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Meniscus, articular cartilage, and nucleus pulposus: a comparative review of cartilage-like tissues in anatomy, development, and function

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Abstract

The degradation of cartilage in the human body is impacted by aging, disease, genetic predisposition, and continued insults resulting from daily activity. The burden of cartilage defects (osteoarthritis, rheumatoid arthritis, intervertebral disc damage, knee replacement surgeries, etc.) is daunting in light of substantial economic and social stresses. This review strives to broaden the scope of regenerative medicine and tissue engineering approaches used for cartilage repair by comparing and contrasting the anatomical and functional nature of the meniscus, articular cartilage (AC), and nucleus pulposus (NP). Many review papers have provided detailed evaluations of these cartilages and cartilage-like tissues individually, but none have comprehensively examined the parallels and inconsistencies in signaling, genetic expression, and extracellular matrix (ECM) composition between tissues. For the first time, this review outlines the importance of understanding these three tissues as unique entities, providing a comparative analysis of anatomy, ultrastructure, biochemistry, and function for each tissue. This novel approach highlights the similarities and differences between tissues, progressing research toward an understanding of what defines each tissue as distinctive. The goal of this paper is to provide researchers with the

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fundamental knowledge to correctly engineer the meniscus, AC, and NP without inadvertently developing the wrong tissue function or biochemistry.

Keywords

Articular Cartilage; Meniscus; Nucleus Pulposus; Development; Tissue Engineering

Introduction

The meniscus, articular cartilage (AC), and nucleus pulposus (NP) are all significant tissues in the progression of pathologies such as osteoarthritis (OA) (Loeser *et al.*, 2012), rheumatoid arthritis (RA) (Goldring, 2003), meniscus tears (Fox *et al.*, 2015), and degenerative disc diseases (Tian *et al.*, 2013). What defines these three tissues as unique compared to other tissues, and subject to regenerative approaches, is their overall avascularity, inability to heal properly *in vivo*, and difficult clinical and translational remediation (Fox *et al.*, 2015; Hunziker, 2002; Tian *et al.*, 2013). Because these tissues assume similar functions (distribution and transfer of weight across surfaces) and are composed of similar cell types (fibrochondrocytes, chondrocytes, and chondrocyte-like cells), it may be presumed that regenerative approaches would also be similar. The purpose of this paper is to thoroughly explicate upon these parallels, illustrating how these tissues appear comparable, but have far ranging disparities in their development, anatomy, tissue and cell structure, and function. For researchers interested in tissue engineering and regenerative medicine approaches, this review provides a compare and contrast analysis between the meniscus, AC, and NP.

Developing the appropriate tissue is not only subject to achieving a specific cell phenotype, but also to regulating extracellular matrix (ECM) composition and production levels (Shine *et al.*, 2009), vascularization and innervation (Johnson *et al.*, 2001), growth factors (Pei *et al.*, 2002), proper molecular signaling (Zhang *et al.*, 2014), and ability to correctly respond to pressure/tension stimulation (Zhang *et al.*, 2016a). Each tissue exhibits differing levels of ultrastructure, composed of varying cellular components, ECM, and levels of oxygen distribution; the meniscus with red-red, red-white, and white-white zones (Fox *et al.*, 2015), AC with four layers (superficial, transitional, deep, and calcified) (Becerra *et al.*, 2010), and NP with central and peripheral regions (Roberts *et al.*, 1995). As surgical procedures become less invasive and more easily conducted (Frank and Cole, 2013), the field of regenerative medicine will see increased opportunities for tissue explants (Musumeci *et al.*, 2014). This review offers an assessment of anatomical differences between these three tissues to better understand their commonality and diversity, providing the reader with the knowledge of more efficient differentiation studies in the meniscus, AC, and NP. By increasing the quality of cartilage and cartilage-like grafts, the graft/host homology should allow for more efficient assimilation and comparable functionality thereby limiting tissue explant deterioration (Chen *et al.*, 2012; Jackson, 2015;).

Vascular, Neural, and Basic Anatomy

The uniqueness of the meniscus, AC, and NP as discrete cartilage-like tissues begins in development, through site-dependent signaling and extraneous environmental stimuli casting the shape, and terminates in the vascularization and innervation that directly impacts the layering of each tissue. Signaling, vascularization, innervation, stem cell source, and location in the body all present slight nuances to tissue development; these distinctions direct the subset of cells that will eventually populate the tissue.

Meniscus

Deriving from a condensation of mesenchymal cells within the intermediate layer, called interzone cells, the meniscus develops its typical shape from the eighth to tenth week of conception (Gardner and O'Rahilly, 1968). The immature menisci are rife with cells and blood vessels, with a blood supply through the whole menisci (Clark and Ogden, 1983); as the fetus develops, cellularity continues to decrease in the menisci, while the collagen content continues to increase in a circumferential arrangement (Clark and Ogden, 1983). Besides partial vascularization in the periphery provided by branches of the popliteal artery, the meniscus comparatively is a tissue without blood vessels. Antiangiogenic factors are important not only for the development but also for the maintenance of avascular zones in the meniscus. The antiangiogenic peptide endostatin/collagen XVIII was detected in the menisci of both human fetus and adult; however, in the adult, endostatin/collagen XVIII mainly existed in the inner two-thirds avascular region of the meniscus, whereas, in the fetus, endostatin/collagen XVIII was mainly distributed in the outer one-third (Pufe *et al.*, 2004). Blood supply for the lateral meniscus ranges from the peripheral 10% to 25% and 10% to 30% for the medial meniscus, functioning significantly for self-healing (Danzig *et al.*, 1983). The rest of the meniscus absorbs nutrition through synovial diffusion or joint motion.

The nerve fibers following the blood supply are detected mainly in the peripheral vascular area of the meniscus (Kennedy *et al.*, 1982). The mechanoreceptors within the menisci could convert mechanical stimulation into a unique electrical nerve impulse. Three morphologically distinguishing mechanoreceptors have been found within the human meniscus: "Ruffini endings, Pacinian corpuscles, and Golgi tendon organs", particularly in the meniscal horns (Zimny *et al.*, 1988). It is believed that proprioception can be acquired from free nerve endings (nociceptors) (Mine *et al.*, 2000) stimulated on the anterior and posterior horns in the process of knee flexion and extension (O'Connor, 1984; O'Connor and McConnaughey, 1978). (Figure 1A) (Table 1)

Articular Cartilage

Like the meniscus, the AC also originates from the interzone (Archer *et al.*, 2003). Recent evidence indicates that a continuous influx of GDF5 (growth differentiation factor 5) positive cells contributes to joint development (Ray *et al.*, 2015; Shwartz *et al.*, 2016). With the upregulation of unique molecules such as Wnt9A (Wingless-Type MMTV Integration Site Family, Member 9A), GDF5, Erg (ets related gene), Gli3 (GLI Family Zinc Finger 3), CD44 (cluster differentiation 44), and type IIA/I collagen (Iwamoto *et al.*, 2007; Koyama *et*

al., 2008; Pacifici *et al.*, 2006), cavitation appears within the interzone (Archer *et al.*, 2003). In the meantime, the joint capsule, consisting of the outer ligaments and the inner synovium, promotes the connection of the two cartilaginous constituents (Merida-Velasco *et al.*, 1997). From the top surface of the AC, chondrocyte size becomes larger toward the secondary ossification center, ending with calcified and vascularized hypertrophic cells (Hunziker *et al.*, 2007). The mature AC consists of four sequential layers; superficial, transitional (middle), deep (radial), and calcified zones (Becerra *et al.*, 2010). The tidemark is a transition zone between the non-calcified and calcified layer (Meirer *et al.*, 2011). Due to its avascular and aneural properties, AC depends on diffusion to acquire its nutrition and oxygen supply, which results in limitations in self-repairing capacity. (Figure 1B) (Table 2)

