A Standard Surgical Protocol for a Rabbit Ulnar Osteotomy Model

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Summary
Critical size defects in the long bones of rabbits have been used for years as an experimental model for the investigation of different bone substitute materials. However, no standard surgical protocol exists in the literature. This is a source of misunderstandings and makes results from different studies hardly comparable. This technical note attempts to present a standard surgical technique for the creation of a segmental critical size ulnar defect in the New Zealand white rabbit.

Introduction
A critical size bony defect (CSD) is defined as the smallest intra-osseous wound characterized by an absence of spontaneous healing, which would not heal by bone formation during the lifetime (*Schmitz & Hollinger, 1986*). In the preclinical field of orthopedic and trauma surgery, CSD creation is the method of choice for in vivo testing of bone repair materials (BRM) (*Hollinger & Kleinschmidt, 1990*). Besides in vitro screening methods it is essential to evaluate the biocompatibility of BRMs by in vivo investigations before subjecting them to the rigors of clinical testing. Only the complex organism of an animal allows an investigation of systemic responses induced by the complex implant-/transplant-tissue interactions like elimination and bioabsorption kinetics, inflammation reactions, tumour genesis and biomechanical effects. Considering this, bone defect models play a crucial role for experimental testing of orthopaedic implants, transplants and growth factors. However, a review of the bone research literature reveals an obvious lack of either uniformity or consistency among the various researchers concerning animal models. CSDs have been determined in various bones of rats, rabbits, cats, dogs, swine, horses, and nonhuman primate species. Rabbits have been widely used in experimental orthopedics and traumatology, but only a few scientific data exist concerning a standard, generally accepted surgical technique of CSD creation in long bones, and in particular the ulnar leporine bone.
Our experience with different surgical techniques in a large number of various experimental animal species has led us to develop of a standard, safe, reliable, and repeatable operative protocol for CSD creation in the ulna of New Zealand white rabbits. The aim of this article is to describe the technical details of this surgical protocol.

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Materials and methods
General considerations
All animal experiments were performed in accordance with The Council Directive of the European Communities on the Protection of Animals Used for Experimental and Other Scientific Purposes, 1986. Permission was granted from the ethics committee in Karlsruhe (AZ 35-9185.81/54/01). Each

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study has its own design which is specific for the aims of the experiment: That is why we shall deliberately limit the subject of this work to a description of the surgical technique and medications directly related to the procedure of the creation of a CSD.

Medication

The operative procedure requires general anesthesia. It is induced by atropine (Atropinsulfate Braun®; B Braun, Melsungen, Germany) 0.1mg/kg s.c., xylazine 2% (Rompun®; Bayer, Leverkusen, Germany) 4mg/kg s.c., and ketamine 10% (Ketanest®; WDT, Garbsen, Germany) 60mg/kg i.m.. Together with premedication, the first dose of antibiotic prophylaxis is given: enrofloxacin 2.5% sol. (Baytril®; Bayer, Leverkusen, Germany) 5mg/kg i.m. and the same dose should be repeated on the first and the second postoperative days. Anesthesia is further maintained by isoflurane-nitrous oxide-oxygen mixture administered by mask. Isoflurane (Forene®; Abbott Rungis, France) 2Vol% is used initially for about 5 minutes, then 1Vol% for the next 5 minutes and 0.8Vol% for the rest of the time. Oxygen is supplied at 800ml/min, and nitrous oxide at 1000ml/min. It is important to note that pure oxygen should be given alone for the last three minutes of anesthesia. As anaesthesiology device we use the Anesthesia Gas Machine "Narkomat" (Heyer Medical Co., Bad Ems, Germany). Dexpanthenol (Bepanthen® 5g ophthalmic ointment; Roche, Eppstein-Bremthal, Germany) is placed gently onto the conjunctiva of each eye as corneal protection. Carprofen (Rimadyl®; Pfizer, Karlsruhe, Germany) 4mg/kg s.c. once a day is used as analgesic for the first two postoperative days.

