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# MICROMECHANICAL PROPERTIES OF CHONDROCYTES AND CHONDRONS: RELEVANCE TO ARTICULAR CARTILAGE TISSUE ENGINEERING

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Articular cartilage is a highly mechanical tissue, performing multiple functions to ensure proper joint movement. Degradation of this tissue may be due to improper loading conditions that lead to a debilitating condition known as osteoarthritis. Furthermore, it is believed that mechanical signals transmitted from the tissue to cellular levels are necessary for the production of essential extracellular matrix components responsible for cartilage viability. Examinations of the tissue on its most rudimentary level elucidate mechanical regimens related to cartilage health and disease. A fundamental unit approach has been employed to study the biomechanical properties of single cells with discrete pericellular and extracellular matrix layers. This approach enables researchers to develop definitive relationships between mechanical stimulation and changes in gene expression corresponding to regenerative or catabolic processes. The knowledge gained from these studies sheds light on the etiology of osteoarthritis and elucidate the mechanical loading regimens useful for promoting articular cartilage health. This review article discusses the micromechanical environment of the cartilage cell, the chondrocyte, and the mechanical models and experimental techniques utilized to examine its physical characteristics. This information is then related to changes in cellular behavior and its potential toward tissue engineering of articular cartilage.

## 1. Introduction

Articular cartilage is the load-bearing material lining diarthrodial joints. It is a specialized type of hyaline cartilage and a highly versatile tissue, serving multiple functions to ensure proper joint movement. Together with the synovial fluid, it provides a lubricating and wear resistant surface, facilitating nearly frictionless motion about the articulating joints of our body. In addition, articular cartilage works to resist and distribute high compressive loads from one subchondral bone to another. Due to its biomechanical nature, articular cartilage health is largely determined by a variety of mechanical factors. It is well known that mechanical forces can elicit particular changes in the viability of articular cartilage, from the cellular to tissue levels [Freeman et al. 1994; Buschmann et al. 1995; Smith et al. 1995; Chen and Sah 1998; Knight et al. 1998; Lee et al. 1998; Carver and Heath 1999; Ragan et al. 1999; Smith et al. 2000b; Smith et al. 2000a; Roberts et al. 2001; Chowdhury et al. 2003]. Mechanical stimuli can induce modulations in cartilage tissue metabolism, in either a catabolic or anabolic manner. Therefore, the goal of much current research is to elucidate specific regimens of mechanical forces that will lead to the growth and strengthening of articular cartilage.

Under conditions of improper joint loading, the signaling pathways leading to normal gene regulation may not be stimulated correctly, and articular cartilage will degenerate. It has been shown that an

*Keywords:* articular cartilage, chondrocyte, chondron, tissue engineering, mechanical modeling, mechanotransduction, gene expression.

abnormal mechanical environment within the tissue, created in cases of high joint impact or due to an accumulation of small repeated loading events, significantly affects cellular behavior and may result in pathological extracellular matrix (ECM) synthesis and apoptosis [Dekel and Weissman 1978; Radin et al. 1984; Ehrlich et al. 1987; Radin et al. 1991; Jeffrey et al. 1995; Kurz et al. 2001; J. Borrelli et al. 2003]. These detrimental tissue changes lead to a condition known as osteoarthritis, which afflicts millions of Americans and significantly affects the United States economy [Arthritis Foundation 2006].

This review will begin by briefly discussing the physiological and structural properties of articular cartilage and the need to pursue tissue engineering techniques in order to treat patients with osteoarthritis. If the reader is interested, a more comprehensive discussion of articular cartilage physiology can be found in several excellent reviews [Buckwalter et al. 1991; Mow and Ratcliffe 1997; Hu and Athanasiou 2003] Due to the heterogeneous nature of the tissue, it is important to look individually at articular cartilage's most fundamental units in order to develop definitive relationships between applied stimuli and changes in cellular behavior. Therefore, to formulate effective treatment methodologies for osteoarthritis, it is critical to first study articular cartilage on its most basic level, the *chondrocyte* cell, so that regulatory mechanisms within cartilage can be directly correlated with mechanical factors. Researchers may then move upwards toward the tissue level, building upon single cell research by including discrete ECM regions, to elucidate on all fronts the role of mechanical stimulation in cartilage heath and disease. As such, this review will primarily focus on the mechanical environment necessary to bring about a favorable response from the cell and the *chondron*, which is the cell within its local microenvironment, and its tissue engineering implications.

The single cell, chondron, microexplant, and tissue construct are metabolic units of increasing complexity. This review utilizes the concept of a *fundamental unit approach* to demonstrate how research conducted at each of these phases may be interrelated. The fundamental unit approach is a modular template that facilitates research and understanding of these phases, and consists of modeling, experimental validation, and stimulating the unit of interest. Information gained from this research can be used in the formulation of necessary treatment methodologies, such as tissue replacement strategies, for patients suffering from osteoarthritis.

## 2. Articular cartilage structure and tissue engineering

**2.1.** ***Heterogeneous tissue properties.*** Articular cartilage tissue is nonuniform in its composition, varying in terms of matrix components, matrix organization, cellular phenotypes, and organization. It is largely composed of chondrocytes sparsely interdispersed within an intricate network of collagen fibrils, proteoglycans, lipids, and various ionic and nonionic solutes [Mow and Ratcliffe 1997]. Due to the inherent heterogeneity of articular cartilage, chondrocytes in one location may sense and respond to forces differently than cells in another location. Therefore observations of the tissue on the bulk level may not accurately reflect the intrinsic phenotypic and morphological differences existing within various subpopulations of chondrocytes in cartilage [Darling et al. 2004]. As will be further discussed in detail, chondrocytes perceive stimuli within their individual mechanical microenvironments. To begin with, an understanding of the structural properties of articular cartilage is necessary to appreciate the scope of the cellular microenvironment and grasp the heterogeneous nature of this tissue.

Articular cartilage is often considered to consist of both a solid and fluid phase. The interaction and arrangement of these various tissue components and phases provide articular cartilage with its unique functional capabilities. The primary component of articular cartilage is interstitial water that accounts for 60–85% of its wet weight. Water and various electrolytes in the tissue are denoted as the fluid phase. Collagens, particularly type II collagen, make up approximately 50%–75% of the solid phase and are responsible for the tensile characteristics of the tissue [Buckwalter et al. 1991]. Proteoglycans and other glycoproteins compose a majority of the remaining solid phase [Mow and Ratcliffe 1997], and contribute to the compressive and flow-dependent viscoelastic properties of articular cartilage [Mak 1986]. Proteoglycans are a highly specific type of glycoprotein, containing long, unbranched, negatively charged chains of glycosaminoglycans (GAGs) attached to a central link protein. Aggrecan is the most predominant proteoglycan in articular cartilage [Buckwalter et al. 1991; Mow and Ratcliffe 1997].

The spatial arrangement of collagens and proteoglycans has been shown to affect cellular metabolism [Muir 1983]. The thickness of the collagen fibers is determined by a fixed charge density created by the adjacent GAG chains [Katz et al. 1986]. The interaction of electrostatic charges from the GAGs and cations from the fluid phase contribute to the regulation of interstitial water content through a swelling pressure following the Donnan osmotic pressure law [Buschmann and Grodzinsky 1995]. The swelling generated by the resulting outward osmotic pressure is fettered by the cross-linked network of collagen and aggregan [Maroudas 1976]. When articular cartilage is compressed, fluid exits the tissue and experiences a drag via osmotic pressure. This drag enables the tissue to deform viscoelastically and act as a damper and distributor of applied forces.

Articular cartilage consists of four distinct zones: superficial, middle, deep, and calcified layers. Each layer is known to vary in matrix composition, and cellular, metabolic and mechanical properties [Aydelotte and Kuettner 1988; Aydelotte et al. 1988; Guilak et al. 1995; Wong et al. 1996; Freemont et al. 1997; Lee et al. 1998; Scott et al. 2005; Darling et al. 2006; Shieh and Athanasiou 2006; Youn et al. 2006]. Transitioning from the superficial layer to the deep layer, water content decreases and collagen fiber alignment changes from a tangential orientation to a radial direction along the tissue [Minns and Steven 1977; Buckwalter et al. 1991; Mow and Ratcliffe 1997]. The superficial layer is the outermost level of cartilage, encompassing the upper 10–20% of the tissue, and is characterized by a high tensile strength due to the tangential alignment of collagen fibrils [Verteramo and Seedhom 2004]. Chondrocytes in this region are stiffest and produce a specific superficial zone protein that aids in providing articular cartilage with its lubricating surface and prevents undesirable cell adhesion in this region [Flannery et al. 1999]. The middle layer contains more rounded chondrocytes and the greatest proteoglycan levels compared to the other layers. The deep layer is the thickest region of articular cartilage, although it contains the lowest amounts of interstitial fluid and collagen. Despite their low abundance in the deep layer, collagen fibers in this region are the greatest in diameter and function as a connection between articular cartilage and the underlying subchondral bone. The high compressive mechanical properties of this region can also be attributed to the radial orientation of these fibers [Mow and Ratcliffe 1997]. Lining the underside of the deep layer is a tidemark, conspicuously separating the region from the calcified layer below. In this final layer, chondrocytes are mostly inert and sheathed within a calcium fortified milieu [Marles et al. 1991].