The avascular nature of AC is attributable to its biochemical composition that antagonizes vascular invasion. The breakdown of the antiangiogenic barrier can cause undesirable vascular invasion of AC and irreversible cartilage degeneration. Of the components encompassed in AC, thrombospondin-1 (TSP1), chondromodulin-I (ChM-I), endostatin/collagen XVIII, secreted protein acidic and rich in cysteine (SPARC), and the type II collagen-derived N-terminal propeptide (PIIBNP) have demonstrated antiangiogenic properties *in vitro* and *in vivo* (Patra and Sandell, 2012). Additionally, tissue inhibitor of metalloproteinases-2 (TIMP2) was also present at high levels in normal articular chondrocytes as an antiangiogenic factor (Mi *et al.*, 2012).

Nucleus Pulposus

The emergence of the intervertebral disc (IVD) begins during the third week of embryogenic development (Rodrigues-Pinto *et al.*, 2014). The axial mesoderm, or notochord, goes through two transitions – first a mesenchymal to epithelial transition (MET) (to allow for correct formation of the neural tube and somites) and then an epithelial to mesenchymal transition (EMT) (to allow for appropriate differentiation) (Hay, 2005; Nakaya and Sheng, 2008). At week four, when the cells readapt this mesenchymal phenotype, the somites that surround the notochord begin to associate into new layers: the dermomyotome (muscle and skin), non-condensed sclerotome (vertebral bodies), and condensed sclerotome (annulus fibrosus, AF) (Rodrigues-Pinto *et al.*, 2014) (Figure 1C). At week five, the dermomyotome begins to dissociate from the notochord, leaving only the notochord and sclerotome cells (Peacock, 1951).

In the sixth and seventh week, notochord cells start their migration to the central portions of the condensed sclerotome (Rodrigues-Pinto *et al.*, 2014) (Figure 1C). By the tenth week, the notochord derived cells, confined within the condensed sclerotome, will begin transition into large, immature NP cells (Figure 1C) (Rodrigues-Pinto *et al.*, 2014; Smith *et al.*, 2011). The notochord's transition into the NP, as well as the direction of other mesenchymal cell populations during development, is controlled through Brachyury (T), Sonic hedgehog (Shh), Noggin (Nog), transforming growth factor beta (TGFβ), and other signaling molecules (Chan *et al.*, 2014). The Shh-dependent expression of Paired box 1 and 9 (Pax1/9) synergistically regulate vertebral column development (Peters *et al.*, 1999), whereas TGFβ is involved in the differentiation of the sclerotome into AF cells (Hayes *et al.*, 2011).

Few blood vessels are available for mature discs and they mainly exist in the longitudinal ligaments alongside the disc and in young cartilaginous endplates (CEP) which are branches of the spinal artery (Crock *et al.*, 1988; Roberts *et al.*, 1995). The disc acquires most nutrition through diffusion *via* the CEP or from the restricted blood supply in the outer layers of the AF. Fas ligand, a type II transmembrane protein of the tumor necrosis factor family, expressed by normal NP cells, could cause apoptosis in vascular endothelial cells and subsequently inhibit blood vessel infiltration (Sun *et al.*, 2013). Additionally, Nog and chondroitin sulfate released from notochordal cells inhibited angiogenesis by suppressing vascular endothelial growth factor signaling (Cornejo *et al.*, 2015). Nerves in the discs, either accompanying the vessels or occurring independently, are branches of the sinuvertebral nerve or the gray rami communicantes (Johnson *et al.*, 2001; Raj, 2008). (Table 3)

Cell Property and Phenotype

The physical environment (oxygen and nutrient supply, microenvironment composition, and biomechanical stress) directly modifies the shape and function of cells within the meniscus, AC, and NP. Each cell population demonstrates an exclusive phenotype within these cartilage-like tissues, having distinctive cell surface profiles, progenitor cell lines, and responses to injury or inflammation.

Meniscus

The cells in the meniscus were initially classified as “chondrocytes, fibroblasts, or cells of intermediate morphology” (Ghadially *et al.*, 1978). However, the characteristic description of meniscus cells seems disputed in the literature, with various terms being applied, such as fibrocytes, fibroblasts, meniscus cells, fibrochondrocytes, and chondrocytes (Nakata *et al.*, 2001). Despite the diverse terminology used, the inner zone cells are apparently round to oval shaped and display a distinct cell associated matrix (CAM) including a mass of cartilaginous type II collagen and a lower, but remarkable, quantity of type I collagen and aggrecan. These properties lead meniscus cells to be termed “fibrochondrocytes” in comparison with hyaline chondrocytes that produce primarily type II collagen and aggrecan (Melrose *et al.*, 2005). In contrast, the cells in the outer portion of the tissue were named fibroblast-like cells due to their similarity to fibroblasts in appearance and behavior; they are encircled in the ECM predominantly by type I collagen, have fewer glycoproteins, and less type III and type V collagen (Melrose *et al.*, 2005). However, mRNA levels of SRY (Sex Determining Region Y)-Box 9 (*SOX9*), an important transcription factor of type II collagen synthesis and chondrogenesis (Lefebvre and de Crombrugghe, 1998), were similar in the meniscus between the inner and outer regions (Upton *et al.*, 2006).

A third cell group, characterized as $CD34^+$ and identified in the outer area of the meniscus (with the majority of meniscal cells exhibiting a $CD34^-/CD31^-$ phenotype), is flat and fusiform-like shaped without cell extensions (Verdonk *et al.*, 2005). This cell group has been proposed to be specific progenitor cells for therapeutic and regenerative purposes (Declercq *et al.*, 2012). As CD34 is regarded as a marker of mesenchymal stem cells (MSCs) (Kopher *et al.*, 2010) which express smooth muscle actin (SMA) (Cai *et al.*, 2001), $CD34^+$ and SMA^+

meniscus cells might participate in the reparative process of pathological menisci (Declercq *et al.*, 2012). α-SMA$^+$ cells were reported to align with collagen fibers in a meniscus crevice three weeks after injury (Kambic *et al.*, 2000) thus indicating their involvement in the differentiation process.

Articular Cartilage

Distributed throughout the matrix, chondrocytes comprise less than 5% wet weight of the AC (Buckwalter and Mankin, 1998). The chondrocyte and its pericellular matrix (PCM) together constitute the chondron, which is recognized as the main structural, functional, and metabolic unit of the AC (Poole, 1997). Investigations show that the cells harvested from the surface of postnatal bovine or mouse AC have stem cell traits such as a high capability of colony formation and expression of MSC markers (Dowthwaite *et al.*, 2004; Hattori *et al.*, 2007), acquiring and expressing chondrogenic phenotypes after multiple passages (Yasuhara *et al.*, 2011). The existence of stem cells in the superficial zone of human AC has also been verified by their positive reaction to TGFβs (Dowthwaite *et al.*, 2004; Hattori *et al.*, 2007), such as boosting production of proteoglycan 4 (PRG4) proteins [also named superficial zone proteins (SZPs) or lubricin] and cartilage matrix aggrecan and type II collagen (Muinos-Lopez *et al.*, 2012). The SOX9 protein is necessary but not sufficient for induction and maintenance of chondrocytic phenotypes; it may act in concert with SOX5 and SOX6, to induce transcription of type II collagen and aggrecan (Ikeda *et al.*, 2004). It is worth noting that SOX9 expression does not correlate with type II collagen expression in AC cells (Aigner *et al.*, 2003) and has been shown to suppress type II collagen transcription in de-differentiated chondrocytes (Kypriotou *et al.*, 2003).

