Biomechanical Characterization of Single Chondrocytes

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Abstract Normal cartilage functions to cushion and distribute loads throughout the joint. The tissue's constitutive cells, chondrocytes, experience a variety of stresses as a result of these functional aspects, but the effects of these stresses on the individual cells are largely unknown. To understand the mechanical integrity of chondrocytes and how these properties change in response to various stimuli, mechanical testing systems for single cells have been developed. These systems are able to apply a wide variety of load types to characterize cellular biomechanics, and must rely on complex mathematical models to calculate these properties. This chapter reviews the five major mechanical testing systems that are used to test single chondrocytes, their distinct advantages, and discusses the salient results they have produced relating to chondrocyte mechanics and mechanosensitivity. Using these testing systems, it is clear that mechanical signals play a major role in chondrocyte gene expression, and these changes are essential to understand when developing functional cartilage replacements.

1 Introduction

Beginning with Aristotle's book *On the Movement of Animals*, biomechanics has sought to explain the complex processes of locomotion. As our understanding of the inner workings of the human body increased, a subset of the field emerged to

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closely investigate the mechanical role of individual tissues and cells. By focusing in on smaller and smaller subcomponents, biomechanics is able to explain how mechanical perturbations affect the normal and diseased states of tissues and how these stimuli can be employed in tissue engineering strategies. Employing this micro-scale approach is especially useful in studying tissues such as articular cartilage, given its major mechanical role in the body, inability to self-repair following injury, and need for functional replacement therapies.

1.1 The Mechanical Role of Cartilage

Lining the ends of bones in articulating joints such as the knee, articular cartilage facilitates smooth joint movement as well as bearing and distributing mechanical loads. Within the knee joint, cartilage routinely experiences compressive loads of three times body weight depending on the joint flexion angle and activity. Shear forces in the knee are also significant, and can reach a third of body weight at a knee flexion angle of 40° [1]. These forces are further magnified during activities such as running or jumping. As the articular cartilage lining is only between 1 and 2.55 mm thick in the joints of the lower limb, the tissue must be highly specialized to withstand its mechanical environment [2].

To achieve mechanical integrity, articular cartilage relies on a network of collagen and proteoglycans produced by its constitutive cells, chondrocytes. The collagen present in the tissue is mainly type II and provides tensile strength, while negatively charged proteoglycans such as aggrecan attract water molecules and resist tissue compression. Containing mostly water and proteins, the tissue can be modeled as biphasic material [3–6]. Mechanical testing of the tissue reveals that the tensile and compressive moduli of articular cartilage vary with depth and joint type, with the aggregate compressive modulus ranging from 0.8 to 2 MPa, and tensile modulus between 5 and 25 MPa [7–13]. But as mechanically robust as cartilage is, injury and disease can compromise its integrity and, lacking vasculature, the tissue is unable to self-repair. In response to this problem, tissue engineering strategies and biomechanical characterization techniques have emerged to further understand the role of chondrocytes in cartilage and to apply this knowledge to cartilage replacement and repair strategies.

1.2 Functional Tissue Engineering

Due to the aforementioned forces it must bear, engineered cartilage must reflect the native tissue's functional characteristics, especially its compressive and tensile integrity. To this end, the field of functional tissue engineering has emerged and spurred the creation of mechanical stimulation bioreactors to produce mechanically robust engineered tissue, and enhance purely biochemical approaches to

tissue engineering cartilage. These bioreactors use hydrostatic pressure, direct compression, shear, and combinations thereof to recapitulate the native mechanical environment in vitro and cause engineered constructs to become more like native cartilage [14–18]. While these mechanical stimulation strategies have improved matrix deposition and mechanical strength, the exact mechanisms of their action are ill-understood and optimal parameters for stimulation have yet to be determined. It is clear, however, that construct changes in response to mechanical stimuli are caused by cells, the most basic functional unit of any engineered tissue. Therefore, by studying individual cells it is possible to tease out the microscopic phenomena that, in combination, give rise to macroscopic changes in engineered constructs.

Applying the functional unit approach to understand the effects of mechanical stimulation begins with mechanically characterizing single cells. By determining the mechanical properties of single chondrocytes, the material limitations of the cells can be used to define the upper and lower limits of stimulation. Using these limits, the effects of various mechanical perturbations on the gene expression of single cells can be studied. And finally, mechanical stimulation parameters resulting in ideal gene expression changes can be applied to more complex arrangements of cells in tissue engineered constructs. Thus, understanding the response of the single cell to various mechanical stimuli can provide useful information for developing tissue engineering strategies.

2 Mechanical Testing of Single Cells

A variety of techniques have been developed to study the unique mechanical characteristics of single chondrocytes. Because the chondrocyte's diameter is on the order of 10 μm, mechanical testing machines must be especially sensitive to small changes in force and displacement. For compressive and shear testing, this is often achieved by the use of a cantilever to probe the cell and some mechanism to detect the cantilever's position over time. To test the tensile properties of chondrocytes, micropipette aspiration is the most common method and relies on pressure differentials to deform the cell. The following sections will explore in more detail the most prominent techniques used to elucidate single chondrocyte compressive, shear, and tensile mechanics.