With the cell as the center, distinct matrix divisions also exist concentrically within articular cartilage. Extending radially from the cell, pericellular, territorial, and interterritorial matrices each play a role in

chondrocyte maintenance and articular cartilage function. The ECM region consists of both the territorial and interterritorial matrices and is known to contain the vast majority of collagen and aggrecan proteins in cartilage tissue. The pericellular matrix (PCM) is most prominent in the middle and deep layers of the tissue and immediately surrounds the chondrocyte within a lacuna. The PCM, together with its enclosed cell, is defined as the *chondron* and is considered to be the smallest metabolic and functional unit of articular cartilage [Poole 1997]. Examinations of isolated chondrons have provided researchers with vast insight into the micromechanical environment of the chondrocyte in vivo. It has been found that the PCM is important in directly conveying biomechanical and biochemical stimuli on to the chondrocyte and will be discussed in greater detail later.

**2.2.** ***Tissue engineering potential for articular cartilage.*** Though primarily serving a mechanical function, articular cartilage lacks the vasculature and lymphatic system to repair itself under conditions of wear and tear or traumatic injury [Buckwalter et al. 1991; Mow and Ratcliffe 1997]. Since articular cartilage is unable to naturally restore its original structure and functionality after damage, ample research has focused toward understanding the etiology of osteoarthritis and to tissue engineer fully functional neotissue as a replacement for diseased cartilage. In cases of severe articular cartilage pathology, tissue replacement may become necessary. In the laboratory, it is now possible to engineer cartilage of clinically relevant dimensions and properties [Graff et al. 2003; Hu and Athanasiou 2006; Kang et al. 2006], and this may have profound implications for orthopedics in the near future.

Due to the inherent biomechanical nature and function of articular cartilage, the majority of tissue engineering techniques employ various mechanical stimuli as promoters of ECM synthesis and tissue growth. While external loading regimens are applied on the bulk tissue construct level, it is the chondrocyte itself which produces the proteins necessary for healthy and viable cartilage. Therefore, an understanding of chondrocyte mechanotransduction and its mechanical environment is paramount toward elucidating the ideal methodologies for stimulating cartilage tissue. As will be later discussed, forces and deformations applied onto the construct will transduce down through the chondrocyte microenvironment and can effect gene expression. Therefore, when considering a tissue engineering approach to articular cartilage, it is critical to understand how mechanical signals are interpreted by the individual cell.

While unified by a realm of exogenous stimuli, such as mechanical factors, which modulate cartilage development and growth, current tissue engineering techniques can themselves vary in their methods to coalesce isolated cells. Traditionally, tissue engineering techniques employ a degradable polymer scaffold upon which to seed a desired cell density. Since chondrocytes are already able to create cartilage ECM elements, such as the collagen and GAG types found in cartilage, they immediately come to mind as the appropriate cell type to be used in tissue engineering of articular cartilage. As the scaffold degrades, it is replaced by a natural matrix produced by the cells. Some common scaffold types include collagen [Wakitani et al. 1998], poly(DL-lactic acid) and poly(glycolic acid) [Sittinger et al. 1996], and more recently, poly(1,8-octanediol citrate) [Kang et al. 2006], with promising results.

Despite the vast potential of scaffold-based tissue engineering approaches, they do include several notable drawbacks. Whenever dealing with degradable polymers, researchers must consider issues of biodegradability, degradation products' toxicity, stress-shielding, and hindrance of cell-to-cell communication. To avoid such scaffold-related concerns, many scaffoldless approaches have been tested and utilized in the past few years. Autologous chondrocyte implantation is the most common technique of

this sort and the leading method to treat osteoarthritic patients under the age of 55. In this procedure, the defected cartilage region is cleared of debris and covered by a periosteum flap. The surgeon then injects a high density of the patient's own chondrocyte cells underneath the flap where they may develop into healthy cartilage tissue [Romeo et al. 2002; Micheli et al. 2006]. In contrast, scaffoldless procedures have also been employed to grow articular cartilage outside the body with the goal of then transplanting the neotissue into the defective region. Pellet culture [Graff et al. 2003] and aggregate culture [Horton et al. 1987; Furukawa et al. 2003] techniques have been implemented to grow articular cartilage constructs and tested under various mechanical conditions. Another scaffoldless approach, known as the self-assembling process, has recently been validated by Hu and Athanasiou [Hu and Athanasiou 2006] to produce cartilage tissue constructs with biomechanical and biochemical properties nearing those of native tissue. This novel approach, which involves seeding of primary chondrocytes at high-density over agarose wells, can be employed using various molds to create the desired shape for an articular cartilage implant.

**2.3.** ***Fundamental unit approach.*** Elucidating the mechanical properties of single cells and chondrons and of their behavior in response to mechanical stimuli will provide great insight into the most rudimentary level of articular cartilage. This is a necessary first step toward tissue engineering of articular cartilage and understanding the regimens of mechanical stimuli needed to elicit favorable gene responses, in terms of ECM production and cellular proliferation, from within a tissue construct. Information on how chondrocytes respond to various forces within their own microenvironment will greatly aid researchers toward developing a functional tissue replacement for deteriorated cartilage in patients suffering from osteoarthritis.

The majority of previous studies investigating chondrocyte mechanotransduction have looked at the mechanical response of an entire population of cells in their ECM. Though these experiments have been essential in furthering our knowledge of mechanotransduction and of the influence of mechanical stimuli on cellular behavior, they are subject to significant limitations. Most notably, testing cells in a bulk manner does not take into consideration variables such as individual cell shape, position, orientation, or local ECM characteristics. Cells within the same population may experience different stress-strain patterns under similar testing modalities. Therefore, the observed response may not accurately reflect a true response to the presumed applied force. Moreover, past research has strongly suggested that an individual chondrocyte responds to mechanical stimuli within its local environment, as opposed to the aggregate mechanical environment of the whole tissue [Mow et al. 1994; Guilak and Mow 2000].

To overcome such drawbacks, a fundamental unit approach can be utilized, wherein mechanical forces are applied to a single metabolic unit and the resulting changes in cellular behavior are recorded. By applying a well-defined force and recording the subsequent changes in the same unit, definitive relationships between mechanical stimuli and their intracellular effect can be developed. Therefore, the effects of the magnitude, duration, and frequency of a certain load regimen application can be examined unequivocally. This approach presents the additional advantage of uncovering the direct role of biomechanical forces as potential stimulators of tissue healing or degeneration via observation of particular changes in gene regulation related to regenerative, catabolic, or apoptotic cell behavior (Figure 1). Forces precipitating the onset of osteoarthritis may be observed and studied to further our knowledge of this degenerative

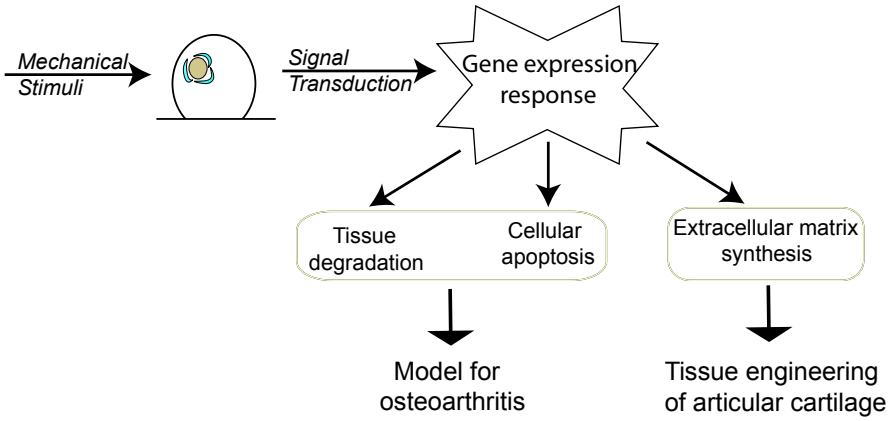


**Figure 1.** Response of a single cell to mechanical forces. Mechanical stimuli inducing tissue degradation and cellular apoptosis may be used at models for osteoarthritis. In contrast, stimuli leading to ECM synthesis should be included in any tissue engineering approach for articular cartilage.

disease. Similarly, loading regimens inducing favorable gene responses can be easily identified and utilized in further work to develop articular cartilage neotissue. Furthermore, mechanical stimuli may also be examined in conjunction with additional exogenous factors, such as growth factors or oxygen tension levels. Combinations of these factors have exhibited a synergistic effect on chondrocyte metabolism and may prove to be quite useful in tissue engineering articular cartilage [Gooch et al. 2001; Hansen et al. 2001; Jin et al. 2003; Mauck et al. 2003; Scherer et al. 2004], yet are beyond the scope of this review.

