A comparative study of articular cartilage thickness in the stifle of animal species used in human pre-clinical studies compared to articular cartilage thickness in the human knee

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Summary
Histological measurements of the thickness of non-calcified and calcified cartilage, as well as the subchondral bone plate, in five locations on the femoral trochlea and medial femoral condyle of species were used in pre-clinical studies of articular cartilage and compared to those of the human knee. Cadaver specimens were obtained from six human knees, six equine, six goat, six dog, six sheep and six rabbit stifle joints (the animal equivalent of the human knee). Specimens were taken from the lateral trochlear ridge, medial trochlear ridge and medial femoral condyle. After histopathological processing, the thickness of non-calcified and calcified cartilage layers, as well as the subchondral bone plate, was measured. Average articular cartilage thickness over five locations were 2.2–2.5 mm for human, 0.3 mm for rabbit, 0.4–0.5 mm for sheep, 0.6–1.3 mm for dog, 0.7–1.5 mm for goat and 1.5–2 mm for horse. The horse provides the closest approximation to humans in terms of articular cartilage thickness, and this approximation is considered relevant in pre-clinical studies of cartilage healing.

Keywords
Reticular cartilage thickness, stifle

Introduction
From a clinical point of view, there are two distinct goals for cartilage repair: restoration of joint function (which includes pain relief), and prevention of, or at least, delay of the onset of osteoarthritis (1). These goals can potentially be achieved through replacement of damaged or lost articular cartilage with a substance capable of functioning under normal physiologic environments for an extended period. Screening of potential procedures for human clinical use is done by pre-clinical studies, which assess restoration of a joint surface by its appearance anatomically, histologically, biologically, and mechanically relative to the original tissue, hyaline cartilage. Pre-clinical studies use animal models, and the choice of animal models is one of the most frequently discussed and controversial areas. It has been stated that the key issue in the selection of the appropriate model, is to match the model to the question being investigated and the hypothesis being tested (2).

In 1929, a Danish physiologist named August Krogh wrote, ‘for a large number of problems there will be some animal of choice, or a few such animals, in which it can be most conveniently studied’ (now known as the ‘August Krogh Principle’) (3). It has, however, also been pointed out that the uncritical application of this principal could lead to inaccurate generalizations, because extrapolating findings from one species to another is not always without flaws (4). The researcher must consider which animal model(s) most accurately represents(s) the human condition being investigated and to what extent might the results obtained from these models be extrapolated to humans (5). The obvious critical questions with regard to joint defect repair are:

1) Which animal model(s) most accurately represents(s) the critical chondral defect in humans, and

2) To what extent can pre-clinical research results in this model be extrapolated to humans (1).

If an animal model, while economically convenient, is totally inappropriate, then the research achieves nothing. The equine stifle also suffers considerable naturally occurring clinical disease and can answer questions for equine clinical patients as well as, potentially, the human patient. Osteoarthritis with erosion of articular cartilage occurs naturally on the medial condyle of the femur. The femoral trochlear ridges are commonly affected with osteochondritis dissecans (OCD).

Articular cartilage lesions encountered within the human joint typically arise as a consequence of trauma (usually a sports injury) or during the course of diseases such as osteoarthritis and osteochondritis dissecans. While changes in the subchondral bone (including sclerosis, cyst formation and osteophyte formation) occur, the defects rarely encroach significantly beyond the cartilage-bone interface into the subchondral bone compartment (5). Repair strategies should focus on re-establishing the articular cartilage compartment rather than the bony one (5). Anatomical studies on the thickness of human articular cartilage on the medial femoral condyle (a common site for clinical lesions) have reported thickness ranging from 1.65 to 2.65 mm with anat-
omical measurements (6) (and average thickness of 1.69 mm in a recent study using digitized photographs). Studies of the healing of articular cartilage defects have been done in numerous animal models including the rabbit, dog, sheep, goat, and horse. The criticism of many of these models is that the defect extends through subchondral bone and into cancellous bone below, and does not emulate the human lesion. The difficulty in emulating the human articular defect in small experimental animals not only depends on cartilage thickness, but also the volume with the majority of the defect being in bone (5).