2.1 Compression

Physiologically, cartilage tissue undergoes compressive forces on a regular basis. According to its viscoelastic nature, compressive loads are initially borne by the fluid within the tissue, but over time this load transfers to the solid portion of the matrix as the fluid is forced out [3]. Trapped within their collagen and proteoglycan

matrix, chondrocytes also deform under the load. Three major tools have been used to test the compressive properties of single chondrocytes: the cytocompressor, cytoindenter, and the atomic force microscope. The basic principles of these three apparatuses will be detailed in this section.

2.1.1 Cytocompressor

The cytocompressor device is a tool to determine the compressive properties of single cells, and has been used extensively to characterize the bulk mechanical behavior of chondrocytes. In this setup, unconfined compression is applied to single cells seeded on a glass slide via a large, flat, nonporous probe of around 50 μm in diameter. This probe is attached to the end of a cantilever beam, which is controlled by a piezoelectric actuator (Fig. 1).

For each test, the probe is positioned directly over the cell which is determined by concurrent focusing of the cell and probe in the microscope. With the probe positioned over the cell, the piezoelectric actuator moves the cantilever a set distance toward the cell surface, causing the probe to compress it. This position is held until the cell reaches equilibrium. The cantilever is then retracted from the cell surface, and the volume recovery of the cell is observed. The entire compression event is recorded via a CCD camera. Video post-processing of each compression event allows for the measurement of key parameters, namely the initial, compressed, and recovery geometries of the cell at different time points, and the position of the probe. These measurements allow for the determination of the cell's compressive modulus, Poisson's ratio, and recovered volume fraction, among others.

To determine the compressive modulus of the cell, the relationship between stress and strain must be known. The following equations are used to determine the cell's stress (σ) from a cytocompression experiment:

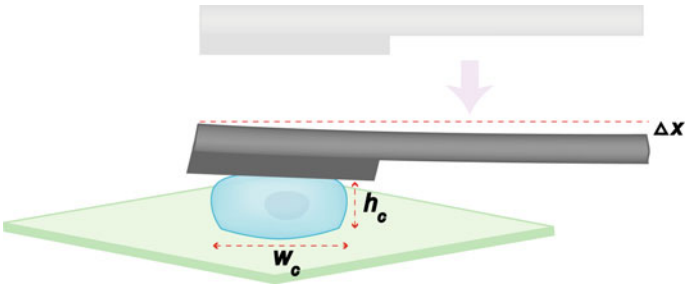

Fig. 1 *Schematic diagram of the cytocompressor.* To test single cells under unconfined compression, the cytocompressor uses a cantilever beam with a wide probe attached to its end. A piezoelectric actuator precisely moves the cantilever probe assembly toward the cell surface, and compresses the cell a set amount. Compression events are recorded via a CCD camera, and cell height (h_c) and width (w_c) are determined from extracted frames

$$\sigma = \frac{3EI(\Delta x)}{L^3 A}$$

where E and I are the Young's modulus and moment of inertia of the cantilever, L is the length of the cantilever, Δx is the difference between actual and prescribed translation of the cantilever, and A is the contact area of the cell and probe. The strain can be written as:

$$\varepsilon = \frac{h_i - h_f}{h_i}$$

where h_i is the initial height of the cell, and h_f is the height of the cell at maximum compression. Using the cytocompressor, a range of strains can be applied to single cells, and the resultant stresses can be calculated from the deformation of the cantilever beam and the contact area of the probe with the cell. These stresses and strains can then be plotted and fitted with a line, the slope of which gives the compressive modulus of the cell [19–22].

In addition to the modulus of the cell, the geometric data during the compression event allow for the determination of the cell's compressibility and recovery behavior over time. The apparent Poisson's ratio (v) for the cell can be calculated as follows [23]:

$$v = \frac{\frac{w_f}{w_i} - 1}{1 - \frac{h_f}{h_i}}$$

where w_i and h_i are the cell's initial width and height, and w_f and h_f are the cell's width and height at equilibrium compression. Recovery behavior can be determined by tracking the volumetric changes of the cell over time, and can indicate whether the cell was permanently changed as a result of the applied force. For chondrocytes, which remain mostly rounded after initial seeding, cell volume can be approximated as an ellipsoid with two identical axes. Approximating the cell's volume initially and after it has recovered from the compression, a measure of recovered volume fraction (V_r) can be determined as follows:

$$V_r = \frac{V_i - V_f}{V_i}$$

where V_i and V_f are the cell's initial and final volume, respectively.

By measuring the compressive stiffness, apparent Poisson's ratio, and recovered volume fraction, the cytocompressor is able to provide quantitative data to help understand not only the mechanical behavior of cells themselves, but also their ability to recover from mechanical stresses. It is a system that is capable of applying varying stresses to cells, at varying rates, and can even be programmed to apply dynamic strain. This system is also unique in that it performs unconfined compression on single cells. This test is particularly relevant to chondrocytes, as these cells live in a tissue that is regularly compressed.

It is important to note, however, that the cytocompressor has some limitations. Because its mechanism tests cells in a semi-rounded morphology, for cells that do not normally exist in this geometry the data may not be as relevant. Moreover, teasing out the mechanical characteristics of cells using this setup requires that some approximations in geometrical models be made. These approximations undoubtedly introduce error into the calculations, and careful measurements must be made in order to minimize this error.