Operative procedure

The animal is placed in a true lateral position on the surgery table on a warming pad, so that the leg to be operated upon is on top, leaving plenty of room on the table near the head (Figure 1A). The rabbit's foreleg is shaved and prepared for surgery by three times using a chlorhexidine scrub brush working

Figure 1A: Position of the animal on the table.

from the incision site outward. The shaved area is finally sprayed with betadine (Octanisept), and the incision site is carefully draped to create a sterile area. A non-sterile assistant holds up the rabbit's leg while a sterile surgeon wraps the pad in a small ster-

Figure 1B: Covering the operative field.

ile drape towel (Figure 1B). With the rabbit's leg still held up, a sterile drape is placed longitudinally beneath the leg and a second drape towel is used to cover the rabbit to keep fur out of the sterile area as shown at the picture (Figure 1C), while ensuring the drapes do not stick to the animal's whiskers (vibrissae) or the mask. Finally, a large laparotomy drape is placed over the rabbit exposing only the rabbit's leg and ensuring a spacious aseptic working area.

To start the operation, the olecranon should be first palpated as a point of orientation. Distally the proximal part of the shaft of the ulna is palpated and a

Figure 1C: The operative field is already covered.

one inch long, anterolateral slightly curved incision; through skin is made over it with the scalpel. The superficial and deep fasciae are identified and cut separately in line with the skin incision, then a 10mm segment of the bone is exposed by dissection between the flexion and extension musculature overlying it (Figure 2A). To get a better view of the

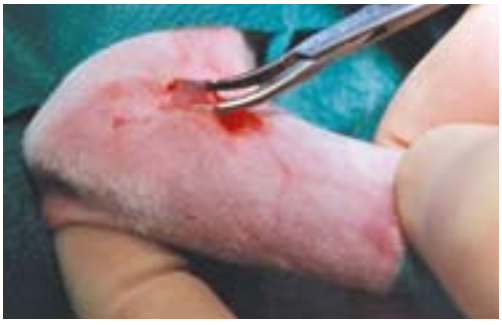

Figure 2A: Access to the ulna.

situs we use a small, subperiostal Hohmann's elevators and Langenbeck's retractor to hold apart muscles and soft tissue. The periosteum is incised circumferentially and removed by scraping and cleaning with a gauze. This is essential for a CSD, since periosteum includes in its cambium layer a high potential for bone regeneration. Following exposure of the bone a cut of 7 mm length and 5 mm depth is made using an oscillating bone saw with trephine and trephine guide, thus creating a segmental ulnar defect (Figure 2B, 2C). The trephine is fixed with

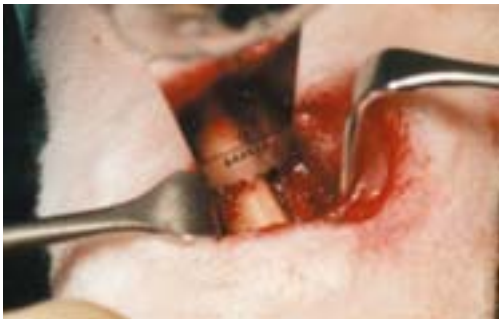

Figure 2B: Performing osteotomy.

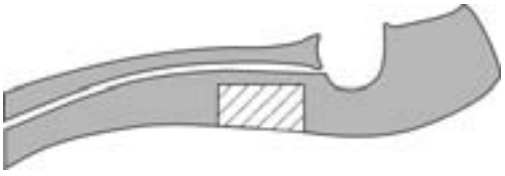

Figure 2C: Schematic presentation of the critical size defect in a rabbit's ulna.

the help of a template, taking care to avoid injury to the radius. The bone marrow is removed with a sharp spoon leaving a CSD surrounded by a thin layer of cortical bone (Figure 2D). Constant copious irrigation with physiological saline solution is used to reduce local heat and protect soft tissue from bony debris contamination. A three-hole titanium mini-plate is then fixed onto the bone to ensure the stability of the defect (Synthes / Oberndorf-Switzerland). Proximally, a 1.1mm hole

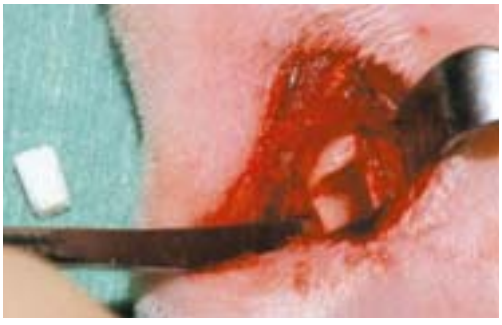

Figure 2D: CSD – intraoperative situs without a filling.

for the screw (1.5mm diameter) is drilled without cutting a thread for it. Then the plate is proximally screwed, but loose so it can be rotated cranially to allow filling the defect with the studied bone substitute in the form of cement, paste etc (Figure 2E).

