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Clinical anatomy of the meniscus in animal models: pros and cons.

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ABSTRACT:

Nowadays, despite the possibility to use in vitro or computer models in research, animal models are still essential. Different animal models are available for meniscal repair investigation. Although a unique perfect model for the structure of the human's knee does not exist, the choice of the proper animal model is crucial for a correct research. The principal animal models in the meniscal repair are sheep, goats, pigs and dogs. Each of these has pros and cons for their utilization. Analysing each pro and con is essential for optimizing the choice of the animal model, which depends on the experimental question, avoiding unnecessary waste of resources and minimizing the animal suffering, according to the Russell and Burch's three "Rs" principles (Reduce, Refine and Recycle). In this concise review, we resume the meniscus anatomical features of the main large animals, in order to help choose the most suitable animal model for subsequent studies on meniscal repair.

Key words: meniscal repair, animal models, meniscus.

INTRODUCTION

Despite the promising results of alternative procedures such as cell, tissue and organ cultures, animal models are still essential for scientific, economical, ethical, and regulatory reasons in many research fields, and orthopaedics is one of them. In fact, animal models are a unique tool for testing tissue repair and regeneration solutions, as well as for validating specific tissue engineering methods after in vitro testing and before moving to clinical application. The meniscus is a challenging tissue from the healing point of view, since it often undergoes a time-dependent degeneration and exhibits a very low healing potential. On the other hand, it is frequently affected by injuries, especially tears. These lesions are extremely frequent and represent the most common cause of surgical procedures performed by orthopaedic surgeons (1). They are also often responsible for the consequent development of osteoarthritis. The functionality of different therapeutic devices and the efficacy of engineered tissues for meniscal healing have been also evaluated in different species. Since the ultimate goal of research is achieving a functionally efficient clinical outcome, species-specific features must be taken into account in the different experimental questions. Therefore, several animal models turn out to be more or less valuable in the different scientific issues.

THE MENISCUS: GROSS ANATOMY, STRUCTURE, COMPOSITION AND PHYSIOLOGY.

The menisci are fibro-cartilaginous structures located in the knee joint, in simultaneous contact with the femoral condyles and the tibial plateau. They are C-shaped, and they can be anatomically divided into three different regions: a body and two horns. Each meniscus has a thick convex peripheral margin in contact with the inner capsule of the joint, and an opposite thin- and free-edged margin (2). The meniscal body is made of a dense scaffolding of type I collagen fibers characterized by circumferential orientation. Throughout the tissue, a lower number of radial fibers can also be found. Radial fibers seem to hold circumferential fibers together, opposing longitudinal stresses (2). The meniscal tissue is composed of water (72%), collagen (22%), and glycosaminoglycans (GAGs) (0.8%) (2). On a dry weight basis, normal adult menisci contain 78% collagen, 8% noncollagenous proteins and 1% hexosamine (2).

Collagen type I is the major collagen component (90%), followed by collagen types II, III and V (2). Regional variations exist in the distribution of collagens: collagen type I is mostly represented in the peripheral area, while collagen type II is restricted to the inner zone, which shows cartilage-like properties (2). Normal human meniscal proteoglycans contain 40% chondroitin 6-sulphate, 10–20% chondroitin 4-sulphate, 20–30% dermatan sulphate and 15% keratan sulphate (2). The most represented cell type in the meniscus is the fibrochondrocyte (2).

Moreover, the vascularization of the meniscus is provided by a perimeniscal capillary plexus which forms from the lateral and medial geniculate arteries (2).

Structure and composition exhibit numerous interspecific differences that will be considered later.

ANIMAL MODELS:

The need of using animal models despite the existence of new techniques and resources that virtually seem to allow the non-use of animals in research, is due to the fact that all in vitro studies have some limitations because they focus on aspects that cannot be transferred to the in vivo scenario (3). Unfortunately, a perfect model for meniscal repair in the animal reign does not exist. The principal animals employed in studies on meniscal repair are sheep, goats, pigs and dogs. A few studies have used the cow meniscus which, however, has been proved to be significantly bigger than the human meniscus based on anatomical analysis. Each model has pros and cons for its use, as summarized in table I, including different anatomy and behavioral habits.