2.1.2 Cytoindenter

Closely related to the cytocompressor is the cytoindenter (Fig. 2). This apparatus applies many of the same principles as the cytocompressor, but there are a few key differences. Like the cytocompressor, the cytoindenter uses a probe attached to a cantilever beam controlled by a piezoelectric actuator for load application, but here the probe is much smaller than the cell (approximately one quarter of its diameter). Unlike the cytocompressor, the cytoindenter does not rely on video capture to determine probe position and deflection. Originally these measurements were made using a dual photodiode detector, a technique that is used in the cytodetacher apparatus and will be discussed in more detail later [24, 25]. The current system, however, monitors the displacement of the cantilever via a laser reflected off the free end of the cantilever [26]. This information is transmitted to the control system and integrated with the displacement data of the

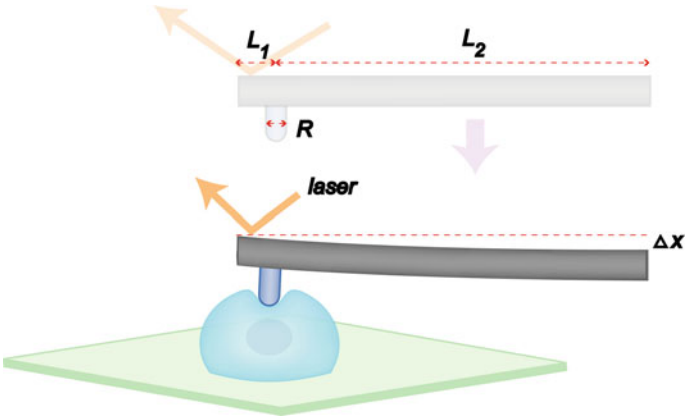

Fig. 2 *Schematic diagram of the cytoindenter.* The cytoindenter tests cells under creep indentation, using a thin probe of radius R attached to a cantilever beam. Like the cytocompressor, the cantilever-probe assembly is controlled by a piezoelectric actuator. The probe placement on the cantilever (described by lengths L_1 and L_2), material properties of the cantilever, and data from the laser micrometer allow for the application of constant force to the cell surface. Probe displacement data over time are recorded and used to extract viscoelastic material properties of the cell

piezoelectric actuator. Together, the laser displacement meter and piezoelectric actuator are able to keep constant the force applied to the cell, resulting in creep indentation testing.

As the system does not measure force outright, it must be calculated based on the measured cantilever displacement by the laser and the intrinsic geometry of the apparatus. This is achieved by combining laser displacement data with the force equation for a cantilever beam, as shown below [26]:

$$F = \frac{3EI\Delta x}{\left(L_1^3 + \frac{3}{2}L_1^2 L_2\right)}$$

where E is the Young's modulus of the cantilever, I is its moment of inertia, L_1 and L_2 add up to the length of the cantilever and are determined by the position of the probe, and Δx is the deflection of the cantilever beam, as measured by the laser micrometer. This equation is similar to that for pure end-loading of a cantilever, but is complicated by the fact that the force is applied a short distance from the end and the laser micrometer measures the displacement of the beam at its end. Nevertheless, using this equation the force can be monitored in real time and used to apply creep indentation to single chondrocytes.

Once a creep curve is produced, it must be analyzed using a mathematical model in order to obtain the material properties of the cell. To model cell indentation, the punch problem can be used in which the cell is assumed to be a linearly elastic, isotropic, and homogeneous half-space which is indented with a flat, rigid punch. The basic equations for this model have been adapted by Koay et al. [26] to account for viscoelasticity in the cell. The resultant equations from this analysis can define three material properties of the cell: the apparent viscosity (μ), instantaneous modulus (E_0), and relaxed modulus (E_∞). These properties are determined by fitting the following equation to the displacement versus time curve from each experiment:

$$\Delta x(t) = \frac{3F}{8RE_0}\left(-\frac{E_0}{E_x}e^{\frac{-E_x t}{3\mu}} + \frac{E_0}{E_x} + 1\right)$$

where R is the radius of the indenting probe and E_x is an elastic constant. The relaxed modulus can then be calculated from the following equation:

$$E_\infty = \frac{E_0 E_x}{E_0 + E_x}$$

where the variables are as mentioned previously.

The cytoindentation apparatus possesses several advantages that allow it to characterize the mechanical behavior of individual chondrocytes. Most importantly, it is capable of performing creep indentation on single cells, a test that is able to elucidate the viscoelastic properties of single cells. Due to the simplicity of

sample preparation in this setup, any type of anchorage dependent cell type may be tested. The system may also be adapted to use different shaped probes to apply different types of load to the cell. These characteristics all contribute to the system's versatility and applicability.

Along with its many advantages, there are some limitations that must be considered when using the cytoindenter. As with the cytocompressor, assumptions about the geometry and homogeneity of the material must be made in order to solve for material properties. In the case of indentation of single cells, the assumption of cell homogeneity may not be accurate as the mechanical properties of subcellular components can vary. Additionally, this system is unable to record recovery data for mechanical tests due to inherent noise in the system. Cell analysis using cytoindentation must be therefore be combined with recovery data from the cytocompressor.