While examinations on a single cell level present several distinct advantages in tissue engineering, they are not without their own caveats. The response of individual cells seeded on a dish can potentially be quite different from that of cells interdispersed within an extracellular matrix or polymer scaffold. In these situations, which are more related to the chondrocyte's environment in vivo, mechanical stimuli are not directly applied onto the cell but rather are a result of a secondary response, such as gel compression or shear due to induced fluid flow. Therefore, the methods used to stimulate and mechanically test individual cells, which will be discussed in detail later, can never fully reproduce the true mechanical environment within articular cartilage. It is also known that cells within articular cartilage are in constant communication with each other and these molecular signals can also affect chondrocyte behavior and gene response [D'Andrea and Vittur 1996; D'Andrea et al. 1998; D'Andrea et al. 2000]. However, it should be noted that single cell research is simply the first step in a modular approach to tissue engineering of articular cartilage. The research performed with individual cells can provide great insight into the mechanical environment necessary to elicit favorable cellular responses, which can then be translated upwards toward the tissue level. Findings which elucidate loading parameters favorable to the individual cell can be utilized in subsequent phases, which will eventually involve cells embedded within a matrix. This knowledge may include the ideal type of loading regimen (hydrostatic pressure, direct compression, shear, and so on) and their corresponding force levels, applied to the tissue construct, which will bring about the production of essential matrix components from the cells.

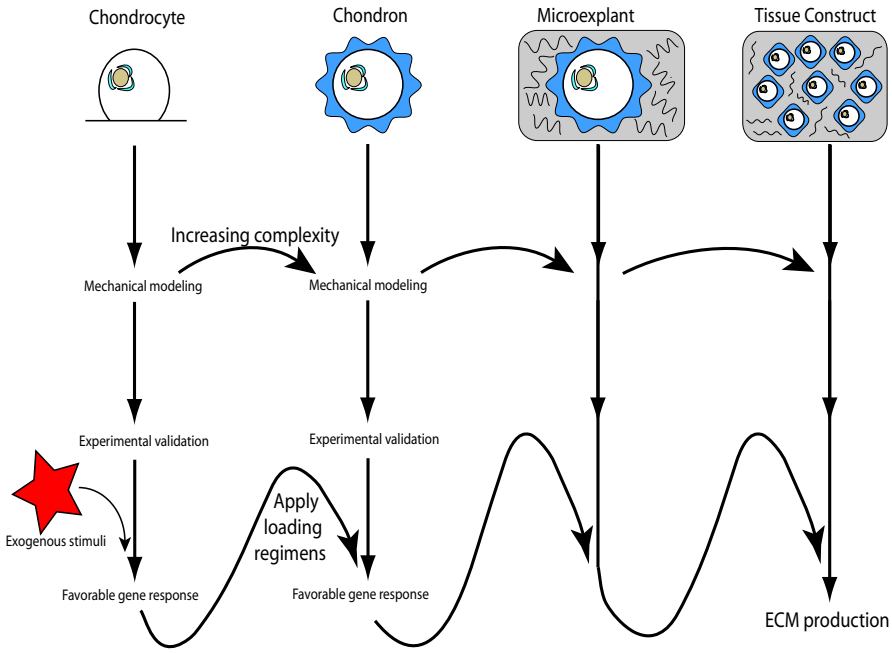


**Figure 2.** Functional unit approach. Research with a single cell will provide an excellent starting point for subsequent research of the chondron, microexplant, and cartilage tissue. Each phase consists of three steps which examine the biomechanical properties of individual units within articular cartilage. With each added level of complexity, researchers gain invaluable insight toward understanding the effects of mechanical forces on cellular behavior. This knowledge may be used to elucidate regimens of mechanical stimulation necessary toward tissue engineering articular cartilage.

The fundamental unit approach to tissue engineering of articular cartilage consists of four interrelated phases: single cells, single chondrons, microexplants, and tissue constructs, each of which thoroughly examines mechanical behavior through increasingly complex models and uses a series of steps that are translatable from one phase to the next. Each phase contains three main steps. Researchers first need to develop mathematical models for the properties and characteristics of each unit. These models then need to be validated through direct experimental techniques. Finally, various combinations of the previously described exogenous factors can be applied to the single unit in order to determine ideal stimulation levels to produce a well-developed neotissue (Figure 2). Moving stepwise through each phase, researchers could adapt this knowledge to account for the additional PCM and ECM regions to reach desired levels for gene expression. Advanced mechanical modeling will enable researchers to correlate the applied stresses or strains back down to the cellular level for each phase. Multiscale models have recently been developed to account for the intrinsic matrix variation and cellular spatial arrangement within articular cartilage. These models can be utilized to predict the local mechanical environment of chondrocytes under various loading conditions [Guilak and Mow 2000; Breuls et al. 2002; Wang et al. 2002].

Moreover, models used to examine the properties of individual cells can be translated to the tissue level by maintaining the same underlying physical principles and assumptions governing cellular properties.

With each added level of complexity, the mechanical model for chondrocytes will not change, enabling researchers to translate these properties to different spatial scales. Therefore, those mechanical loading regimens which elicit the upregulation of ECM genes can be applied and tailored to each subsequent phase in the approach, in order to work toward the greatest ECM production due to stimulation at the tissue level. The fundamental unit approach provides researchers with an indispensable tool for translating the effect of mechanical forces back down to the cellular level and for developing strong and healthy cartilage tissue in vitro.

## 3. Cellular microenvironment

To accurately model the mechanical characteristics of single cells and chondrons, an appreciation of the cellular microenvironment is necessary. This is an essential component of the first step of the fundamental unit approach and will enable researchers to develop appropriate mechanical models of increasing complexity describing each matrix layer surrounding the cell, as well as the cell itself. All components within the immediate surroundings of the chondrocyte can potentially act as force transducers onto the cell and affect cellular transcriptional changes. Mechanical forces applied to cartilage tissue are conveyed down to the subcellular levels via linkages between the ECM, PCM, cytoskeleton, and nuclear lamina. The cellular microenvironment consists of the latter three connections, which serve as the principal mechanical components of the chondron.

**3.1.** ***PCM composition and function.*** The chondron, that is, the chondrocyte together with its PCM, has been studied for years and much progress has been made to establish its structure. The PCM is known to contain collagen types II, VI, and IX [Poole et al. 1988a; Poole et al. 1988b], and significant concentrations of decorin [Poole et al. 1996], hyaluronan [Goldberg and Toole 1984], and sulphated glycosaminoglycans [Poole et al. 1990]. Of the aforementioned proteins, type VI collagen is considered a key molecular marker of chondron microanatomy [Poole et al. 1988a; Poole et al. 1992] and is essential in maintaining the microenvironment of the cell. The glycoprotein fibronectin is also localized within the pericellular region in adult articular cartilage [Poole et al. 1990] and has been shown to interact directly with type VI collagen to create a scaffold for the assembly of other essential PCM proteins [Chang et al. 1997].

The biochemical and biomechanical contributions of the PCM to the chondrocyte microenvironment have also been studied. In particular, it is known that the PCM surrounding the chondrocyte organizes and constructs collagen fibrils [Poole 1997] regulates cellular osmolarity [Hing et al. 2002] and modulates growth factor interactions with the enclosed cell [Ruoslahti and Yamaguchi 1991]. The mechanical properties of the PCM are known to differ from the larger territorial and interterritorial matrices inside the cartilage tissue and are approximately 10-fold greater in stiffness than the enclosed chondrocyte [Guilak et al. 1999; Guilak and Mow 2000; Alexopoulos et al. 2003; Alexopoulos et al. 2005b; Alexopoulos et al. 2005a]. In this manner, the PCM acts as a biomechanical buffer of stresses applied onto the chondrocyte.