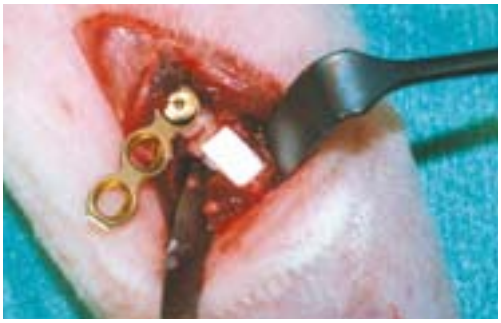

Figure 2E: Stable plate osteosynthesis.

Then, the free end of the plate is rotated back and adjusted to the distal end of the bone segment, where a second 1.1mm hole is prepared for the second 1.5mm screw. Both screws are then firmly tightened. The proximal screw should be 6-8mm long, while the distal one should not exceed 6-7mm (Figure 2F).

Figure 2F: A screw for the osteosynthesis.

The multilayer closure of the wound is done with fast absorbable Vicryl Rapide® (Ethicon Inc.) or Biosyn® sutures (Braun-Dexon, Tuttlingen, Germany). The deep and superficial fasciae are carefully restored with 4-0 single sutures, and 3-0 single sutures are used for the skin (Figure 2G). The

Figure 2G: Wound closure – final view.

area is sprayed with betadine. An elastic bandage is placed onto the leg and stuck with plaster. The plaster should be stuck not at a slant to avoid compression of the leg (Figure 3). Free movement of the ani-

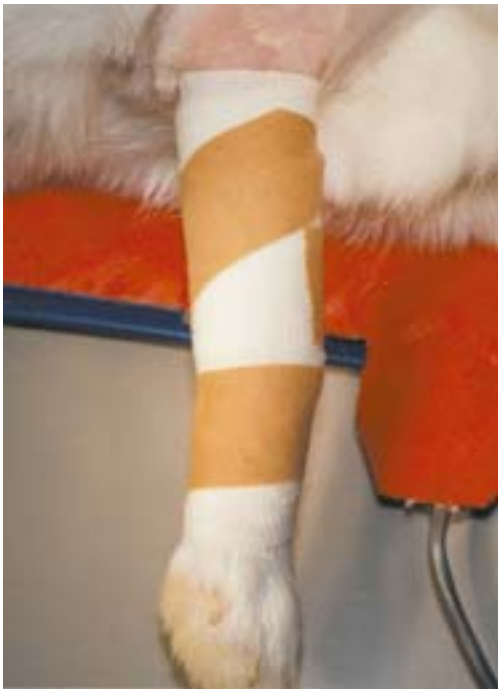

Figure 3: Wound dressing with bandage.

mal is allowed immediately after the operation. The dressing is checked daily for the first 48 hours and then removed. Conventional radiographs are conducted postoperatively. One surgeon should perform all procedures per experiment to achieve what is considered to be a uniform technique.

Results

In a series of 32 consecutive animals we found that the above described technique gave excellent intra- and postoperative results. The mean body weight of the rabbits was 4.3 (± 0.2) kg. All of them were preoperatively radiographically screened for bone maturity. The mean operative time was 37.5 (± 4.8) minutes. The clinical progression of the rabbits was free of complications. Intraoperative problems occurred only in two cases – the point of the screw drill broke, but was easily extracted from the hole. Other intraoperative complications and problems were not encountered. There was no postoperative mortality for a period of 60 days. Morbidity was presented by two cases of delayed wound healing due to bandage friction. The animals were able to move freely after the operation. Unproblematic wound healing and bone formation allowed tissue sampling at the end of the study period in 100% of the animals. Titanium plates were removed without technical difficulties.

Discussion

Testing of BRMs has had an interesting and diverse history. Bone researchers today cannot agree on the best model for testing BRMs. Groups studying ceramic compounds have taken a somewhat different route from those testing osteoinductive proteins, which is different from those testing electrical stimuli. Frame (1980) provided the classic study on the model of rabbit's calvaria when he described the healing of 5, 10, 15, and 20mm defects in rabbits' calvariae and determined that calvarial defects 15mm or greater would not heal by bone formation. Creation of CSDs in the long bones of New Zealand white rabbits was first studied by Ben-Fu and Xue-Ming in Shanghai (*Ben-fu & Xue-Ming, 1986*). They created 6mm defects in the leporine distal radius, which showed no bony growth at 150 days and thus proved that relatively small osteotomies in the long bones of rabbits are an excellent model for a CSD. These defects are large enough that dose response measurements can be made to optimize the material for more rigorous testing in later models.