2.1.3 Atomic Force Microscopy

Atomic force microscopy (AFM) has a wide variety of applications including scanning material surfaces, measuring intermolecular forces, and testing the mechanical properties of single cells. This technology relies on the use of a cantilever beam, similar to the cytocompressor and cytoindenter, but the tip of the cantilever on the AFM is much smaller (Fig. 3). For use in testing single chondrocytes, a 5 μm diameter spherical tip is attached to AFM cantilevers and used for indentation of the cell surface [27, 28]. The deflection of the cantilever as it

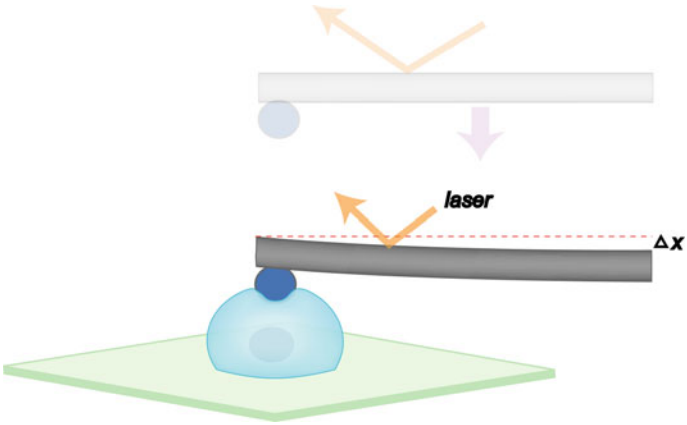

Fig. 3 *Schematic diagram of the atomic force microscope*. In this setup, stress relaxation experiments on single chondrocytes are performed by using a spherical probe attached to a cantilever beam. The probe displacement throughout the experiment is monitored by reflecting a laser off the cantilever and monitoring the angle of reflection over time. This information is then fed back into the actuator system that moves the cantilever to apply a constant strain on the cell. The resultant force versus time graph is then used to determine cellular mechanical properties

indents the cell is monitored via a laser reflecting off the cantilever into a photodiode detection system. Small changes in the position of the laser beam on the photodiodes indicate how far the cantilever is deflected, thereby allowing for the calculation of applied strain on the cell. For stress–relaxation testing using the AFM, a feedback loop is used to apply a set strain and measure the deflection of the beam over time. Using the appropriate model to fit the data, it is possible to gain both stress and strain data from these tests, and ascertain single cell mechanical properties.

Taking into account the shape and hardness of the indenting probe, and viscoelastic nature of cells, Darling et al. [27] developed a model to fit the data obtained from AFM stress–relaxation tests of single chondrocytes. Beginning with a modified Hertz equation for the force of a rigid sphere on a deformable substrate, the elastic and viscoelastic stress–strain relationships are derived assuming the cell surface is isotropic and incompressible. Combining the viscoelastic and elastic responses and specifying a step displacement for the stress–relaxation test, the following force equation can be obtained:

$$F(t) = \frac{4R^{1/2}\delta_0^{3/2}E_R}{3(1-\nu)}\left(1 + \frac{\tau_\sigma - \tau_\varepsilon}{\tau_\varepsilon}e^{-t/\tau_\varepsilon}\right)$$

where R is the relative radius of the probe tip and cell, E_R is the relaxed modulus of the cell, δ_0 is the prescribed step displacement, ν is the cell's Poisson's ratio, and τ_ε and τ_σ are relaxation time constants under constant deformation and load. This equation can then be fit to a force displacement curve to obtain viscoelastic properties such as the instantaneous and Young's moduli of the cell. The equations for these properties are as follows:

$$E_0 = E_R\left(1 + \frac{\tau_\sigma - \tau_\varepsilon}{\tau_\varepsilon}\right)$$

$$E_Y = \frac{3}{2}E_R$$

where E_0 is the instantaneous modulus, and E_Y is the Young's modulus of the cell.

AFM technology allows for very precise measurement of forces, and has been used to study many types of materials and surfaces [29, 30]. The system is capable of testing in a variety of modalities including scanning, tapping, and controlled displacement, and can accommodate many tip geometries including conical and spherical. The tips have even been functionalized to study interaction forces between molecules, demonstrating the AFM's ability to study nanoscale events on a cell's surface or between a cell and a substrate [31–33]. Indenting cells with the AFM can produce data that, when combined with an appropriate mathematical model, is a powerful characterization tool.

The development of a mathematical model to describe single cell testing with the AFM can be a challenging task. As all variables cannot be controlled, assumptions about the cell's geometry, homogeneity, and compressibility must be

made in order to solve the constitutive equations involved. Because the cell contains organelles and cytoskeleton, and may assume different shapes when attached to a surface, these assumptions may introduce error in calculating the mechanical properties. Careful consideration must be made, therefore, to ensure the applicability of various models to single cell AFM mechanics data.

