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Histological and Ultrastructural Differences between Normal and Softened Articular Cartilage

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Articular cartilage is a highly compressive load-bearing tissue by virtue of the functional interplay between a 3-dimensional (3-D) structure of collagen fibrils and the entrapped water-swollen proteoglycan macromolecules. Any loss of interconnectivity in the collagen network that might reduce the constraints on the swelling tendency of the proteoglycan domain will lead to swelling and low matrix stiffness. The 3-D fibrillar architecture of the cartilage matrix strongly influences its stiffness and therefore its ability to maintain a sufficient hydrated thickness under load.

One of the pathologies of osteoarthritis is softening and swelling of the articular cartilage. Such degenerative process is thought to involve a major disruption or breakdown of the collagen network. Any rigorous description of the mechanisms responsible for the degenerative breakdown of the healthy cartilage matrix will include a detailed understanding of the ultrastructural steps that transform the normal fibrillar architecture into one that is unable to resist the normal pattern of forces transmitted through the joint system.

The purpose of the present study is to investigate structural and ultrastructural differences between the normal and softened matrix. Under Nomarski light microscopy, the general matrix of normal cartilage was characterized by an almost non-directional, ground glass or amorphous texture. In contrast, the general matrix of the softened cartilage exhibited a fine, fibrous texture and with a strong radial alignment which often contained a superimposed crimp or waveform. The tail of the cells in this softened cartilage appeared more evident than in the normal cartilage. Under transmission electron microscopy, the normal matrix appeared with repeating fibril segment obliquity and thus creating a pseudo-random network. In contrast, the general matrix of the softened cartilage featured the presence of extensive, parallel and relatively unentwined fibril segments. The result of this study provides a guideline for further research on histological and ultrastructural changes of the articular cartilage that might be affected by many environmental factors. (J Rehab Med Assoc ROC 2000; 28(1): 1 – 10 )

Key words: articular cartilage, bovine, ultrastructure

INTRODUCTION

Articular cartilage functions as a load bearing material, which covers on the surface of bones. It can transmit and distribute high compressive loads and shearing forces to the subchondral bones and provide a smooth, lubricative surface that facilitate low friction movement between the articulating surfaces. It also provides a dimensionally stable thickness of compliant
tissue whose primary role is to deform in a controlled manner so as to spread the compressive forces over a sufficiently large contact area thus reducing the stress to non-damaging levels. The cartilage layer must therefore have sufficient intrinsic strength to resist both excessive swelling and rupture, and yet be compliant enough to deform in a controlled manner. All of these physico-mechanical requirements arise as a result of the unique coupling between the 3-dimensional collagen framework and the high-swelling proteoglycan complex.

Cartilage from the macroscopically intact joint can exhibit a wide range of morphological and mechanical properties [1]. Softening and roughening of the articular surface is palpable on the tibial condyles of large mammals, especially in those regions not covered by the meniscus [2,3]. Anatomically, soft cartilage is consistently found on the tibial plateau of the normal bovine joint, especially the area not covered by meniscus.

Broom had examined many bovine knee joints and had occasionally observed a softened and thickened cartilage removed from the patella [3]. There are also some structural similarity between the softened tibial (and/or patellar) cartilage and that of both the chondromalacia matrix and the osteoarthritic (OA) matrix [4,5,6]. This softened cartilage is almost invariably of greater thickness than that of the normal cartilage taken from contiguous or opposing joint sites. In the standard Outerbridge classification of articular cartilage lesion [7], chondromalacia represent grade I, which malacic cartilage is soft and perhaps dull in appearance. Grade II lesion is fibrillation which is similar to softening and swelling OA cartilage. The OA process is thought to involve a major disruption or breakdown of the collagen network.

