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The Rabbit Model of Femoral Bone Defects: Steps to Overcome Potential Pitfalls

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ID and AAN participated in the surgery and wrote the manuscript. Authors CZ, NS, CN and AMV conducted the surgical bone defects. Authors TP, DM and NP designed the study. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: To identify the potential pitfalls and indicate procedures to prevent them, during the evaluation of biomaterials for orthopaedic and craniofacial research in the New Zealand White (NZW) rabbit animal model of femoral bone defects.
Place and Duration of Study: Laboratory for Research of the Musculoskeletal System, School of Medicine, University of Athens, between June 2014 and July 2015.

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Materials and Methods: Pre-emptive analgesia (carprofen 2.2 mg/kg sc), chemoprophylaxis (enrofloxacin 10 mg/kg sc) and anaesthesia (ketamine/xylazine 30/5 mg/kg im) were administered to NZW rabbits (body weight 3.3±0.2 kg, mean ± SD) for the aseptic surgical creation of drilled bone defects of 6 mm diameter ("critical size defect") in the external femoral condyle of the left limb. All rabbits recovered without post-surgical complications from the first postoperative day.
Results and Discussion: Although the research group consisted of Veterinarians and Orthopaedic Surgeons with experience in this model, they were challenged with potential pitfalls which were overcome step by step. Among them is the precise localization of the defect to be drilled. Intra-operative palpation of the external femoral condyle assists in determining the site, and post-operative X-ray evaluation confirms it. Additionally the correct width and depth of the bone defect are important to adhere to, which was achieved by using a 5.5 mm diameter bone drill and observing its depth marks. Another challenge is to have the specific amount of biomaterial implanted confined to the defect. Its potential distribution in the femoral shaft, diffusion in the metaphysial trabecular bone or excessive covering of the bone surface, are also pitfalls to be avoided.
Conclusions: The increased use of this animal model in the evaluation of biomaterials in orthopaedic and craniofacial research requires knowledge, skills, surgical accuracy and attention to a sequence of steps, in order to achieve homogenous results and high repeatability of the implantation technique. With the fulfillment of these conditions, the extraction of valid scientific results and reduction of the number of animals used are possible.

Keywords: Animal model; critical size defect; femur; New Zealand White; rabbit.

ABBREVIATION

New Zealand White (NZW) rabbits.

1. INTRODUCTION

The necessity for animal testing prior to clinical trials of any novel treatment is well established, as it fills the "knowledge gap" between the theoretical and the practical part [1]. It has been estimated that 26-35% of orthopaedic research is carried out on rabbits [2,3]. Among the reasons for this selection are their size and easiness to handle, which facilitate surgery and analysis on a radiographic, histologic and mechanical level [2].

An experimental technique often used to evaluate new biomaterials for the treatment of bone defects consists of their surgical implantation in trabecular or cortical bone sites. Several animal species have been used to this end, such as dogs, sheep, goats, pigs and rabbits [2,3]. Rabbits are often selected as appropriate animal models for these studies, and in particular the New Zealand White (NZW) rabbit [2,4,5,6]. The NZW rabbit is commercially bred for research purposes and as such it is the most studied breed in biomedical research generally and even more so in biomaterial research.

Our research group, consisting of veterinarians, orthopedic surgeons and biomaterial specialists, evaluates new biomaterials for the treatment of bone defects by their surgical implantation in a bone defect created on the external distal femoral condyle of the NZW rabbit; a well-established technique. The rabbit femoral condyle is usually selected by researchers due to the anatomical predominance of trabecular bone over cortical bone, which presents a higher remodelling rate compared to cortical bone providing early results in biomaterial evaluation [3,7]. The "critical size" of a femoral bone defect in the rabbit femoral condyle has been determined to have a 6 mm diameter [2,8,9] and was selected in the present study. This paper aims to identify key points that are important to take into consideration when using this animal model in order to avoid potential pitfalls, such as the incorrect localization of the femoral site or the defect dimensions, or the unsuccessful confinement of the material implanted to the defect, based on our personal experience.

