Chapter 1
Articular Cartilage: Structure and Restoration

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Function and Significance

Articular cartilage is a highly specialized (osteochondral unit) connective tissue found at the epiphyses of synovial joints. Glassy and light blue in appearance, the articular cartilage layer is 2–4 mm thick contingent on its location. Articular cartilage is composed of hyaline cartilage, which functions to protect the underlying subchondral bone and, in combination with the synovial fluid, reduce the friction between movable joints to levels less than water on ice. The articular cartilage layer also functions to redistribute the daily loads applied to the synovial joints and acts as a shock absorber. These loads are prevalent during everyday walking, jumping, running, and kneeling. During these movements, load and shear forces are being redistributed from the articular cartilage layer to the ends of long bones. Therefore, the articular cartilage layer

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A. B. Yanke, B. J. Cole (eds.), *Joint Preservation of the Knee*,
https://doi.org/10.1007/978-3-030-01491-9_1

acts as a safeguard to maintain the strength of the entire bone-cartilage interface, the osteochondral unit.

Articular cartilage lesions are one of the most consistently encountered conditions in orthopedics, leading to significant long-term sequelae. In a retrospective study of 31,516 knee arthroscopies performed, chondral lesions were reported in an astounding 19,827 (63%) patients across all age groups [1, 2]. Once osteochondral unit defects occur, many will progress to osteoarthritis (OA) dependent on a multifactorial process. OA is a very common injury and can occur because of genetic predisposition and/or can be induced by trauma, by obesity/immobility, and through normal wear and tear. OA may cause pain and serious disability, decreasing the quality of life for those it affects. As OA progresses, bone spurs form, and inflammation leads to further degeneration of the articular cartilage. This ultimately leads to painful bone-on-bone interactions and a vicious cycle of continual and worsening damage. Radiographically, more than 80% of people above the age of 65 have signs of OA in at least one joint of the hand, hip, knee, or spine [3]. The associated annual costs for the treatment of OA and inflammatory arthritis exceed $100 billion dollars in the United States, and healthcare costs account for ~2% of the US gross domestic product [3–6].

Understanding the normal function and structure of articular cartilage is required to adequately understand chondral lesions and the osteochondral unit in its diseased state. Moreover, understanding normal function and anatomy of the articular cartilage may lead to improved operative and non-operative treatments. Because of the complex structure of articular cartilage, restoration to its normal state is difficult to achieve. Artificial constructs have yet to satisfactorily replicate the effectiveness of the osteochondral unit, thus highlighting the importance of preserving its original structure and continuing research aimed at improving current conservative and surgical treatments.

Structure of the Osteochondral Unit

Cartilage Structure

The preservation of articular cartilage is critical to maintain the osteochondral unit's function. Articular cartilage is aneural, alymphatic, and avascular, which limits its regenerative healing capacity. Due to the avascular nature of articular cartilage, the cartilage must receive nutrients and oxygen by diffusion from the synovial fluid and the subchondral bone.

Articular cartilage is composed of an extracellular matrix (ECM) and chondrocytes. The ECM is mainly composed of water, collagens, and proteoglycans, although there are also other proteins, glycoproteins, and lipids found in sparse concentrations [7]. If one includes the surrounding pericellular matrix of chondrocytes, this is referred to as the chondron [8]. Articular cartilage has low chondrocyte cellularity, and the chondrocytes are encapsulated within a dense matrix, further reducing its capacity to regenerate. The structure of articular cartilage is represented in Fig. 1.1.

Extracellular Matrix

The largest component of the ECM is water, contributing ~65–80% of its wet weight. Traversing through the zones of cartilage, the water content decreases from ~80% at the superficial zone to ~65% at the deep zone [2, 9]. Water's main function is to hydrate the proteoglycans, which along with water molecules themselves expands the collagen network, lubricates the joint, and aids in the flow of nutrition to the cartilage. Water is maintained in the matrix by the proteoglycans and collagens. Electrolytes including potassium, sodium, calcium, and chloride are also dissolved in the water [7]. The water content of articular cartilage generally diminishes over the lifetime but rises to ~90% in those with OA. An increase in the water content of articular cartilage leads to decreases in strength and increases in the permeability of the cartilage layer.