Considering these biochemical and biomechanical functional roles, it is no surprise that the PCM has received special attention in tissue engineering of articular cartilage and research toward maintaining a healthy, well-developed ECM [Larson et al. 2002; Kelly et al. 2004; Graff et al. 2003; Fraser et al. 2006].

**3.2.** ***Cytoskeleton.*** The cytoskeleton provides a framework for cellular structure and plays a key role in the mechanical characteristics of the cell. It is primarily composed of three main structural proteins: microtubules, microfilaments, and intermediate filaments [Becker et al. 2003]. Understanding the properties and behavioral changes of cytoskeletal components is important for discerning the mechanobiology of individual chondrocytes.

**3.2.1.** *Cytoskeletal components.* Microtubules extend over the entire cytoplasm of the cell, forming a scaffold upon which other cytoskeletal elements can branch out [Langelier et al. 2000]. Due to their omnipresence, microtubules function to support cellular shape and the movement of organelles. They are also the largest fibers of the cytoskeleton and consist of hollow cylindrical tubes of the globular protein tubulin [Becker et al. 2003].

Intermediate filaments span the entirety of the cell and serve primarily to mechanically link the nuclear lamina with integrin receptors on the cell surface [Maniotis et al. 1997]. They are considered the most stable of cytoskeletal elements, comprised of protofilaments or fibrous proteins. Intermediate filaments are also involved in providing additional support in maintaining cell shape and structure [Becker et al. 2003].

Microfilaments, which are the smallest of cytoskeletal elements and are composed of two threaded polymer chains of $F$-actin, are involved primarily in cell motility [Becker et al. 2003]. These proteins are located cortically, with many focal adhesion points along the cell membrane. In addition, microfilaments are important in resisting cell deformation due to applied mechanical forces, particularly shear stresses [Janmey et al. 1991].

**3.2.2.** *Tensegrity model.* The organization of the cytoskeleton has been described using a tensegrity model [Wang et al. 1993; Ingber 1997; Chen and Ingber 1999; Volokh 2003]. This approach attempts to explain cellular structure and behavior by modeling the cytoskeleton as an interwoven mesh of discrete compressive and tensile elements in a three-dimensional configuration. Applying the tensegrity model to the cytoskeleton, the microtubules serve as struts which resist compression and which can reorient their position due to prestresses produced by the contractility of both microfilaments and intermediate filaments. The various mesh elements can be anchored to each other or the ECM. Applied stresses are distributed and transmitted through this lattice, depending on the preferred directionality of the mechanical coupling between elements [Chen and Ingber 1999].

**3.2.3.** *Response to mechanical forces.* It is believed that the cytoskeleton plays a direct role in mechanotransduction within the cell [Wang et al. 1993; Ingber 1997; Wang and Stamenovic 2000]. The structure and organization of cytoskeletal proteins has been shown to have a strong influence in force transmission onto the nucleus [Wang et al. 1993] and the mechanical properties of the chondrocyte as a whole [Trickey et al. 2004; Leipzig et al. 2006]. It has further been reported that the cytoskeleton remodels and thickens in response to applied mechanical loads [Durrant et al. 1999; Jortikka et al. 2000; Langelier et al. 2000], suggesting that these organizational or conformational changes may effect mechanical signal transduction within the cell, and thereby alter the regulation of essential ECM proteins [Jortikka et al. 2000].

**3.3.** ***Nucleus.*** The mechanical properties of the chondrocyte's nucleus are also of great interest. The chondrocyte nucleus has been described as a viscoelastic structure [Guilak et al. 2000], containing both solid and fluid elements. It is known to be considerably stiffer than the cell itself, potentially indicating a

unique nuclear function to mediate mechanical forces with the enclosed genomic DNA. Moreover, it has been shown that the nucleus will respond, in terms of its physical dimensions and volumetric properties, to an induced strain applied on the cartilage tissue level [Guilak 1995], and that these changes correlate with the synthesis of certain ECM proteins [Buschmann et al. 1996].

The nucleus is supported by a filamentous meshwork known as the nuclear lamina, positioned underneath the nuclear envelope. This lamina provides a framework for nuclear structure and serves as an intermediary between the cytoskeletal network and chromatin [Aebi et al. 1986]. Hence, deformation of the nucleus is often seen as the most direct transducer of cellular mechanotransduction. Alterations in the structural characteristics of the nucleus can cause changes in chromosome alignment via the lamina network, affecting the accessibility of genomic sequences to various transcriptional factors or other molecular signals.

## 4. Single cell approach

While studies of cartilage mechanobiology can quantify stress and strain at the bulk tissue level, few studies have attempted to quantify the local mechanical environment around the cell. Doing so would help to identify levels of mechanical stimuli at the cellular level relevant to cartilage health and disease [Stockwell 1987]. By applying different mechanical models and making the necessary geometric and physical assumptions, researchers can describe with growing accuracy the mechanical characteristics of single cells. This first phase of the fundamental unit approach consists of mathematical modeling, validating these models through direct experimentation, and determining appropriate mechanical loading regimens to elicit favorable gene responses on the single cell level.

**4.1.** ***Cellular mathematical modeling.*** Three models, of increasing complexity, have been used in recent literature to determine the mechanical properties of individual chondrocytes: 1) punch model, 2) viscoelastic model, and 3) linear biphasic model. While all of the models arrive at different constitutive relations between stress and strain within the continuum, all have been developed using basic physical principles and can accurately predict the equilibrium deformation of a cell due to an applied force. The various models serve as a foundation for later mathematical models describing more complex situations such as those with the chondron or tissue microexplants.

**4.1.1.** *Punch model.* The punch model is one of the simplest in single cell mechanics, treating the cell as a linearly elastic, homogeneous, isotropic, and incompressible half-space. Although the punch model does not account for strain- or time-dependent responses by the cell, it is useful in ascertaining the elastic modulus of stiffer, anchorage-dependent cells, namely chondrocytes. It has been applied in both the testing modalities of cell indentation and micropipette aspiration to determine the mechanical properties of single cells.

The solution to indentation experiments can be obtained by solving Cauchy's equations of motion under conditions of axial symmetry. Neglecting inertial forces, the equations governing stress distributions

reduce to

$$\frac{d\sigma_{rr}}{dr} + \frac{d\sigma_{rz}}{dr} + \frac{1}{r}(\sigma_{rr} - \sigma_{\theta\theta}) = 0, \tag{1}$$

$$\frac{d\sigma_{rz}}{dr} + \frac{d\sigma_{zz}}{dz} + \frac{\sigma_{rz}}{r} = 0. \tag{2}$$

Then by representing the stress components by a single function and applying the method of Hankel transforms, a relationship between applied force and Young's modulus can be derived for a given indenter displacement [Harding and N. 1944; Tran-Cong 1997]. There are three common indenter shapes that can be used to prod the cell: conical, spherical, and flat-ended cylindrical. For the rigid conical punch, the solution for Young's modulus is

$$E = \frac{2P(1-\upsilon^2)}{\pi\varepsilon a}, \tag{3}$$

where $P$ is the applied force, $\upsilon$ is the Poisson's ratio of the cell, $\varepsilon$ is the indentation depth, and $a$ is the indenter radius at the cell's surface [Harding and N. 1944]. For a rigid spherical indenter, the Young's modulus is shown to be

$$E = \frac{3P(1-\upsilon^2)}{4R^{1/2}\varepsilon^{3/2}}, \tag{4}$$

where $R$ is the radius of the indenting sphere [Harding and N. 1944]. The last case of a cylindrical end is the most common among indentation experiments. In this situation, the cell's Young's modulus is

$$E = \frac{P(1-\upsilon^2)}{2\varepsilon a}. \tag{5}$$

The punch model solution under the experimental modality of micropipette aspiration has been previously described by Theret et al. [1988], and later compared to more complex continuum mechanics models [Jones et al. 1999; Haider and Guilak 2002; Youn et al. 2006]. This model describes the Young's modulus of the cell as

$$E = \frac{3a\Delta P}{2\pi L}\phi_p(\eta), \tag{6}$$

where $\Delta P$ is the applied external suction pressure, $a$ is the inner radius of the micropipette, $L$ is the distance the cell is aspirated in the micropipette, and $\phi_p(\eta)$ is a function of the inner and outer radii of the micropipette.