2. MATERIALS AND METHODS

2.1 Laboratory Animals

New Zealand White male rabbits, obtained by a commercial breeder (Kounker, Greece) 3-4 months old with a mean body weight of 3.3±0.2 kg (mean ± SD), quarantined and acclimatized for 10 days in the conventional facilities of the Laboratory prior to surgery, were studied. In addition to the above inclusion criteria regarding sex, age and weight, an accompanying health report from the breeder was also required.

It was calculated that a sample size of 10 rabbits per group was required in order to have a 90% probability of demonstrating a between group difference of more than 10% (30% vs 40%, SD 6.5) in percent of newly formed bone at 12 weeks, with a significance of <5% (two-tailed test).

It was decided to include 12 animals per group, in case an anesthetic or surgical casualty would reduce the desired minimum number [10] of animals. In each group a different biomaterial was to be implanted. The exclusion criteria set were loss of appetite and body weight, infection of the surgical site and fracture of the femur either during creation of the defect or post-operatively.

The study application, including the power analysis and animal number, was approved by the Institutional Protocol Evaluation Committee and was granted a project license (No. 3009/15.05.2014), by the Prefecture Veterinary Directorate, according to national legislation (Greek Presidential Decree 56/2013) in conformance with the Directive 2010/63/EU.

All procedures followed were according to the P.D. 56/2013. Rabbits were housed in individual stainless steel cages under standard environmental conditions and were fed a high-quality commercial balanced pelleted rabbit diet; tap water in bottles was provided *ad libitum*.

2.2 Anesthesia and Surgery

Pre-emptive analgesia (carprofen 2.2 mg/kg sc), chemoprophylaxis (enrofloxacin 10 mg/kg sc) and general anaesthesia (ketamine / xylazine 30/5 mg/kg im) are administered to the rabbits preoperatively. Preoperative analgesia reduces the required anesthetic dose and promotes optimal recovery. Induction of anesthesia, preparation of the surgical site by shaving the extended left knee region of both femur and tibia and aspiration of the fur, are followed by transfer of the rabbit to the aseptic operating room, scrubbing with povidone iodine and aseptic draping of the site.

The first aim is to be accurate in the localization of the defect. Identification of the femoral condyles and the patella is achieved by palpation (Fig. 1), while determination of the site to be drilled follows after a 2 cm dermal incision 0.5 cm proximally from the patella and parallel to the femur's axis, and observation of the external distal femoral condyle (Fig. 2). The underlying fascia is incised parallel to the skin incision and the femur shaft. The external condyle is exposed by spreading the muscle fibers. The site to be drilled is 2 cm proximally to the external condyle and in the middle of the femoral metaphysis.

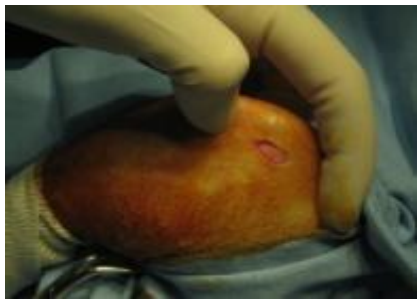

Fig. 1. Palpation of the distal femoral condyles and patella

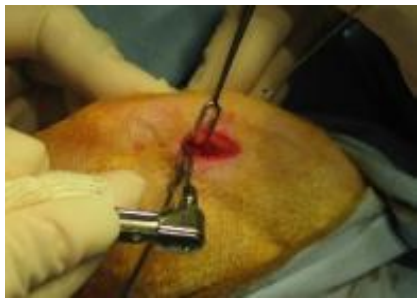

Fig. 2. Determination of the site to be drilled

The second aim is to be accurate in the width and depth of the defect. In order to achieve the desired critical size round defect of 6 mm diameter, drilling with the use of gradually increasing diameter drills is applied consecutively, starting with a 2 mm diameter (Drill 2 mmD x 3-13 mmL) until a 5.5 mm diameter drill (DL1356 Twist Step Drill 5.2/5.6 mmD x 6-13 mmL) is finally used in order to achieve the 6 mm diameter. The drill moter type used in our Laboratory is the NSK surgic XT and the handpiece is the NSK ER 20i.