Multiple techniques evaluating either genetic or surface protein expression have been implemented to distinguish AC from cells of the meniscus and NP. In studying AC and NP cells, AC cells were identified positive for fibulin-1 (FBLN1) and integrin-binding sialoprotein (IBSP), with minor NP expression. Interestingly, sources of more compromised, degraded NP expressed higher levels of FBLN1, purporting potential problems for cross-tissue regeneration approaches (Minogue *et al.*, 2010b). Later studies by Minogue involved more genome analysis but with a switch from bovine NP tissue to human. Analysis of human NP cells revealed similar findings for FBLN1 and IBSP, but also revealed novel markers, cytokine-like-1 (CYTL1) and GDF10 (Minogue *et al.*, 2010a). These factors were shown to be increased more than 100-fold in AC compared to NP cells. Other studies have suggested cartilage oligomeric matrix protein (COMP) and matrix gla protein (MGP) as possible identification markers distinguishing AC cells. Again comparing AC and NP cells, AC more highly expressed COMP and MGP (Rutges *et al.*, 2010). COMP is known to be associated as a biomarker for OA cartilage turnover (Pearle *et al.*, 2005) , but it is also suggested that COMP may play a role in suppressing vascularization (Rutges *et al.*, 2010).

Nucleus Pulposus

In early childhood, the cells within the NP are shaped like those that make up the embryonic notochord (large – 25–85 μm, intracellular vacuole-like structures, “immature” mitochondria, and large endoplasmic reticulum) (Hunter *et al.*, 2004; Risbud *et al.*, 2015). In humans, it is reported that these large vacuolated notochordal cells decrease during the first

decade of life, gradually being replaced by smaller (around 10 μm in diameter) and non-vacuolated round cells in the NP (Hunter *et al.*, 2003). The mature NP cells have morphological similarities with articular chondrocytes, even being termed “chondrocyte-like cells” (Risbud *et al.*, 2015; Urban and Roberts, 1995). However, a small proportion of cells which express notochordal biomarkers persisting until maturity (Stosiek *et al.*, 1988) and retaining a distinct phenotype (Clouet *et al.*, 2009; Minogue *et al.*, 2010a) makes NP cells distinct from articular chondrocytes.

The importance of notochordal cells has been demonstrated in the synthesis of functional ECM and in the survival of chondrocyte-like cells. Connective tissue growth factor (CTGF/CCN2), one of the growth factors synthesized by notochordal cells, stimulated the proliferation of chondrocyte-like cells and the production of type II collagen and aggrecan (Erwin *et al.*, 2006). Furthermore, the secretome of notochordal cells could protect chondrocyte-like cells from apoptosis (inhibiting caspases-3 and -9 and favoring aggrecan and type II collagen expression) (Erwin *et al.*, 2011). A recent study reported that the expression of CTGF/CCN2 in notochordal cells could be controlled by oxygen tension (Tran *et al.*, 2013). Thus NP degeneration can be initiated with the gradual disappearance of notochordal cells during skeletal maturation.

Like many other tissues, tissue-specific stem cells were also identified in the IVD (Blanco *et al.*, 2010). A subpopulation of cells distinguished by expression of $Tie2^+$ and $GD2^+$ was shown to be multipotent in the NP tissues because of their ability for differentiation to both mesenchymal and NP lineages (Sakai *et al.*, 2012). The similarity between articular chondrocytes and NP cells, such as sharing common markers, Sox9, type II collagen, and aggrecan (Sive *et al.*, 2002), facilitates a hypothesis that differentiation of MSCs to NP cells with a chondrocyte-like phenotype would be enough to imitate the IVD environment. Interestingly, anabolism of type I collagen and catabolism of type II collagen in the NP may diminish the differentiation into NP cells and ECM biosynthesis of transplanted stem cells (Tao *et al.*, 2016). However, this view has been questioned in a study that determined that AC and NP cells synthesized an obviously different ECM in terms of the ratio of proteoglycan to collagen (Mwale *et al.*, 2004). Moreover, a report showed that, compared to the AC, aggrecan in the NP was highly enriched with keratan sulfate and less aggregated with smaller, more degraded fragments (Donohue *et al.*, 1988). In addition, autologous chondrocyte implantation (harvested from the AC) of the same rabbit’s IVD led to hyaline-like cartilage formation (Gorensek *et al.*, 2004).

Matrix Microenvironment

Evaluation of the meniscus, AC, and NP through water, collagen, proteoglycan, and glycoprotein content illustrates significant compositional changes between tissues. Figure 2 develops a gradient, starting with a low hydrated, low aggrecan, high collagen tissue, the meniscus, and progressing toward a more highly hydrated, high aggrecan, low collagen tissue, the NP. This section provides further detail about the unique tissue-specific environment of the meniscus, AC, and NP. In developing a high-quality tissue graft, the microenvironment must be sufficiently capable of handling biomechanical, oxidative, and matrix remodeling stresses, while retaining proper genome expression.

Meniscus

Water, constituting 72% of the wet weight in mature meniscus, contributes to hydraulic pressures (HP) by binding with proteoglycans to overcome the friction drag of forcing fluid flow through the meniscus (Fox *et al.*, 2012; Herwig *et al.*, 1984). Collagen, another major matrix component, constitutes up to 22% of the wet weight in the meniscus, primarily for type I collagen (Eyre and Wu, 1983; Herwig *et al.*, 1984; McDevitt and Webber, 1990). The unique collagen fiber arrangement in the meniscus, oriented circumferentially in the deeper layers and more radially in the superficial region (Aspden *et al.*, 1985; Fithian *et al.*, 1990; Skaggs *et al.*, 1994), contributes a vertical compressive load transferred into circumferential "hoop" stresses (Ghosh *et al.*, 1975).

Proteoglycans, constituting 1–2% of the dry weight in mature meniscus, initiate hydration for the resistance of compressive loads (Ghosh and Taylor, 1987). The density in the meniscus is significantly diverse at the sample site and is patient age dependent (Fithian *et al.*, 1990). As the major proteoglycan in human menisci, aggrecan largely contributes to the viscoelastic compressive properties by binding with chondroitin sulfate and keratan sulfate of glycosaminoglycan (GAG) (Herwig *et al.*, 1984). Regarding glycoproteins, fibronectin, constituting 8–13% of the dry weight in the meniscus, takes part in tissue repair, embryogenesis, and cell migration/adhesion (Fox *et al.*, 2012). Elastin, which accounts for less than 0.6% of the dry weight in the meniscus (Höpker *et al.*, 1986), most likely interacts directly with collagen to provide resiliency to the tissue (Fithian *et al.*, 1990). Link protein (LP) can stabilize proteoglycan-hyaluronic acid aggregates that are situated around the collagen bunches in the interterritorial matrix (Fife, 1985). ChM-I, a 25 kDa glycoprotein, is involved in the inhibition of endothelial cell proliferation (Hiraki *et al.*, 1997). Larger amounts of ChM-I in the inner meniscus inhibited endothelial cell proliferation, suggesting that ChM-I may be a key antiangiogenic factor for maintaining the avascularity of the inner meniscus (Fujii *et al.*, 2013).

