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ORIGINAL PAPER

Biomechanics of single chondrocytes under direct shear

Gidon Ofek · Enda P. Dowling · Robert M. Raphael · J. Patrick McGarry · Kyriacos A. Athanasiou

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Abstract Articular chondrocytes experience a variety of mechanical stimuli during daily activity. One such stimulus, direct shear, is known to affect chondrocyte homeostasis and induce catabolic or anabolic pathways. Understanding how single chondrocytes respond biomechanically and morphologically to various levels of applied shear is an important first step toward elucidating tissue level responses and disease etiology. To this end, a novel videocapture method was developed in this study to examine the effect of direct shear on single chondrocytes, applied via the controlled lateral displacement of a shearing probe. Through this approach, precise force and deformation measurements could be obtained during the shear event, as well as clear pictures of the initial cell-to-probe contact configuration. To further study the non-uniform shear characteristics of single chondrocytes, the probe was positioned in three different placement ranges along the cell height. It was observed that the apparent shear modulus of single chondrocytes decreased as the probe transitioned from being close to the cell base (4.1 ± 1.3 kPa), to the middle of the cell (2.6 ± 1.1 kPa), and then near its top (1.7 ± 0.8 kPa). In addition, cells experienced the greatest peak forward displacement ($\sim$30% of their initial diameter) when the probe was placed low, near the base. Forward cell movement during shear, regardless of its magnitude, continued until it reached a plateau at $\sim$35% shear strain for all probe positions, suggesting that focal adhesions become activated at this shear level to firmly adhere the cell to its substrate. Based on intracellular staining, the observed height-specific variation in cell shear stiffness and plateau in forward cell movement appeared to be due to a rearrangement of focal adhesions and actin at higher shear strains. Understanding the fundamental mechanisms at play during shear of single cells will help elucidate potential treatments for chondrocyte pathology and loading regimens related to cartilage health and disease.

Keywords Cell mechanics · Actin · Focal adhesions · Articular cartilage

G. Ofek · R. M. Raphael · K. A. Athanasiou (✉)
Department of Bioengineering, Rice University,
P.O. Box 1892, Houston, TX 77251-1892, USA
e-mail: athanasiou@rice.edu

E. P. Dowling · J. P. McGarry
Department of Mechanical and Biomedical Engineering,
National University of Ireland, Galway, Ireland

1 Introduction

Articular cartilage is a highly mechanical tissue that lines diarthrodial joints and functions to ensure proper joint movement. Due to its biomechanical nature, cartilage physiology is largely maintained by proper mechanical stimulation during normal activity. A variety of forces are present within cartilage and have been shown to affect tissue composition and integrity, including compression (Quinn et al. 1998; Kurz et al. 2001), tension (Maroudas 1976; Akagi et al. 2006), shear (Smith et al. 2000; Jin et al. 2001), and hydrostatic pressure (Hall et al. 1991; Elder and Athanasiou 2009). The particular stress and strain fields that the chondrocyte experiences within its microenvironment are influenced by its physical properties relative to its immediate tissue matrix surroundings (Guilak and Mow 2000). Cellular interpretations of these mechanical signals through mechanotransductive pathways may induce either catabolic or anabolic gene

expression changes, thereby altering the essential extracellular matrix synthesis for cartilage tissue (Ofek and Athanasiou 2007). In addition, chondrocyte mechanical characteristics will affect cellular deformations and the transmission of direct strain to the nucleus (Guilak 1995; Ofek et al. 2009), which also alters biosynthetic activity (Buschmann et al. 1996; Leipzig and Athanasiou 2008). Therefore, much cartilage research has specifically focused on studying the mechanical properties and behavior of individual chondrocytes toward understanding the role of mechanical forces in precipitating tissue regeneration or degeneration (Shieh et al. 2006; Shieh and Athanasiou 2007).

Various single cell testing modalities have been developed to simulate certain aspects of the chondrocyte biomechanical milieu. While the exact in vivo stress and strain environments of chondrocytes can never be fully replicated with any existing apparatus, these approaches present the distinct advantage of identifying the precise levels of force necessary to initiate changes in cellular activity or physical characteristics (Shieh and Athanasiou 2002). Moreover, the information obtained through these experiments may be coupled with multi-scale finite element models, which describe the local mechanical environment of chondrocytes, to gain insight to the response of cartilage to various loading conditions (Guilak and Mow 2000; Breuls et al. 2002; Wang et al. 2002). The majority of previous research efforts have utilized such experimental modalities as atomic force microscopy (Darling et al. 2006), unconfined compression (Leipzig and Athanasiou 2005; Shieh and Athanasiou 2006), or micropipette aspiration (Jones et al. 1999; Trickey et al. 2000) to yield indicators for single chondrocyte biomechanics under compression or tension. To also examine the adhesive strength of the connections between a cell and its substratum, recent efforts in our group have examined the maximum force necessary to detach a cell from various biomaterials (Athanasiou et al. 1999; Huang et al. 2003). However, to date, little work has been performed to singly investigate the mechanical characteristics of chondrocytes under direct shear.