2.2 Shear

In addition to compressive forces, chondrocytes also experience shear as loads are distributed within the joint space. Understanding the shear characteristics of single chondrocytes will allow for a better understanding of their contribution to the tissue as a whole. In this section, two related systems will be reviewed that are able to measure cell adhesion and the apparent shear modulus of single chondrocytes.

2.2.1 Cytodetacher and Cytoshear

The cytodetacher was first developed to measure adhesion forces of cells to various substrates, and has since broadened its applications to measure the bulk shear properties of chondrocytes and other cells [24]. The system consists of a 75 μm diameter horizontal cantilever probe attached at the top to a piezoelectric actuator setup (Fig. 4).

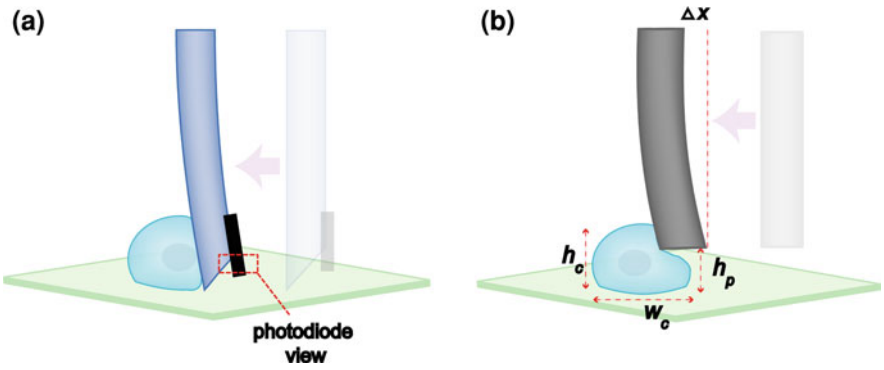

Fig. 4 *Schematic diagrams of the cytodetacher and cytoshear devices.* Both the cytodetacher (**a**) and cytoshear (**b**) systems rely on a piezoelectric actuator to move the probe toward the cell and cause deformation. The cytodetacher system uses a glass probe with an attached carbon filament positioned at the base of the cell, and monitors the carbon filament displacement via a dual photodiode. Attachment force is then calculated using cantilever beam theory. The cytoshear device records each event using a CCD camera, and positions its probe some distance above the base of the cell. Shear properties of the cell are then determined using the cell width (w_c) and height (h_c) over time combined with the knowledge of the probe height (h_p) and probe displacement (Δx)

Once the horizontal cantilever is positioned at the edge of a cell attached to a flat vertical substrate, the piezoelectric actuator moves a precise distance across the cell-seeded surface, detaching the cell from its substrate. The displacement of the probe is measured by a dual photodiode which detects small changes in the transmitted light in the microscope's view field resulting from the movement of a carbon filament attached to the side of the vertical probe. Using the displacement data from the photodiode, and the mechanical properties of the cantilever probe itself, it is possible to calculate the reaction force of the cell during detachment. From cantilever beam theory, this force can be written as:

$$F = \frac{3EI\Delta x}{L^3}$$

where E is the material stiffness of the horizontal probe, I is the probe's moment of inertia, Δx is the difference between the actual displacement of the probe and its prescribed displacement, and L is the length of the probe.

While the elements of data analysis remain the same, this system has been modified to allow for cells to be seeded on a horizontal surface [34]. This modification was achieved by rotating the probe 90° while maintaining the carbon filament horizontal to the cell seeded surface for photodiode detection. This provided a significant improvement in the system's ease of use, and initiated further modifications to enhance its ability to measure cell stresses and strains.

This system has most recently been modified to measure the shear properties of cells [35]. In this modification, the vertical probe is represented by a 50.8 μm diameter tungsten wire and displacement measurements are made by analyzing individual frames from video-captured shear events. As in the first iteration of this system, cantilever beam theory is used to calculate applied force from the apparent and prescribed displacements. In the case of cell shearing, however, the probe is placed a set distance from the substrate and translated resulting in shearing of the cell rather than simple detachment. The necessary data for the cellular deformation are also provided via analyzing frames extracted from video-captured events. Throughout the shearing event the cell's leading edge, trailing edge, and the probe are tracked providing data to calculate the cell-probe contact area (needed to calculate stress), and the cell's elongation. The contact area of the probe on the cell can be calculated by assuming the area is a half-ellipse:

$$A = \frac{1}{4}\pi(w_c)\left(h_c - h_p\right)$$

where w_c represents the width of the cell, and $h_c - h_p$ denotes the difference in height of the cell and probe from the surface, respectively. Using this contact area and the applied force from cantilever beam theory, a measure of the applied stress can be calculated using the relation:

$$\sigma = \frac{F}{A}$$

To calculate the shear strain (ε) experienced by the cell throughout the shearing event, the following relationship can be used:

$$\varepsilon = \frac{w_i}{w_c}$$

where w_i denotes the indentation depth of the probe into the cell, and w_c is the initial cell width as before. By plotting the stress versus strain curve and fitting a line to the data, it is possible to calculate the apparent shear modulus of the cell.