It is important to understand the architecture in the normal cartilage matrix and changes of collagen network in the softened matrix. The mechanisms responsible for the degenerative breakdown of the healthy cartilage matrix will explain how to transform the normal fibrillar architecture into one that is unable to resist the normal pattern of forces transmitted through the joint system. Such degenerative change is thought to result from fibrillation of the collagen network, which then becomes less able to restrain proteoglycan-swelling forces. However, there is still little understanding of how the fibrillar network works particularly in the early stages of the disease. Nor is it known to what extent early structural changes to the fibrillar network might reduce the intrinsic strength of the cartilage thereby increasing its susceptibility to further breakdown even under relatively normal levels of joint loading.

The purpose of this study aims at investigating the histological and ultrastructural appearances of both the normal and softened cartilages under examinations of Nomarski light microscopy and transmission electron microscopy (TEM), respectively.

### MATERIALS AND METHODS

#### Materials

Twenty-five cartilage-on-bone samples were collected from fresh femoral condyles and tibial plateau of butchered bovine animals approximately 2-3 years of age from the city slaughterhouse. The specimens were fixed in 2.5% glutaraldehyde for 24 hours.

#### Preparation for histological observation

Using vacuum infiltration processing, the specimens were dehydrated with graded ethanol, cleared with xylol and impregnated with molten paraffin wax. Rotary microtome was used to obtain wax sections approximately 15 μm thick for routine histology. Ribbons of sections were floated onto a warm waterbath to remove wrinkles. Selected sections were collected on albuminised slides and dried at 60 °C. They were then stained using solutions of hematoxylin and eosin and observed under Nomarski light microscopy.

#### Preparation for TEM observation

Additional glutaraldehyde fixed samples of cartilage adjacent to the slices for histological observation were prepared for TEM. After postfixed in 1% osmium tetroxide, the specimens were dehydrated with graded concentration of ethanol and embedded with epoxy resin.

Thin/Thin sections 0.2 μm in thickness and incorporating the full depth of thickness of the articular cartilage in the radial direction of the embedded block were prepared using conventional microtome techniques. Some embedded blocks were trimmed so as to sample only the deep general matrix centered in the lower third of the full
cartilage thickness. These sections were then stained in aqueous 2% uranyl acetate for 5 minutes. After washed with distilled water, after which sections were stained with 4% lead citrate for 5 minutes, washed with distilled water, and then examined using TEM JEM1210 at 120 kV.

## RESULTS

**Macroscopic differences between normal and softened cartilage**

The normal cartilage obtained from the femoral condyle was generally smooth (Fig. 1A) and relatively stiff in response to gentle probing. By contrast, the softened cartilage obtained from the central part of the medial tibial plateau not covered by the meniscus were consistently less smooth and dramatically softened in response to gentle probing and in part, fibrillated (Fig. 1B, 1C). Similar findings are observed in all of the 25 knee joints.

**Histological appearances of cartilage stained with hematoxylin and eosin**

**Normal cartilage**

The cartilage obtained from each of the femoral condyle is about 1 mm in thickness. A full depth radial slice of the normal cartilage was shown in figure 2a. The articular surface is smooth. The outermost fibrils form a macroscopically smooth, glistening hypocellular layer called the lamina splendens. The superficial layer of the normal cartilage occupies about 5-10% of the thickness of the full-depth and the cells in this layer are distinctly flattened and oriented parallel to the articular surface (Fig. 2A, 2B). The intermediate layer occupies 40-45% of the thickness and the cells in this zone are more spherical and randomly oriented (Fig. 2A, 2C). The deep layer occupies 40-45% of the thickness and the cells are grouped in clusters and tend towards a radial alignment (Fig. 2A, 2D). The term 'general matrix' refers to the interterritorial matrix that is most distant from the chondrocytes and their associated extracellular matrix. At higher magnification, the general matrix of normal cartilage in the femoral condyle and the patellar groove was characterized by an almost non-directional, ground glass or amorphous texture (Fig. 2D) as noted earlier by

![Fig. 1A](image1.png)

![Fig. 1B](image2.png)

![Fig. 1C](image3.png)