Cooling of the bone by normal saline irrigation is continuous during the drilling process. If the localization is correct, trabecular bone can be observed during drilling and the minor bleeding can be controlled by 20-40 seconds of pressure application of a sterile cotton swab. It is equally important to continuously observe the drill's depth marks, in order to achieve the desired defect depth (10 mm) and not to extend the drilling to the cortex of the other side.

The next challenge is to have the specific amount of biomaterial implanted to be confined to the femoral defect. Slow and careful filling of the defect follows. Depending on the consistency of the biomaterial to be tested, its potential distribution in the femoral shaft, diffusion in the metaphysial trabecular bone or excessive covering of the bone surface, are pitfalls to be avoided. These undesirable cases or a successful implantation can be diagnosed by radiology following the implantation or after euthanasia (Figs. 3, 4 and 5).

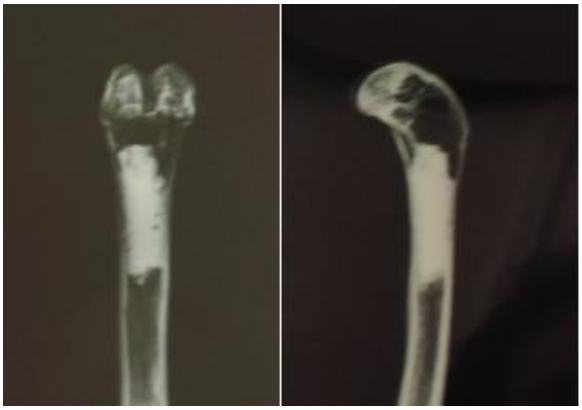

Fig. 3. Undesirable distribution of the implanted biomaterial into the femoral shaft proximally to the defect, shown in front/back and lateral X-rays of the lower femur *ex vivo*

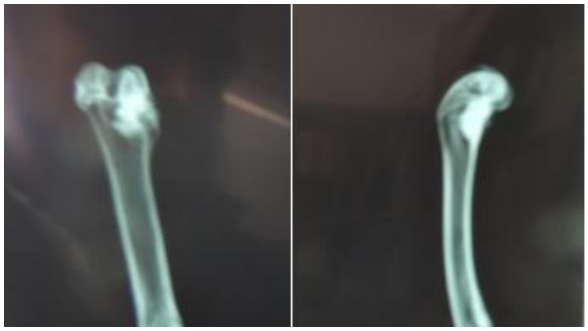

Fig. 4. Undesirable diffusion of the implanted biomaterial in the femoral condyle shown in front/back and lateral X-rays of the lower femur *ex vivo*

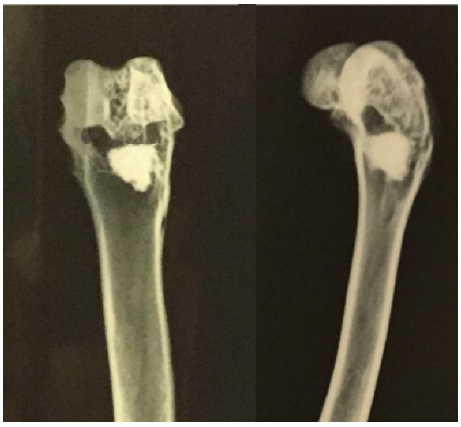

Fig. 5. Successful biomaterial implantation in a critical sized femoral condyle defect, without distribution in the femoral shaft or significant diffusion in the condyle, shown in front/back and lateral X-rays of the lower femur *ex vivo*