While previous research has demonstrated that shear forces directly influence cellular homeostasis and matrix production (Smith et al. 1995; Frank et al. 2000; Jin et al. 2003; Raimondi et al. 2006), the fundamental mechanisms at play during these processes remain elusive. Using a single cell approach, a lateral mechanical force can be applied onto a single adherent cell to simulate shearing behavior. Unlike fluid flow-induced shear that populations of cells experience in a flow or perfusion chamber, direct shear allows the precise measurement of cell strain and shear forces, as well as the control of the contact region for the cell experiencing the shear force. A concrete understanding of chondrocyte behavior under shear can also aid computational models that are predictive of intracellular signaling and structural changes under mechanical perturbations (Deshpande et al. 2006). In this manner, a detailed insight into the cellular responses and mechanical characteristics during shear may shed light on loading regimens related to cartilage health and disease.

The overall objective of this study was to investigate the biomechanical properties and intracellular structural changes during direct shear of single chondrocytes. A novel video-capture method was developed to visualize the morphological alterations of the single cell under shear and to acquire precise measurements of force and strain. Individual chondrocytes were sheared using a probe placed at various positions along their height to further examine spatial variations in cellular shear properties. The intracellular organization of the actin cytoskeleton and focal adhesions under mechanical shear were additionally investigated toward understanding the response of single chondrocytes under shear. Based on prior observations demonstrating a correlation between cell stiffness and actin abundance (Leipzig et al. 2006), it was hypothesized that chondrocytes would exhibit non-uniformity in their mechanical properties related to changes in their actin network during shear. It was further hypothesized that focal adhesions and actin would reorganize to the trailing side of the cell, since this region is experiencing the highest levels of stress.

2 Materials and methods

2.1 Cell isolation and seeding

Articular cartilage was harvested from the fetlock joint of mature steers obtained from a local abattoir (Doreck and Sons Packing Company, Santa Fe, Texas). Single chondrocytes were isolated from the middle/deep region of the tissue through overnight digestion in 0.2% collagenase and 0.3% dispase, as previously described (Darling et al. 2004). After tissue digestion, chondrocytes were counted and resuspended in supplemented DMEM (0.1 mM NEAA, 100 U/ml penicillin/streptomycin, 0.25 μg/ml fungizone) at a concentration of 2.0×10^5 cells/ml. Cut pieces ($\sim 5 \times 20$ mm) of untreated Superfrost® Plus glass microscopy slides (VWR, West Chester, PA) were placed in a single well of a 6-well plate and covered with 3 ml of the cell suspension, yielding an areal cell seeding density of approximately 10^5 cells/cm^2. Untreated glass slides were chosen for this experiment based on prior results which demonstrated that chondrocytes adhered well to this substrate, with a minimal baseline expression of focal adhesions and the actin cytoskeleton (Athanasiou et al. 1999). The 6-well plates were incubated at 37°C and 10% CO_2 for 3 h. The 3 h seeding time was determined in prior studies to be sufficient for proper chondrocyte attachment, as well as closely maintaining the cell's in vivo morphology (Leipzig et al. 2006; Shieh and Athanasiou 2006).

2.2 Direct shear of single cells and videocapture

Direct shearing of single chondrocytes was performed using a modified cytodetachment apparatus (Athanasiou et al. 1999; Hoben et al. 2002). This device was adapted from its original setup to allow for the videocapture of cells experiencing mechanical shear. Essentially, the glass slides were transferred to a Petri dish which was placed on an IMT-2 inverted microscope (Olympus America, Melville, NY, USA), such that the cells could be viewed perpendicularly to the original seeding plane at 400x magnification. Supplemented DMEM with 30 mM HEPES, pre-heated to 37°C, was added to the dish throughout the duration of the experiment. The HEPES buffer was used to prevent pH changes in the ambient conditions. A 50.8 μm diameter tungsten probe (Advanced Probing Systems, Inc., Boulder, CO, USA) was then aligned to the side of an attached cell (Fig. 1). Three general ranges ($n = 8 - 10$ cells/group) were examined for the vertical distance of the probe from the glass: 20–35% of original cell height ("low"), 35–50% of original cell height ("medium"), and 50–65% of original cell height ("high"). Control over the distance between the bottom of the probe and the glass slides was achieved via fine linear positioners, as confirmed by visualization on the microscope. A piezoelectric motor then drove the probe a prescribed distance (chosen between 10–15 μm) laterally toward the cells at a rate of 4 μm/s. The entire shear event was recorded through an AVC-D7 CCD camera (Sony USA) connected to the microscope. Videos were saved as an AVI file at 640 × 480 resolution for subsequent analysis.

