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The Sheep as an Animal Model in Orthopaedic Research

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ABSTRACT: The aim of this paper is to describe a technical sequence of procedures, including anaesthesia and surgery steps, that we are using in our facilities as standard surgical procedures in the sheep as an experimental model to evaluate the mechanical behaviour of new concepts of hip implant, rapidly manufactured in the surgery time, and to study the osteointegration phenomenon through histological analysis.

KEYWORDS: Animal-models, Biomaterials, Implants, Experimental Surgery, Sheep

INTRODUCTION

According Davidson et al. (1), the selection of animal models, for research should be based on the following considerations: 1) appropriateness as an analog, 2) transferability of information, 3) genetic uniformity of organisms, where applicable, 4) background knowledge of biological properties, 5) cost and availability, 6) generalizability of the results, 7) ease of and adaptability to experimental manipulation, 8) ecological consequences, and 9) ethical implications. The criteria for selection or rejection of particular animal models also include customary practice within a particular discipline, the existence of diseases or conditions that might complicate results, the existing body of knowledge on the problem under consideration, and special features of the animal, that may make a particular species useful (1).

Sheep are a convenient large-animal model for biomedical research because of availability, ease of handling and housing, animal cost, and acceptance to society as a research animal (2). In orthopaedic research, sheep are a well accepted model for in vivo studies: although the anatomy of quadrupeds is quite different than humans, sheep is useful to address the biomechanical, biochemical and histological processes of bone biology, due to similarities with humans in weight, size, bone and joint structure and bone remodeling process (3-7); on the other hand, although rodents may be less expensive, they have different bone morphology, and often are too small in size to test degradable materials in bone especially in combination with internal fixation and fracture repair (8) as they are too small to easily be used as a model to study joint replacement implants. However sheep are not suitable for studies involving oral absorption of drugs, because of their different gastrointestinal system, and the lack of natural menopause, the normal estrus cycles restricted to fall and winter and seasonal changes in bone metabolism are physiological disadvantages if sheep are used to establish an animal model for osteoporosis (3,7,9).

All materials intended for application as biomaterials, medical devices, or prostheses undergo tissue responses when implanted into living tissues (10). Recent advancements in biomaterials science have focused on the control of those biological responses (11) and the understanding of cellular interactions with synthetic surfaces, particularly in the context of inflammatory and healing responses. The development of new methods to evaluate the interactions between bone-implant surfaces has been a major goal of orthopaedic and oral surgery (12) and several phenomena have been investigated, such as biocompatibility, osteointegration, cell adhesion, osteoinduction and osteoconduction, all of which have broad applications on in vitro and in vivo studies. On the other hand, there is a search for the best implant surface and geometry to increase bone adhesion and growth (12). For this reason the use of animal models is often an essential step in the testing of orthopaedic and dental implants prior to clinical use in humans (13-15). The use of sheep as model in remodeling process in cancelous and cortical

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bone for the assessment of new orthopaedic biomaterials and implants [16-21], in biomechanical studies (5,22) and as model for tissue-engineered bone constructs (23) has been described.

This paper describes surgical procedures using in sheep as an animal model to study the biocompatibility of new biomaterials and test new concepts of hip implant built in PEEK-Carbon (24) or manufactured in the surgery time (25) and also to evaluate the bone conduction or guided bone regeneration in the implant-bone interface using several different scaffolds and nanotopographies.

MATERIALS AND METHODS

Animal model and surgery

The trials were performed by strictly following Portuguese laws on animal experimentation (Portaria 1005/92) and approved by the official veterinary authorities. Animals were provided from the flock of the C.E.E.H.M. of the University of Évora, healthy mature, with an age of 3 to 6 years and bodyweight of 45 to 65 kg, females, alentejano merino local breed. The implantation sites were the crest of the tibia for the implants or the scaffolds and the proximal epiphysis of the femur for the hip implant. Food was withdrawn 48 hours and water 6 hours prior to anaesthesia. The operation sites were prepared in the standard manner, shorn and cleaned with polividona iodine and alcohol. Before surgery animals were pre-medicated with xilazine (Rompum®) 0,05-0,1 mg/kg IM and atropine sulfate 0,7mg/kg SC. General anaesthesia was induced with sodium thiopental 5-10 mg/kg IV and after an endotracheal intubation, maintained with isofluorane in 100% Oxigen. During the operation the animals were infused with saline solution and monitored through a pulsoximeter and eletrocardiography. Analgesia was maintained perioperatively, through butorphanol (Torbugesic®) of 0,01mg/kg IV and postoperatively for 3 days. They also received antibioterapy for 5 days after surgery.