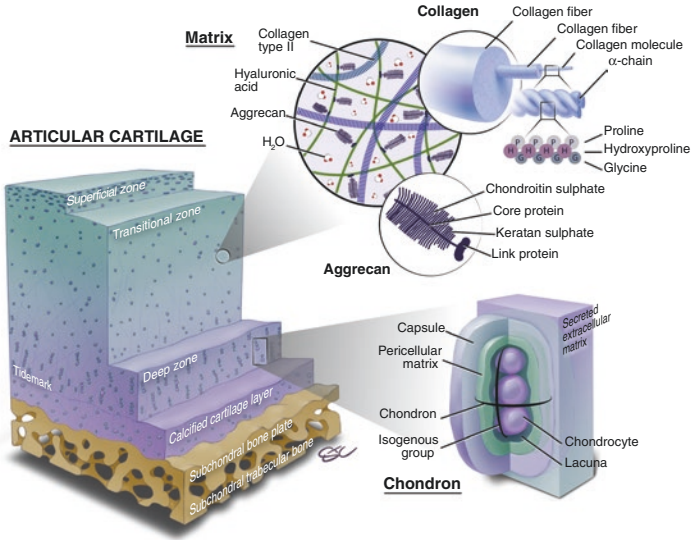

FIGURE 1.1 Structure of the osteochondral unit and the unit's individual components

The second largest component of the ECM is collagen. Collagen is a fibrous tough structural protein found throughout the body, namely, the connective tissues. Collagen develops its tensile strength from its sophisticated triple-helix structure. Composed of three polypeptides wound together by hydrogen bonds, collagen forms a tight right-handed triple helix. Each polypeptide is primarily comprised of a repeating trimer of amino acids: glycine, proline, and hydroxyproline. This repeating trimer forms a left-handed helical structure formed by hydrogen bonds [10]. The predominant collagen found in articular cartilage is Type II collagen, ~95% [9]. Little attention has been paid to other collagen fibers present in articular cartilage; Types IV, VI, IX, X, XI, XII, XIII, and XIV. The monitoring of the breakdown of these collagens could generate new biomarkers to further understand disease progression and elucidate improved therapeutic treatments [11]. Collagen is dispersed throughout the ECM, and its

distribution is dependent on regional differences of the articular cartilage (articular cartilage zones). Moreover, the collagen organization at the apical surface of a chondron is denser than that on the basal side [12]. Collagen is found associated and crosslinked with proteoglycans, forming the structural unit of the ECM.

Proteoglycans are found throughout the connective tissues, and their negative charges help attract water to the articular cartilage, further strengthening the matrix. In articular cartilage, the most prevalent proteoglycan and the largest in size is aggrecan. Proteoglycans are proteins covalently attached to glycosaminoglycans (GAGs), long repetitive dimers of a hexosamine and a uronic acid. The major GAGs attached to the aggrecan link protein are chondroitin sulfate and keratin sulfate. Another GAG highly important to the function and structure of articular cartilage is hyaluronic acid (HA). HA is extremely large and does not form covalent attachments to proteins; therefore, it is not a formal constituent of proteoglycans. However, HA serves an important function by forming non-covalent complexes with proteoglycans via proteoglycan link proteins. Together, HA and proteoglycans, such as aggrecan, form extensive proteoglycan-HA aggregates. These aggregates bind to the surface of collagen II fibers via their side chains, linking all the constituents of the ECM forming the strong backbone of articular cartilage.

Chondrocytes

Chondrocytes are the viable cells of cartilage and they reside in lacunae. These spheroidal cells contribute to only ~5% of the articular cartilage volume [13]. Chondrocytes are formed in clusters among one another, known as isogenous groups, and the cell's metabolism is critical for the preservation of the ECM. Due to the hypoxic nature of the cartilage, much of the metabolism is anaerobic [2]. Originating from mesenchymal stem cells, chondroblasts form and secrete the collagens and proteoglycans of the ECM. Once chondroblasts are completely engulfed by their secreted matrix, they are referred to

as chondrocytes. The surrounding ECM protects the chondrocytes from the forces and friction applied to the joint. Growth factors and cytokines play a critical role in the control of chondrogenesis, directing the differentiation of mesenchymal stem cells into mature chondrocytes. Essential growth factors for chondrogenesis include insulin-like growth factor 1 (IGF-1), members of the fibroblast growth factor (FGF) family, and members of the transforming growth factor-beta (TGF-β) superfamily, which includes the bone morphogenic proteins (BMP) [14]. This chondrogenesis is termed appositional growth and occurs near the apical surface of the articular cartilage in the superficial zone. Load on the articular cartilage allows for chondrocyte maturation, differentiation, and proliferation [15].