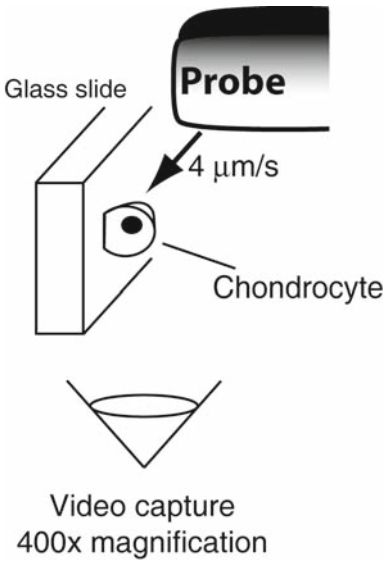

Fig. 1 Illustration of the experimental setup for studying single cells experiencing direct shear. A piezoelectric actuator drives a shearing probe laterally toward an articular chondrocyte seeded onto glass slides. Through visualization on an inverted microscope, the probe height can be controlled during the experiment. The entire mechanical event is further recorded through a CCD video camera connected to the microscope. The figure is not drawn to scale

2.3 Video analysis and biomechanical measurements

Individual frames from the videos were extracted using Videomach 4.0.2 software (Gromada.com). Images of the initial cell-probe configuration, initial cell-probe contact, and the shear event every 0.25 s thereafter were examined. The dimensions of the cell and probe positions in each frame were marked using Microsoft Paint 5.1 (Microsoft Corporation, Redmond, WA, USA). A pixel-to-micron ratio of 7.0 was employed in all image subsequent analysis and the accuracy of the measurements was deemed to be 2 pixels or 0.29 μm.

Immediately prior to initial cell-probe contact, the cell width ($W_C(0)$), cell height (H_C), and probe height from the base (H_P) were measured (Fig. 2). The relative positive of the probe onto the cell was calculated as:

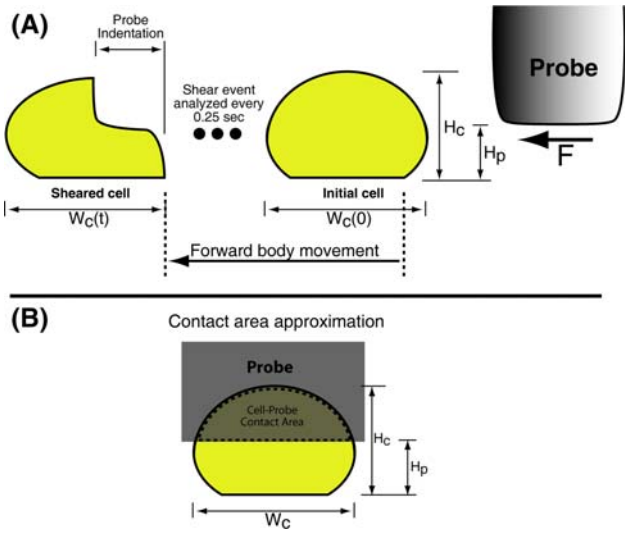

Fig. 2 Biomechanical analysis of single cells experiencing direct shear. Through an examination of the video for each mechanical event, precise measurements of cell dimensions and probe positions can be obtained as the cell experiences direct shear (**a**). In its initial configuration, the cell width ($W_c(0)$), cell height (H_c), and probe height (H_P) are recorded. Then, every 0.25 s after the probe contacts the cell, the cell's width ($W_c(t)$) is measured and subsequently used in the calculation for cell elongation. The cell's trailing edge is also tracked as an indicator for forward body movement. The probe indentation, defined as the forward-most position of the probe minus the trailing edge of the cell, is further measured over time and used in the determination of shear strain. In addition, the applied force at each time interval can be computed by comparing this forward-most position of the probe to the prescribed probe displacement. The force values are then normalized to an estimated contact area between the cell and the probe to yield an applied stress. Looking head-on (**b**), this contact area can be defined as the upper half of an ellipse, with half of the cell's initial width and the distance of the probe base to cell top as major and minor radii, respectively

$$\text{Probe position } (\%) = \frac{H_P}{H_C} \tag{1}$$

Upon shear application, the probe indentation onto the cell was defined as the forward movement of the probe less the movement of backmost part of the cell (Fig. 2a). The shear strain (ε) at each time interval was then calculated as probe indentation divided by $W_c(0)$. Cantilever beam theory was further employed to determine the reaction force of the cell at the various strain levels:

$$F = \frac{3EI}{L^3}\delta \tag{2}$$

where E (Young's modulus), I (moment of inertia), and L (cantilever beam length) are known parameters of the tungsten probe. The deflection of the cantilever (δ) was calculated by comparing the true displacement of the probe (via video analysis) with the prescribed piezoelectric displacement for each time step. The cell-probe contact area for this reaction force was estimated as the area of the upper half of an ellipse (Fig. 2b):

$$A = \frac{1}{2}\pi\left(\frac{W_C(0)}{2}\right)(H_C - H_P) \tag{3}$$

where $\frac{W_C(0)}{2}$ and $(H_C - H_P)$ represent major and minor radii of the ellipse, respectively. Then considering only the significant linear portion of the force versus shear strain plot, this slope was normalized to the cell-probe contact area to yield an apparent shear modulus.