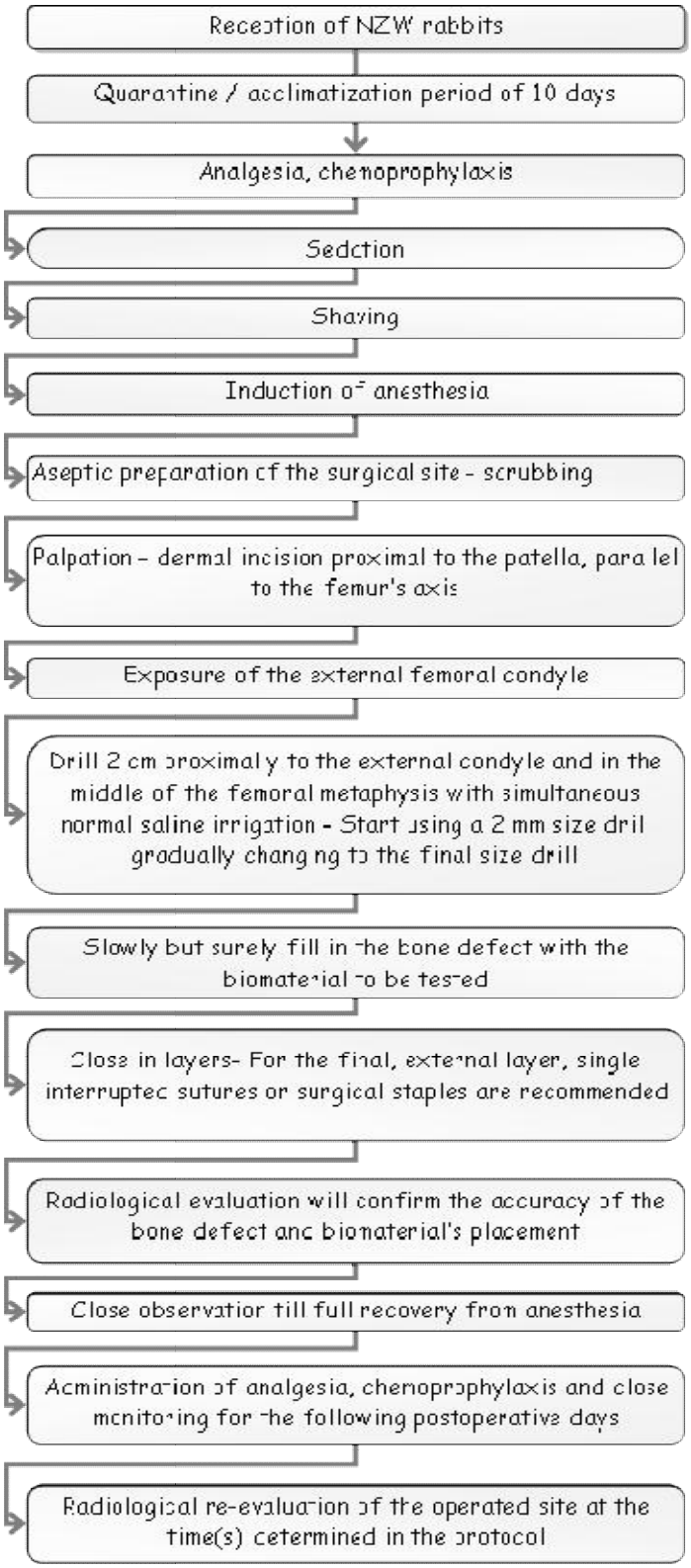

Fig. 6. Description of the sequential steps in a research study of biomaterial implantation in the rabbit animal model of femoral bone defects

The surgical site is closed in layers, with interrupted single sutures or surgical staples for the skin closure.

2.3 Post-operative Course

With the above anesthetic and analgetic scheme, all rabbits recover uneventfully with normal weight-bearing and appetite from the first postoperative day and continue to receive analgesia and chemoprophylaxis for 2 and 4 days post-operatively respectively. As rabbits have been observed to post-operatively remove the incision stitches, aseptic surgical stapling is often applied as a skin closure with a very good healing result. Depending on the biomaterial studied, they are euthanized between 6 and 12 weeks post-operatively, after which the femur is removed, evaluated radiologically and processed for pathology.

