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Establishment of a femoral critical-size bone defect model in immunodeficient mice

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ABSTRACT

Background: The development of innovative therapies for bone regeneration requires the use of advanced site-specific bone defect small-animal models. The achievement of proper fixation with a murine model is challenging due to the small dimensions of the murine femur. The aim of this investigation was to find the optimal defect size for a murine critical-size bone defect model using external fixation method.

Methods: An external fixation device was attached to the right femur of 30 mice. Femoral bone defects of 1 mm ($n = 10$), 2 mm ($n = 10$), and 3 mm ($n = 10$) were created. Wounds were closed without any additional treatment. To investigate bone healing during the 12-wk observation period, x-ray analysis, histomorphology, immunohistochemistry, and μCT scans were performed.

Results: MicroCT analyses after 12 wk showed that 3/8 1-mm defects, 5/8 2-mm defects, and 8/8 3-mm defects remained as nonunions. The defect volumes were 0.36 ± 0.42 mm^3 (1-mm group), 1.40 ± 0.88 mm^3 (2-mm group), and 2.88 ± 0.28 mm^3 (3-mm group; $P < 0.001$, between all groups).

Conclusion: Using external fixation, a defect size of 3 mm is necessary to reliably create a persisting femoral bone defect in nude mice.

1. Introduction

Localized bone loss associated with trauma, tumor, infection, periprosthetic osteolysis, or congenital musculoskeletal disorders, with all of these conditions requiring surgical intervention, is a major worldwide socioeconomic problem. Immunodeficient small-animal models are of particular interest for translational research strategies, as they allow for

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the use of human cells—a critical step on the path from bench to bedside.

So far, both tibial and femoral murine segmental bone defect models have been used. The tibial fracture model was first described by Hiltunen *et al*. in 1993 [1]. The disadvantages of this model are the triangular, distally declining caliber of the tibia and the bent longitudinal axis. Additionally, the close proximity of the fibula can influence the fracture repair process [2]. In contrast to the tibia, the murine femur is a tubular bone with a relatively consistent inner and outer diameter and a straight longitudinal axis [2].

In order to develop cell-based tissue engineering strategies for bone repair, there is a need for high-quality bone defect models in small animals. The small dimensions of the murine femur make it difficult to achieve proper stabilization of a critical-size bone defect. The aim of this study was to find the optimal defect size for a murine critical-size bone defect using external bony fixation method. The defect size has to be large enough to get reliable nonunions, while at the same time being small enough to achieve proper stabilization when using an external fixation device. Our hypothesis was that a segmental osseous defect size of a minimum of 2 mm would be required to generate a reliable nonunion.

2. Methods

2.1. Experimental procedure

Mice were randomized to three groups. One surgeon implanted the external fixation device (Fig. 1A, MouseExFix, RISystem; AO Research Institute, Davos, Switzerland) onto the right femur of each mouse. A defect of 1 mm, 2 mm, and 3 mm was created in groups 1 ($n = 10$), 2 ($n = 10$), and 3 ($n = 10$), respectively. After wound closure no additional treatment was provided in an effort to avoid influencing the natural pattern of bone regeneration. All of the operations were performed within a 12-d period. The mean operative time was 40 min, independent of the defect size being created. The weight of the complete external fixator, including the four pins and the body, was measured to be 0.20 g. The postoperative observation period was 12 wk. X-ray films were obtained immediately after surgery and every 2 wk during the 12-wk postoperative period. The animals were then euthanized and histomorphometry, immunohistochemistry, and μCT analysis was performed on the femura.

2.2. Animals

For the *in vivo* investigation 30 male nu/nu nude mice (40.7 ± 2.8 g, 95 ± 2.6 d old) were used. Mice were bred at the Animal Experimental Center of the Medical Faculty of the Technical University of Dresden, Germany. The animals were kept on a 12-h light-and-dark cycle and were fed a standard diet with food and water *ad libitum*. All experiments were performed in adherence to the National Institutes of Health Guidelines for the Use of Experimental Animals and were approved by the Local Animal Care Committee (protocol no. 24-9168.11-1/2010-29).

2.3. Surgical procedure

2.3.1. Preoperative care

Animals were anesthetized by inhalation of 2% isoflurane for the surgical procedure. The mice were first placed in an inhalation chamber. Upon adequate anesthesia, the animals were transferred to a warming pad and the head and front paws were placed inside a plastic tube that was connected to the anesthesia apparatus (Fig. 1B).

2.3.2. Surgical technique

The operations were performed under surgical aseptic conditions. All mounting pins were prethreaded into the plastic bodies of the external fixation device and removed in order to create a preformed thread, thereby facilitating smooth drilling during the surgical procedure. Each mouse was placed in the prone position. The right rear extremity was extended and rotated inward at the hip joint (Fig. 1B). Exact locations of the hip and knee joints were detected by flexion and extension of the hip and of the knee. A 12-mm incision was then performed along the lateral upper leg, giving exposure to the greater trochanter (Fig. 1C). An incision was then made along the fascia lata, following a line from the greater trochanter to the knee joint. The quadriceps femoris muscle was mobilized anteriorly towards the knee and the hip using two spatulas (Fig. 1D). A sharp elevator was used to expose the distal femur and the first hole (diameter 0.45 mm) was drilled into the distal femur (Fig. 1E). The first pin was put into one of the lateral holes of the plastic body of the external fixation device and was subsequently orthogonally drilled into the distal femur, penetrating both the lateral and the medial cortex (Fig. 1F). Subsequently, the proximal part of the femur was exposed (Fig. 1G). The second hole was drilled through the remaining lateral hole on the plastic body and into the proximal femur or the lateral femoral neck as described above (Fig. 1H). The second pin was then placed through this hole. The inner two pins were then placed after drilling the inner two guide holes (Fig. 1I). After having fixed all four pins, two Gigli wires were placed around the femur and placed into the fixed saw guide on the body of the external fixator (Fig. 1J–M). A defect was cut using two Gigli wires while the body of the fixation device was stabilized with the help of two clamps (Fig. 1M). The clamps were then removed and the femur and surrounding structures were all guided back into their natural anatomic positions (Fig. 1N). Finally, the skin was closed with interrupted sutures (Fig. 1O).

2.3.3. Postoperative care

Two and a half micrograms of buprenorphine in 100 μL saline per animal was used for postoperative pain control and injected subcutaneously. A single dose of 0.75 mg amoxicillin in 300 μL saline was injected subcutaneously for postoperative infection prophylaxis. Both of these injections were repeated on the first and second postoperative day. For the x-ray procedures, the animals were anesthetized with single intraperitoneal injections of 100 mg/kg ketamine and 10 mg/kg xylazine.

2.4. Preparation of the specimens for μCT and histologic analysis

After 12 weeks all mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine and then euthanized by