**Fig. 1.** (A) Gross view of normal femoral condyle cartilage. (B) Coronal view of tibial plateau (left side) with meniscus attached. The medial central region (arrows) is softened. (C) Gross view of tibial plateau (left side) after removal of the meniscus showing structural differences in the central and the peripheral area. Note the continuous and gradual structural changes, the medial central area (arrows) is softened whereas the peripheral cartilage, which normally lies beneath the medial meniscus, is smooth.
Broom [11]. The chondrocyte and cells processes are continuous with a weakly stained pericellular matrix which is limited by an increasingly compacted pericellular capsule, and terminate abruptly at the interface with the territorial matrix (Fig. 2D). The dominant fibrillar organization imposes a radial alignment on the chondrons and a clear distinction can be made in the organization of the capsule at the articular and basal poles of the chondron. Invariably, the pericellular capsule at the articular pole, which faces the surface, forms a dense, compact 'cupola' over the articular and lateral aspects of the chondrocyte and its pericellular matrix. Conversely, the basal pole, which faces the tidemark, is loosely woven and less intensely stained, and often formed an extended ‘tail’ that tapers for several microns from the base of the chondron.

Fig. 2. (A) Full depth of radial slice of the normal cartilage observed under Nomarski light microscopy. H&E, x197. (B) Superficial layer of the normal cartilage showing flattened chondrocytes oriented parallel to the surface. Nomarski light microscopy, H&E, x1239. (C) Intermediate layer of normal articular cartilage showing more spherical and randomly oriented chondrocytes. Nomarski light microscopy, H&E, x1239. (D) Deep layer of normal articular cartilage showing radially aligned chondrocytes with matrix of amorphous texture. Nomarski light microscopy, H&E, x1239.
**Softened cartilage**

The cartilage obtained from the central region of each of the tibial plateau was significantly thicker (3-3.5 mm). The superficial layer comprised about 5% of the full cartilage depth and the cells in this layer were not as dense as those in the normal cartilage and tended to be spherical rather than flattened (Fig. 3A). This low density of chondrocytes may indicate a less compact matrix. The intermediate layer occupied about 25-40% of the full depth and cells are spherical and randomly aligned and transformed gradually to a columnar alignment with increasing depth (Fig. 3A). The deep layer occupied 50-60% of the cartilage thickness, and the cells in this zone were grouped in pronounced, radially aligned columns (Fig. 3B). At this lower magnification, a repeating undulating texture was observed approximately perpendicular to the radial direction. At higher magnification, the general matrix exhibited a fine, fibrous texture and with a strong radial alignment which often contained a superimposed crimp or wave form (Fig. 3C) as was noted in earlier studies by Broom [31]. The ‘tail’ of the cell in this softened cartilage (Fig. 3D) appeared more evident than that in the normal cartilage.

![Fig. 3](image)

(A) Superficial layer of softened articular cartilage showing lower density and more spherical chondrocytes as compared with the normal cartilage. Nomarski light microscopy, H&E, x197. (B) Deep layer of softened articular cartilage showing radially aligned columns of chondrocytes. Nomarski light microscopy, H&E, x197. (C) Radially aligned and waveform texture of the softened general matrix in the tibial plateau. Nomarski light microscopy, H&E, x1239. (D) Chondrocyte with long tail and radially aligned and waveform texture of the softened general matrix in the tibial plateau. Nomarski light microscopy, H&E, x1239.
**TEM appearances of the normal and the softened general matrix**

**Normal matrix**

The normal matrix (Fig. 4) appeared with repeating fibril segment obliquity and thus creating a pseudo-random network from continuous or near-continuous radial fibrils, is one of the most fundamental ultrastructural characteristics of the normal general matrix. This pseudo-dispersed arrangement of fibrils based on the above radial model cannot be resolved optically because each structural element is below the limit of resolution. This is consistent with the ‘amorphous’ appearance of the normal matrix observed by using Nomarski light microscopy (Fig. 2D).