When a defect is perceived by the osteochondral unit, chondroblasts migrate to locations of cartilage injury where chondrocytes are damaged. Chondrocytes at the site of injury can then divide and form chondroblasts that will secrete a surrounding matrix and heal the injured cartilage. Ultimately, these chondroblasts will become chondrocytes. Chondrocytes and chondroblasts, however, have an extremely limited ability to replicate or regenerate. Mitotic rates in the adult chondrocyte are at levels 1/20th of those found in the epiphyseal growth plate during development, resulting in an inadequate healing capacity for articular cartilage [16]. Immune responses against chondrocytes are limited due to the aneural and alymphatic nature of articular cartilage. Furthermore, the ECM of articular cartilage guards against major histocompatibility complex (MHC) I antigen recognition of host cells [17].

Zones

Articular cartilage can be separated into four anatomically and functionally distinct zones: superficial, transitional, deep, and the calcified cartilage layer (CCL). Collectively these zones function in syncytium to provide the highly specialized functions of articular cartilage.

The superficial zone, also known as the tangential fiber zone, is the outermost zone of the cartilage and is in immediate contact with the synovial fluid of articular joints. This zone can be further divided into the lamina splendens and the cellular layer. Preservation of the lamina splendens is required to maintain the integrity of the entire joint as it provides the friction-free surface that allows for joint mobility. The cellularity in the superficial zone is more robust, and the chondrocytes are flatter relative to more basal zones [8]. The superficial zone has the highest collagen and water content. It comprises 10–20% of the thickness of the cartilage, and the collagen fibers in this zone are highly organized [18]. These fibers are arranged parallel to the surface of the joint in order to resist shear forces from friction produced by motion between the articular surfaces [9].

Immediately beneath the superficial zone is the transitional or middle zone. In this layer, collagen fibers are much thicker and are organized obliquely [9]. As its name implies, the transitional zone is a transition point between the highly specialized superficial and deep zones. The chondrons in the transitional zone are less prominent than the apical superficial zone, and the cells are more spheroidal [8]. The transitional zone accounts for ~50% of the depth of the cartilage and by nature is responsible for resistance to compressive forces. The transitional zone has higher levels of proteoglycans and less collagen content than the superficial layer. As mentioned above, the water content of the transitional layer is less than that of the superficial layer and is more than that of the deep layer.

Basal to the transitional zone is the deep zone, also called the radial fiber zone. In the deep zone, the collagen fibers are organized perpendicular to the surface to provide the greatest resistance to compressive forces applied to the joints. Chondrocytes are arranged in columnar orientation, parallel to the collagen fibers and perpendicular to the tidemark. The chondrons in this layer are scarcer and are more elongated in shape. The deep zone accounts for ~35% of the depth of the cartilage. The water content in the deep zone is the lowest of

the zones, ~65%. The deep zone has the largest proteoglycan content and the largest diameter of collagen fibrils.

The calcified cartilage layer is a thin layer, ~20 to ~250 microns, located directly above the subchondral bone and below the deep zone [19]. The CCL anchors the cartilaginous zones to the subchondral bone and serves as a transitional buffer to compensate for the discontinuity of stiffness between the cartilage and subchondral bone [20, 21]. The thickness and intermediate stiffness of the CCL aid in the transfer of load by reducing the stress concentrations between the articular cartilage and the subchondral bone. The CCL has undulating vascularity, and the cellularity in this level is extremely low; thus, there are trace amounts of metabolism present [7]. A tidemark is present that delimits the CCL from the deep zone. The tidemark acts to inhibit vascular penetration of the above zones [22]. This tidemark can be clearly seen histologically by most stains, including hematoxylin and eosin.

Subchondral Bone

Although the subchondral bone is not a constituent of articular cartilage, together, they form the osteochondral unit. Therefore, subchondral bone is incredibly important for the functioning of articular cartilage and the pathogenesis of OA [23]. In severe abnormalities of the cartilage, such as in an International Cartilage Repair Society (ICRS) grade 4 lesion, the subchondral bone is affected yet is still habitually neglected in basic science reviews of articular cartilage. Further, some conditions that affect the entire osteochondral unit, such as osteochondritis dissecans (OCD) and spontaneous osteonecrosis, originate in the subchondral bone and progress to the articular cartilage [24]. Thus, to fully understand the structure of articular cartilage and the entire osteochondral unit, the subchondral bone must be appreciated.