The cell's width ($W_c(t)$) and position of the cell's trailing edge at each time interval were also recorded as indicators for physical changes in the cell's morphology and location during the shear event. In particular, the cell's elongation was determined as:

$$\text{Elongation} = \frac{W_C(0)}{W_C(t)} - 1 \tag{4}$$

In addition, the forward distance its trailing edge traveled during shear application, divided by the initial cell diameter, represented a normalized forward body movement (nFBM) for each cell.

2.4 Immunocytochemistry

Fluorescent staining was performed on four separate cells to observe alterations in focal adhesion organization and the actin cytoskeleton in response to direct shear, with consistent changes observed among the sheared cells. Chondrocytes were seeded using the protocol described above, except onto microscope slides that were etched with an indelible marker along their underside. Immediately prior to experimentation, a digital image was taken of the cell seeding pattern in one of the etched regions on the slide, in order to identify specific chondrocyte locations. After 3 h of culture, the slides were placed flat inside a Petri dish on the IMT-2 microscope such that the cells could be viewed bottom-up. The same region of the slide that was previously imaged digitally was located on the microscope and a single chondrocyte within this region was selected for shearing and fixation. A probe was placed immediately adjacent to this cell and translated laterally toward the cell, similar to the protocol described above. The cell was held at a high shear strain level for 10 min, at which point 4% paraformaldehyde was carefully added to the dish and fixation was allowed to occur for an additional 10 min.

After the cell was fixed, the microscope slide was removed from the Petri dish, washed with PBS, blocked with 10% FBS, and permeabilized with 0.1% Triton X-100 for cell staining. Slides were then incubated with AlexaFluor 647 Phalloidin (Invitrogen, Carlsbad, CA) for actin visualization, mouse anti-Vinculin primary antibody (Sigma-Aldrich, St. Louis, MO) followed by a goat anti-mouse secondary antibody (AlexaFluor 488; Invitrogen, Carlsbad, CA) for focal adhesion imaging, and Hoescht's dye (Invitrogen, Carlsbad, CA) for nuclei staining. The slide was viewed on a LSM 510 confocal microscope (Carl Zeiss, Oberkochen, Germany). Images of the sheared cell were acquired using a 63x objective with Z-stacks (at 0.2μm) through the cell. Unperturbed cells were similarly imaged on the same slide for use as controls.

2.5 Data analysis

All statistical analysis was performed using Matlab R2007b (Mathworks, Natick, MA, USA). A single factor ANOVA was used with a Fisher's Least Significant Difference post-hoc test (when warranted) to identify differences in mechanical characteristics obtained with the different probe heights. Significance was defined as $p < 0.05$ throughout the study.

3 Results

In this study, single chondrocytes were examined for their biomechanical characteristics and morphological changes under shear through video analysis of the mechanical event (Fig. 3). Substantially different trends in the forward movement of the cell's trailing edge and deflection of the probe (and thus, applied force) could be observed for probes placed in the low, medium, and high positions during the shear application.

3.1 Mechanical response during shear

Shearing cells at each probe position resulted in different trends in force accumulation (Fig. 4). Force values were generally negligible until around 15–20% applied strain for all groups, after which the force levels increased significantly.