**4.1.2.** *Viscoelastic model.* The viscoelastic model accurately depicts the behavioral response of chondrocyte cells to an applied stress or strain, by describing the cell as containing both fluid-like and solid-like structural elements. The most common viscoelastic model for the chondrocyte is the standard linear solid (SLS), where the cell is represented using a circuit analog of springs and dashpots. The springs are strain-dependent elements, whereas the dashpots are varying according to strain rate. In the SLS model, the spring is in series with an element consisting of a spring and dashpot in parallel. Using this one-dimensional analog, the constitutive equation describing the stress and stress relationship is written

$$(E_1 + E_2)\sigma + \eta\frac{d\sigma}{dt} = E_1E_2\varepsilon + E_1\eta\frac{d\varepsilon}{dt}, \tag{7}$$

where $E_1$ and $E_2$ are the elastic constants for the two springs, $\eta$ is the coefficient of viscosity for the fluid element, and $\sigma$ and $\varepsilon$ represent the stress and strain within the system, respectively [Ozkaya and Nordin c1999].

By including characteristics of both viscous fluids and elastic solids, this model can predict the cellular creep response to an applied constant stress and stress-relaxation response to a step strain. The SLS model has been used to describe chondrocyte behavior in many testing modalities, including cell indentation, unconfined compression, and micropipette aspiration. The indentation solution was first developed by Ting [1966] to describe the behavior of a viscoelastic half-space. A more recent creep indentation solution was developed by Cheng et al. [2000] and used by Koay et al. [2003] to test the material properties of single chondrocytes. The SLS model has also been applied to micropipette aspiration experiments using a solution originally developed by Sato et al. [1990] and to unconfined compression creep tests on single cells using a relationship described by Leipzig and Athanasiou [2005]. This model allows researchers to elucidate three mechanical parameters describing the individual chondrocyte: instantaneous modulus ($E_0$), relaxed modulus ($E_\infty$), and apparent viscosity ($\mu$) of the continuum [Koay et al. 2003].

**4.1.3.** *Linear biphasic model.* Developed by Mow et al. [1980], the biphasic model describes a material as having both a fluid and solid phase. Though initially difficult to conceive, this theoretical model treats each spatial point within the continuum as a mixture of both a liquid and a solid. To reduce the relations of conservation of mass and momentum describing this continuum to workable equations, several assumptions need to be made. The solid matrix is assumed to be linearly elastic, isotropic, nondissipative, and incompressible, and the fluid phase is inviscid and also incompressible. Additionally, the fluid is assumed to flow through the porous solid medium, creating a frictional drag force which accounts for the time-dependent behavior of the continuum. These assumptions enable the following constitutive relationships to be made for the solid matrix and fluid components:

$$T^s = -\phi^s pI + \lambda_s eI + 2\mu_s E,$$
$$T^f = -\phi^f pI,$$

where $T^s$ and $T^f$ are the stress tensors for the solid and fluid phases respectively, $\phi$ denotes the volume fraction for each phase, and $p$ is the hydrostatic pressure within the medium. Further denoted for the solid matrix, $E$ is the infinitesimal strain tensor and $e$ is its volumetric change, and $\lambda$ and $\mu$ are the Lamé constants [Mak et al. 1987].

Applied to single cells, this model correctly depicts the interaction of both fluid and solid behavioral components inside the confines of the cell membrane. Solid elements, such as cytoskeletal proteins, the nucleus, and organelles create a porous continuum for the free flowing cytoplasmic fluid. Through cell indentation [Shin and Athanasiou 1999], unconfined compression [Leipzig and Athanasiou 2005], and micropipette aspiration [Trickey et al. 2006] experimental techniques, the biphasic model has been used to extract the following three intrinsic mechanical properties of the material's solid phase: the aggregate modulus ($H_A$), permeability ($k$) and Poisson's ratio ($\upsilon_s$). In addition, more complex versions of the biphasic model have recently been developed to predict cellular behavior within articular cartilage tissue [Wu et al. 1999; Guilak and Mow 2000].

**4.2.** ***Experimental validation.*** Several experimental modalities have been used on single chondrocytes in recent years. Applying the three mechanical models described in the first step of the single cell

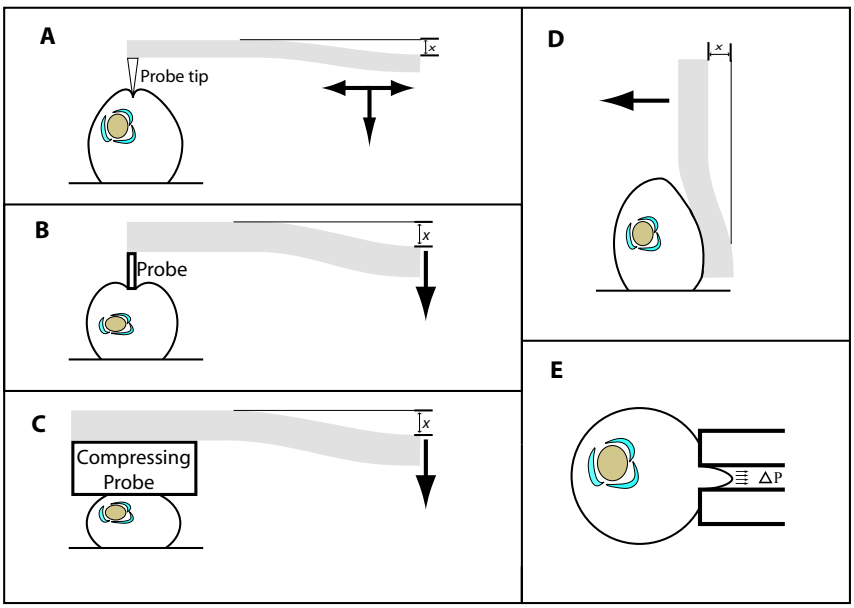


**Figure 3.** Experimental modalities for mechanically testing single chondrocytes. Techniques of (A) atomic force microscopy, (B) cytoindentation, (C) unconfined compression, and (D) cytodetachment utilize the anchorage-dependent characteristics of chondrocytes to obtain relevant mechanical properties. Each of these four techniques applies a controlled force or displacement to the cell through a probe attached to a cantilever. The probe deflection $x$ due to the cell's reactive force is as shown. In contrast, (E) micropipette aspiration employs a micropipette to apply a controlled suction pressure $\Delta P$ on the cell, and the resulting deformation is recorded.

approach, researchers have obtained cellular characteristics through either stress- or strain-controlled experiments. Major advancements in high precision systems have made it possible to apply forces or deformations on a scale relevant to that of a single cell. While not an all-inclusive list, several of the more prominent single cell experimental techniques include:

- atomic force microscopy
- cytoindentation
- cytodetachment
- unconfined compression
- micropipette aspiration (Figure 3).

The first four methods take advantage of the anchorage-dependent characteristics of chondrocytes to elucidate salient mechanical properties, while micropipette aspiration tests generally treat the cells as free-floating within a fluid medium. A summary of the salient mechanical properties obtained through the aforementioned experimental techniques is delineated in 1.

**4.2.1.** *Atomic force microscopy.* Atomic force microscopy (AFM) has been used to provide researchers with a high-resolution topographic description of variations along the cell surface. With accuracy on the order of picometers, AFM is a popular technique to obtain a detailed spatial map regarding local pressure points on the cell. As an example, Bader et al. [2002] used AFM to examine the nonlinear force–displacement response of single chondrocytes at various positions on the cell surface. Their results showed that the center of the cell was most resistant to an applied indentation. Therefore, AFM can be quite advantageous in studying heterogeneous materials, such as chondrocytes and their associated matrix, where highly localized differences exist.

Current AFM techniques generally consist of a pyramidal-shaped probe, with a diameter on the order of nanometers, attached to cantilever beam indenting the cell. The minute contact area of the probe and the cell provides AFM its high local precision. A piezoelectric motor displaces the probe toward the cell and force levels upon indentation are measured using Hooke's law, $F = kx$, based on the deflection, $x$, and stiffness, $k$, of the cantilever beam. Controlled feed back loops are used to maintain either desired constant force or displacement levels. Force and displacement data, over time, can then be fitted to elastic, viscoelastic, or biphasic models to acquire specific material properties.

A study by Darling et al. [2006] examined the viscoelastic nature of porcine articular chondrocytes from both superficial and middle/deep zones using AFM. The authors derived a viscoelastic solution for the punch problem with a spherical indenter (see Equation (4)). Applying a ramp velocity of 6.25 $\mu$m/s to reach a target force of 2.5 nN, held for 60 seconds at the center of the cell, the stress-relaxation experiments yielded relevant mechanical properties. It was found that superficial zone chondrocytes were stiffer than middle/deep cells in terms of instantaneous moduli (0.55 kPa versus 0.29 kPa) and relaxed moduli (0.31 kPa versus 0.09 kPa), and had a greater apparent viscosity (1.15 kPa-s versus 0.61 kPa-s).