The following study presents the histological measurements of the thickness of non-calcified and calcified cartilage, as well as the subchondral bone plate in five locations on the femoral trochlea and medial femoral condyles of the rabbit, dog, sheep, goat and horse (all species in which preclinical studies have been performed) and comparison with specimens from the same locations in the human knee.

Methods

Cadaver specimens were obtained of six different human knees, as well as six different equine, six goat, six dog, six sheep and six rabbit stifles joint (the animal equivalent of the human knee). All of the joints of the horse, sheep, rabbit, and dog were from animals that were skeletally mature, less than three years old, and did not show any indication of disease. Each animal was of similar weight, age, and fitness and rolled as a control in an experimental study. Human specimens were donated by ‘Allosource’ in Denver, CO; dated specimens were from an allograft bank and were from mature donors, again without joint disease. One specimen was taken from the lateral trochlear ridge of the femur and two from the medial trochlear ridge of the femur as depicted in Fig. 1. This figure shows how the samples were consistently taken from each trochlea. A total measurement was made of the trochlear ridge from the anterior articular cartilage margin (a) to the trochlea-condyle junction (b). The consistency of location of the samples was achieved by relating the position of the specimens to the top of the trochlear groove and the top of each trochlear ridge. Similarly, two specimens were taken from the medial condyle of the femur, and the right diagram of Fig. 1 depicts the position of the specimen sites (29% and 41%, respectively) along the line from the trochlea-condyle junction to the articular cartilage margin caudally. The medial femoral condylar site was chosen because it is a common area of clinical articular cartilage loss in humans, and has been used by the authors in articular cartilage repair studies of microfracture in the horse (8–10). Simi-

![Fig. 1](image_url)
larly, the authors have used the same locations on the trochlear ridges in studies of articular cartilage repair using autologous chondrocytes (11) and morcellized autogenous cartilage transplantation (12).

Each of the animal tissue specimens was obtained within at least 24 hours of death and frozen until sections were cut (except for the goat, which was sent whole-leg frozen, defrosted on arrival and sectioned). The human specimens were frozen, transported with dry ice, and specimens were cut within 24 hours of receiving them. The specimens were 2.5 cm x 1 cm in surface area and 3.5 cm deep (Fig. 1). These blocks were placed in 10% formalin and then decalcified. After decalcification the block specimens were then cut into 2–3 mm thick sections to make histological sectioning easier. Each of these specimens were embedded in paraffin wax, sectioned with a microtome to create a 5 μm sections which were placed on a slide, and stained with H & E (Haematoxylin and Eosin). Each section was subjected to a similar protocol so that any shrinkage and/or swelling could be controlled for by the comparison nature of the study.

The sections were then examined using a microscope coupled to a computerized digital analysis software package (Bioquant, Version 310, R & M Biometrics Inc., Nashville, TN). The thickness of non-calcified cartilage, calcified cartilage, and subchondral bone layers were then measured using this system. The thickness of each layer was measured in 10 different locations on each section. The H & E staining method easily detected a clear edge between the calcified and non-calcified layers. The subchondral bone layer was less distinct, but it was quite possible to consistently measure its thickness (Fig. 2). The measurements of the non-calcified and calcified layers were made separately on each individual section, but could be added together for total articular cartilage thickness.

The thickness measurements taken 10 locations in each section from the distal femurs of six different individual subjects (horse, goat, sheep, rabbit, dog, and human) and then averaged followed by analysis using a mixed model of variance (SAS version 8e, Cary, NC) that considered species and location as independent variables and the individual/animal as a random variable. When specific comparisons were made a Least Squares means procedure was utilized. A p-value < 0.05 was considered statistically significant. Reports are reported as means +/- standard error of the mean.