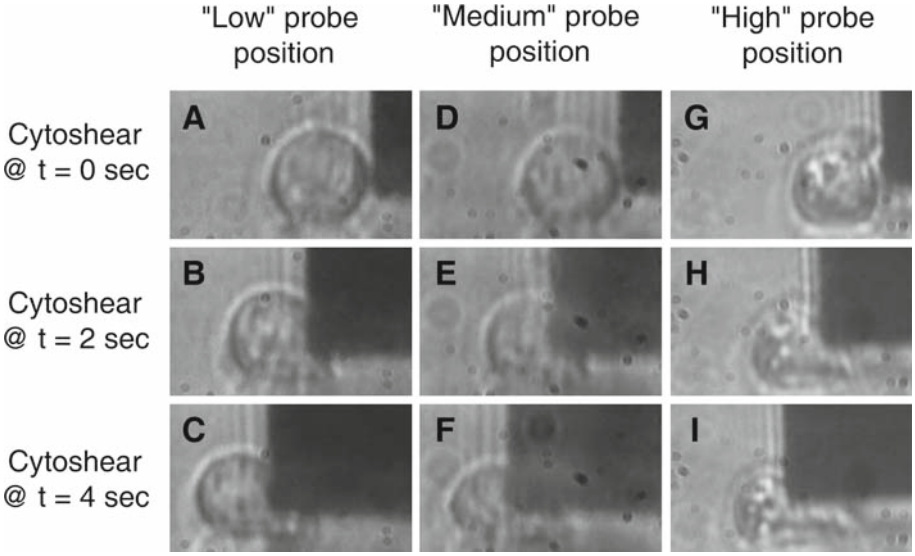

Fig. 3 Alterations in cellular morphology and position of chondrocytes experiencing direct shear. The entire mechanical event is video recorded and subsequently analyzed to yield mechanical properties and indicators for cell movement. Differences can be observed when the probe is placed in its low position (**a**–**c**), medium position (**d**–**f**), and high position (**g**–**i**) during the time course of shear application. Most notably, the cell's trailing edge experiences a substantial forward movement when the probe is in the low position compared to the high position. In addition, through this videocapture methodology, information on applied shear strain and the cell's reaction force is obtained by tracking the movement of the probe over time and measuring the initial dimensions of the cell

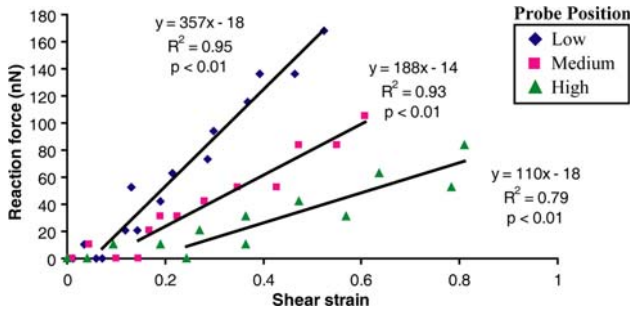

Fig. 4 Representative force plots of single chondrocytes experiencing shear from different probe heights. Force values are typically minimal until around 15–20% applied strain, after which a significant increase in force is observed. In addition, the slope of force versus shear strain is noticeably different among the three probe heights, with the low probe height generally inducing the greatest slope in force accumulation

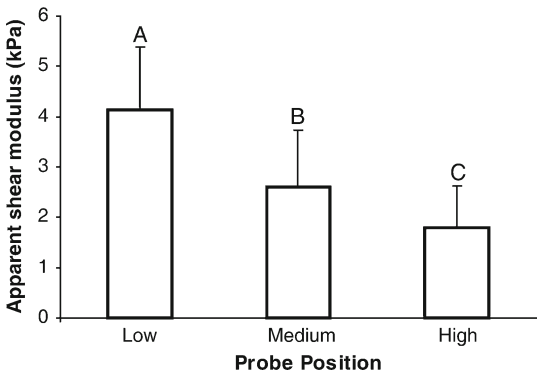

Fig. 5 Apparent shear moduli as a function of probe height. Single chondrocytes are perceived to be stiffer when the probe is placed lower to their base, with significant differences ($p < 0.05$) observed among all three groups. This suggests that the cell is non-uniform in its mechanical properties along its vertical axis. Data presented as mean ± standard deviations

However, the slope of force versus shear strain was substantially different among cells being sheared from the three probe heights. The low probe height typically induced the greatest slope in force accumulation, while the high probe height generally led to a lower slope. After normalizing this slope of the linear region in each force plot to the cell-probe contact area, variations in apparent shear moduli along the cell's height became evident (Fig. 5). The apparent shear moduli values were 4.1 ± 1.3, 2.6 ± 1.1, 1.7 ± 0.8 kPa for cells experiencing shear from probes in the low, medium, and high positions, respectively. Significant differences were observed between the moduli of all three groups ($p < 0.05$).

3.2 Physical cell alterations during shear

The average height and diameter of chondrocytes were found to be 10.9 ± 1.2 and 11.4 ± 1.0 μm, respectively. Moreover, the cells consistently exhibited an initial elliptical morphology during all trials, when viewed from their side using the

Table 1 Salient physical alterations of single chondrocytes experiencing direct shear

	"Low" probe height (20–35%)	"Medium" probe height (35–50%)	"High" probe height (50–65%)
Cell forward movement			
Peak normalized forward body movement (nFBM)	0.31 ± 0.14[a]	0.14 ± 0.10[a]	0.05 ± 0.04[a]
Time to plateau (s)	2.42 ± 0.48[a]	2.06 ± 0.72[a]	1.63 ± 0.26[a]
Shear strain for plateau (ε, %)	32.4 ± 5.6	34.0 ± 12.9	35.7 ± 7.6
Cell forward expansion			
Elongation ε = 25% (% increase)	7.0 ± 4.4	6.4 ± 6.2	7.9 ± 6.4
Elongation ε = 50% (% increase)	21.8 ± 5.8	24.3 ± 7.9	22.9 ± 10.4
Elongation ε = 75% (% increase)	38.4 ± 6.1	37.3 ± 17.9	34.4 ± 15.6

[a] Significant differences of $p < 0.05$

videocapture technique, as demonstrated by a bulging along the cell periphery.

