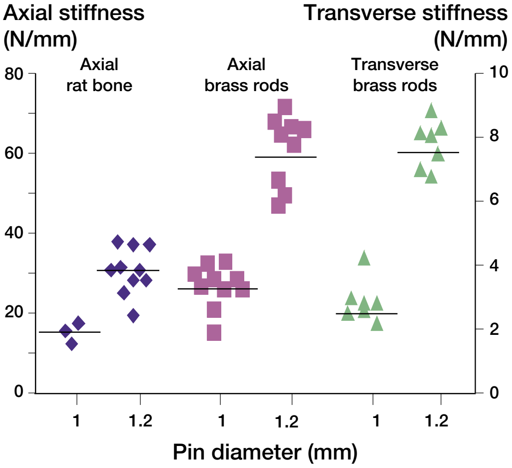

Figure 5. Effects of pin diameter in brass rods and rat bone on stiffness. The diagram shows the difference in stiffness in axial loading between 1.0 mm (n = 3) (14.78, SD 2.59 N/mm) and 1.2 mm (n = 10) (30.70, SD 5.93 N/mm) pins in rat bone (p= 0.007) and between 1.0 mm (n = 10) (27.04, SD 5.41 N/mm) and 1.2 mm (n = 9) (60.75, SD 8.87 N/mm) pins in brass rods ($p < 0.001$). The diagram also shows the difference in stiffness in transverse loading in brass rods between 1.0 mm (n = 7) (2.86, SD 0.66 N/mm) and 1.2 mm (n = 7) (7.77, SD 0.74 N/mm) pins ($p < 0.001$).

In a last set-up, based on the findings in 164 rats, using a 1.2 mm pin diameter and 6 mm offset, we assessed the frequency of complications such as: 1) broken pins, 2) wound healing, 3) bone healing and 4) well-being. Wound healing refers to open and/or infected wounds, bone healing to clinical major misalignment (> 20 degrees) in dissected bones after the animals were killed and well-being to a gain in weight and normal movement throughout healing.

Statistics

In the statistical analyses, Fisher's nonparametric permutation test was used to compare the groups. Pitman's nonparametric permutation test was used for correlation analysis and calculation of Pearson's correlation coefficient (r) for descriptive purposes. All significance tests were two-tailed and done at the 5% significance level.

Results

Mechanical testing

In the first set-up, we evaluated the stiffness of the external device in brass rods. In all parameters

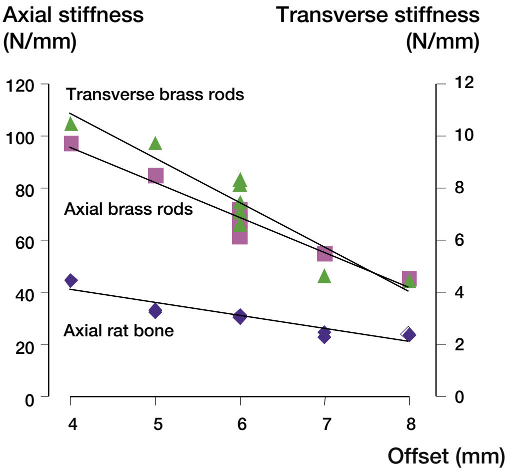

Figure 6. Effects of offsets on stiffness in brass rods and rat bone. The diagram shows linear relationships between offset and stiffness, using 1.2 mm pins in axial loading in rat bone (n = 8), ($p<0.001$) and brass rods (n = 8), ($p < 0.001$) and transverse loading in brass rods (n = 9), ($p < 0.001$).

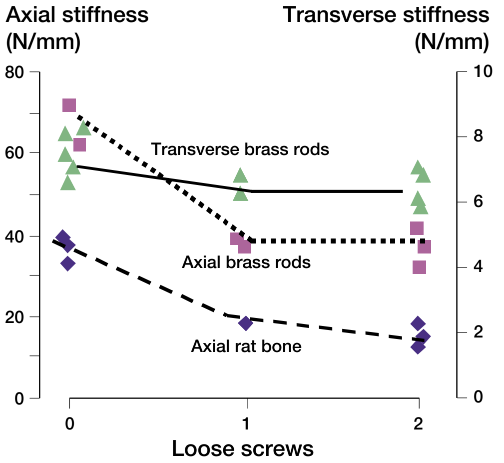

Figure 7. Effects of loose screws on stiffness. The diagram shows a significant correlation between a reduction in stiffness and 1 and 2 loose screws in axial loading in rat bone (n = 7), ($p = 0.009$) and brass rods (n = 11), ($p = 0.048$).