Ng et al. [2007] recently utilized AFM to investigate chondrocyte viscoelastic properties with its developing PCM. Individual chondrocytes, with and without their associated matrix, were placed in custom-made pyramidal wells in preparation for testing. This provides the significant advantage of testing individual cells or chondrons without requiring them to be adherent on a substrate. Accurate spatial maps were obtained and finite element and Hertzian modeling was used to obtain biomechanical properties. In addition, a hysteresis upon unloading the probe was also observed, confirming previous findings regarding the time-dependent behavior of chondrocyte cells.

**4.2.2.** *Cytoindentation.* One increasingly popular technique to study the mechanical properties of single cells is cytoindentation. This recently developed procedure investigates a cell's ability to withstand controlled indentations by a flat-ended miniature borosilicate glass probe 5 $\mu$m in diameter, which is attached to a much larger cantilever beam. Cytoindentation utilizes similar cantilever principles as in AFM to determine the applied force based upon the deflection of the beam. A force transducing system is then employed to measure the cell's reactive force, on the order a single nanonewton, for a given displacement of the probe. The earliest model of the cytoindenter was developed by Shin and Athanasiou [1999] to study the biomechanical properties of individual MG63 osteosarcoma cells. In this version of the device, the deflection of the cantilever was measured by a dual photodiode system, enhanced by light microscopy. This system monitors the displacement of the end of the cantilever beam as a function of the voltage difference between the two photodiodes and is designated as the displacement on the cell. A piezoelectric motor applied a controlled ramp displacement with 0.275 $\mu$m increments to a 2.0 $\mu$m final

depth and a linear biphasic finite element model was used to determine cellular characteristics based upon recorded force measurements. The results showed that the cells had a permeability of $1.18 \times 10^{-10}$ m $^4N^{-1}s^{-1}$, which is strikingly greater than that of articular cartilage [Ateshian et al. 1997]. Additionally, the cells had an aggregate modulus of 2.05 kPa, shear modulus of 0.41 kPa, and Poisson's ratio of 0.37.

Recently, Koay et al. [2003] modified the cytoindenter setup to enable a laser micrometer to record the displacement at the end of the cantilever. Using a closed loop algorithm, the authors applied creep tests on single chondrocytes at a test load of 50 nN for either 15 or 20 seconds. Both punch and viscoelastic continuum models were used to determine the mechanical properties of the chondrocytes. The punch model (see Equation (5)) yielded an average Young's modulus of 1.10 kPa. The viscoelastic model resulted in an instantaneous modulus of 8 kPa, relaxed modulus of 1.01 kPa, and apparent viscosity of 1.5 kPa-s for the chondrocyte.

**4.2.3.** *Cytodetachment.* The cytodetacher measures the adhesive properties of cells attached to various substrata and also can be used to examine the response in cellular behavior to an applied shearing force [Athanasiou et al. 1999]. In this device, a piezoelectric translator drives a small diameter carbon filament, attached to a larger glass cantilever, parallel to the cell substratum. Similar to experimentation with the cytoindenter, cantilever beam theory is utilized to calculate the reactive forced based upon the deflection of the beam recorded by dual photodiodes. The first study employing the cytodetacher examined the force necessary to detach articular chondrocytes after seeding for 2 hours on untreated glass, bovine serum albumin-coated glass, and fibronectin coated glass. It was observed that the quantified adhesiveness of the cells on fibronectin (72.6 nN) were significantly greater than both the bovine serum albumin (20.3 nN) and untreated glass (38.9 nN) experimental groups [Athanasiou et al. 1999]. Follow-up studies further modified the cytodetacher and examined the temporal characteristics of chondrocyte adhesive properties. It was observed that cellular adhesion strength increased significantly with seeding time and could be attributed to a spreading of the actin cytoskeleton [Hoben et al. 2002; Huang et al. 2003]. Quantifying cellular adhesion strength will prove to be quite useful when examining cell-matrix interfaces in more complex situations in subsequent phases of the fundamental unit approach.

**4.2.4.** *Unconfined compression.* Additional simple modifications of the cytoindenter and cytodetacher have made it possible to examine the effects of unconfined compression on single chondrocytes. This testing modality has been extensively utilized to study both the material properties and mechanical behavior of chondrocytes [Leipzig and Athanasiou 2005; Leipzig et al. 2006; Shieh and Athanasiou 2006; Shieh et al. 2006]. In order to adapt the mechanical models to extract properties via this experimental technique, a suitable geometry for the chondrocyte was identified to accurately depict the anchorage-dependent properties and structure of the cell. Using vertical scanning interferometry, Scott et al. [2005] determined that a disc-shape would be an appropriate model for the cell, and Leipzig and Athanasiou [2005] used this shape to develop unconfined creep compression solutions for elastic, standard linear solid, and linear biphasic mechanical models. Using the cytoindenter device, the original 5 $\mu$m diameter indenter was replaced with a wider 50.8 $\mu$m diameter tungsten probe to apply constant loads of 75 nN for 45 seconds onto the chondrocyte. It was observed that viscoelastic model best fit the creep behavior of the individual cell, most notably in the early response phase to the applied force. Leipzig and Athanasiou [2005] also determined the elastic modulus for chondrocyte to be 2.55 kPa. Curvefitting the data to the viscoelastic model resulted in an instantaneous modulus of 2.47 kPa, relaxed modulus of 1.48 kPa, and

apparent viscosity of 1.92 kPa-s. Finally the biphasic fit yielded an aggregate modulus of 2.58 kPa, cell permeability of $2.57 \times 10^{-12}$ $m^4N^{-1}s^{-1}$ and Poisson's ratio of 0.069.

Shieh and Athanasiou [2006] expanded unconfined compression experiments to look at zonal variations and attachment times in chondrocyte viscoelastic mechanical properties. Similar to later findings by Darling et al. [2006], it was determined that superficial zone cells were significantly stiffer than those from the middle/deep region in terms of instantaneous moduli (1.59 kPa versus 0.69 kPa), relaxed moduli (1.20 kPa versus 0.49 kPa), and apparent viscosity (6.32 kPa-s versus 0.18 kPa-s) after 18 hours of seeding. Similar trends were also observed in the experimental group given three hours to attach.

Shieh et al. [2006] also modified the original cytodetacher setup to study the response of single chondrocytes to various levels of strain under unconfined compression. By placing seeded chondrocytes perpendicular to the advancing probe, the authors were able to apply specific deformations onto the individual cell for 30 seconds and to record the subsequent cellular recovery behavior through videocapture. Quite interestingly, the cells exhibited a change in their response around 25–30% strain levels. This critical point in chondrocyte behavior may have vast implications toward determining threshold levels for eliciting particular biochemical responses from the cell in terms of tissue engineering articular cartilage. Moreover, this yield strain may be analogous to an intrinsic threshold where bulk tissue is damaged and chondrocytes no longer experience their normal in vivo mechanical environment.

**4.2.5.** *Micropipette aspiration.* One of the earlier methods to measure the mechanical characteristics of individual cells is through micropipette aspiration. In this technique, a miniature pipette applies a negative suction pressure onto the surface of the cell, thereby deforming its membrane inward through the pipette. By recording this event, researchers are able to calculate salient mechanical properties of the cell based upon the amount of membrane deformation, the required suction pressure levels, and the geometric relations pertaining to the shape of the pipette. Similar to the cytodetacher, this technique can also be used to quantify the mechanical adhesiveness of a cell attached to a particular substratum.

Micropipette aspiration has frequently been used to determine the mechanical properties of chondrocytes harvested from both normal and osteoarthritic articular cartilage. Jones et al. [1999] first used this approach to compare cellular mechanical characteristics, such as Young's modulus and volumetric properties, from both healthy and diseased tissue. They applied maximum pressures between 0.05 and 1 kPa through a micropipette with an inner diameter of approximately 5 $\mu$m. By modeling the cell as a homogeneous elastic half-space (see Equation (6)), they determined that no differences existed between the Young's modulus of normal and osteoarthritic chondrocytes (0.65 kPa versus 0.67 kPa). However, significant differences were observed in cell volume changes immediately and 600 seconds after complete aspiration of the cells into the micropipette. Normal chondrocytes only exhibited an 11% volume change, while osteoarthritic cells lost 20% of their volume. A similar study by Trickey et al. [2000] applied the standard linear solid model to micropipette experiments on normal and osteoarthritic chondrocytes. Their results showed that cells from diseased cartilage tissue were stiffer and more viscous than healthy cells, with regards to instantaneous moduli (0.63 kPa versus 0.41 kPa), relaxed moduli (0.33 kPa versus 0.24 kPa), and apparent viscosity (5.8 kPa-s versus 3.0 kPa-s). Trickey et al. [2006] recently applied a biphasic model to compare the recovery behavior of chondrocytes from the two sources. While there were no significant differences between the Poisson's ratio of normal and osteoarthritic cells (0.38 versus

0.36), osteoarthritic chondrocytes did exhibit a greater characteristic recovery time upon release of the suction pressure.