Table I. Pros and Cons among different animal models for meniscus studies MODEL	**PROS**	**CONS**
Sheep	Similar anatomy, vascularization and mechanical properties; Possibility to utilize like chronic models; easy to handle and not aggressive.	Difficult post-operative immobilization; Different force distribution.
Goat	The most similar anatomy; Joint easy accessible by arthroscopy; Less expensive than other large animal models and quite easy to handle.	Difficult post-operative immobilization; Small dimensions of the knee.
Pig	Similar vascularization patterns, cell density, collagen structures and biomechanical properties; Similar weight; Possibility to fast translate the information to human field.	Expensive and difficult to host; Can be aggressive.
Dog	Anatomy, histology, and biochemistry similar to that of the human knee; Joint easy accessible; Causes, symptoms, and therapies for osteo-arthritis similar to those in people; Easy handling; Different available sizes.	Strong intrinsic response to tissue damage by deposition of fibrous tissue; Elevated tibial plateau; More ethical issues.

Sheep

Sheep are often chosen as model (2) for the analysis of the effect of a meniscectomy in the joint environment, for meniscal repair (with growth factors) or replacement (meniscus allograft and engineering).

Compared anatomy:

The sheep menisci are shorter than the human ones, but they are the most similar in size among the different animal model species (4). In particular, the lateral sheep meniscus has been found to have a size not statistically different compared to the lateral human meniscus. In fact, except for circumference, dimensions like width, peripheral height, articulating height, superior articular length matched those of the human meniscus. Also, most of the dimensions of the sheep medial meniscus showed no statistical difference in comparison to the human menisci. Both medial and lateral meniscus have smaller average wet weight and volume than human menisci (5).

The medial and lateral menisci have a small connection, which does not hide their anterior insertion sites (4). Microscopically (Figure 1), the sheep meniscus presents fusiform cells at the surface and round cells in deeper layers (2). The water content of ovine meniscus is 68%, and the remaining dry weight being is largely composed of collagen (75%). Sheep menisci show a deeper Safranin O stain compared to human menisci, but GAGs distribution is similar between the two species. GAGs are present mainly in the inner zone (6). Collagen type I throughout the entire matrix except for the inner tip (2) and collagen type II with an age-dependent distribution: in the young ovine it is located only in the inner zone (7) while in the adult it is located in the inner main body (2).

Pros:

Sheep have the most similar meniscal anatomy (4) with vascularization patterns similar to those of human being (2). Mechanical properties of sheep menisci are comparable to those of men and can be utilized to simulate chronic meniscal injuries that better represent the type of lesions found in human clinical practice (8). This species is handy and not aggressive.

Cons:

The distribution of shearing and compression forces during the gait is different between human and sheep (2), because the latter usually loads knee in flexion (2) while human does in extension. Sheep generally do not tolerate the post-op immobilization and, even if immobilized in a cast, the full weight bearing could not be completely avoided (2).

Goat

Goats are used as model for meniscal repair with sutures and cell therapy and for testing new devices or techniques for meniscal replacement with allograft or tissue engineering-based strategies (2).

Compared anatomy

The goat is an animal model very close to humans. In fact, the medial meniscus size and proportions are extremely similar to those of humans (4). A menisco-tibial coronary ligament is also present (4). The main differences are the absence of a full-length fibula/proximal tibiofibular joint, replaced by a fibular head fused to the lateral side of the tibial plateau, serving as an attachment site for the lateral meniscus (4).

Pros:

Considering macroscopic anatomy, the goat is the ideal model. The size of caprine knee is similar to humans and the joint, thanks to its structure and dimension, is easily accessible by arthrotomy and arthroscopic procedures (2). Moreover, the proportion of cartilage to subchondral bone and the subchondral bone consistency is closer to those of humans than those of other models (2,9). Since goats rapidly develop osteoarthritic signs following meniscectomy/meniscal replacement (2), they are considered suitable models for the evaluation of the consequences of meniscal substitution on articular cartilage. Goats are less expensive than other large animal models and quite easy to handle (2).

Cons:

Despite the ideal dimension of the knee (very similar to human), the size of the caprine tibiofemoral joint is still significantly smaller compared to human (2). Functionally, it is recognized that the fused tibiofibula in the goat could potentially make its bony anatomy more stable than the human knee in a posterolateral knee injury (10). Moreover, the goat meniscus has been considered too small to provide a realistic model for arrow insertion (11). Behavioral factors limit the use of this model due to rehabilitation concerns. Goats are browsers, not grazers like sheep, so they often stand on their hind legs while foraging. Thus, excessive weight bearing may lead to early damage to meniscal implants or sutures (6). Finally, despite the favorable cost, very few studies on meniscus replacement/repair have been performed on goats compared to sheep: this probably also reflects a partial lack of facilities for goat housing and surgery.