Results

The thickness of the non-calcified cartilage, calcified cartilage and subchondral bone in each location and for each species is depicted in Figs. 3–5 as well as summarized for total cartilage thickness (calcified plus non-calcified cartilage) in Table 1. In all locations, the thickness of human non-calcified articular cartilage was the greatest and...
was always over 2 mm. In all instances, the horse was the closest in thickness, with the non-calcified cartilage being 2 mm in thickness in both locations on the medial femoral condyle and between 1.5–2.0 mm in thickness on the trochlear ridges. The calcified cartilage layer was slightly greater in the horse than it was in the human in all five locations, meaning that the non-calcified and calcified cartilage thickness, when combined, almost equaled that of the human. The goat had the next thickest articular cartilage, being between 1.1–1.4 mm, respectively, in the two locations on the femoral condyle, but only ranging from 0.5–0.7 mm in the three locations on the trochlear ridge.

**Discussion**

Because of their size and relatively low cost, (compared to other species) rabbits have been commonly used in articular cartilage defect studies. However, emulating a human clinical defect in thickness and volume is different (5) as defects are typically at least 3 mm deep (7–10). Hunziker cited the example that if you created a 3 mm deep lesion in rabbit articular cartilage (the majority of which would be in bone), 93–95% of the volume of this defect would be enshrouded by bone, bone marrow space and vasculature (yielding an abundance of different cell types, growth factors and signaling substances and only 5–7% of the defect volume would abut on cartilage) (5). In some instances, specific questions can be answered with the rabbit model such as studies of donor cell fate (11) or when defects are filled with a plug (12, 13). In a classic study on continuous passive motion (CPM), full-thickness defects that were 1 mm in diameter and 4 mm deep at four different locations were created (and therefore they were principally in bone), marked differences in healing of the defects at 3 weeks in adult rabbits was demonstrated using CPM (13).

In a study that evaluated cartilage chondrocyte transplantation in the rabbit, a 3 mm diameter chondral defect which more closely emulated human defects was made creating a core by means of a sharpened, stainless steel punch and curetting down to ‘while not violating, the subchondral plate’. However, histological examination revealed failure to completely remove calcified cartilage (14) and this is one of the caveats also noted with similar defects in the dog (15, 16). In other studies the authors have found that, unless there is careful monitoring of the depth and use of arthroscopy to differentiate calcified cartilage from subchondral bone, calcified cartilage will often remain. Retention of the latter changes the nature of healing (17).

In this study, average articular cartilage and subchondral thicknesses over five anatomical locations were as follows: rabbit: 0.3 mm, sheep: 0.4 to 0.5 mm, dog: 0.6 to 1.3 mm, goat: 0.7 to 1.5 mm, and horse: 1.5 to 2 mm. Samples from human femurs showed a range of 2.2 to 2.5 mm. These data confirm that the majority of a 3 mm deep defect in the rabbit is indeed in the bone. The thickness of cartilage in the sheep is also an issue. Studies reported in the sheep have typically involved cylindrical defects penetrating deeply into the bone and the testing of osteochondral plug grafts (18).

Based on these thickness measurements, the goat is the next animal after the horse which comes close to the size of human articular cartilage thickness. Chondral defects were created in the goat by Driesang and Hunziker that were 5 mm in width and 10 mm in breadth and 0.5 mm in depth and so would be an appropriate chondral defect model (19). Other studies in goats have typically used deep osteochondral defects and evaluated osteochondral plug grafts (20). Jackson et al. have also described spontaneous repair of full-thickness 6 mm diameter and 6 mm depth defects in goats but progressive subchondral bone resorption and cyst formation occurred (21).

This study shows that the horse, among the species studied, provides the closest approximation to man in terms of articular cartilage thickness in the stifle (knee) joint. Also patent of OA lesions, at least on the medial femoral condyle, is similar in horses
and humans and the occurrence of OCD on the trochlear ridges of the femur leaves large articular defects in clinical cases. In addition, the equine femoropatellar and femorotibial joints are large and this facilitates easy access arthroscopically, both for the creation of experimental defects and for subsequent monitoring. In other work, the authors have shown that it is possible to leave the entire calcified cartilage layer, creating a defect to the tide mark, or, in turn, removing the calcified cartilage layer down to the subchondral bone (17). A recent study has demonstrated the importance of removing the calcified cartilage to obtain the ultimate endogenous healing response (17). On the other hand, if one wants to evaluate the potential contribution of a grafted cartilage construct to produce repair tissue, retention of calcified cartilage, which minimizes contributions from cancellous bone is also a useful model (22).

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References


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