![Figure 4. Ultrastructure of normal articular cartilage matrix showing pseudo-random network of fibril segment obliquity. Transmission EM, x15,000, side bar~200 nm.](image)

**Softened matrix**

The most distinctive feature of the softened matrix was the presence of extensive, parallel and relatively untwined fibril segments, strongly aligned in the radial direction (Fig. 5). These fibrils frequently formed discrete aggregates or bundles separated by regions of low electron density. These bundles were exhibited a pronounced waviness or crimp. The presence of an optically resolvable fibrous texture in the softened cartilage matrices, whether straight or crimped, therefore indicates the presence of discrete bundles of closely packed and aligned fibrils at the ultrastructural level of organization. The close packing of adjacent fibrils in their parallel arrays suggests some form of regular secondary interaction between them.

Conversely, in the normal cartilage general matrix the general absence of such texture is consistent with a much greater degree of interconnectedness and related short-range obliquity in the fibrillar architecture as is illustrated in Fig. 4.

![Figure 5. Ultrastructure of softened articular cartilage matrix showing parallel and relatively untwined fibril segments. Transmission electron microscopy, x15,000, side bar~200 nm.](image)

**DISCUSSION**

The abnormally softened cartilage in this study was found consistently on the medial tibial plateau, especially the central part not covered by the meniscus. It is interesting to speculate why this central region is softened and why the medial side consistently exhibits a greater degree of softening than the lateral side. The question therefore arises as to whether there is a connection between the location of this abnormal softening and the pattern of biomechanical loading across this complex joint system.

Earlier investigators have noted that cartilage is fibrillated in areas that seldom carry load [9]. It was found that the central part of cartilage not covered by the meniscus was most frequently fibrillated, therefore Bullough et al [8] argued that in the knee joint the meniscus must carry much of the load. Walker et al [9] demonstrated that the cartilage beneath the meniscus would be under load most of the time, whereas the exposed cartilage would carry load when the joint is highly loaded.

The consistent presence of abnormally softened cartilage on the tibial plateau may therefore be a direct
consequence of the rather complex pattern of stresses operating in the joint due to the presence of the menisci.

Different degrees of mobility were found in the medial and lateral menisci. Benjamin et al. [90] recognized the greater displacement of the lateral meniscus. They demonstrated similar differences in movement between the lateral and medial menisci. Both menisci slide backwards as the knee moves from full extension to flexion, but the medial meniscus moves only a few millimeters whereas the lateral meniscus moves about 10 mm.

It is therefore that the more pronounced softening of cartilage on the uncovered central region of the medial tibial condyle is a direct consequence of its more constant exposure to underloading arising from the relative immobility of its meniscus. It is assumed that the meniscus is the predominantly loaded tissue, then the central region of the medial tibial condyle will be mostly unloaded due to the relative mobility of its meniscus. By contrast, the lateral meniscus, because of its greater mobility, will tend to expose a larger part of the central region to at least some intermittent component of loading.

Previous studies of experimental models of osteoarthritis may be relevant to an understanding of why cartilage might soften. It has been proposed that the degenerative changes in joint cartilage induced by immobilization are a consequence of the lack of joint movement [111]. It has also been shown that joint motion in the absence of normal loading may bring about degenerative cartilage [12,13]. Yan and Kering [13] proposed that degenerative articular cartilage induced by immobilization resulted from a combination of abnormally low stress and lack of joint motion.

There are some opposing points of view. Kettelkamp and Jacobs [14] argued that with a relatively small medial plateau area the knee may be susceptible to functional overloading. Armstrong et al. [15] suspected that the differences observed between the medial and lateral tibial plateau may be a consequence of being less well protected from loading by the meniscus than the articular cartilage on the lateral tibia.

However, a canine model of osteoarthritis induced by transection of the anterior cruciate ligament has been studied extensively. In this model which involved destabilizing the knee, there is increased hydration of the cartilage [16]. Clinical studies in patients with anterior cruciate ligament deficiency also showed significantly decreased external knee flexion and this may contribute to the development of degenerative arthritis of the knee [17]. Maitland et al. pointed out that the arthritic changes associated with deficiency of the anterior cruciate ligament may be a consequence of an interaction between knee instability and functional muscular stabilization [18]. The increase in hydration is similar to the softened cartilage in central part of the medial tibial plateau. It is therefore possible that the abnormal underloading pattern in the central part of the medial tibial plateau is an important factor leading to its softening.