**4.3.** ***Effects of mechanical stimuli on single cells.*** The last step in the single cell approach is to determine regimens of mechanical stimulation that elicit favorable gene responses. Using the knowledge attained through modeling and experimental validation, researchers can tailor the applied stimuli to the individual cell. Leipzig and Athanasiou [2006] and Shieh and Athanasiou [2007] have most recently used the technique of single cell reverse transcriptase polymerase chain reaction (scRT-PCR) to qualify gene expression changes due to direct mechanical stimulation. scRT-PCR has recently been validated as a highly sensitive and effective means to detect alterations in gene regulation within a single cell [Eleswarapu et al. 2007]. Briefly, their experimental set-up involved using the previously described unconfined compression device to apply particular loading regimens upon adherent chondrocytes. Then through a custom designed micropipette aspirator, the same cell was removed from its substrate and placed into a lysis buffer in order to disrupt the cell membrane. Following protocols essentially similar to that of traditional RT-PCR, the RNA for each individual cell was isolated and reverse transcribed into cDNA. Specific primers and probes for DNA sequences of interest were optimized in conjunction with real-time PCR to measure relative gene expression levels.

Shieh and Athanasiou [2007] examined the effects of static, intermittent, and dynamic compression cycles on the gene expression levels for type II collagen, aggrecan, tissue inhibitor of metalloproteinase-1 (TIMP-1), and matrix metalloproteinase-1 (MMP-1) on individual chondrocytes. Both type II collagen and aggrecan genes are considered related to regenerative pathways for articular cartilage. In contrast, TIMP-1 and MMP-1 are catabolic genes, known to be involved in matrix degeneration. A housekeeping gene of GAPDH was used to identify any variability between samples. It was found that static compression of 50 and 100 nN downregulated type II collagen and aggrecan, while dynamic loading at the same force levels significantly alleviated these negative effects. Leipzig and Athanasiou [2006] also looked at the effects of mechanical stimulation on chondrocyte gene expression. Adding an additional complication, the authors examined 25 nN, 50 nN, and 100 nN static loading in the presence of known growth factors, TGF-$\beta$1 and IGF-1. It was observed that increased static loading significantly decreased type II collagen and aggrecan levels, and increased TIMP-1 abundance. Furthermore, the addition of soluble growth factors provided a form of mechanoprotection against the detrimental effects of static loading. Taken together, these results clearly demonstrate that it is possible to measure gene expression on the single cell level, providing credence to future experiments on the chondron level. Moreover, these findings correlate very well with tissue engineering studies involving large number of chondrocytes in dynamically loaded matrices, with and without the addition of growth factors [Mauck et al. 2003; Kisiday et al. 2004; Ng et al. 2006]. Research on the single cell level may therefore prove to be useful toward identifying the ideal frequency of these dynamic loads applied to the tissue construct, and their associated magnitudes.

## 5. Single chondron approach

After studying the biomechanical properties and behavior of single cells, the next logical progression within the fundamental approach is to look at single chondrons. Examinations of the characteristics of the chondron include cell-matrix interactions and the three-dimensional configuration of the chondrocyte

embedded in articular cartilage tissue. The information gained from experiments with single chondrons sheds light on how the tissue functions and responds in its mechanical environment, and contributes to the overall goal of generating a tissue that more closely mimics the properties of native cartilage. By including the PCM in these experiments, researchers gain a physiologically relevant model of chondrocyte behavior and structure in vitro, while maintaining all the previously mentioned advantages as in the single cell approach. To date, little research has been completed to directly correlate the effect of mechanical stimuli and gene expression on single chondrons. However, the potential for this research is extensive, and significant strides have already been made in chondron mathematical modeling and experimental techniques.

**5.1.** ***Chondron mathematical modeling.*** To accurately depict the mechanical effect of the PCM on chondrocyte behavior, mathematical models for the chondron need to include a cell-matrix interface to describe the bilayered nature of this continuum. There are currently two main models used in the mechanical testing of single chondrons: 1) the layered half-space model, and 2) the multiscale biphasic model. Modeling the interface of the cell and its immediate surroundings presents several significant advantages in articular cartilage micromechanics. First, researchers can accurately portray the role of the PCM as an immediate transducer of mechanical forces onto the enclosed chondrocyte. Second, peak stress and flow conditions around the cell may be predicted. Third, the micromechanical environment of the cell can be directly linked to cellular metabolic and structural changes to further understand chondron mechanobiology.

**5.1.1.** *Layered half-space model.* The layered elastic half-space model is an extension of the previously described punch problem, including an elastic layer lining the elastic half space. The outer layer (corresponding to the PCM) is assumed to be infinite, isotropic, and homogeneous. Similarly, the enclosed region, corresponding to the chondrocyte, is treated as an isotropic, homogeneous, and semiinfinite half-space. Each layer is assumed to be a linearly elastic solid, with different Lamé elastic constants, and both layers are in perfect contact with each other [Dhaliwal 1970]. Solutions have been developed for the stress distribution within a bilayered material undergoing static deformation by a flat-ended rigid circular indenter [Dhaliwal 1970; Schwarzer 2000] or by a given force application [Horton et al. 1987]. These solutions can be potentially applied to the indentation problem of individual chondrons, although presenting practical experimental hurdles. Kumar and Hiremath [1982] have solved the punch problem for an annular shaped indenter and Alexopoulos et al. [2003] have extended this solution in order to determine the mechanical characteristics of single chondrons using micropipette aspiration.

**5.1.2.** *Multiscale biphasic model.* The multiscale biphasic model utilizes a finite element approach to elucidate the mechanical characteristics of the individual matrix layers extending radially outward from the chondrocyte. This approach considers cell-PCM and PCM-ECM interactions within articular cartilage and has been employed to determine the mechanical properties of the chondrocyte's PCM. Bachrach et al. [1995] originally described the cell-matrix interface within soft biological tissues under confined compression conditions. Later, Wu and Herzog [2000] developed a model to determine the effect of time and position under unconfined loading conditions in the local mechanical environment of the chondrocytes embedded within the articular cartilage matrix. A further advancement of this approach was the inclusion of distinct matrix layers surrounding the cell, particularly pertaining to the PCM and ECM, into a biphasic finite element model by Guilak and Mow [2000]. Each level, including the cell, is assumed

to be a biphasic material with continuous boundary conditions at each interface. Most recently, a radial biphasic model has been developed by Haider [2004] to describe the transmission of mechanical forces throughout the chondron.

**5.2.** ***Experimental validation.*** Due to practical limitations, the majority of single chondron mechanical experiments have utilized the technique of micropipette aspiration. This is in large part due to the non-adhesive properties of the PCM, thus making it difficult to apply a constant load without the chondron slipping away. Micropipette aspiration has been extensively used to elucidate the osteoarthritic and zonal differences in chondrocyte biomechanical characteristics and its associated PCM [Alexopoulos et al. 2003; Alexopoulos et al. 2005b; Guilak et al. 2005]. Alexopoulos et al. [2003] developed an analytical solution for the layered half-space model for chondron micropipette aspiration. This was the first reported study to directly measure PCM mechanical properties of single chondrons. The authors compared their newly developed layered half-space model to elastic half-space and shell models for the PCM and chondron, respectively. Single chondrons were isolated mechanically from the superficial and middle/deep zones of both healthy and osteoarthritic human articular cartilage tissue. The layered half-space model yielded Young's moduli of 68.9 kPa and 39.1 kPa for normal and osteoarthritic chondrons, respectively, from the superficial zone, and 62.0 kPa and 43.9 kPa, respectively, from the middle/deep zone. Alexopoulos et al. [2005b] later applied a biphasic model to similar single chondron micropipette aspiration experiments and found that osteoarthritic PCM was less stiff and more permeable than normal PCM. These results bolster the observation that PCM, in addition to ECM, degrades in terms of its mechanical integrity upon the onset of osteoarthritis. The layered half-space model was again used by Guilak et al. [2005] to study the zonal variations in chondron mechanical properties within canine articular cartilage. Confirming previous observations, there was little difference in the Young's modulus of the PCM between superficial and middle/deep zones (24.0 kPa versus 23.2 kPa). The aforementioned results for PCM mechanical properties are outlined in Table 1.