Pig

Swine models (2) are usually employed for testing meniscal sutures and cell therapy (12,13).

Compared anatomy:

The two menisci present a small connection between the two anterior insertions that, however, does not hide the distinct anterior insertion sites (4). Furthermore, the lateral porcine meniscus is longer than the medial one (4). The meniscal weight and the meniscal volume in pig are significantly larger than those of humans. The pig meniscus is bigger in size than the human meniscus in most of the dimensional aspects (5). Microscopically, the pig meniscus present chondrocyte-like cells in the inner and middle zones (2). Collagen type I is organized as circumferential fibers and mainly located in the external zone while collagen type II is organized as radial fibers and is principally located in the inner and intermediate zones (2).

Pros:

The porcine meniscus is similar to the human meniscus in its shape and structure (2), and, in particular, it is comparable to the young adult human meniscus. The pig model, like human, is characterized by the absence of spontaneous healing (2). The miniature swine bred are also able to maintain an adult weight and size comparable to adult men (2). This model features allow the reasonable translation of the results to clinical applications (14).

Cons:

Porcine menisci have higher mechanical properties than adult human menisci, displaying minimal deformation and equilibrium displacement in response to the visco-elastic creep response compared to human menisci (2). For these reasons, the clinical relevance of the data originating from this species has to be carefully considered. In addition, pig breeding involves high costs and the facilities that can host this large and potentially aggressive animal are limited (2).

Dog

Canine models are often utilized like models in meniscectomies, cell therapies and meniscal replacement techniques (2). The dog model has been used in many reports because of the high animal compliance which allows better control in postoperative care, but it is getting more and more unpopular due to the pressure coming from the animal rights organizations.

Compared anatomy:

Dogs have a relatively high tibial slope (range: 19-28°) with respect to human (range: -1°-19°) (15). The insertion areas of the medial meniscus are smaller than those of men (2). Microscopically, meniscus cells are round shaped and, particularly in the anterior and posterior horns of the inner third, chondrocyte-like while in the outer third fibrocytes are present (2). Aggrecan, as an organized network, is more concentrated in the inner zone (16). Collagen type I forms circumferential fibers throughout the matrix from the peripheral to the inner area but not radial ones (2). Collagen type II is markedly deposited in the inner body in an organized network composed of both radial and circumferential fibers (2).

Pros:

Anatomy, histology, and biochemistry of the dog's knee are very similar to those of human. Furthermore, causes, symptoms, and therapies for osteo-arthritis in dogs are similar to those in people (17). The dog model is characterized by easy handling, a variety of available sizes and increasing information on meniscal repair and replacement. Dogs have an exposed stifle joint that makes the arthroscopic approach easier with respect to the other large animal models.

Cons:

This animal model is characterized by a strong intrinsic response to tissue damage by deposition of fibrous tissue (2). The elevated tibial plateau can compromise the translation of the results to men. Finally, there are more ethical issues when dogs are utilized in the research, because of the domestic relevance that this animal has conquered in the course of human history.

DISCUSION AND CONCLUSIONS:

Animal models are fundamental in meniscal repair and regeneration studies. In particular, large animal models are more suitable for anatomical and biomechanical studies, as they are very similar to humans as far as these characteristics are concerned. Moreover, they proved to be excellent models for the evaluation of surgical biomaterials and procedures. Nevertheless, further aspects besides the anatomical one, should be considered when choosing the animal model for exploration of new strategies and clinical translation of novel techniques. For example, the innate regenerative capacity shown by menisci of some animal models, could jeopardize the outcome, thus representing a limitation in their use. Menisci in dogs, pigs, and sheep, like in humans, do not have innate regenerative capacity which makes them suitable animal models. Furthermore, as the vascularity and cellularity of the meniscus decrease on maturation, the animals used have to be skeletally mature. The different gait pattern compared to humans and the difficulties in postoperative weight bearing management in these species make them still unsuitable for the evaluation of the short-term efficacy of fragile materials (18).

This review may serve as a guide to help choosing the most suitable animal model among different large animals for meniscus studies, taking into account remarkable anatomical similarities to humans and reasonable handling costs.

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Figure Legends

Figure 1. Safranin-O histochemical staining: orange/pink color for GAGs deposition and green color for fibers. Both pictures have the same scale bar located in Figure 1a. 1a) anterior horn of an adult pig with positive staining for extracellular matrix and chondrocytes (orange); b) anterior horn of an adult sheep with positive immunostaining for extracellular matrix (orange) and negative staining for fibers (green).