The subchondral bone is separated from the CCL by the cement line and can be further separated into the subchondral bone plate and subchondral trabecular bone.

The subchondral bone plate is a thin bone layer that separates the CCL from the marrow spaces of the subchondral trabecular bone. Composed of cortical bone, the subchondral bone plate is nonporous and strong [25].

The trabecular bone of the subchondral bone operates as a shock absorber for the rest of the long bone and functions to retain the shape of the joint. The trabecular bone has higher metabolism than the subchondral bone plate. Additionally, the trabecular bone has bone marrow present. The bone marrow of trabecular bone houses mesenchymal stem cells (MSCs) with chondrogenic potential [26].

Vascular channels run from the marrow of the trabecular layer to the CCL. As mentioned earlier, the tidemark inhibits vascular penetration of the apical zones [22]. Apical diffusion then allows for nourishment of the avascular cartilage layers not receiving nourishment from the synovial fluid. Additionally, trabecular bone is responsible for the nourishment of the subchondral bone plate [27]. While the articular cartilage is limited in its immune response, the subchondral bone is not. The subchondral bone expresses MHC antigens [17].

Aging

With normal aging, the articular cartilage structure develops a host of changes. Although the incidence of OA increases exponentially with age, the symptoms of normal aging are not synonymous with the symptoms seen in OA. What exactly facilitates articular cartilage changes is not yet fully understood. Chondrocyte levels remain mostly unchanged with aging; however, there is a reported loss of chondrocytes from more superficial layers and a rise in chondrocyte levels closer to the subchondral bone. In addition, there is a reported thinning of the CCL with age, and the ECM typically experiences a loss of water and, as such, an intrinsic gain in stiffness [19]. Considering these changes, the entire osteochondral unit is more susceptible to damage, has a reduced ability to bear loads of the joint, and has an increased likelihood for the development of OA.

Chondral Lesions

As time has passed, it has become increasingly evident that chondral lesions must be evaluated from a perspective considering the entire osteochondral unit, rather than solely the articular cartilage [24]. Lesions of the articular cartilage provide a challenge to clinicians as it is difficult to resurface the joint. However, in young active patients, resurfacing of the defect is desirable to reduce pain levels, improve function, and increase activities and sports levels. Additionally, it may prevent the early onset of OA and avert serious disability.

The treatment plan for chondral lesions is principally determined by the size of the defect and the grading of the lesion but is also predicated on the experiences of the physician.

The International Cartilage Repair Society (ICRS) Hyaline Cartilage Lesion Classification System is the international standard for classifying the severity of chondral lesions [28, 29]. An ICRS grade 0 lesion is when the articular cartilage surface is normal. Grade 1 lesions are nearly normal; however, there may be slight indentations of the articular cartilage surface, and the cartilage may have superficial fissures. ICRS Grade 2 lesions are abnormal and extend to <50% of the depth of the cartilage, into the middle zone. Lesions of Grade 3 extend to >50% of the depth of the cartilage, which can go down to the calcified layer or subchondral bone (but not through the subchondral bone). Blisters are included in Grade 3. Grade 4 are lesions that go through the subchondral bone. The distinction between Grade 3 and 4 lesions is that Grade 4 lesions traverse through the subchondral bone [28, 29]. Figure 1.2 displays a schematic diagram, and Fig. 1.3 displays corresponding arthroscopic imagery of cartilage lesions to help further clarify the ICRS classification of cartilage lesions.

Imaging

Magnetic resonance imaging (MRI) is a useful and noninvasive tool to assess and diagnose chondral lesions. In MRI, one is also able to visualize the health of the soft tissue and the