Salient changes in the forward movement and elongation of the cell for the different probes heights are summarized in Table 1. Cells sheared from all three probe positions experienced forward movement of their trailing edge, as indicated by their nFBM. An accumulation of nFBM did not occur until soon after the probe crossed the original vertical plane of the cell's trailing edge, typically around 15–25% strain. The nFBM for each cell then increased until it reached a plateau at approximately 35% strain (Fig. 6). This plateau strain did not change between probe heights. However, the magnitudes of the peak nFBM did vary among the three probe heights, with a significant difference observed between the low and medium probe positions. In addition, the time for the cell to reach this plateau changed as a function of initial probe height, with significant differences observed between the low and high probe positions. Moreover, the elongation of the cell significantly increased at greater applied strain levels and was unaffected by the initial probe position.

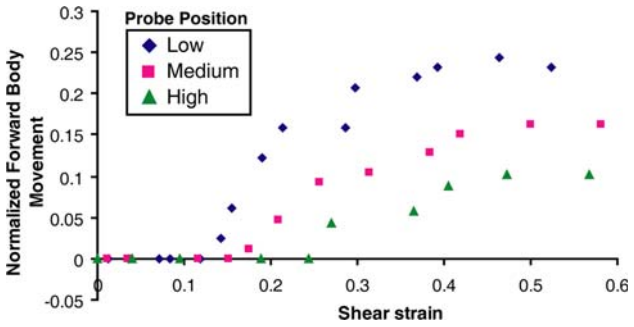

Fig. 6 Representative normalized forward body movement (nFBM) plots of single chondrocytes experiencing shear from different probe heights. The nFBM of each cell indicates the forward distance its trailing edge travels during the shear event, divided by the initial cell diameter. These values are typically minimal until around 15–25% applied strain, since the probe has not yet crossed the vertical plane of the cell's trailing edge. After this point, a substantial increase in nFBM can be observed until it plateaus at approximately 35% strain. However, the magnitudes of this movement are noticeably different among the three probe heights, with the low probe height generally inducing the greatest forward displacement during shear

3.3 Intracellular changes during shear

Cells, either sheared or control, were subsequently imaged using immunocytochemistry to identify alterations in their intracellular structure, with consistent changes observed among the sheared cells. Distinct modifications in the actin network and focal adhesion organization occurred as chondrocytes individually experienced direct shear (Fig. 7). Control cells typically displayed rounded morphologies at all focal planes through the cell's height, when viewed top-down on the glass slides through the confocal microscope. In addition, actin filaments and focal adhesions were equally distributed throughout the control cells' periphery. In contrast, the sheared cells generally sloped downward and became narrower along their trailing side. The actin network in the sheared cells was predominately identified behind the nucleus on the trailing side of the cell and near the base of the cells. Lastly, focal adhesions were consistently concentrated along the trailing edge of the cells.

4 Discussion

The study was designed to examine the effect of direct shear, which is prevalent within cartilage, on chondrocyte biomechanical characteristics. Confirming our hypotheses, this study presents several notable findings relating to chondrocyte biomechanics and demonstrates a clear connection between physical cellular properties and intracellular organization. First, we identify that the shear stiffness of individual chondrocytes, as measured by an apparent shear modulus, is highly non-homogeneous, increasing toward the cell's base. Second, cell forward movement during shear continues until a critical strain is reached, consistent among all