The cells in the superficial layer of the softened cartilage were less dense than those in the normal cartilage and tended to be spherical rather than flattened. The tail of the chondrocyte in the softened cartilage was evident than that of the cell in the normal cartilage. The function of the tail is not yet known. Previous histochemical and immunohistochemical studies of isolated chondrons suggest that the tail contains high concentrations of sulfated proteoglycans and may provide a reservoir of synthesized material beneath the cell [19]. This reservoir could act as a hydrostatic cushion beneath or between adjacent chondrocytes and may play a role in load bearing.

Combined microscopic and micro-rupture conducted on fully hydrated slices of cartilage have shown that in the softened matrix there is a greatly reduced resistance to rupture propagation in the radial direction relative to that of the normal matrix [20]. In our present findings, a reduction in the density of fibril interconnection in the softened matrix may explain its reduction in mechanical force bearing. Khalsa et al. [21] found that tensile force in the collagen network play a role in determining tissue behavior in confined compression even for relatively large volume changes. This is in consistent with the work by Zhu et al. [22] who also investigated the dynamic viscoelastic shear properties of articular cartilage and declared that the mechanical properties of articular cartilage depend not only on the relative amount of collagen and proteoglycan but also on their structural organization and physical interactions, which concept of cartilage mechanics has been emphasized earlier by Broom [2,20].

Articular cartilage is a highly specialized connective tissue, particularly suited to compressive load bearing.
Like other connective tissues such as tendon, ligament and meniscus, articular cartilage shows its functional properties from its extracellular matrix. Nomarski imaging provides a rapid and convenient means of assessing the cartilage matrix up to medium level of structural resolution.

The present study showed histological and ultrastructural differences between the normal and softened articular cartilage. The altered structure of the extracellular matrix also prove the explanation of previous findings of the lower intrinsic mechanical strength in the softened matrix compared to the normal matrix.

REFERENCES


正常與軟化關節軟骨之組織與超微結構差異研究

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關節軟骨是一個承受壓力的組織，藉著特殊三度空間結構的膠原纖維以及嵌在其中含等份的多醣蛋白大分子，以具有的相互協作作用達成髒物承受壓力的特性。膠原纖維網絡遭受外力而喪失特殊結構後，將會減低對多醣蛋白區域表層的耐力，進而導致組織表層、軟骨基質的硬度降低，甚至組織在承受的情形下尚能維持含水厚度能力的減退。

退化關節炎的一個重要表徵，就是軟骨的軟化及水腫，而退化過程的病理發現就是膠原網路的崩解或破損。本研究的目的，就是在探尋正常與軟化軟骨基質的一般組織結構與超微結構之差異。我們選取了 25 組牛的股骨頭及股骨端軟骨層，針對其正常與軟化的軟骨加以觀察分析。在 Nomarski 立體光學顯微鏡下，正常的軟骨基質幾乎是無特定方向及形狀的紋理；而軟化的軟骨基質則出現微細的纖維結構，形狀上呈現放射狀排列及充滿重疊的纖維與波浪狀，深層軟骨細胞層的形狀較正常更為明顯。進一步，在穿透式電子顯微鏡下，正常的軟骨基質顯現重疊斜式交叉的纖維節段，構成仿似倒僞排列的網絡；相對地，在軟化的軟骨上則存在著擴張、平行且不緊密纖維的節段。

本篇深入而詳細的觀察組織及超微結構，是連串退化軟骨研究的起點，可進一步做為將來觀察各種環境因素對軟骨造成變化之基準。（中華復健醫誌 2000; 28(1): 1 - 10）

關鍵詞：關節軟骨(articul cartilage)，牛(bovine)，超微結構(ultrastructure)