All of the modifications of the cytodetacher have provided some improvement in the ability to quantify cell adhesion forces and shear properties. As chondrocytes rapidly de-differentiate in monolayer, adhesiveness of these cells to various substrates can provide a quantitative measure of phenotypic changes over time. Adhesion forces of chondrocytes to various substrates is also an important measure of the cell's interaction with materials used in tissue engineering strategies. The modification of the cytodetacher for measurement of the apparent shear modulus of chondrocytes also provides a useful tool to measure the biomechanical properties of the cell itself. This method allows for measurement of the apparent shear modulus, which can be used to ascertain characteristics of the cell under a biomechanically relevant load.

The cytodetacher, while useful for studying anchorage-dependent cells, was not designed to study floating cells given that its setup necessitates cell adhesion to a substrate. Additionally, care must be taken to apply the correct geometrical model to each experiment as different cell types may appear more rounded than others when adhered to a surface.

2.3 Tension

Chondrocytes also experience tensile forces from matrix proteins around them pulling in the direction of local compressive or frictional loads. Tensile forces may also be generated in mechanical stimulation of tissue engineered cartilage constructs. In this section, the use of micropipette aspiration will be examined as it relates to chondrocyte biomechanics.

2.3.1 Micropipette Aspiration

Micropipette aspiration uses pressure differentials to calculate the force the cell experiences, and relates that to the observed strain, as seen in Fig. 5.

To perform this type of experiment, a cell is suspended in fluid of pressure p_1 and a micropipette is placed on the cell membrane. The pressure within the pipette is then reduced to p_0 and the cell membrane extends into the pipette at a distance l_p. Given the radius of the micropipette, r_p, the relationship between stiffness and pressure differential for an infinite homogeneous half-space aspirated into a pipette is:

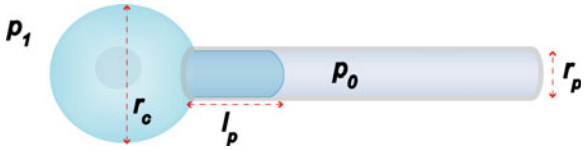

Fig. 5 *Schematic diagram of micropipette aspiration.* In this setup, the cell is suspended in fluid of pressure p_1 and a micropipette of radius r_p is placed on the cell membrane. The pressure inside of the pipette is then lowered to p_0 and the resulting deformation of the cell membrane, l_p, into the pipette is measured. This information, along with the radius of the cell outside of the pipette (r_c), can be used to determine the cell's tensile modulus and viscosity

$$\Delta P = \frac{2\pi}{3} E \frac{r_p}{l_p} \phi$$

where ΔP is the pressure differential between p_o and p_1, E is the Young's modulus of the cell, and φ is approximately 2.1 and depends on the geometric properties of the pipette itself [36]. Solving for the Young's modulus, and substituting for φ, this equation reduces to:

$$E = 0.22\left(\frac{r_p \Delta P}{l_p}\right).$$

Another useful parameter to gain from micropipette aspiration experiments is the viscosity of the cell. Given that cells are viscoelastic materials, cell viscosity can be a useful measure of its phenotype. Modeling the cell as a homogeneous, semi-infinite half-space, to calculate cell viscosity (μ) in this setup the following equation may be used [37]:

$$\mu = \frac{r_p \Delta P}{6\frac{dl_p}{dt}\left(1 - \frac{r_p}{r_c}\right)}$$

where ΔP, r_p, and l_p are the same as before, and r_c is the radius of the cell outside of the micropipette. In this equation, the rate of change of membrane extension into the pipette (dl_p/dt) must also be measured, and can be attained by varying the pressure within the micropipette and recording the resulting deformation with time. This method can also be used to obtain the instantaneous and relaxed modulus of the cell [27].

Micropipette aspiration can be used on both anchorage dependent and floating cells, making it a widely useful mechanical testing tool. It can produce forces between 10 pN and 10^4 nN, and can reach pressures as low as 0.1 pN/μm^2 [38]. This method can also determine whether a cell behaves as a liquid drop or a solid, which becomes useful when deciding on a model for further analysis of cell biomechanics. Mechanical parameters drawn from micropipette aspiration tests can be used to characterize cells and understand their mechanical role in the body.

Along with the many attributes to this method, there are some important considerations that must be made when analyzing its resulting data. Due to the nature of the experiment, measures of viscosity and stiffness are heavily influenced by the mechanical properties of the cell membrane, and thus may not reflect the bulk properties of the cell. Given the microenvironment of the chondrocyte, and that its deformation occurs mostly in compression, testing a portion of the cell in tension will provide some indication of cell properties, but those properties may not be as physiologically relevant.

3 Chondrocyte Biomechanics

All of the aforementioned single cell mechanical testing systems have been used to mechanically characterize chondrocytes, providing information on a wide variety of previously unknown cellular properties. Considering the mechanical properties of chondrocytes can help to understand the mechanical limitations of the cells within their native environment.