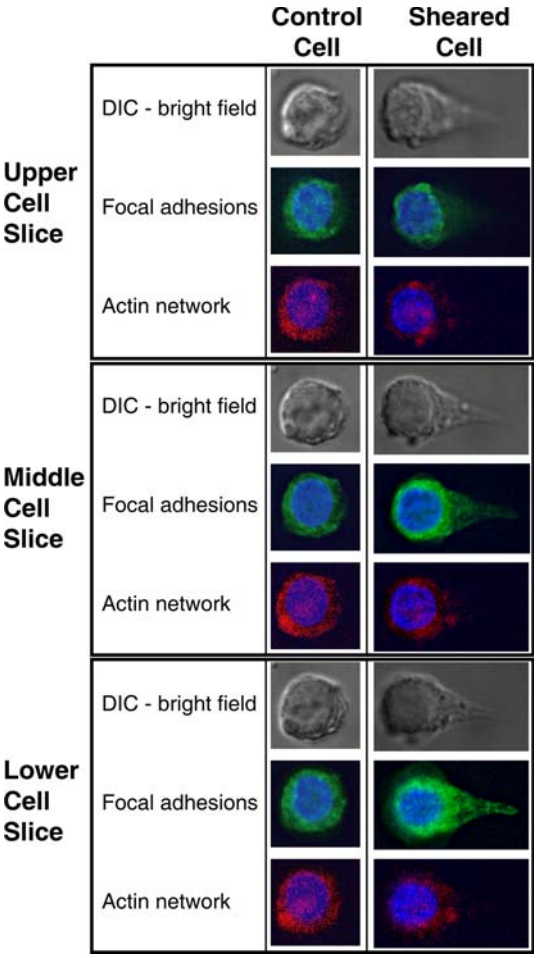

Fig. 7 Alterations in cell structure in response to direct shear. The bright field image, nuclei (displayed in *blue*), actin network (displayed in *red*), and focal adhesions (displayed in *green*) are shown for a representative control and sheared cell at different focal planes in the cell's height. A clear change in cellular morphology and intracellular organization of actin and focal adhesions can be observed for the cell experiencing the shear force. The control cell exhibits a rounded morphology, when viewed top-down on the glass slides, with actin and focal adhesions equally distributed along its periphery at all focal planes. In contrast, the sheared cell undertakes a 'sloped' morphology along its trailing side, which converges akin to a 'comet's tail'. The actin network in the sheared cell can be identified concentrated close to the cell's base, on the trailing side of the cell directly behind the nucleus. In addition, strong pockets of focal adhesions can be observed beneath the trailing edge of the cell, near its base. For representation, this sheared cell experiences 75% applied shear strain

probe heights, wherein the cell becomes strongly adhered onto the substrate. This result suggests that focal adhesion activation may be a strain-dependent process within chondrocytes. Lastly, this study describes the development of a novel videocapture methodology to study the shear characteristics of single chondrocytes.

To our knowledge, this is the first study to examine the mechanical properties of single chondrocytes experiencing direct shear. This is a necessary step in understanding the complete biomechanical milieu of chondrocytes within cartilage, which includes shear forces, among other mechanical pressure modalities (Mow et al. 1994; Guilak and Mow 2000). It is found that the apparent shear modulus increases (∼1.5-fold) as the probe is repositioned from the cell top to its middle, and again increases (∼1.5-fold) as the probe is further repositioned toward the cell base. This observed nonuniformity in cell mechanical properties, along its height can be attributed to spatial variations in the concentration of the actin cytoskeleton during shear. Actin filaments are highly sensitive to mechanical loading and dynamically adapt to perturbations in the cellular microenvironment through Rho-kinase mediated signaling mechanisms (Chrzanowska-Wodnicka and Burridge 1996; Amano et al. 1997; Knight et al. 2006; Campbell et al. 2007). In our study, sheared chondrocytes exhibit a reorganization of their actin filaments, which become localized on the trailing side of the cell, near its base. Moreover, it has been previously established that actin contributes significantly to cell stiffness parameters under compression or tension testing modalities (Trickey et al. 2004; Leipzig et al. 2006; Hemmer et al. 2009). Thus, based on the results in this study, similar connections can be drawn between actin filaments and the spatial shear characteristics within a single cell. Elucidating the mechanisms at play in determining chondrocyte shear stiffness may have important ramifications in future tissue engineering efforts, which employ shear stress as a means toward improving cellular viability and matrix production (Raimondi et al. 2006; Timmins et al. 2007). Furthermore, through a precise knowledge of the apparent shear moduli of single chondrocytes, regimens can be developed to induce desired cell strains (Chung et al. 2007).

Studying the range of shear strains, which induce desired changes in cellular behavior, is critical toward understanding the mechanosensitivity of single chondrocytes and identifying potential loading modalities that promote tissue growth. Single chondrocytes begin to exhibit a forward movement along their substrate at ∼15–25% applied shear, which continues until a critical strain of ∼35% is reached. This forward movement may involve both a rolling and sliding component as the cell displaces in the direction of the probe, which may intrinsically entail the breakage of some focal contacts between the cell and its substrate. While the peak nFBM for chondrocytes increases as the probe is repositioned closer to the base of the cell, the critical strain wherein the nFBM reaches its plateau is not affected by the probe height. At this critical strain, the cell-substrate bonds may strengthen through focal adhesion activation mechanisms (Riveline et al. 2001), thereby preventing additional movement of the cell's trailing edge during shear. This result is supported by cytoimmunochemical staining of chondrocytes experiencing shear above this critical strain, where a clear rearrangement of focal adhesions to the cell's trailing side is evident.