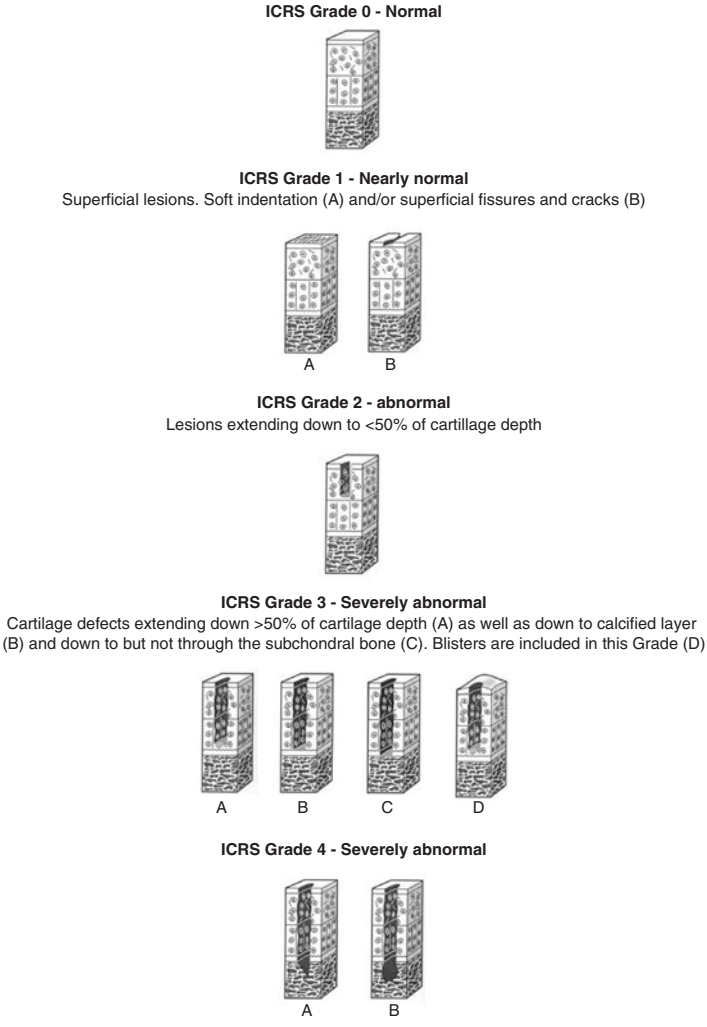

FIGURE 1.2 ICRS articular cartilage injury classification system. (Image kindly provided and reprinted with permission by the International Cartilage Repair Society)

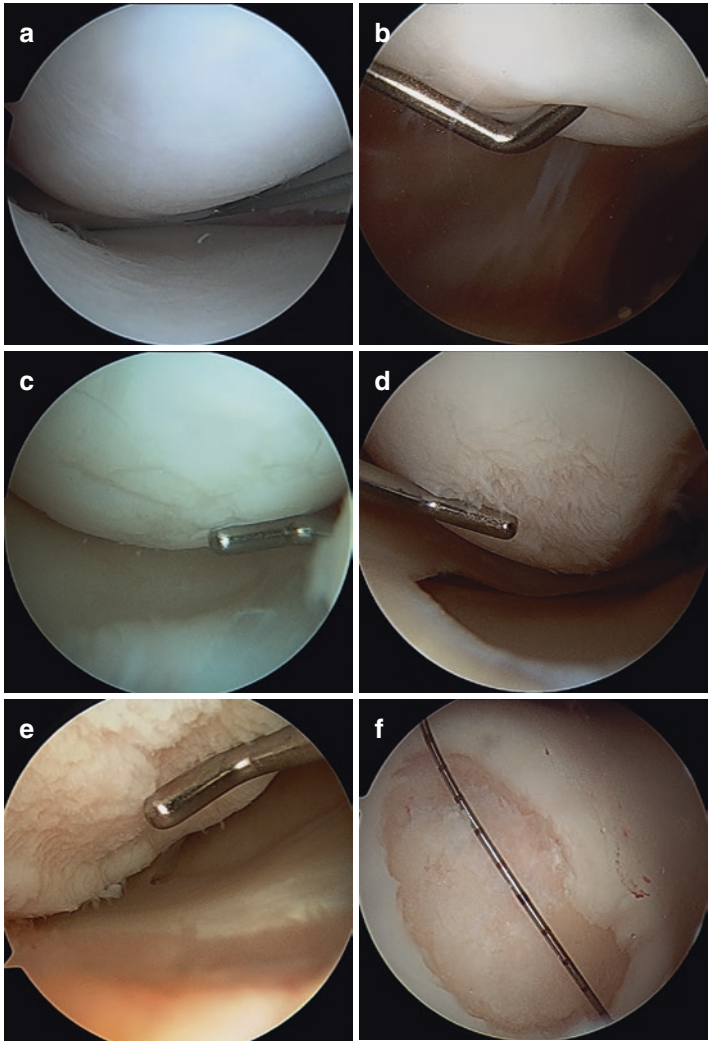

FIGURE 1.3 Representative arthroscopic images of the ICRS articular cartilage injury classification system. (**a**) Grade 0, (**b**) Grade 1A, (**c**) Grade 1B, (**d**) Grade 2, (**e**) Grade 3A, (**f**) Grade 3B, (**g**) Grade 3C, (**h**) Grade 3D, (**i**) Grade 4AB

