Interspecies Comparison Of Subchondral Bone Properties Important For Cartilage Repair

Anik Chevrier, PhD¹, Ahou SM Kouao¹, Mark B. Hurtig, DVM², Michael D. Buschmann, PhD¹.
¹Ecole Polytechnique de Montreal, Montreal, QC, Canada, ²University of Guelph, Guelph, ON, Canada.

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Introduction: In the knee, articular cartilage lesions occur mostly in the weight-bearing medial femoral condyle while the trochlea is much less affected. Treatment by bone marrow stimulation techniques such as microfracture provides access channels to the subchondral bone to initiate repair. Clinical practice involves debridement of the lesion and perforation of bone to 2-4 mm deep. The mechanisms of bone marrow stimulation have been well studied in various animal models. Age-, species- and site-related differences in repair outcomes have been reported. Repair outcomes are better in young adult humans and rabbit trochlea than in corresponding ovine or horse models or in the rabbit medial femoral condyle (MFC). Since such differences may be related to properties of the subchondral bone, the purpose of this study was to characterize and compare the bone structure of rabbits, sheep, horse and humans. We hypothesized that the subchondral bone from rabbit trochlea is similar to that of young adult human MFC.

Methods: Rabbit joints were processed whole (n = 8 joints; 8-12 months). Cylindrical cores of 3.5 mm dia were drilled from the MFC and trochlea of sheep (n = 7 joints; 2-3 years) and horse (n = 6 joints; 3-7 years). Tubular chisels were used to collect 3.5 and 4.5 mm dia cores from the MFC of human cadaveric distal femurs provided by RTI Surgical (n = 3 females and 5 males; 35-45 years). Additionally, large 10 mm human MFC samples were received already cored by RTI Surgical (n = 3 females; 40-47 years). Although none of the human donors had reported joint pathologies, small articular cartilage lesions were present in some of the femurs and graded according to the ICRS system (Ref 1). Cores were collected from both normal (n = 11 cores with ICRS lesion grade 0) and lesional areas (n = 8 cores with ICRS lesion grade 1 and n = 2 cores with ICRS lesion grade 2) on the MFC. Samples were fixed in 10% NBF and scanned by micro-CT (Skyscan X-ray Microtomography 1172, Skyscan, Kontich, Belgium). The acquired micro-CT images were reconstructed with NRecon (Skyscan) and repositioned with Dataviewer (Skyscan) for analysis. The subchondral bone properties were analysed at 3 sites for each sample using CTAn (Skyscan): 1) A 1 mm x 1 mm region of interest (ROI) encompassing the bone plate and calcified cartilage layer was defined. This area is concave in the rabbit trochlea (Fig 1b), slightly convex in rabbit MFC (Fig 1h) and is usually flat in cylindrical cores (Fig 1d, f, j, l, n, p); 2) A 1 mm3 ROI with a surface adapted to that of the bone plate was positioned 1 mm below the bone plate; 3) The 1 mm3 ROI was moved to 3 mm below the bone plate. Samples were decalcified in mild HCl with trace fixative for embedding, sectioning and Safranin O/Fast Green staining (Ref 2). Stained sections were scanned with a Nanozoomer R5 system (model C10730-02, Hamamatsu, Japan) and scored using the Histopathological/Histochemical Grading System developed by Mankin (Ref 3). Uncalcified cartilage thickness was measured on images exported with NDPView (Hamamatsu) using Image J. Statistical analyses were performed with Statistica (version 10, Statsoft Inc, USA).

Results: As expected and previously reported by other groups, uncalcified cartilage was thinner in rabbits and sheep, compared to horse and humans (Fig 1). Only the MFC in the horse had similar thickness to human MFC (Fig 2a). There was a trend of decreased cartilage thickness in lesional cores (Average Mankin score 5) compared to normal cores (Average Mankin score 1) (Fig 2a). In micro-CT images, the bone plate and calcified cartilage layers form a continuous mineralized layer (Fig 1). This mineralized layer was thick in rabbit MFC as well as sheep and horse joints compared to human (Fig 2b). There was a trend of increased calcified cartilage and bone plate thickness in lesional cores (Fig 2b). The properties of the subchondral bone in the human MFC were most similar to those of the rabbit trochlea (Fig 1 m, n vs a, b), where both had a relatively thin layer of calcified cartilage and bone plate and a more porous and less dense character of subchondral bone with thinner trabeculae (quantified in Fig 2c to h). The larger domesticated animals (horse and sheep) and the rabbit MFC all displayed thicker layers of calcified cartilage and bone plate, denser subchondral bone and thicker trabeculae than human MFC and rabbit trochlea.

Discussion: No animal model had structural properties identical to the human MFC. The structural properties of the subchondral bone and bone plate were most similar in human MFC and rabbit trochlea. Larger animal species (sheep, horse) and rabbit MFC had thicker bone plates, higher bone volume and mineral density and thicker trabeculae, which may be more representative of older/OA patients. The choice of an appropriate animal model of cartilage repair should take into account such properties when repair is to be derived from subchondral bone, as in microfracture and microfracture augmentation.

Significance: Animal models are used to develop new cartilage repair therapies which will be translated to humans. Our data expand on the knowledge of what constitutes the most appropriate animal model for cartilage repair studies.

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3. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritis
Figure 1. Safranin O/Fast Green stained histological sections and 2-D micro-CT slices of trochlea from rabbit, sheep and horse (a-b, c-d and e-f respectively) and MFC from rabbit, sheep, horse (g-h, i-j and k-l respectively). Sections from human MFC taken from normal (m-n) and lesional (o-p) areas. Subchondral bone structure in human normal MFC is similar to rabbit trochlea (compare blue boxes). The blue boxes go the 3 mm deep under the bone plate, the deepest area analyzed.