Our observation that the critical strain in nFBM is not altered by probe position suggests that focal adhesion recruitment may occur primarily through strain-dependent, rather than force-dependent, mechanisms. Previous research has found that focal adhesion complexes are indeed sensitive to mechanical stimulation and may develop at the location of the applied load (Riveline et al. 2001; Galbraith et al. 2002; Sniadecki et al. 2007). In the scenario where a critical shear strain results in a plateau of forward movement, stretch-activated ion channels may induce intracellular signaling pathways once a critical strain is reached, which then leads to the development of strong focal adhesion complexes (Lee et al. 2000; Millward-Sadler et al. 2000; Riveline et al. 2001). These results lay the groundwork for future research to examine the real-time reorganization of focal adhesions or specific signaling activation pathways prevalent during shear of single cells, toward a better understanding of the levels of strain and intracellular mechanisms affecting cellular physiology.

A novel experimental approach is presented in this study to examine the shear characteristics of single cells. Through video analysis of the mechanical event, clear measurements of cellular deformations and movements can be obtained as the cell experiences direct shear. These physical cell alterations are further correlated with applied stresses or strains to yield fundamental properties for single chondrocytes. Our current experimental modality improves upon previous work in our laboratory which studied the lateral detachment force for individual adherent chondrocytes (Athanasiou et al. 1999; Hoben et al. 2002; Huang et al. 2003), by enabling control of the probe height and capturing cell morphological changes via video recordings. Despite technological advances, an inherent limitation remains that chondrocytes must be removed from their native mechanical microenvironment for single cell testing. In essence, this sidesteps the natural transmission of forces through the chondrocyte pericellular matrix (Knight et al. 1998; Alexopoulos et al. 2005). However, chondrocytes have been shown to retain their cytoskeletal organization (Trickey et al. 2004) and the biophysical differences between pathologic or regenerative states (Trickey et al. 2000; Cross et al. 2007; Titushkin and Cho 2007) when isolated in vitro. Furthermore, our experimental approach presents the unique advantage of singly investigating how cellular shear properties may be altered on various biomimetic materials, under different culture conditions, and attributed to specific intracellular structures. For instance, single chondrocytes may be seeded on different coated substrates, such as with aggrecan or collagen, which are frequently utilized in tissue engineering scaffolds (Revell et al. 2006); or for different seeding durations, which may alter chondrocyte morphology, cytoskeleton, and adhesiveness (Huang et al. 2003). Future experimental work may also employ cytoskeletal inhibitors or growth factors to study the specific role of the actin network or focal adhesions in spatial shear properties (Riveline et al. 2001; Trickey et al. 2004; Leipzig et al. 2006). In addition, chondrocytes were not mechanically preconditioned in this study. Since pre-conditioning may have an effect, future studies may investigate this aspect of the cell's shear behavior.

In addition, studying the biomechanics of single chondrocytes undergoing shear has the potential to benefit future computational studies of cellular behavior. A number of studies in recent years have utilized theoretical approaches to replicate fundamental biological phenomena, including contractility and spreading characteristics on various surfaces, with remarkable similarity to experimental measurements (Tan et al. 2003; Deshpande et al. 2008; Pathak et al. 2008). Furthermore, computational models have focused specifically on single chondrocytes experiencing various mechanical perturbations, such as the application of a lateral force (McGarry and McHugh 2008), compressive load (Ofek et al. 2009), or local membrane aspiration (Vaziri and Mofrad 2007). These combinations of experimental and computational approaches have yielded important observations regarding the cell-substrate interface and intracellular contribution to cell stiffness, which could not be demonstrated in the experimental results alone. Our current experimental method for cell shear can provide inputs for three-dimensional computational models which simulate the rearrangement of the cytoskeleton via intracellular signaling mechanisms. In particular, an active bio-chemical-mechanical model has been developed to simulate the stress-dependent stress fiber activation and active focal adhesion assembly in single cells (Deshpande et al. 2006). These models may then be used to examine the thresholds of shear strains exerted onto chondrocytes that induce a rearrangement of the cytoskeleton and potentially alter cellular homeostasis or biosynthetic capacity (Shieh et al. 2006). Thus, the future utilization of active cell modeling in parallel with in vitro examination of subcellular biomechanical phenomena provides a powerful novel tool to elucidate the mechanisms underlying cellular responses to their physical environment.

This study provides new insight into the biomechanical characteristics of single chondrocytes under shear. The apparent shear modulus of individual adherent chondrocytes is non-homogeneous since it is observed to increase toward the cell's base, correlating with alterations in the actin network during shear. In addition, chondrocytes exhibit a forward movement in the direction of the shear force, until an applied shear strain is reached, regardless of initial probe position. Above this critical strain level, focal adhesions reorganize into strong contacts along the cell's trailing edge to prevent additional forward movement. These results improve our knowledge of the chondrocyte micromechanical environment and intracellular changes in response to applied loads. A firm understanding of the physical characteristics of

single chondrocytes may shed light on the role of mechanical forces in promoting cartilage regeneration or degeneration (Ofek and Athanasiou 2007; Shieh and Athanasiou 2007).

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