Proximal femoral epiphysis preparation for hip hemi-arthroplasty

The skin incision was made just cranial to the grater trochanter and over the cranial border of the shaft of the femur. Another incision was made in the fascia lata to free it and its tensor muscle cranially, and the biceps femoris muscle caudally. Blunt dissection and separation along the neck of the femur with the finger tip allows visualization of a triangle bounded dorsally by the middle and deep gluteal muscles, laterally by the vastus lateralis muscle, and medially by the rectus femoris muscle. After identification of the joint capsule, an incision was made and continued laterally along the femoral neck through the origin of the vastus lateralis muscle on the neck and lesser trochanter. The exposition can be improved by the tenotomy of a portion of the deep gluteal tendon close to the trochanter. Two Hohmann retractors were placed intracapsularly, ventrally and caudally to the neck, to allow visualization of the femoral head and make the osteotomy through its neck. The opening into the femoral canal was started with a drill a bit smaller size than the femoral stem. The final preparation of the femoral channel was enlarged by hand with reamers prepared for the effect. After cleansing of the bone marrow cavity and stopping of bleeding, slowly, retrogradely the femoral component was introduced into the femoral channel taking care achieving the correct position (Figure 1)

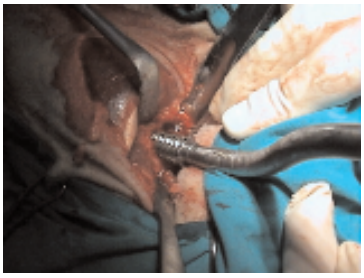

Figure 1. Enlargement with reamer

Proximal tibial epiphysis preparation

The skin incision was centered along the longitudinal axis bone, in the craniomedial face of tibial tuberosity. The underlying fascia was opened, and through blunt dissection exposed the cortical bone, that was drilled with the help of a drill guide, placed perpendicularly to the bone long axis and firmly pressed to avoid slippage. All bone perforations and drillings were performed under constant irrigation with saline solution (Figure 2).

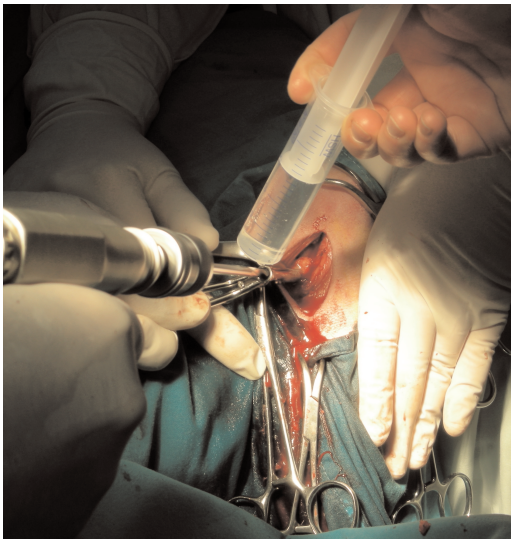

Figure 2. Intra-operative modelling of the stem hip-prosthesis

This predrilled hole can be enlarged according to the implant design (Figure 3). The sites of implantation could also be in the medial proximal epiphysis for cortico-trabecular bone studies or in the medial diaphysis for cortical ones, if they were drilled medial to lat-

eral direction.

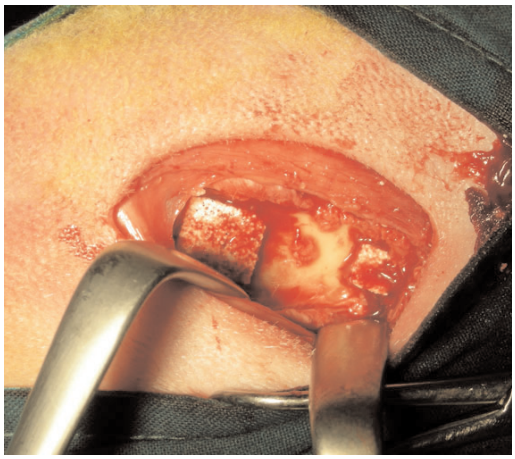

Figure 3. Drilling the holes

All of the animals recovered well from the anaesthesia and surgeries without signs of infections, lameness or other discomfort. After all surgical procedures, the animals were kept in boxes for large animals in groups, the lameness and health conditions were monitored. Although some of the biomaterials are radiolucent, periodically the sheep were radiographed (Figure 4). Animals were sacrificed at different times, depending on the original study, with overdose of pentobarbital.

