Biomechanical properties and mechanobiology of the articular chondrocyte

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Chen C, Tambe DT, Deng L, Yang L. Biomechanical properties and mechanobiology of the articular chondrocyte. Am J Physiol Cell Physiol 149: C1202–C1208, 2013. First published September 25, 2013; doi:10.1152/ajpcell.00242.2013.—To withstand physiological loading over a lifetime, human synovial joints are covered and protected by articular cartilage, a layer of low-friction, load-bearing tissue. The unique mechanical function of articular cartilage largely depends on the composition and structural integrity of the cartilage matrix. The matrix is produced by highly specialized resident cells called chondrocytes. Under physiological loading, chondrocytes maintain the balance between degradation and synthesis of matrix macromolecules. Under excessive loading or injury, however, degradation exceeds synthesis, causing joint degeneration and, eventually, osteoarthritis (OA). Hence, the mechanoresponses of chondrocytes play an important role in the development of OA. Despite its clear importance, the mechanobiology of articular chondrocytes is not well understood. To summarize our current understanding, here we review studies of the effect of mechanical forces on mechanical and biological properties of articular chondrocytes. First, we present the viscoelastic properties of the cell nucleus, chondrocyte, pericellular matrix, and chondron. Then we discuss how these properties change in OA. Finally, we discuss the responses of normal and osteoarthritic chondrocytes to a variety of mechanical stimuli. Studies reviewed here may provide novel insights into the pathogenesis of OA and may help in development of effective biophysical treatment.

articular cartilage; chondrocyte; osteoarthritis; joint loading; mechanobiology

THE HUMAN SKELETON is composed of stiff bones and flexible joints. The joints, especially the weight-bearing joints, are continuously subjected to mechanical loading. To withstand these mechanical demands, each of the bony ends in diarthrodial joints is covered and protected by articular cartilage, a layer of connective tissue that has a low friction coefficient, high load-bearing capacity, and can diffuse shock loads onto the subchondral bone (8). However, when the articular cartilage is injured by trauma or excessive mechanical loading, focal degradation of cartilage and remodeling of subchondral bone occur, resulting in joint pain and dysfunction, clinically identified as osteoarthritis (OA) (6, 22).

The articular cartilage is maintained in a healthy state by its resident cells, “chondrocytes” (88). In particular, the chondrocyte is not exposed to the extracellular matrix (ECM) but is surrounded by the pericellular matrix (PCM), which is predominantly composed of type VI collagen and proteoglycans (73). The PCM transmits physical forces between the chondrocyte and its surrounding ECM (35). The PCM together with the ECM constitutes the cartilage matrix. Under physiological loading, the chondrocyte maintains homeostasis of the cartilage matrix by maintaining a balance between catabolic and anabolic events; under abnormal mechanical stimuli (excessive loading, joint trauma, and joint malalignment), this balance of chondrocyte metabolism is disturbed, which accelerates matrix loss, induces degeneration of cartilaginous tissue, and, finally, leads to OA (30, 32). As such, mechanical stimuli have been recognized as a key factor in the initiation and progression of OA (19, 62). In this scenario, the chondrocyte as the sole type of cell in articular cartilage, its mechanical properties have become of primary significance. Besides, articular cartilage has a very limited ability to self-repair because of its avascular and aneural nature (7), and current clinical treatments cannot completely repair osteoarthritic cartilage (23). Therefore, investigation of biophysical therapies for OA, which requires a thorough understanding of mechanotransduction at the cellular level for articular cartilage, is under way.

Indeed, chondrocyte mechanobiology has been studied extensively in vitro (9, 47, 54) and in situ (40, 52, 61). These studies establish that biomechanical properties of the chondrocyte play a crucial role in the structure and function of articular cartilage. In this review, we first describe the mechanical properties of the articular chondrocyte in the normal and osteoarthritic states. Then we discuss biological responses of the chondrocyte in response to mechanical signals and its underlying mechanotransduction mechanism.

Elastic and Viscoelastic Properties of the Chondrocyte

Techniques for measurement of mechanical properties of the chondrocyte include indirect determination of cell stiffness through measurement of cell deformation within compressed...
hydrogels (agarose gel or alginate gel), micropipette aspiration (34), atomic force microscopy (AFM) (4, 45), cytoindentation (51, 80), and unconfined creep compression (57, 78).