Articular Cartilage

Water, constituting 65–80% of the wet weight of the AC, is approximately 15% more concentrated at the surface than in the deeper zone (Buckwalter and Mankin, 1998). Collagens make up about 10–20% of the wet weight of the AC (Pearle *et al.*, 2005). Of at least 15 distinguishing types of collagen in the AC, type II collagen accounts for 90–95% of the collagens in AC matrix (Eyre *et al.*, 1978). Despite contributing only a minor proportion, types I, IV, V, VI, IX, and XI collagen help create and maintain a fibril meshwork formed by type II collagen (Buckwalter and Mankin, 1998; Hunziker, 2010). This meshwork helps withstand the swelling pressure produced by proteoglycans and supply the tissue's tensile strength. Types I and III collagen are undetectable in healthy AC, but the expression is upregulated in degeneration (Gouttenoire *et al.*, 2004). Type VI collagen is the primary element of the PCM and solely identified within the PCM in adult AC (Poole *et al.*, 2001), despite the fact that it is ubiquitous in AC ECM of the newborn (Poole, 1997).

Proteoglycans, constituting 10–15% of the wet weight of the AC, are the second largest category of macromolecules in the matrix (Oldberg *et al.*, 1990; Pearle *et al.*, 2005). The major proteoglycan (aggrecan) and small leucine-rich proteoglycans [biglycan,

fibromodulin, decorin, aspirin, and parathyroid hormone-like hormone (PTHLH)] (arcOGEN Consortium. *et al.*, 2012; Heinegard and Oldberg,1989; Kizawa *et al.*, 2005) interact with type II collagen and regulate fibril formation to modify the tissue structure and characters. In the AC, the distribution and arrangement of these matrix components are not even. For example, compared to the surface with flattened chondrocytes, a relatively small quantity of proteoglycans and high amounts of collagen fibrils are arranged parallel to the articular surface (Schumacher *et al.*, 1994); the middle zone, on the contrary, has round chondrocytes, the largest quantity of proteoglycans among the four areas, and a random arrangement of collagen (Lorenzo *et al.*, 1998). The deep zone is distinguished by collagen fibrils accompanied by columns of chondrocytes which are perpendicular to the underlying bone (Schmid and Linsenmayer, 1985). The calcified zone is mineralized to some extent and serves as a transformation between cartilage and the underlying subchondral bone (Schmid and Linsenmayer, 1985).

Glycoprotein concentration in the AC decreases depending on disease states (Noyes and Stabler, 1989). During fetal development, AC chondrocytes have been shown to express α6β1 integrins, which associate with laminin in the ECM and promote cell proliferation, differentiation, and polarization (Durr *et al.*, 1996). Through development and into adulthood, laminin becomes less important as a glycoprotein for sustaining the AC, while clusterin begins to play a more important role. Clusterin, excreted from chondrocytes of the superficial zone, activates the complementary pathway, resulting in immune response, cell death, and potentially tissue destruction (Khan *et al.*, 2001). For daily load-bearing activities, lubricin, another important glycoprotein excreted from chondrocytes of the superficial zone, is responsible for reducing friction within the joint; a decrease in lubricin is also associated with OA progression (Musumeci *et al.*, 2013). ChM-I is expressed specifically in cartilage as a functional matrix component (Hiraki *et al.*, 1991). ChM-1 null mice exhibit retarded chondrocyte maturation in the periosteal callus, aberrant cartilage formation during fracture repair (Yukata *et al.*, 2008), and marked reduction in bone remodeling (Nakamichi *et al.*, 2003). A recent study suggested that ChM-1 governed stable chondrocyte phenotypes and maintained cartilage homeostasis possibly by inhibiting hypoxia inducible factor-2 alpha (HIF-2α) induced catabolic activity (Zhang *et al.*, 2016b).

Nucleus Pulposus

Water, constituting about 80% of the wet weight in the NP compared to 70% of the wet weight in the AF (Choi *et al.*, 2015; Raj, 2008), along with type II collagen, allows NP to be elastic and deform under stress. In the IVD, the outer AF contains highly organized type I collagen fibers (about 70% of dry weight), which becomes progressively richer in type II collagen fibers toward the inner AF and the central gelatinous NP (Eyre and Muir, 1977). The primary collagen in the NP is type II (about 20% of dry weight), while types VI, IX, and XI only occur in small amounts (Sive *et al.*, 2002). The arrangement of collagen fibers within the disc is random, interspersing throughout the ECM environment (Inoue, 1981).

Proteoglycans constitute around 14% of the wet weight in the NP and about 5% of the wet weight in the AF (Raj, 2008). Unlike collagen that mainly contributes to the tensile strength of the disc, proteoglycans are the primary components resisting compression and providing

resilience (Greenwald *et al.*, 1978). In young discs, the major macromolecules are chondroitin sulfate A and C, which are strongly hydrophilic and promote disc viscosity (Freeman and Meachim, 1979); during the early 20s, however, these macromolecules start to break down into smaller molecules, such as chondroitin sulfate B and keratan sulfate, which bind less water (Holm *et al.*, 1981). Of them, aggrecan is responsible for sustaining tissue hydration (Bogduk and Twomey, 1987; Johnstone and Bayliss, 1995) *via* osmotic pressure supplied by chondroitin and keratan sulfate chains (Urban *et al.*, 1979). Despite expression of aggrecan and type II collagen in both chondrocytes and NP cells, the ratio of GAG to hydroxyproline was reported to be around 27:1 in young adult NP and about 2:1 in the AC (Mwale *et al.*, 2004).

For glycoprotein, elastin by dry weight in nondegenerated human disc was 2% on average with no site dependent difference (Cloyd and Elliott, 2007). Elastic fibers, aligned with fibrillin-rich microfibrils in the disc (Yu *et al.*, 2007), are important for the recovery of collagen fibers after deformation. Fibronectin plays a key role in matrix organization by interacting with integrins such as α5β1 on cell surfaces, as well as ECM compositions such as collagen, fibrin, and heparin sulfate proteoglycans (Hynes and Yamada, 1982). Lubricin found in both NP and AF is suggestive of its role in inter-lamellar tribology (Shine *et al.*, 2009). As with the AC, three LPs have also been detected in human IVD (Mort *et al.*, 1985; Tengblad *et al.*, 1984). The largest, LP1, is the predominant form in immature discs, whereas the smallest form (LP3), a proteolytic cleaved product of LP1 and LP2 (Mort *et al.*, 1985), is more abundant in mature discs (Pearce *et al.*, 1989). ChM-I was detected in both the ECM and chondrocytes in the zone of hypertrophic cartilage, the zone of proliferative cartilage, and the zone of resting cartilage in human fetal discs as well as in the AF, NP, and CEP in human mature discs (Takao *et al.*, 2000).