3.1 Mechanical Properties of Single Chondrocytes

Often, the same mechanical property can be obtained using a variety of different testing methods. The properties that do overlap, however, do not always match between systems. For example, the instantaneous modulus of chondrocytes using the AFM is around 0.29 kPa, while the same parameter measured using cytoindentation is around 8 kPa [26, 27]. Similar disparities are observed in relaxed modulus, viscosity, and equilibrium time constant measurements between systems. From Table 1, it is apparent that the AFM measures relatively low viscosity compared to measurements from micropipette aspiration, while the viscosity measured by cytoindentation falls between the two. Moreover, equilibrium time constants, when measured with micropipette aspiration can be more than an order of magnitude higher than values obtained from cytoindentation. This wide range could be a result of many different factors, including the constitutive model used, geometric assumptions, biological variability, the source of the tested chondrocytes, and of course the fact that the cell may or may not be anchored during testing.

Perhaps the biggest contributor to these apparent inconsistencies, however, is the type of test performed on the cell. Though literature for chondrocyte mechanics may report the same cellular properties, the particularities of each apparatus and the mathematical model used to calculate those values may only yield a certain aspect of those properties. For example, while the cell's instantaneous modulus can be obtained by AFM and cytoindentation, the different probe geometries and testing modalities may be testing different areas of the cell. More research must be done to better understand these differences, which will inevitably be helped by more rigorous mathematical models for the approximation of the cell.

Table 1 Reported mechanical properties of articular chondrocytes using various testing devices

Mechanical testing device	Modulus (kPa)[a]	Viscosity (μ, kPa·s)	Time constant (τ, s)	References
Cytocompressor	1.63 ± 0.31 (E)	–	1.6 ± 1.3 (recovery)	[20–22]
Cytoindenter	8.0 ± 4.41(E_0) 1.09 ± 0.54 (E_∞)	1.5 ± 0.92	1.32 ± 0.65	[26]
AFM	0.29 ± 0.14 (E_0) 0.17 ± 0.9 (E_∞)	0.61 ± 0.69	9 ± 6.2	[27]
Cytoshear	4.1 ± 1.3 (*low*) 2.6 ± 1.1 (*med*) 1.7 ± 0.8 (*high*)	–	–	[35]
Micropipette aspiration	0.41 ± 0.17 (E_0) 0.24 ± 0.11 (E_∞)	3 ± 0.18	33 ± 20	[44]
	0.45 ± 0.14 (E_0) 0.14 ± 0.05 (E_∞) 0.2 ± 0.07 (E_Y)	2.57 ± 1.83	37 ± 26	[27]

[a] Modulus abbreviations: instantaneous (E_0), relaxed (E_∞), compressive (E), Young's (E_Y), shear (*low, med, high* probe positions)

3.2 Contributors to Chondrocyte Mechanics

Though comparing properties from different mechanical testing modalities may not yield meaningful results, comparing cells tested in one modality is a powerful tool and has been used to understand the source of chondrocyte mechanical integrity. Using the cytocompressor, Ofek et al. [20–22] showed that knocking out different cytoskeletal components within the chondrocyte can vary the mechanical properties of the cell. Specifically, actin filaments emerged as the greatest contributor to compressive stiffness as actin disruption decreased the compressive modulus of the cell by nearly 40%. Additionally, absence of any of the cytoskeletal components doubled the residual strain of the cell following compression, indicating that the cell's cytoskeleton is important for recovery from mechanical perturbation. This study also showed that cytoskeletal components greatly influence the apparent Poisson's ratio. The results indicate that at a certain strain threshold, the microtubules that normally serve as rods holding the cell shape are broken down, reducing cell volume and Poisson's ratio. Termed the critical-strain threshold, this change in mechanical behavior of the cell with applied strain may indicate modifications in gene expression and matrix production in response to, or as a result of, cellular deformation.

Along with the cytoskeleton, the nucleus can contribute largely to the overall mechanical integrity of the cell. Chondrocytes, because they exist in a rounded morphology, also have rounded nuclei which respond to loading by changing volume and modifying gene expression [39]. Therefore, nuclear deformation may be necessary for the cell to respond to mechanical load, and the determination of its stiffness could play a major role in identifying effective cartilage stimulation regimens. Nuclear mechanical properties have been measured with a few different

methods. Using micropipette aspiration, the free-floating nucleus appears to be three to four times stiffer than the cell, while modeling techniques used to fit chondrocyte cytocompression data indicate that the nucleus may be only about 1.4 times stiffer than the rest of the cell [20–22, 40]. Despite the inconsistencies between micropipette aspiration data and theoretical modeling of nuclear stiffness, the methods do agree that the nucleus is somewhat stiffer than the rest of the cell, and therefore can affect its overall mechanics. With more investigation, nuclear mechanics data may prove to be a powerful tool in understanding the underlying mechanotransduction of stimulated chondrocytes.

3.3 Chondrocyte Mechanosensitivity

Chondrocytes are known to modify gene expression patterns in response to both biochemical and mechanical perturbations. While research in the area of chondrocyte mechanosensitivity is still in its early stages, the present data indicate that these cells are particularly sensitive to mechanical loading.