## 6. Conclusions

The fundamental unit approach provides several advantages toward elucidating and effecting tissue engineering of articular cartilage. The ability to develop definitive relationships between mechanical stimulation and cellular behavior will enable researchers to define appropriate loading regimens that are favorable to the cell as it develops within a tissue engineered construct. Conversely, an understanding of the mechanical forces pertaining to degenerative processes, that is, osteoarthritis, may allow researchers to devise intervention regimens and other possible treatment modalities. Advanced mechanical modeling enables researchers to correlate mechanical forces between layers and has progressed substantially in recent years. As previously described, the punch model for single cells can be related to the layered half-space model for chondrons and the biphasic model can be adapted to account for discrete matrix layers. Mechanical models on the cellular level can be scaled up to the tissue level by maintaining the same governing principles of chondrocyte behavior and physical properties. Loading regimens can then be translated between phases until one eventually determines an ideal regimen on the tissue construct level which will elicit the production of necessary ECM proteins.

Of further special interest is the possibility of using one of the previously described tissue engineering approaches, particularly on the scale of a single chondron, as a model for articular cartilage development.

| Tissue source | Experimental technique | Mechanical model | Material properties |
|---|---|---|---|
| Porcine femoral joints [Darling et al. 2006] | AFM | Viscoelastic | $E_o = 0.55$ kPa, $E_\infty = 0.31$ kPa, $\mu = 1.15$ kPa-s $^{++}$ |
| | | | $E_o = 0.29$ kPa, $E_\infty = 0.17$ kPa, $\mu = 0.41$ kPa-s |
| | Micropipette aspiration | Viscoelastic | $E_o = 0.45$ kPa, $E_\infty = 0.14$ kPa, $\mu = 2.570$ kPa-s |
| Bovine metatarsal joints | Cytoindentation [Koay et al. 2003] | Elastic | $E_Y = 1.10$ kPa |
| | | Viscoelastic | $E_o = 8.00$ kPa, $E_\infty = 1.09$ kPa, $\mu = 1.50$ kPa-s |
| Bovine metatarsal joints | Unconfined compression [Leipzig and Athanasiou 2005] | Elastic | $E_Y = 2.55$ kPa |
| | | Viscoelastic | $E_o = 2.47$ kPa, $E_\infty = 1.48$ kPa, $\mu = 1.92$ kPa-s |
| | | Biphasic | $H_A = 2.58$ kPa, $k = 2.57 \times 10^{-12}$ m$^4$N$^{-1}$s$^{-1}$, $\upsilon_s = 0.069$ |
| Bovine metatarsal joints | Unconfined compression [Shieh and Athanasiou 2006] | Viscoelastic | $E_o = 1.59$ kPa, $E_\infty = 1.20$ kPa, $\mu = 6.32$ kPa-s $^{++}$ |
| | | | $E_o = 0.69$ kPa, $E_\infty = 0.49$ kPa, $\mu = 3.18$ kPa-s |
| Canine femoral articular cartilage [Guilak et al. 2005] | Micropipette aspiration | Layered half-space | $E_Y = 24.0$ kPa $^{\#\#++}$ |
| | | | $E_Y = 23.2$ kPa $^{\#\#}$ |
| Healthy human articular cartilage | Micropipette aspiration | Punch [Jones et al. 1999] | $E_Y = 0.65$ kPa |
| | | Viscoelastic [Trickey et al. 2000] | $E_o = 0.41$ kPa, $E_\infty = 0.24$ kPa, $\mu = 3.0$ kPa-s |
| | | Biphasic [Trickey et al. 2006] | $\upsilon_s = 0.38$ |
| | | Layered half-space [Alexopoulos et al. 2003] | $E_Y = 68.9$ kPa $^{\#\#++}$ |
| | | | $E_Y = 62.0$ kPa $^{\#\#}$ |
| | | Multiscale biphasic [Alexopoulos et al. 2005b] | $E_Y = 39.7$ kPa, $k = 4.71 \times 10^{-17}$ m$^4$N$^{-1}$s$^{-1}$ $^{\#\#++}$ |
| | | | $E_Y = 36.8$ kPa, $k = 3.69 \times 10^{-17}$ m$^4$N$^{-1}$s$^{-1}$ $^{\#\#}$ |
| Osteoarthritic human articular cartilage | Micropipette aspiration | Punch [Jones et al. 1999] | $E_Y = 0.67$ kPa |
| | | Viscoelastic [Trickey et al. 2000] | $E_o = 0.63$ kPa, $E_\infty = 0.33$ kPa, $\mu = 5.80$ kPa-s |
| | | Biphasic [Trickey et al. 2006] | $\upsilon_s = 0.38$ |
| | | Layered half-space [Alexopoulos et al. 2003] | $E_Y = 39.1$ kPa $^{\#\#++}$ |
| | | | $E_Y = 43.9$ kPa $^{\#\#}$ |
| | | Multiscale biphasic [Alexopoulos et al. 2005b] | $E_Y = 20.8$ kPa, $k = 10.46 \times 10^{-17}$ m$^4$N$^{-1}$s$^{-1}$ $^{\#\#++}$ |
| | | | $E_Y = 24.4$ kPa, $k = 9.91 \times 10^{-17}$ m$^4$N$^{-1}$s$^{-1}$ $^{\#\#}$ |

**Table 1.** Overview of published mechanical properties for the individual chondrocyte and its associated PCM. All material properties are for chondrocytes isolated from the middle/deep region of articular cartilage unless otherwise denoted. Legend: ++ Superficial zone characteristics; ## PCM mechanical parameters.

On this level, researchers can elucidate the role of the pericellular and territorial matrices in the formation of strong and healthy cartilage tissue. Research in this field may enable investigators to answer several pertinent questions regarding articular cartilage development:

1) How does the expansion of the PCM affect the growth of other ECM regions?
2) Do the thresholds for ideal cartilage mechanical stimulation change as the PCM creates a more robust mechanical buffer around the chondrocyte?
3) How do these local matrix changes facilitate or modulate the passage of cytokines and growth factors among the cells in the construct?

While ample research has already been performed on single chondrocytes, there are several areas for future studies aside from the mechanical aspects described in this review. Additional combinations of various exogenous factors, including oxygen tension levels and growth factors, may be used in conjunction with mechanical stimulation to optimize the biochemical response of individual cells. It would also be of great interest to examine the temporal effects of gene expression after mechanical stimulation. This knowledge could potentially provide researchers with a working time frame for eliciting particular cellular responses. For instance, after a certain time point, changes in gene expression may return to baseline values or remain permanently changed. Lastly, deformations on the cellular and nuclear level can be directly linked to changes in gene expression to further our understanding of chondrocyte mechanobiology.

Mechanical testing of single chondrons can be seen as a logical next step after single cell experiments. Many single chondrocyte experiments require seeding the cells onto a substrate, which does not account for their three-dimensional configuration in vivo and may alter cytoskeletal structure and cellular organization. Studying single cells with their associated matrix allows researchers to observe changes in cellular behavior more indicative of an in vivo response. However, as yet, micropipette aspiration is the only technique used to directly measure the mechanical properties of single chondrons. Despite the ability of micropipette aspiration to quantify cellular and PCM properties, it cannot be used to apply compressive forces or controlled deformations on single chondrons. This can be seen as a significant drawback, as it prevents researchers from performing conventional stress relaxation experiments. Furthermore, the types of forces normally experienced by chondrocytes in vivo, namely compressive, shear, or hydrostatic forces, cannot be achieved using micropipette aspiration alone. This leaves the window wide open for new techniques to be developed, which may be used to directly stimulate individual chondrons and measure their gene expression response.

As previously described, Ng et al. [2007] recently fabricated microwells that are capable of holding chondrons in place during AFM experimentation, thereby providing a potentially significant experimental modality to be used on single chondrons. In addition, other techniques may employ a type of matrix glue to attach the chondrons onto a given substrate prior to mechanical testing. Coverslips coated with monoclonal antibodies specific to PCM proteins can be used to immobilize chondrons for direct compression experiments. Similarly, micropipette aspiration also may be employed to apply a small tare load to hold the chondrons in position while a probe compresses the sample on the other end. In short, the potentials for chondron mechanical testing and stimulation are vast, with profound implications in articular cartilage micromechanics. Future experiments will need to examine the possibilities of various new approaches and study the threshold levels necessary to elicit positive responses from single chondrons.

Then following the fundamental unit approach, researchers can work upwards toward the overarching goal of tissue engineering articular cartilage.

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