Collagen Network

Examining microenvironments of the meniscus, AC, and NP, it is important to understand how posttranslational modifications, enzyme activity during expansion, and changes in gene expression can alter the composition of the tissues produced. Since collagen meshwork plays an important role in the biomechanical characteristics of cartilage (Bastiaansen-Jenniskens *et al.*, 2008), the collagen meshwork should be a focus in cartilage engineering and regeneration (Maroudas and Venn, 1977; Maroudas, 1976). Modifications in the collagen network involving hydroxypryidinoline, percent of hydroxylysine (Hyl), and pentosidine content are all important in determining the function of the tissue. Hydroxypryidinoline and Hyl are both more highly expressed in type II collagen fibers (Bank *et al.*, 2002), showing a greater percentage in the AC than the meniscus and NP; these modifications are also involved in cross-linking collagen fibers together, increasing the mechanical integrity of the tissue (Bastiaansen-Jenniskens *et al.*, 2008). Pentosidine, an advanced-glycation end-product (AGE) found on collagen fibers, allows for more collagen aggregates, but increases during aging and can result in stiff and brittle cartilage (Brama *et al.*, 1999; Duance *et al.*, 1998).

In alginate microbead-expanded cells from the meniscus, AC, and NP, levels of matrix synthesis and degradation proteins change, e.g. procollagenlysine 2-oxoglutarate 5-dioxygenase 3 (*PLOD3*), matrix metalloproteinase 13 (*MMP13*), serpin peptidase inhibitor,

clade H (heat shock protein 47), member 1 (*SERPINH1*), and a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 2 (*ADAMTS2*), from levels observed in native meniscus, AC, and NP tissues (Vonk *et al.*, 2010). Vonk *et al.* (Vonk *et al.*, 2010) measured genes including proteoglycans, collagens, and enzymes, in collagen synthesizing or degradation (16 genes are assessed in Table 4). Each meniscal, AC, and NP cell displays a specific mode of aggrecan (*ACAN*), biglycan (*BGN*), α1 (I) procollagen (*COL1A1*), and α1 (II) procollagen (*COL2A1*). Lysyl hydroxylation levels also changed during expansion of cells (Vonk *et al.*, 2010). The results of this study indicate that expanding cells can drastically alter the characteristics of tissues produced when compared to the *in vivo* condition. Two specific examples include a 15–150 fold increase in MMP13 expression and decrease of lysyl hydroxylation within the meniscus, AC, and NP tissue (Bastiaansen-Jenniskens *et al.*, 2008; Vonk *et al.*, 2010). MMP13 is also known to increase in OA affected tissue (Kevorkian *et al.*, 2004). Changes associated with type I and II collagen ratios, amount of remodeling present, and specific posttranslational modification in each group were all seen during cellular expansion (Vonk *et al.*, 2010).

Biomechanics and Function

Developing a functional tissue requires biomechanical stimulation that invokes proper cell-ECM signaling, gene activation, and ultimately, ECM remodeling. Because some functions of the meniscus (stability), AC (articulation), and NP (compression) are unique to their location, biomechanical stimulation needs to be specifically targeted for each tissue, involving regulating fluid flow mechanisms, ECM organization, and autocrine/paracrine signaling.

Meniscus

Elaborating on biomechanical properties is very important for acknowledging meniscus functionality *in situ*. The biomechanical function of the meniscus is determined by its fibrocartilaginous structures and semilunar shape as well as its relationship to the surrounding tissues, including load transmission (King, 1936) and load bearing functions (Fairbanks, 1948). Load transmission stems from the wedge shape of the meniscus, on which the hoop stresses from circumferentially oriented collagen fibers balance the shear force from radially oriented collagen fibers when a load is applied (Aspden *et al.*, 1985; Shrive, 1974; Shrive *et al.*, 1978); through this mechanism, the meniscus faces compressive, shear, and tensile forces. The posterior horns of the meniscus carry more load than the anterior horns though both connect to the tibial plateau by intertwining collagen fibers which convey forces from the meniscus to the tibial plateau (Gao *et al.*, 1998). Either total or partial meniscectomy (Baratz *et al.*, 1986) and subsequent malalignment of the joint would decrease the contact areas and increase the peak stresses in the knee joints (Bargar *et al.*, 1980). The meniscus also functions to increase joint stability and congruity by virtue of its unique concave surface that can accommodate the convexity of the femoral condyles (Renström and Johnson, 1990; Walker and Erkman, 1975; Warren *et al.*, 1986).

Articular Cartilage

In 1970, for the first time, Kempson *et al.* characterized the correlation between biochemical composition and mechanical parameters of human femoral head cartilage and found that the two-second creep modulus strongly correlated with chondroitin and keratan sulfates, but weakly correlated with collagen content, indicating that the compressive stiffness of human AC was mainly determined by both GAGs rather than by collagen (Kempson *et al.*, 1970). The positive correlation between sulfated GAG and the equilibrium shear and equilibrium aggregate modulus was further confirmed by other research groups (Jurvelin *et al.*, 1988; Treppo *et al.*, 2000; Williamson *et al.*, 2001). During maturation, external forces assist to regulate innate mechanical properties *via* matrix adjustment (Responte *et al.*, 2012), such as compressive and shear strain, stress, hydrostatic pressure, and fluid flow, which are assigned to the anisotropic, zonal organization of AC matrix (Wong and Carter, 2003). A recent report suggested that mechanical motion induced PRG4 expression in the superficial zone of articular cartilage (Ogawa *et al.*, 2014). Age-dependent and regional variation were found in the compressive and tensile properties of bovine fetal, newborn, and adult cartilage tissues (Williamson *et al.*, 2003). Evidence indicates the physiological magnitude of stresses in AC (Hodge *et al.*, 1989; Afoke *et al.*, 1987), such as hydrostatic pressure and compression, varied from 3 to 10 MPa with a frequency of 1 Hz (Waters *et al.*, 1988). In human AC, PCM has a significantly lower modulus than that of the ECM (Darling *et al.*, 2010). PCM has an important influence on the stress-strain environment of the chondrocytes that potentially varies with the depth of AC (Alexopoulos *et al.*, 2003). AC deformation under compressive loading is highly dependent on the relative mechanical properties of the chondrocytes, PCM, and ECM (Choi *et al.*, 2007).

Nucleus Pulposus

As a critical factor for the flexibility and stability of the spine, NP mechanics is primarily dependent on compressive and shear stresses *in vivo* (Nerurkar *et al.*, 2010). The main function of the NP is to absorb the loads acting on the spine and redistribute them radially to the inner layers of the AF and vertically to the cartilaginous endplates (Nixon, 1986; Pattappa *et al.*, 2012). The swelling pressure in human discs was approximately 0.1–0.2 MPa in the supine position (Wilke *et al.*, 2001) but increased up to 2.3 MPa when lifting a heavy weight (Wilke *et al.*, 1999). In degenerated discs, the fragmentation of aggrecan increased but its effective negative charge decreased, resulting in a decrease of intradiscal pressure (Sato *et al.*, 1999) and the ability to retain water under compressive forces (Lee *et al.*, 2013), which led to a reduction of disc height (Iatridis *et al.*, 2013; Vergroesen *et al.*, 2014).

For all human spines tested, proteoglycan and collagen contents could be used to predict the correlation between equilibrium hydration and swelling pressure (Urban and McMullin, 1988). Proteoglycan content was reported with an age- and site-dependent decrease and was lowest in the L5-S1 disc (Urban and McMullin, 1988) and/or L4/L5 (Adams *et al.*, 1996). Age-related degenerative changes were also found in a switch of size and pressure of the NP and AF; with a decrease of the diameter and pressure of the NP region, the width of the AF and the height of compressive “stress peaks” increased, indicating that anatomic changes within the AF and cartilaginous endplate led to a shift of load from the NP to AF (Adams *et al.*, 1996).