Rabbits offer some obvious advantages as a model. They are relatively inexpensive, and plentiful. Compared to rats, which indicate the lower limit appropriate for bone experiments, leporine long bones are big enough to allow surgery without the use of microinstruments. Furthermore, rats grow constantly throughout their life, which is not the case with rabbits and humans. Because of the technically demanding surgical procedure many rats are lost before the end of the experiment. Compared to larger animals, rabbits are easier to handle, lower in costs, and widespread spread experimental models in biocompatibility testing. BRMs designed for orthopaedic use can be tested in the long bones of rabbits, instead of dogs and cats. The rising public sentiment against the using of "pet" animals such as dogs and cats in laboratory testing may require us to look at the rabbit in more depth as a bone healing model.

Fixation of osteotomied bones is an issue that deserves special attention. Although the pioneers Ben-fu and Xue-Ming, as well as many others after them, used no fixation, we consider rigid fixation essential for the accuracy and reproducibility of the method. It is important when developing a CSD model because it is in the first few days of bony healing that a critical period exists in which cells are migrating, attaching, and differentiating into osteoblasts needed to propagate bone growth. During this time, rigid fixation must be present to optimize this process and assure maximal bone regeneration (*Hollinger & Kleinschmidt, 1990; Reddi et al., 1987*). While rigid fixation is needed for a CSD, each type of fixation has advantages and disadvantages. The intramedulary fixation device has the disadvantage of occluding the bone marrow

and possibly blocking the source of osteoprogenitor cells needed for a BRM to be active. External fixation is undesirable in most animals because they pick and chew on the fixator, resulting in movement of the bone fragments. Infectious complications with all devices are possible as well. Our results showed that internal plates and screws function well and are preferable not only in humans, but in our experimental leporine model too.

We want to pay special attention to the suture technique used for restoring the soft tissues at the end of the operation. Our personal experience and communications with other authors make us believe that a key to minimizing postoperative morbidity, mainly wound infections, is the layer-by-layer careful and atraumatic wound-closure. Pedantic asepsis, meticulous preparation technique and anatomical restoration of the dissected structures are crucial in such models, based on implantation of foreign materials.

A limitation of the method, characteristic for all animal bone models, is the varying degree of bone wound repair that is seen along the phylogenetic scale. Any testing scheme would have to take into consideration the lesser ability of spontaneous bony wound healing by higher order species (*Enneking et al., 1975*).

Another important detail is the necessity to prove the skeletal maturity of the rabbit before the operation. The animals should be with closed epiphyseal plates. Immature animals of most species heal at a faster rate than adults. By testing BRMs in immature animals, falsely high expectations of the materials could be realized. Therefore, it is prudent to assure closure of the epiphysis in all animals radiographically prior to their use in an experiment. Weight charts often give a premature indication of skeletal maturity.

In conclusion, we should assess every animal model form the standpoint of the Three R's principles – reduce, refine and replace. It should always be emphasized and complied with to ensure the use of a minimum number of animals, the highest well-being of involved animals, and the use of alternative in-vitro methods (*Russell & Burch, 1992*). The first principle is achieved in our method by the ability to use the animals further for other experiments, to use ulna bilaterally, by avoiding unnecessary duplication of prior research, and by reducing postoperative mortality and morbidity, thus decreasing the number of animals needed per experiment. The use of rabbits replaces the use of "pet" animals such as cats, dogs, horses and non-human primates while preserving the opportunity to perform a study in a whole organism. Our model focuses mainly on the refinement of the experiment procedures, especially the surgical technique used to minimize pain and distress.

Searching for an ideal animal experimental model in bone research will continue in the future, despite the significant progress in alternative models and in vitro experiments. Testing a bone substitute material in a living organism is a necessary evil before further application in humans. Scientists should establish standard study protocols, and in particular operative protocols, in order to make results from the constantly increasing number of experimental studies comparable and thus more useful.

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