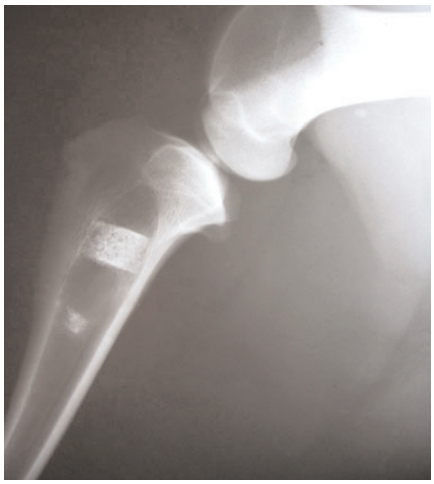

Figure 4. Xray with the scaffolds

Evaluation of samples

Bones were harvested, freed from overlying soft tissues, cut in small blocks (Figure 5) and fixed in 10% (v/v) phosphate buffered formaldehyde for at least 2 weeks, before they proceeded for histological non-decalcified or decalcified bone sections. The non-decalcified sections embedded in methyl methacrylate and prepared in a standard manner (17,24,26), and the EDTA decalcified sections (the implant was gently removed from the surrounding tissue), paraffin embedded and routinely processed, were analysed, by histomorphological-histometrical and immunohistochemical techniques, using light microscopy, as means of assessing the differentiation status of bone deposition and growth, as well as the behaviour of biodegradable materials (Figure 6). For histomorphometrical measurements on cancellous and cortical bone we use the nomenclature approved by the American Society of Bone and Mineral Research (ASBMR) (27). On the other hand, an Index of Affinity (as the ratio of the length of the region in which bone is directly apposed to the implant without the presence of fibrous membrane divided by the total length of the bone-implant interface) and an Affinity Index and Bone Ingrowth (as the amount of bone grown into the implant surface) were also performed.

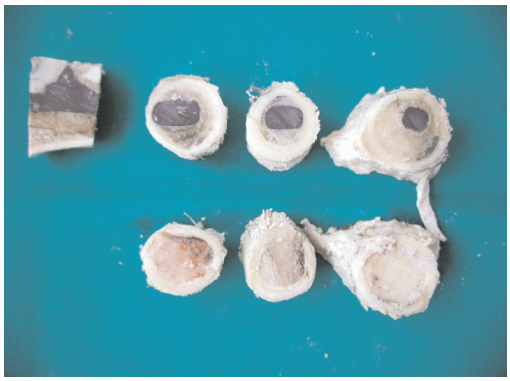

Figure 5. Macroscopic image of the bone fragments with the biomaterial

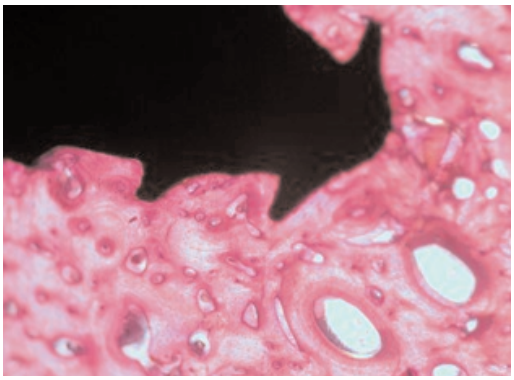

Figure 6. Interface bone-implant

RESULTS AND DISCUSSION

The use of sheep as animal model is now well established for the assessment of new orthopaedic biomaterials and implants. The procedures reported here provide some basic and general information on the suitability of sheep as experimental model for conducting in vivo orthopedic studies. However some aspects should be carefully evaluated when using sheep as an experimental model in orthopedic research. According Bouré et al. (20) sheep have a limited availability of cancellous bone for implantation of biomaterials or surgical implants making it difficult to find multiple comparable sites within a same animal. The authors recommend using the proximal and distal humerus and the proximal and distal femur for the implantation of a maximum of

8 different sites. Concerning the proximal and distal humerus, the distal femur and proximal tibia being used implants of 5 to 8 mm diameter and 15 to 13 mm depth, respectively (20). Additionally Nusss et al. (19) have developed an animal model with sheep that allows the intra-osseous implantation of 8 different site samples per animal in long bones. For use in large animals such sheep, the International Standard ISO recommends cylindrical no larger than 4mm in diameter and 12mm in length and a maximum of 12 implants per animal. This animal model facilitates testing inter and intra-individual differences among different materials, while at the same time, reduce overall used animals, as well as providing necessary numbers to satisfy statistical requirements.

Our results from the histological point of view demonstrated that, besides there were no signs of infections, they contribute to the study of the phenomena that occur at interface bone-implant mainly the osseointegration process and biomechanical aspects of bone remodeling (24,25). The authors hope that this report will contribute to extrapolation of reliable data for use of sheep as animal model in the orthopedics field.

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