Conclusions/Perspectives

This review highlights the similarities and differences between the meniscus, AC, and NP. The vascular, neural, and basic anatomy overview depicts the complex layering that each tissue possesses, ranging from levels of nutrient supply and oxygen distribution to sensitization and proprioception. Each tissue is a unique construct whose cellular ultrastructure and genetic expression further detail its functionality. The cellular composition, along with MSC populations which react distinctly under differentiation conditions, further validates the inconsistencies between tissues. Figure 2 also analyzes the ECM compositional changes between each tissue, showing changes in arrangement between the meniscus, AC, and NP, which are responsible for unique biomechanical stresses of each tissue varying from joint articulation, to gliding, to compression.

Despite the fundamental knowledge provided in this review paper for developing an ideal cartilage or cartilage-like tissue, there are critical and distinct molecular signaling pathways governing tissue regeneration. Regulation of signaling during development in the meniscus (TGFβ and insulin-like growth factor I) (Pazin *et al.*, 2014), the AC (Wnt9A, GDF5, Erg, Gli3, CD44, type IIA collagen, and type I collagen) (Iwamoto *et al.*, 2007; Koyama *et al.*, 2008; Pacifici *et al.*, 2006), and the NP [Brachyury (T), Shh, Nog, and TGFβ] (Chan *et al.*, 2014) all provide insight toward achieving a tissue-specific cell population. Each of the tissues relies on a variety of disparate signaling pathways to achieve maturation; refinement of these pathways can better progress tissue engineering and regenerative medicine approaches. Research to produce better tissue constructs needs to involve a more adequate understanding of the surface expression of host progenitor cells, including $CD34^{+}/CD31^{-}$ cells in the meniscus (Declercq *et al.*, 2012), FBLN1, IBSP, CYTL1, and GDF10 expressing cells in the AC (Minogue *et al.*, 2010a), and $Tie2^{+}$ and $GD2^{+}$ cells in the NP (Sakai *et al.*, 2012). Biomechanical stimuli needed *in vitro* to sustain a tissue-specific, functional population need more defined parameters for each tissue type. While the inner and outer portions of the meniscal cells may respond to variable levels of hydrostatic and tensile strain (Spilker *et al.*, 1992), articular chondrocytes need a specific balance of mechanical loading, potentially different for chondrocytes within any one of the four layers (Jortikka *et al.*, 1997). Likewise, NP cells in culture should be evaluated on matrix synthesis and degradation protein expression correlating to induced pressure gradients (Millward-Sadler *et al.*, 2004).

More serious concerns for tissue engineering are realized during cellular expansion (Vonk *et al.*, 2010), thus understanding how to better regulate cellular responses to *in vitro* stresses is crucial in directing cells toward a specific tissue. Compared to two-dimensional conventional culture, decellularized extracellular matrix (dECM) deposited by stem cells is a three-dimensional nanofibrous scaffold that may alleviate problems of cell senescence during *ex vivo* expansion (Pei *et al.*, 2011b). Using synovium-derived stem cells (SDSCs) to deposit a dECM, it has been demonstrated that SDSC expansion on this substrate increases cell proliferation and chondrogenic capacity (He *et al.*, 2009); likewise, bone marrow-derived stem cells (BMSCs) as a donor cell for a dECM can increase BMSC proliferation and osteogenic differentiation capacity during expansion (Pei *et al.*, 2011a), indicating that a tissue-specific stem cell might provide a unique microenvironment for a lineage-specific

tissue regeneration (Pizzute *et al.*, 2015). For example, SDSCs are tissue-specific stem cells (Jones and Pei, 2012) and currently available research suggests that SDSCs may mimic the regulatory role of notocordal cells for NP regeneration (Shoukry *et al.*, 2013), which might explain how dECM from SDSCs promotes NP rejuvenation (He and Pei, 2012; Pei *et al.*, 2012).

This review hopes to encourage regenerative medicine research through presenting the differences between each tissue, but also explaining levels of commonality that may be utilized for future tissue engineering. The goal is to provide clarity in creating meniscus, AC, and NP tissue that can be produced, not only in high quantity, but also with high biomechanical and functional quality.

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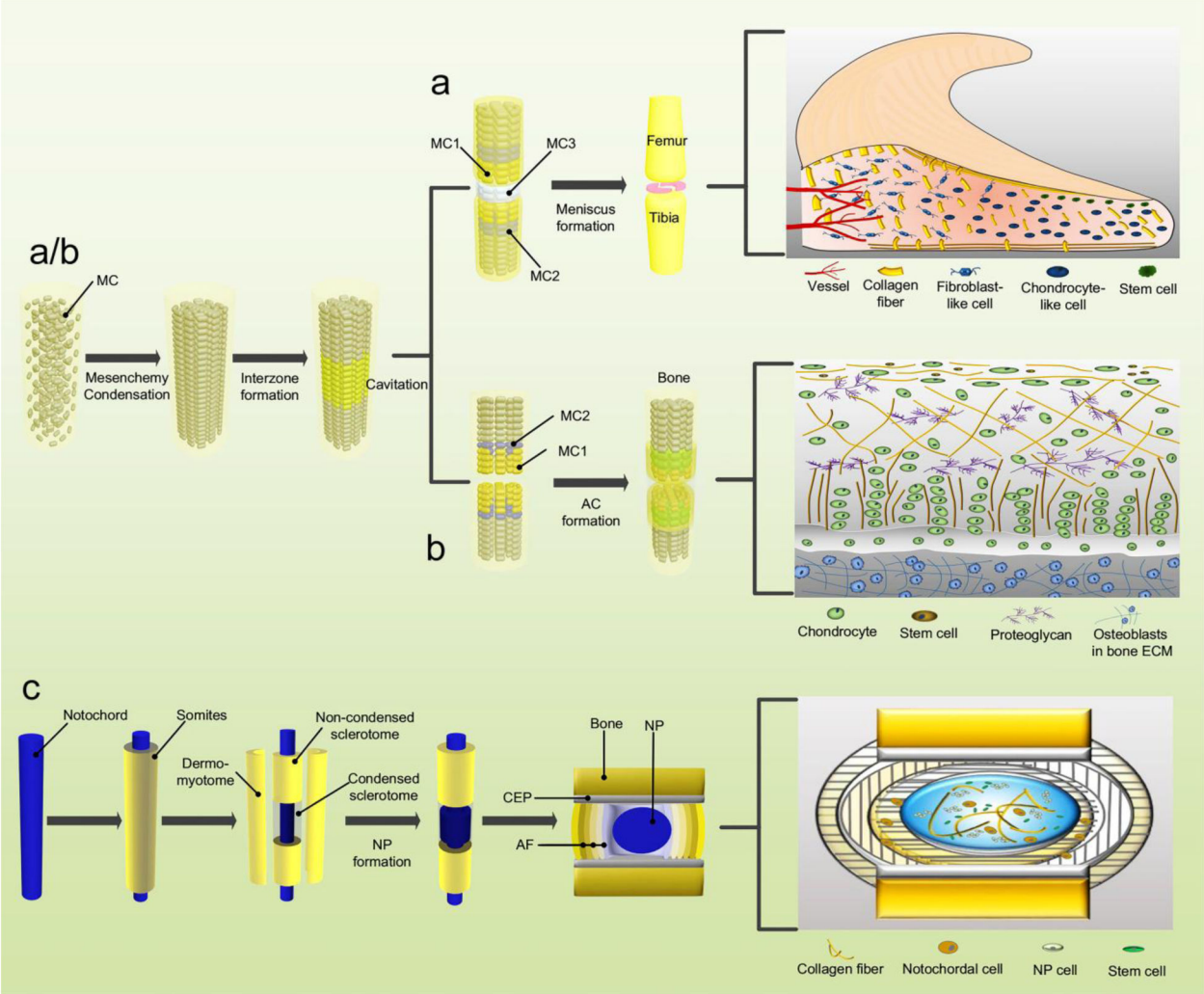

Fig. 1.