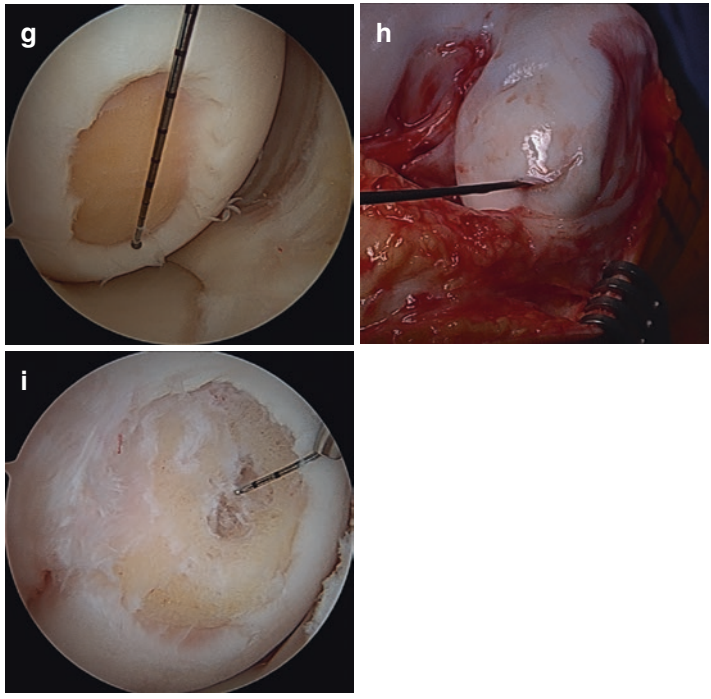

Figure 1.3 (continued)

subchondral bone [7]. Two-dimensional (2D) standard spin-echo (SE) and 2D gradient-recalled echo (GRE) sequences, 2D fast SE sequences, and three-dimensional (3D) SE and GRE sequences of MRI are used to assess the location, depth, and length of cartilage lesions in patients [31]. The ICRS suggests utilizing fast SE imaging for the evaluation of cartilage repair [30]. Newer 3D fast SE sequencing has not replaced the gold-standard 2D fast SE or 2D fast SE in combination with 3D GRE methods [31]. With these techniques, clinicians can clearly envision the morphology of the joint of interest and assess the progression of OA. However, the identification and evaluation of deeper lesions, grades 3 and 4, may be more precise than grades 1 and 2 that can be missed. Further, MRI can often underestimate the median defect

area. On average, defects are ~65% larger than measured by MRI [32]. Most treatment algorithms are dependent on the size of cartilage defects and as such have adverse effects on the choice of treatment for clinicians [32].

The organization of collagen and GAG content can be determined by using certain MRI protocols. Since the normal collagen and proteoglycan organization is known throughout the zones, clinicians can make educated assessments about the health of their patients. When the cartilage degenerates, changes in GAG are among the first detectable manifestations [33]. To assess proteoglycan content and collagen organization and arrangement, clinicians can use a variety of methods such as T2 mapping, delayed gadolinium-enhanced MRI of the cartilage, T1ρ imaging, and sodium imaging [7, 31]. T1ρ has been shown to be the best method at assessing changes in GAG and proteoglycan content, although sodium imaging and delayed gadolinium-enhanced MRI of the cartilage may also be effective [33–35]. T2 imaging of articular cartilage detects changes in collagen content, because these images represent interactions that occur between water molecules and surrounding macromolecules. Increased interactions will result in decreased T2 levels. Another study reported that T1ρ and T2 values are significantly higher for ICRS grade 1 cartilage lesions than for those with grade 0 (normal) [33].