tested, the axial load was about 10 times the transverse for the same degree of deformation (Figures 5 and 6). Only a small difference was noted in the mean stiffness between the fixators used regardless of whether they were loaded in the axial (70, SD 5.2 N/mm, range: 61–78 N/mm, coefficient of variation (C.V.): 7.5%, (n = 12)), or transverse direction (7.2, SD 0.63 N/mm, range: 6.5–8.3 N/mm, C.V.: 8.8% (n = 9)). A linear relationship was shown between the stiffness and fixator setting 1–3 mm (width of fracture gap), when loaded in the transverse direction ($p = 0.008$). The pins of 1.0 mm in diameter in the external fixation set-up differ in stiffness when they are loaded in the axial (27, SD 5.4 N/mm, range: 15–33 N/mm, C.V.: 20% (n = 10)) or transverse direction (2.9, SD 0.66 N/mm, range: 2.13–4.24 N/mm, C.V.: 23.1 % (n = 7)). The pins of 1.2 mm in diameter in the external fixation set-up also differed in mean stiffness when they were loaded in the axial (61, SD 8.9 N/mm, range: 47–71, C.V.: 15%, (n = 9)) or transverse direction (7.8, SD 0.74 N/mm, range: 6.7–8.8, C.V.: 10%, (n = 7)). Thus, the stiffness of an external fixation set-up with a pin diameter of 1.0 mm was about 50% less in both axial ($p < 0.001$) and transverse ($p < 0.001$) loading than the 1.2 mm pins (Figure 5). A linear relationship was also found between the offset and the stiffness of the external fixation in axial ($r = 0.97$, $p < 0.001$) and transverse ($r = 0.94$, $p < 0.001$) loading (Figure 6). We also found a correlation between a reduction in stiffness of the external fixation and if 1 or 2 screws locking the pins into the blocks were loosening when axial ($r = 0.86$), $p = 0.05$), but no transverse, loading was applied (Figure 7). Loosening of 1 locking screw in axial loading reduced the stiffness of the external fixation by about 50%. Loosening of 2 locking screws did not further reduce the stiffness (Figure 7).

In the second set-up, in which we evaluated the stiffness of the construct in osteotomized rat femora, the axial loading was about 50% lower than in brass rods, for the same degree of deformation for all parameters (Figures 5 and 6). The mean axial stiffness was 265, SD 34 N/mm in osteotomized rat femora with offset 6 mm, 1.2 mm pins and fixator setting 0, corresponding to a fracture gap size of 0 mm—i.e., bone ends touching each other. With a fixator setting of 1 mm, corresponding to a fracture gap size of 1 mm, the mean axial stiffness was 30 N/mm, SD 2.1. In axial loading, a difference in stiffness was found between 1.0 mm pins (15, SD 2.6 N/mm, C.V.: 18%) and 1.2 mm pins (31, SD 5.9 N/mm, C.V.: 19%) ($p = 0.007$), and a linear relationship between the offset and the stiffness of the fixation ($p < 0.001$) (Figures 5 and 6) In axial loading, a correlation was shown between a reduction in stiffness and the loosening of 1 or 2 locking screws (p = 0.009). However, the loosening of 2 locking screws, instad of 1, did not further reduce the stiffness (Figure 7).

In vivo performance

The first set of experiments, using pins with a diameter of 1.0 mm and 6 mm offset in 20 rats over a 6-week period, resulted in a complication rate of 65% with broken pins.

In the second set of experiments, using 1.2 mm pins and 6 mm offset in 15 rats, none of the pins broke. The outcome of the third evaluation of complications, using 1.2 mm pins and 6 mm offset in 164 rats, was: a) 0% broken pins, b) 4.3% wound-healing complications, c) 2% bone-healing complications, and d) 98% well-being.