Development of the meniscus (A), AC (B), and NP (C). Abbreviations: AF: annulus fibrosus; CEP: cartilaginous endplate; MC: mesenchymal cells; MC1: MC committed to form superficial layer of AC; MC2: MC with chondrogenic fate; MC3: MC with meniscal fate.

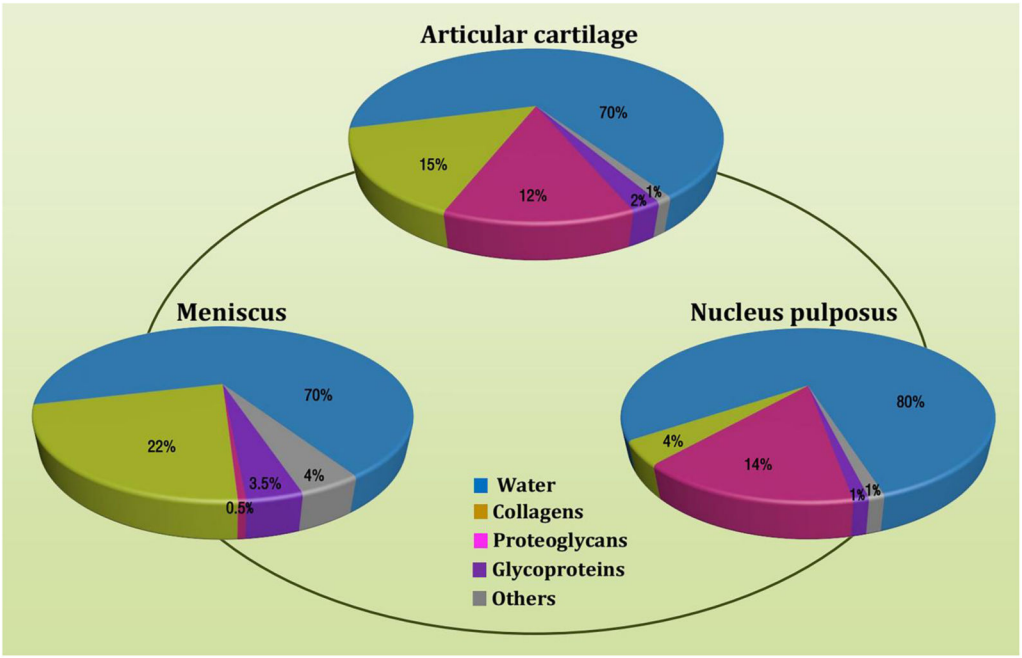

Fig. 2.
ECM composition of the meniscus, AC, and NP.

Table 1

Characterization of meniscus.

Characterization	Embryology and Development	Reference
Origination	Interzone cells	(Gardner and O'Rahilly, 1968)
Shape formation	Between the 8th and 10th week of gestation	(Gardner and O'Rahilly, 1968)
Component switch	Increase in collagen content but decrease in cellularity and vascularity with the development of fetus	(Clark and Ogden, 1983)
	Gross Anatomy	
Medial meniscus	"C"-shaped; 39.8±3.7 mm long and 9.5±0.7 mm wide; anterior horn is attached to the tibia anterior to the ACL; posterior horn is attached immediately anterior to the attachment of the PCL; peripheral border merges with the knee joint capsule	(Fox *et al.*, 2015, Proffen *et al.*, 2012)
Lateral meniscus	"O"-shaped; 33.3±3.5 mm long and 9.8±0.7 mm wide; anterior horn is attached to the intercondylar fossa adjacent to the broad attachment site of the ACL; posterior horn is attached to the PCL and medial femoral condyle	(Fox *et al.*, 2015, Proffen *et al.*, 2012)
	Vascular and Neural Anatomy	
Blood supply	Peripheral 10–25% are vascular for LM and 10–30% for MM	(Danzig *et al.*, 1983)
Intrinsic innervation	Most abundant on the periphery and the anterior and posterior horns	(Kennedy *et al.*, 1982; Zimny *et al.*, 1988)
	Cell Property and Phenotype	
Outer 2/3 region	Elongated fibroblast-like cells	(Melrose *et al.*, 2005)
Inner 1/3 region	Rounded chondrocyte-like cells	(Melrose *et al.*, 2005)
Superficial region	Flattened and fusiform progenitor cells	(Declercq *et al.*, 2012)
Cell density	Vary with regions: 200–2800 cells/mm^2	(Lin *et al.*, 2002)
Phenotypic marker	*C1QR; CA12; COL1A1; COL1A2; ESTs; FLJ20831; HPCAL1; LIMK2; PDLIM1*	(Ochi *et al.*, 2003)
	Matrix Microenvironment	
Water	72% of wet weight and content is higher in the posterior areas	(Herwig *et al.*, 1984)
Collagen	22% of wet weight	(Herwig *et al.*, 1984)
Outer 2/3 region	Type I collagen (80% by dry weight) and other collagen variants (e.g., types II, III, IV, VI, and XVIII) (<1%)	(Fox *et al.*, 2012; Fox *et al.*, 2015)
Inner 1/3 region	Type II (60%) and type I collagen (40%)	(Cheung, 1987)
Proteoglycan	1–2% of dry weight; the major PG (aggrecan) and other smaller PGs (e.g., decorin, biglycan, fibromodulin, and lubricin)	(Ghosh and Taylor, 1987)
Glycoprotein	Type VI collagen, link protein, fibronectin, thrombospondin, elastin, and chondromodulin-I	(Fujii *et al.*, 2013; Höpker *et al.*, 1986; McDevitt and Webber, 1990)
	Tissue Function	
Primary role	Transferring vertical compressive load into circumferential "hoop" stresses	(Ghosh *et al.*, 1975)
Secondary role	Shock absorption, stability, lubrication, nutrition, and proprioception to the knee joint	(Fithian *et al.*, 1990; Renström and Johnson, 1990)

Abbreviations: ACL: anterior cruciate ligament; C1QR: complement component C1q receptor; CA12: carbonic anhydrase XII; COL1A2: collagen, type I, alpha 2; FLJ20831: hypothetical protein FLJ20831; HPCAL1: hippocalcin-like 1; LIMK2: LIM domain kinase 2; LM: lateral meniscus; MM: medial meniscus; PCL: posterior cruciate ligament; PDLIM1: PDZ and LIM domain 1 (elfin); PG: proteoglycan.

Table 2

Characterization of articular cartilage.