Both loading type and loading duration can cause shifts in gene expression of single chondrocytes. When subjected to varying forces applied in unconfined creep compression, chondrocytes display a dose-dependent decrease in collagen type II expression with increasing load, and aggrecan gene expression decreases sharply when force is increased from 25 to 50 nN of load. Accompanying the decrease in matrix protein expression with load, an increase in tissue inhibitor of metalloproteinase-1 (TIMP-1) gene expression levels is observed [39]. Together, these data indicate that when loaded in this manner, chondrocytes adapt their gene expression profiles from matrix production to matrix maintenance. Similar results are observed when chondrocytes are statically loaded with forces of 50 and 100 nN, but these forces applied dynamically result in matrix molecule gene expression recovered to control states [41]. These results suggest that the way a load is applied (static or dynamic) can profoundly affect the chondrocyte gene expression, and can provide valuable insight into useful modes of engineered cartilage stimulation.

Gene expression and mechanics of loaded chondrocytes are also affected by the biomolecules present. In the same creep compression experiment, Leipzig and Athanasiou [39] showed that the application of transforming growth factor beta-1 (TGF-β1) or insulin-like growth factor-I (IGF-I) throughout the experiment significantly decreased the strain experienced by the cell, and resulted in differing gene expression profiles than stimulated cells without growth factors. Applying TGF-β1 increased aggrecan gene expression over controls in most cases, while adding IGF-I kept TIMP-1 levels relatively constant for all loads. It has also been shown that these two biomolecules are able to stiffen cells significantly, resulting in altered deformation patterns under the same loading conditions [19, 42]. Biomolecules are therefore important regulators of chondrocyte mechanosensitivity. In fact, as chondrocytes in the presence of growth factors such as IGF-I are known to both stiffen the cells and increase gene expression for collagen type II and

aggrecan, they can provide a mechano-protective effect by inhibiting both deformation and gene expression changes caused by mechanical loading [19, 43]. More studies need to be performed to determine the precise ways biomolecules and mechanical stimulation can combine to affect chondrocyte gene expression and mechanics. This information will inevitably prove useful when designing cartilage engineering strategies such that they can harness the synthetic capabilities of chondrocytes.

4 Conclusions

The mechanical integrity of cartilage is extremely important to its normal function in the body. Due to its lack of reparative potential, damage to this tissue is usually permanent and can lead to further musculoskeletal complications. Therefore, a functional cartilage replacement is a valuable prospect, and tissue engineering continues to develop new and exciting answers to this problem. Achieving functionality in tissue engineered constructs, however, requires knowledge of the intrinsic biomechanics of cartilage and its cells. By first characterizing the biomechanics of individual chondrocytes, informed decisions can be made regarding the most effective biochemical and mechanical stimulation methods to use in more complex arrangements of these cells.

Healthy cartilage experiences many different types of loads on a daily basis, including compression, shear, and tension. How these forces are transmitted to individual chondrocytes and their effect on the cell's genotype, however, is not well understood. To study this, a number of mechanical testing and stimulation systems have been developed which are able to characterize not only cellular mechanics, but the effect mechanical loading has on gene expression. Additionally, these devices have been able to resolve the effects of growth factors on individual cell biomechanics, showing that chondrocytes become stiffer in the presence of certain biomolecules.

As chondrocyte mechanical properties obtained from different systems do not always agree with one another, it is important to understand each individual system and the type of test it performs. Small differences in load application and the type of model used to fit the data can magnify the discrepancies between mechanical testing systems. Bulk properties of individual chondrocytes are best tested using the cytocompressor and cytoshear devices because non-homogeneities are diminished by the probe being much larger than the cell. In devices where the probe is smaller than the cell, namely the cytoindenter, AFM, and micropipette aspiration systems, these non-homogeneities may be measured, and can provide information about cytoskeletal arrangement and local cell properties. With an understanding of these differences, the mechanical properties of single chondrocytes can be determined and this information can be used to inform tissue engineering strategies. As more information is available about cellular responses to mechanical and biochemical factors, more directed efforts can be made to combine cells with various stimuli to

create functional engineered cartilage. Cellular biomechanics testing and stimulation systems like the ones described here are beginning to make this possible.

5 Future Directions

With the recent advances in chondrocyte biomechanics testing and evaluation, some interesting questions have emerged which deserve further investigation. First, understanding the mechanosensitivity of subcellular components can elucidate the major players in the mechanosensitivity of the cell as a whole. As it is known that the actin cytoskeleton is an important component of cellular stiffness, it may be important in transducing mechanical signals from the cell membrane to the nucleus. Moreover, nuclear mechanical properties are known to differ from the rest of cell, but the importance of this difference in signal mechanotransduction is unknown. These same principles can be applied to any number of chondrocyte subcellular components, and may lead to a more complete understanding of the machinery of chondrocyte mechanotransduction. These types of experiments can be facilitated by the use of targeted fluorescent molecules to track subcellular components throughout a loading regimen, providing graphic evidence of their response to mechanical stresses.

Another area that should be expanded is the application of these cellular mechanical testing devices to other cell types. At present, aside from the AFM, many of these devices have only been used with a few cell types. Mechanical characterization of cells from other mechanically functional tissues such as tendon, ligament, bone, meniscus, and muscle can provide the same benefits as chondrocyte characterization. As most of these systems are highly adaptable, it would be a natural extension of the technology and would provide interesting comparative values for use in reconstructive therapies.

These future applications can add utility to cellular mechanical testing systems, and allow for a more complete understanding of mechanotransduction pathways of single chondrocytes.

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