Using rat articular chondrocytes embedded on agarose gels, Freeman et al. (29) applied 5%, 10%, and 15% compressive strain and estimated Young’s modulus of the chondrocyte to be 4 kPa. Slightly different values of Young’s modulus of bovine articular chondrocytes were calculated by Bader and Knight, who used agarose, as well as alginate, gels: 2.7 kPa on agarose gels (5) and 3 kPa on alginate gels (50). Using micropipette aspiration, Trickey et al. (86) measured viscoelastic properties of a single chondrocyte isolated from normal and osteoarthritic human cartilage and found much higher Young’s modulus and apparent viscosity for an osteoarthritic than a normal chondrocyte: 0.5 vs. 0.36 kPa and 5.8 vs. 3.0 kPa/s, respectively. In other studies that use AFM and confined compression, the human osteoarthritic chondrocyte was softer than the normal chondrocyte (42, 45); in addition, using unconfined compression, Shieh et al. (78) found that a bovine articular chondrocyte from the superficial zone is much stiffer than a chondrocyte from the middle/deep zone. Using AFM indentation, Darling et al. confirmed that viscoelastic properties of porcine articular chondrocytes also differ significantly with the zone of origin (15) and with the cell dedifferentiation state (14); moreover, recent studies showed that aging of the chondrocyte dramatically increases its viscoelastic properties, which include equilibrium modulus, instantaneous modulus, and apparent viscosity (18, 83). Taken together, all these factors (tissue source, experimental technique, zonal location, disease, dedifferentiation, and aging) not only explain why the Young’s modulus of chondrocytes from numerous studies are different by an order of magnitude but also provide evidence that the mechanical properties of the chondrocyte are important references for evaluation of the status of articular cartilage.

Like other eukaryotic cells, mechanical properties of the chondrocyte are largely determined by its cytoskeletal network (27). By disrupting actin filaments with cytochalasin D or disrupting intermediate filaments with acrylamide, Trickey et al. (87) found that the elastic modulus and viscosity of the human articular chondrocyte decline dramatically. In contrast, they found that disruption of microtubules using colchicine does not affect chondrocyte mechanical properties (87). In another study, disruption of vimentin intermediate filaments was found to reduce the stiffness of the primary human chondrocyte by 2.8-fold (42); the mechanism for this reduction was subsequently shown to be indirect alteration of the configuration of the chondrocyte actin cytoskeleton influenced by intermediate filament disruption (11). All these results indicate that the viscoelasticity of the chondrocyte is mainly determined by integrity and organization of the actin filaments and integrity of the intermediate filaments, which is consistent with the tensegrity model (48). In this model, the microtubules serve as struts to resist compression (82), while the actin and intermediate filaments bear the cytoskeletal tension, which is proportional to cell stiffness (Young’s modulus) (90).

Like other adherent cells (17), the chondrocyte also responds to mechanical stimuli by remodeling its actin cytoskeleton. Specifically, dynamic compression causes the chondrocyte to reorganize its actin cytoskeleton in a Rho kinase-dependent manner (43). In addition, the viscoelastic properties and actin remodeling are affected not only by the magnitude, but also by the rate, of applied pressure (74); for high shear strain (~35%), the focal adhesions and actin cytoskeleton of the chondrocyte exhibit extensive rearrangements and are subsequently concentrated on the trailing edge of the cell (68).

Mechanical Properties of the PCM and Chondron

A chondrocyte and its surrounding thin layer of PCM constitute a chondron (71). As we noted above, PCM transfers forces between the chondrocyte cytoskeleton and the ECM. Thus the chondron should be regarded as a mechanical unit. However, difficulty in isolation of chondrons has limited the number of studies. An early isolation procedure that involved low-speed serial homogenization yielded only 1–2% chondron preparations (72). In 1997, Lee et al. (55) developed an enzymatic chondron isolation technique that yielded a much higher (~80%) chondron preparation. Subsequently, the mechanical properties of the chondron were investigated by micropipette aspiration (38) and AFM (64). It was found that the PCM and chondron are generally stiffer (up to 10 times) than the enclosed chondrocyte (2, 65, 66) and, more importantly, that the viscoelastic properties of the PCM and chondron are substantially influenced by OA.