3. RESULTS AND DISCUSSION

Although the research group consists of Veterinarians and Orthopaedic Surgeons with experience in this animal model, they are challenged with potential pitfalls which must be overcome in each animal's operation in order to achieve homogenous and reproducible scientific results consistent with animal welfare. It should be noted that there were no casualties with the use of the previously mentioned anesthetic and analgetic protocol. The induction of general anesthesia includes the administration of xylazine followed by the administration of ketamine, which is considered an effective combination [10]. Ketamine alone as a non-competitive antagonist of the N-methyl-D-aspartate receptor has some analgesic and antihyperalgesic properties. Its combination with xylazine provides good muscle relaxation, analgesia is prolonged and smoothens the recovery from anesthesia [4,11,12]. In addition to the sterile surgery procedures, preoperative chemoprophylaxis with enrofloxacin [13] for a total of 5 days resulted in no adverse effect on the animals' gastrointestinal system, no infection of the operated site or case of osteomyelitis.

Several anatomical sites have been used in the NZW rabbit model of bone defects to evaluate biomaterials, such as the calvaria [5,14], mandible [15] and femur [6,7] of which the latter has been selected by our research group. Among the challenges of this experimental model, the first step to achieve is the precise localization of the defect to be drilled. Intra-operative palpation of the external femoral condyle assists in determining the site to be drilled, and post-operative X-ray evaluation can confirm it.

Additionally the correct size and depth of the bone defect are important to adhere to. That is achieved by initially drilling with a 2 mm diameter drill and gradually changing to larger sized drills, until the use of a 5.5 mm diameter bone drill creates the desired critical size defect of 6 mm diameter. Throughout this procedure, observation of each drill’s depth marks is of utmost importance, in order not to exceed the desired 10 mm depth.

Another challenge is to have the specific amount of biomaterial implanted to be confined to the bone defect. Damage to the trabeculae due to the drilling of the metaphyseal trabecular bone may create a pathway permitting the biomaterial's distribution in the femoral shaft. Similarly, the biomaterial may diffuse into the metaphysial trabecular bone. Alternatively, even a minor excess of the amount of biomaterial implanted may lead to another pitfall, such as excessive covering of the bone surface at the

Table 1. Overview of the challenges of this experimental model and the recommendations to overcome them

Challenges	Recommendations
• Homogenous results	Same region and treatment for all rabbits
• Precise localization of the defect to be drilled	Intra-operative palpation of the external femoral condyles and the patella 2 cm proximally to the external condyle and in the middle of the femoral metaphysis
• Appropriate size and depth of the bone defect	Gradually augment the diameter of the used drill until the desired one
• Proper amount of biomaterial implanted	Depending on the protocol, increasingly fill the bone defect without exceeding the bone surface

defect site. Such failures of the experimental procedure will probably require its repetition in additional rabbits in order to reach the desired number of animals per evaluation group, leading to an unprogrammed increase of the total number of rabbits used, as well as an increase of the associated cost and research duration.

The lack of available radioscopy equipment in the operating room could be considered a limitation to this study. Intra-operative radioscopy could assist in immediate evaluation of a successful or unsuccessful biomaterial implantation instead of using radiology post-operatively or at euthanasia. However, the costs associated with radioscopy may lead researchers to use a similar methodology with radiology examination at the end of the operation and/or of the study. The choice of only male rabbits could be considered another potential limitation. However, our selection was based on the aim to obtain results from the bone biomaterial implantation without female hormonal influences. This choice additionally allowed us to adhere to the principle of Reduction of the 3Rs (Replacement, Reduction, Refinement) by not increasing the number of animals used [16].

4. CONCLUSIONS

The wide use of this animal model in the evaluation of biomaterials in orthopaedic and craniofacial research requires knowledge, skills and surgical accuracy of the research team. These qualities are valuable in order to have high repeatability of the implantation technique. By fulfilling these conditions, the extraction of valid scientific results and reduction of the number of animals used are possible.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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