Perhaps with further discovery and enhancement of techniques, earlier and perhaps reversible signs of degeneration will become more apparent. The reduction of artifacts, decrease in scan time, improvement in lesion sizing, and enhancement in sensitivity of MRI will allow for improved efficacy and utility of this imaging technique [36]. Moreover, the continued and increased usage of MRI techniques such as T1ρ could detect early degeneration of the articular cartilage ECM, even before defects appear on the surface of the articular cartilage.

Macroscopic and Microscopic Evaluations

For macroscopic evaluation of articular joint tissues, staining with India ink allows pathologists to measure the depth of cartilage lesions. India ink adheres to fissured cartilage and can

be easily seen in comparison to the surrounding normal cartilage (i.e., cartilage that does not retain the India ink stain) [37].

For microscopic evaluation of articular cartilage/bone, tissues are sectioned approximately 3 mm in thickness and fixed in 10% neutral buffered formalin (ratio of 10:1 for fixative and specimen, respectively). Once the sections are properly fixed (24–72 h depending on tissue size and bone density), the bone samples are decalcified. A commonly used solution that is gentle to the tissue and maintains cellular detail is a solution of 10% ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (pH 7.2–7.4). The bone samples are kept in 10% EDTA solution till softened (approximately 2–6 weeks based on tissue size, thickness, and bone density, solution changed three times a week) and then embedded in paraffin. To speed up the decalcification process, decalcifying solution can be replaced every day. Once the tissues are processed, there are many histochemical stains available to visualize healthy and degenerative articular cartilage. Each method serves a specific purpose and has its own advantages. Perhaps the most widely used stain in histology is hematoxylin and eosin (H&E). Hematoxylin is a basic dye that stains purple/blue. Hematoxylin attaches to negatively charged elements of the tissue (basophilic), such as the DNA of the chondrocyte. Eosin is an acidic dye that stains pink. Eosin attaches to positively charged elements of the tissue (acidophilic), which includes collagen. H&E stains the nuclei of articular cartilage basophilic and the ECM acidophilic. Areas with high proteoglycan content in the ECM stain bluer due to being highly sulfated and having more negative charges [37]. The orientation of the collagen fibers in the ECM changes the visual orientation of stained chondrocytes, thereby making the individual cartilage zones visible. By using H&E staining, the health of the tissue can be determined by comparing the surface, zones, and staining intensity to baseline (Fig. 1.4).

Another method to visualize the cartilage is to use either Safranin O or Toluidine Blue staining. Safranin O and Toluidine Blue stain proteoglycans and GAG. When using this method, histologists can compare normal articular cartilage to the staining of the patient of interest. By comparing against a

control, a diseased cartilage will have reduced staining of proteoglycans and GAG (Fig. 1.4).

Collagen content and organization can be easily seen with the use of picrosirius red. Picrosirius red staining utilizes polarized light microscopy. Using polarized light microscopy, the color and light visualized are reflective of the collagen organization, alignment, size, and concentration (Fig. 1.4). Therefore, disruptions of the normal collagen arrangement can be seen by comparing the cartilage section to that of a normal articular cartilage section.

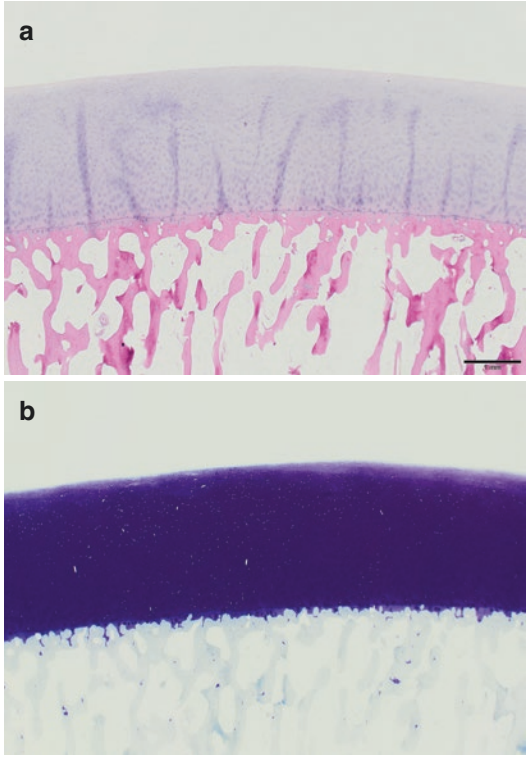

Figure 1.4 Histological photomicrographs (2×) of human femoral condyle. (**a**) Hematoxylin and eosin, (**b**) Toluidine Blue, (**c**) Picrosirius Red (polarized), and (**d**) Safranin O; scale bar, 1 mm