Alexopoulos et al. (1) extracted chondrons from normal and osteoarthritic human cartilage and, using the micropipette aspiration technique coupled with an axisymmetric elastic-layered half-space model, measured the Young’s modulus of the PCM. They found similar Young’s modulus of the PCM from different layers of normal cartilage (66.5 ± 23.3 kPa) but much smaller Young’s modulus of the PCM from osteoarthritic cartilage (41.3 ± 21.1 kPa). Subsequently, they measured the Young’s modulus of PCM again by combining the micropipette aspiration technique with a linear biphasic finite-element model and showed that the Young’s modulus of normal and osteoarthritic human PCM was 38.7 ± 16.2 and 23.5 ± 12.9 kPa, respectively (3). Although the Young’s modulus values reported in these two studies are different because of different modeling methods, the stiffness ratio of osteoarthritic to normal PCM is consistent (~60%).

It is important to note that stiffness of the human PCM is reduced because of matrix loss in OA, but the changes in chondrocyte stiffness in OA are controversial (Table 1). Studies using micropipette aspiration showed an increase in human chondrocyte stiffness (86, 87), while studies using AFM and confined compression reported a decrease in human chondrocyte stiffness with OA progression (42, 45). There are three potential sources of these controversial findings: 1) the micropipette aspiration measurements used chondrocytes that were isolated from the ECM, whereas AFM or compression experiments used chondrocytes that were not isolated from the ECM; 2) each of these studies used human chondrocytes from donors that were not matched for age, sex, and body mass index; and 3) each study harvested cells from sites that were different and were affected by the degree of OA, which also was different. However, future investigation using an in vitro cellular model with a minimum of variable factors is needed to clarify this issue and to provide new insights into the etiology of OA.

Mechanical Properties of the Nucleus

Mechanical forces exerted on the chondrocytes are transmitted through the nucleus of the cell (13). This force transmission
Micropipette aspiration coupled with multiscale modeling (42) and atomic force microscope (45) result in a nearly one-to-one correlation between cellular and nuclear stiffness at 1.4 (equilibrium); 0.36 kPa (tangential) for normal and osteoarthritic chondrocytes. However, in situ studies indicate that the chondrocyte nucleus is less stiff: static compression of single chondrocytes showed a nearly one-to-one correlation between cellular and nuclear strains (56), and finite-element modeling of the chondrocyte estimated the ratio of cellular to nuclear stiffness at 1.4 (69). This discrepancy in nucleus stiffness suggests that the chondrocyte nucleus behaves differently, mechanically, in situ than when isolated.

In a variety of diseases such as muscular dystrophy, dilated cardiomyopathy, and cancer, mechanics of the cell nucleus were found to be systematically affected (93). In OA, by contrast, associated effects on mechanics of the chondrocyte nucleus remain unknown. Such studies would not only help us better understand the role of the nucleus in chondrocyte mechanotransduction but would also contribute to a holistic picture of OA pathogenesis.

Mechanobiology of the Chondrocyte

The articular chondrocyte lives in a dynamic mechanical environment that is a complex combination of compression, shear stress, hydrostatic pressure, osmotic stress, and tensile stretch. The compression results from direct contact between joint surfaces. The static compression applied to human knee joints during standing is 3–10 MPa (20). Osmotic stress is a sudden change in the solute concentration around the articular chondrocyte, and it also results from the influx and efflux of fluid within the cartilage matrix during joint loading (36). Tensile loading is not generally regarded as physiologically relevant for articular cartilage, but it occurs in some physical activities such as stretch gymnastics and yoga.