Discussion

We describe a model for fixation of experimental fractures for research in bone healing in rats, which is well-defined and reproducible (Bonnarens and Einhorn 1984, Einhorn et al. 1984, Hunt et al. 1996). External fixation is an established method for studying bone repair—e.g., fracture healing, healing of segmental defects and distraction osteogenesis. An advantage o external fixation is that a specific stiffness of fracture fixation can be pre-calculated and selected (Wu et al. 1984, Behrens 1989a, b, c, Chao et al. 1989, Goodship et al. 1993). As this is an experimental model, some limitations should be considered—e.g., pin interaction with the bone and surrounding soft tissue, a reduction in bone strength caused by the pins, and the fact that an experimental osteotomy probably is not the same as a fracture in all respects (Hazan and Oron 1993, Hernandez et al. 1995). In our study, the method of external fixation has been adapted for Sprague-Dawley rats weighing 350–450 g. We did several mechanical tests to determine the reproducibility of the method. Two test set-ups were used; 1) application of the external fixator to brass rods and 2) application of the fixator to osteotomized rat femora fragments. The results of tests of the fixation with brass rods showed the mechanical properties of the external fixation equipment, since the brass rods were considered to be extremely stiff, while the corresponding situation with the rat femora evaluated the reliability of the method for experimental studies of bone repair in rats.

The stiffness of all constructs was the same and was most affected by the distance between the two blocks of the fixator, the fixator setting. The axial stiffness of the construct was almost 10 times greater with a fixator setting at 0 mm, corresponding to a 0 mm fracture gap size—i.e., the bone ends touched each other (265 N/mm, SD 34), as compared to a fixator setting of 1 mm, which corresponded to a fracture gap size of 1 mm (31 N/mm, SD 2.1). As regards transverse loading, we found a linear relationship between stiffness and the fixator setting.

The difference among the 1.0 mm and 1.2 mm pins in axial loading may be partly due to a slight difference in diameter and mechanical properties of the pin material. However, the pin diameter, as expected, is very important for the fixation stiffness, 1.0 mm pins resulting in about 50% less stiffness than 1.2 mm pins. The outcome corresponds to the theoretical stiffness of a pin in relation to its diameter. Other parameters shown to be of grat importance for stiffness was the free length of the pins—i.e., the distance between the fixator's two blocks and the bone surface (offset), a relationship that was shown to be linear. Loosening of one or more screws locking the pins to the fixator's blocks was also of great importance for stiffness. Consequently, if one or more screws locking the pins have become loose, such an experiment must be omitted.

It should be noted that the transverse load was much lower than the axial one in the loading tests for the same deformation. Therefore, transverse loading gives less favorable values for stiffness of the fixation than axial loading. With a fixator setting of 2 mm (which corresponds to 2 mm fracture gap), an offset of 6 mm and 1.2 mm pins, the average stiffness of the construct in osteotomized rat femora is about 30 N/mm in an axial direction. Gait analysis in the rat has shown vertical forces from the hind paw of about 50% of body weight (Clarke 1995). Therefore, a 500 g rat (5N) may subject the hind leg to 2.5 N of vertical force on the externally fixated bone. Consequently, the axial interfragmentary movement during loading is calculated as about 0.08 mm or 80 μm. Mechanical tests indicate that the mechanical properties of the construct measured in brass rods were the same as those in osteotomized rat femora. The calculations of the coefficient of variations indicate adequate reproducibility of the method, as would be expected with this type of relatively complicated experiment.

The results of the first evaluation of the in vivo performance showed an unacceptably high of complication rate of broken pins (65%) using those with a diameter of 1.0 mm. This complication was thought to be due to a higher mechanical stress than that calculated and to fatigue properties of the material, which had not been considered. The stress on the pins is related to the radius. Theoretically, 1.2 mm pins reduce the stress by 60%, as compared to 1.0 mm pins. In the second evaluation, 1.2 mm pins were tested and no broken pins were found in the construct. Therefore, 1.2 mm pins are recommended for rats weighing 350–450g and 1.0 mm pins can be used in smaller rats.

The outcome of the third evaluation of in vivo performance of the external fixation method, using 1.2 mm pins and 6 mm offset in 164 rats, had a total complication rate of less than 10%. In view of the precision of the dimensions of the bone-fixator interface and the demanding situation with pin penetration of the skin, this complication rate must be regarded as acceptable. Low complication rates also facilitate studies of bone repair under ethically-safe conditions and with a high degree of accuracy and reproducibility.

In conclusion, the data in the present study provide the basis for a reliable and reproducible method for experimental external fixation of long bones in rats used in investigations of bone repair.

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