Characterization	Embryology and Development	Reference
Origination	Interzone cells	(Archer *et al.*, 2003)
Developing AC	3–4 layers that show a distinct cell shape and size	(Hunziker *et al.*, 2007)
Mature AC	Superficial, middle, deep, and calcified layers	(Becerra *et al.*, 2010)
	Gross Anatomy	
AC thickness	2.4±0.4 mm at the medial femoral condyle and 3.0±0.4 mm at the medial tibial plateau	(Quinn *et al.*, 2013)
	Zonal Organization	
Superficial	Flattened chondrocytes, low quantity of PGs, and high quantity of collagen fibrils arranged parallel to AC surface	(Schumacher *et al.*, 1994)
Middle	Rounded chondrocytes, the highest level of PGs among the four zones, and a random arrangement of collagen	(Lorenzo *et al.*, 1998)
Deep	Chondrocyte columns arrayed along the axis of fibrils, which is perpendicular to the underlying bone	(Schmid and Linsenmayer, 1985)
Calcified	Partly mineralized and acting as the transition between cartilage and the underlying subchondral bone	(Schmid and Linsenmayer, 1985)
Tidemark	The transition zone between the non-calcified and calcified normal AC	(Meirer *et al.*, 2011)
	Vascular and Neural Anatomy	
Blood supply	Avascular	(Buckwalter, 1983)
Innervation	No nerve supply	(Buckwalter, 1983)
	Cell Property and Phenotype	
Chondrocytes	The sole cell in AC	(Buckwalter and Mankin, 1998)
Superficial zone	Progenitor/stem cell	(Muinos-Lopez *et al.*, 2012)
Cell density	1.4×10^4 cells/mm^3	(Stockwell, 1971)
Phenotypic marker	*COMP; CYTL1; FBLN1; GDF10; HIF-1/2α; IBSP; MGP*	(Minogue *et al.*, 2010a&b; Rutges *et al.*, 2010; Wang *et al.*, 2016)
	Matrix Microenvironment	
Water	65–80% of wet weight	(Buckwalter and Mankin, 1998)
Collagen	10–20% of wet weight; 90–95% type II collagen with a small percentage of types I, IV, V, VI, IX, and XI collagen	(Buckwalter and Mankin, 1998; Hunziker, 2010)
Proteoglycan	10–15% of wet weight; the major component (aggrecan) and small leucine-rich PGs (biglycan, fibromodulin, decorin, and lubricin)	(Heinegard and Oldberg, 1989; Oldberg *et al.*, 1990)
Glycoprotein	Clusterin, lubricin, and chondromodulin-I	(Hiraki *et al.*, 1991; Khan *et al.*, 2001; Musumeci *et al.*, 2013)
	Tissue Function	
Primary role	Load transmission and distribution, smooth articulation, lubricating, and wear-resisting structure that facilitates joint motion	(Buckwalter and Mankin, 1998)

Abbreviations: AC: articular cartilage; COMP: cartilage oligomeric matrix protein; CYTL1: cytokine-like 1; FBLN1: fibulin 1; GDF10: growth differentiation factor 10; IBSP: integrin-binding sialoprotein; MGP: matrix gla protein; PG: proteoglycan.

Table 3

Characterization of nucleus pulposus.

Characterization	Embryology and Development	Reference
Origination	Mesodermal somites	(Peacock, 1951; Rodrigues-Pinto *et al.*, 2014)
Shape formation	The tenth week of embryonic development	(Peacock, 1951; Rodrigues-Pinto *et al.*, 2014)
Gross Anatomy		
Property	Gelatinous	(Maroudas *et al.*, 1975)
Microenvironment	Avascular, hypoxia, low pH, low nutrition, low cellularity, high GAG content, and type II collagen	(Agrawal *et al.*, 2007, Rajpurohit *et al.*, 2002)
Vascular and Neural Anatomy		
Blood supply	Avascular	(Crock *et al.*, 1988; Roberts *et al.*, 1995)
Innervation	No innervation	(Crock *et al.*, 1988; Roberts *et al.*, 1995)
Cell Property and Phenotype		
NP cell	Smaller (10 μm), round, and chondrocyte-like cells	(Hunter *et al.*, 2003; Hunter *et al.*, 2004)
Notochordal cell	Large (25–85 μm) and vacuolated	(Hunter *et al.*, 2004; Risbud *et al.*, 2015)
NP progenitor cell	$Tie2^+$ and $GD2^+$ positive	(Sakai *et al.*, 2012)
Cell density	6000 cells/mm^3	(Maroudas *et al.*, 1975)
Phenotypic marker	*HIF1/2α, GLUT1, KRT 18/19, CA-3/12, CD24, A2M*	*#*
Matrix Microenvironment		
Water	80% of the wet weight	(Raj, 2008)
Collagen	About 20% (type II collagen) of dry weight and small amounts of types VI, IX, and XI collagen	(Eyre and Muir, 1977; Sive *et al.*, 2002)
Proteoglycan	15% of wet weight; the major PG (aggrecan) and smaller amounts (decorin, biglycan, and lumican)	(Inkinen *et al.*, 1998; Malrose *et al.*, 2001; Raj, 2008)
Glycoprotein	Elastin, fibronectin, laminin, lubricin, link protein, and chondromodulin-I	*##*
Tissue Function		
Primary role	Absorb the loads and equalize the compressive stress on the vertebral CEP	(Pattappa *et al.*, 2012)

Abbreviations: A2M: alpha-2-macroglobulin; CEP: cartilaginous end-plate; GAG: glycosaminoglycan. GLUT1: glucose transporter 1; HIF1α: hypoxia-inducible factor 1 alpha; KRT18: keratin 18;

#(Fujita *et al.*, 2005; Minogue *et al.*, 2010a; Minogue *et al.*, 2010b; Power *et al.*, 2011; Richardson *et al.*, 2008; Risbud *et al.*, 2006; Risbud *et al.*, 2007; Rutges *et al.*, 2010; Sakai *et al.*, 2012);

##(Chen *et al.*, 2009; Cloyd and Elliott, 2007; Hayes *et al.*, 2001; Hynes and Yamada, 1982; Johnson *et al.*, 1982; Schumacher *et al.*, 1994; Takao *et al.*, 2000)

Table 4

Relative expression levels of 16 genes in freshly isolated chondrocytes from the meniscus, AC, and NP.

Gene	Meniscus	Articular Cartilage	Nucleus Pulposus
ACAN	*b, c*	*a, c*	*a, b*
BGN	*b*	*a, c*	*b*
COL1A1	*b*	*a, c*	*b*
COL2A1	-	-	-
SERPINH1	-	-	-
PLOD1	-	-	-
PLOD2	*b, c*	*a, c*	*a, b*
PLOD3	*b*	*a, c*	*b*
LOX	*b, c*	*a, c*	*a*
P4HA1	*b, c*	*a, c*	*b*
P4HA2	-	-	-
P4HA3	-	-	-
ADAMTS2	-	-	-
ADAMTS3	*b*	*a, c*	*b*
MMP13	-	-	-
MMP14	-	-	-

The expression levels of genes related to synthesis and degradation of the ECM normalized for three housekeeping genes (Vonk *et al.*, 2010). Abbreviations: ACAN: aggrecan; BGN: biglycan; COL1A1: α1(I) procollagen; COL2A1: α1(II)procollagen; SERPINH1: serpin peptidase inhibitor, clade H (heat shock protein 47), member 1; PLOD1, 2, and 3: procollagenlysine 2-oxoglutarate 5-dioxygenase 1, 2, and 3; Lox: lysyl oxidase; P4HA1, 2, and 3: procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide 1, 2, and 3; ADAMTS2, and 3: a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 2, and 3; MMP13, and 14: matrix metallopeptidase 13, and 14.

[a] Significantly different compared to meniscus

[b] Significantly different compared to AC

[c] Significantly different compared to NP

- Not significantly different compared to other cartilage types