Numerous studies have been designed to understand the responses of the chondrocyte to well-defined mechanical forces, including compression (49), shear (91), hydrostatic pressure (60), and osmotic pressure (52). Specifically, dynamic compression upregulates gene expression of aggrecan and type II collagen, while static compression downregulates them (26, 79). More expression of proteoglycans and collagen in bovine articular chondrocytes results from shear stress than from compression (89), and hydrostatic pressure also increases the level of aggrecan and type II collagen (85). Osmotic stress, together with hydrostatic pressure, upregulates the gene expression of anabolic (aggrecan and type II collagen) and catabolic (matrix metalloproteinase-3 and -13) molecules. Release of hydrostatic pressure maintains the anabolic and reduces the catabolic mRNA. In this condition, however, retention of osmotic pressure (450 mosM) maintains upregulation of catabolic mRNA (60). In OA, mechanoresponses of the chondrocyte might change. Dynamic compression still improves biosynthesis of aggrecan and type II collagen (49); however, application of shear stress to osteoarthritic chondrocytes decreases mRNA expression of aggrecan and type II collagen (81), and application of low hydrostatic pressure (1–5 MPa) increases the amount of proteoglycans in osteoarthritic chondrocytes, while high hydrostatic pressure (24 MPa) reduces the amount of proteoglycans (24). Taken together, mechanical stimuli influence metabolism and gene expression patterns of normal and osteoarthritic chondrocytes. However, the precise nature of the chondrocyte response to different mechanical stimuli and the underlying mechanisms of different mechanoresponses between healthy and diseased chondrocytes remain unknown.

Molecular mechanisms considered for transduction of mechanical stimuli include integrin signaling, MAPK/ERK pathway, Ca^{2+} channel, and cell-released soluble mediators (cyto-
kines and growth factors). In chondrocytes, α3β1-integrin is responsible for cell attachment to fibronectin and plays a role in proliferation and adhesion (21). A series of studies (58, 59, 77) demonstrate the interaction between α3β1-integrin, stretch-activated ion channels, and interleukin-4 when chondrocytes were subjected to cyclic mechanical strain. In the presence of a fibronectin fragment, α3β1-integrin also stimulates the MAPK pathway (28), a central conduit to the transduction of shear and compressive forces (25). Another study showed that fluid flow activates the ERK pathway, which leads to reduction of aggrecan gene expression (46). A study using microarray analysis showed that hyperosmotic stress leads to regulation of a wide variety of genes, which involves transduction through p38 MAPK and ERK1/2 pathways (84). A recent study found that, in response to these mechanical stimuli, Ca2+ and integrin-mediated pathways converge on ERK-MAPK, where Ca2+ plays a major role by regulating the integrin-mediated signaling pathway through Src kinase (75).

For a very long time, research in articular chondrocytes has focused on mechanically induced Ca2+ signaling. The mechanosensitive Ca2+ channels in chondrocytes include the intracellular PLC-inositol 1,4,5-trisphosphate pathway (76, 92), stretch-activated ion channels, and the transient receptor potential vanilloid 4 (TRPV4) channel (Fig. 1). TRPV4 was first described as a channel activated by hypotonicity-induced cell swelling (67). In chondrocytes, TRPV4 not only regulates SOX9 expression (63), but it also mediates the response to osmotic stress (37). Block of TRPV4 in vitro makes chondrocytes less responsive to hyposmotic stress and, therefore, impairs the increase in intracellular Ca2+ levels (70). TRPV4 knockout mice also show a loss of the Ca2+ response to hyposmotic challenge (12). Recent improvement of experimental techniques has provided new insights into Ca2+ signaling of chondrocytes. Degala et al. (16) measured the flow-induced Ca2+ signaling response of chondrocytes in three dimensions and found that chondrocytes in three-dimensional alginate culture respond more slowly than chondrocytes in monolayer (2-dimensional) culture; moreover, Han et al. (41) developed a system to measure Ca2+ signaling of chondrocytes in situ and found that Ca2+ signaling occurs more quickly and with greater magnitude when temperature is increased. These studies suggest that Ca2+ signaling of chondrocytes is regulated not only by forces, but also by the cellular environment, such as ECM topography and temperature.

Summary

In conclusion, chondrocytes in articular cartilage are subjected to a host of mechanical stimuli. The mechanical signals are transmitted through the complex network of the ECM, PCM, cytoskeleton, and nucleus of chondrocytes. Chondrocytes respond to these mechanical stimuli by altering gene expression and metabolism. In addition, the chondrocyte also exhibits unique mechanical properties and responds differently.
under normal conditions vs. pathological conditions such as OA. Taken together, the biomechanics of the articular chondrocyte are the central link between the mechanical factors and biological properties of articular cartilage in health and disease. Hence, a better understanding of the mechanobiology of the chondrocyte may lead to effective treatments for OA and other cartilage-related diseases.

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