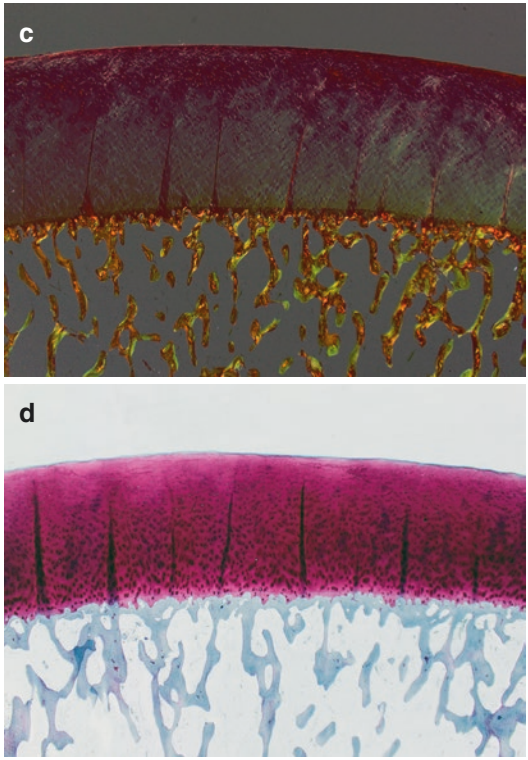

Figure 1.4 (continued)

The health of the osteochondral unit can be further illuminated by assessing the viable chondrocyte density (VCD) of the articular cartilage via fluorescent microscopy. Of clinical interest, the long-term success of operative treatments such as osteochondral allograft (OCA) transplantation is largely dependent on the viability of the chondrocytes of OCAs at the time of implantation [38, 39]. To assess chondrocyte viability, the tissue of interest can be stained for fluorescence using two stains that stain for live and dead cells, respectively, and subsequently imaged using fluorescent microscopy. To determine the VCD using fluorescent microscopy, a homogeneous mixture of the live stain calcein acetoxymethyl (Calcein AM), phosphate-buffered saline (PBS), and either the dead stain SYTOX Blue

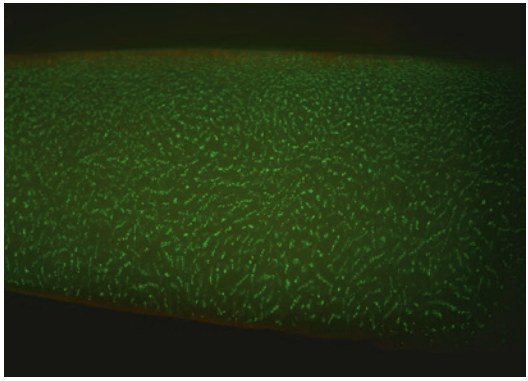

Figure 1.5 Representative (4×) fluorescent chondrocyte viability image of human femoral condyle articular cartilage tissue

(Life Technologies) or the dead stain ethidium homodimer (ETH) can be applied to the tissue of interest. Nonviable cells do not retain Calcein AM as their cell membranes have become weakened and permeable. Neither SYTOX Blue nor Calcein AM is able to cross intact cell membranes; therefore, these dead stains stain chondrocytes whose cell membranes have become compromised but do not stain viable chondrocytes with intact cell membranes. Utilizing these techniques, live cells stain green and dead cells stain red. Photographs can then be taken of the osteochondral unit using fluorescent microscopy, and relative chondrocyte viability can be subsequently visualized, as seen in Fig. 1.5. VCD is found by dividing the number of viable green cells by the area of the cartilage of interest. OA cartilage and cartilage with lesions have dramatically reduced VCD levels compared to those of healthy articular cartilage. This application has been of significant value in assessing articular cartilage in the laboratory, particularly in improving the storage protocols of OCAs for transplantation [40].

Summary

Articular cartilage functions as a specialized connective tissue (osteochondral unit) at the epiphyses of synovial joints. Composed of hyaline cartilage, articular cartilage functions

to reduce the friction between movable joints and acts as a shock absorber. Disorders of articular cartilage are some of the most commonly encountered conditions in orthopedics. Appreciating the normal function and structure of articular cartilage is essential to understanding articular cartilage in its diseased state.

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