Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation

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Collinsworth, Amy M., Sarah Zhang, William E. Kraus, and George A. Truskey. Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation. Am J Physiol Cell Physiol 283: C1219–C1227, 2002. — The effect of differentiation on the transverse mechanical properties of mammalian myocytes was determined by using atomic force microscopy. The apparent elastic modulus increased from 11.5 ± 1.3 kPa for undifferentiated myoblasts to 45.3 ± 4.0 kPa after 8 days of differentiation (P < 0.05). The relative contribution of viscosity, as determined from the normalized hysteresis area, ranged from 0.13 ± 0.02 to 0.21 ± 0.03 and did not change throughout differentiation. Myosin expression correlated with the apparent elastic modulus, but neither myosin nor β-tubulin were associated with hysteresis. Microtubules did not affect mechanical properties because treatment with colchicine did not alter the apparent elastic modulus or hysteresis. Treatment with cytochalasin D or 2,3-butanedione 2-monoxime led to a significant reduction in the apparent elastic modulus but no change in hysteresis. Microtubules did not affect mechanical properties because treatment with colchicine did not alter the apparent elastic modulus or hysteresis. Treatment with cytochalasin D or 2,3-butanedione 2-monoxime led to a significant reduction in the apparent elastic modulus but no change in hysteresis. In summary, skeletal muscle cells exhibited viscoelastic behavior that changed during differentiation, yielding an increase in the transverse elastic modulus. Major contributors to changes in the transverse elastic modulus during differentiation were actin and myosin.

The apparent elastic modulus; viscous properties; myotubes; atomic force microscopy

THE EXPRESSION of cytoskeletal and cell membrane-associated proteins affects the passive mechanical properties of striated muscle. In cardiocytes, collagen and titin are the major contributors to longitudinal passive tension (13). Titin plays a role in the resting longitudinal tension of skinned rabbit muscle fibers (33, 34), and the difference in longitudinal stiffness between cardiac and skeletal myocytes is attributed to the different isoforms of titin expressed in these two types of muscle (13).

Passive properties in the longitudinal direction limit the maximum elongation for a given load. In the transverse direction, linkages between the cytoskeleton and the extracellular matrix maintain fibers in contact with each other and permit synchronous movement. The transverse mechanical properties serve as an internal load acting on the cell that affect the longitudinal extension (16).

Less is known about the molecules affecting the transverse mechanical behavior of myotubes and muscle fibers. Skeletal muscle cells with a dystrophin mutant that does not interact with membrane glycoproteins have a lower transverse elastic modulus than control cells with normal dystrophin (19). The intermediate filament desmin affects the transverse properties (4). Studies with a number of cell types indicate that mechanical properties of cells are affected by microtubules, actin, and myosin (14, 20, 27, 36).

The differentiation of skeletal muscle cells is stimulated by a contact-dependent process, which includes withdrawal from the cell cycle, subsequent fusion of myoblasts to form multinucleated myotubes or myofibers, and expression of differentiation-specific proteins. This fusion process appears to be, at least partly, regulated by growth factors and sequential gene activation (11). Given the morphological and functional differences between skeletal myoblasts and myotubes, we considered whether there may be differences in the mechanical properties of differentiated and undifferentiated skeletal muscle cells. In support of this hypothesis are the observations that mechanical properties depend on both protein expression (19) and cytoskeletal organization (23, 29, 35).

In this study we used atomic force microscopy (AFM) to test the hypothesis that the transverse elastic and viscous properties of skeletal muscle cells change throughout the time course of differentiation of myoblast to myofiber. Transverse properties are likely to be sensitive to the cytoskeleton (15, 24), and the prestress, or intrinsic stress, present in cells in the absence of applied loads depends on microtubules (10) and actin (9). Consequently, we examined actin, myosin, and tubulin as possible contributors to these mechanical properties. The results of this work elucidate the nature of myocyte mechanical properties throughout their development.

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MATERIALS AND METHODS

Cell culture. Murine C2C12 myoblasts (American Type Culture Collection, Rockville, MD), a subclone derived from a cell line that originated from normal adult C3H mouse leg muscle (3, 39), were plated in tissue culture dishes at a density of 3.75 × 10^4 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY) supplemented with 10% calf serum (HyClone Laboratories, Logan, UT), 0.5% chick embryo extract (GIBCO BRL), and 0.5% gentamicin (GIBCO BRL). At 80–90% confluence, the medium was removed and replaced with DMEM, supplemented with 10% horse serum (Intergen, Purchase, NY) with a lower growth factor concentration to promote differentiation, and gentamicin. The point at which the growth medium is replaced with a medium of a lower growth factor concentration is commonly called “shifting.” After shifting, the cells were fed daily with this shift medium.

Western blot analysis. For protein analysis throughout the differentiation of myocytes, cells were harvested at 24-h increments, and the level of myosin or total tubulin was determined as follows. Cells were rinsed twice in cold PBS, scraped with a rubber policeman, and pelleted. Cell pellets were lysed on ice for 15 min in 0.5% SDS lysis buffer consisting of 0.6% Nonidet P-40 (NP-40), 0.15M NaCl, 1 mM EDTA, 10 mM Tris, pH 7.9, and antiproteases (10 µg/ml aprotinin, 10 µg/ml leupeptin, 5 µg/ml pepstatin, and 0.5 mM PMSE) and were either used immediately or stored at −80°C for later analysis. To determine sample protein concentrations, the bicinchoninic acid protein assay (Sigma, St. Louis, MO) was used. Samples were prepared, boiled, and loaded in a 10% resolving gel. Each lane received the same amount of total protein: 15 µg of total protein were added to each lane for detection of myosin, and 40 µg of total protein were added to each lane for detection of tubulin. The gels were run overnight at 3–4 mA for myosin and 10–12 mA for tubulin. A semidy transfer was used (0.8 mA/cm^2) to transfer the proteins to a nitrocellulose membrane (BA-85, 0.45-µm pore size; S&S, Keene, NH). Blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 150 mM Tris, 1.5 M NaCl, pH 7.5) with 0.1% Tween 20 and probed for either myosin or tubulin. Blots were incubated in primary antibody for 1 h (MF20 and E7 for myosin and tubulin, respectively, dilution 1:50; Developmental Studies Hybridoma Bank, Iowa City, IA) and then rinsed for 5 min in TBS-Tween (0.1%) three times. The membranes were then incubated in secondary antibody (antimouse IgG-HRP; Santa Cruz Biotechnology, Santa Cruz, CA) with 0.1% bovine serum albumin (BSA), dilution 1:1,000, for 1 h. After the secondary antibody incubation, the membrane was rinsed twice, 5 min each time in TBS-Tween (0.1%), followed by a third rinse in TBS. Enhanced chemiluminescence (ECL; Amersham, Piscataway, NJ) was used to visualize the proteins on film. Each film was scanned with a GS 300 Transmittance/Reflectance Scanning Densitometer (Hoefer Scientific Instruments, San Francisco, CA). Film background was used for a zero point. Measures of intensity were normalized to shift day 8 for the myosin assays and to pure tubulin controls for the tubulin assays. Control experiments were performed with purified tubulin to establish that electrophoresis and transfer were quantitative.

The harvest of polymerized and total tubulin fractions in taxol and colchicine experiments was adapted from existing protocols (7, 21). To isolate the polymerized fraction of tubulin, cells were rinsed with PBS and microtubule-stabilizing buffer (MTSB; 0.1 M PIPES, 1 mM EGTA, 1 mM MgSO₄, and 2 M glycerol, pH 6.79). The cells were then solubilized with 0.1% Triton X-100 during two 15-min incubations, allowing free tubulin to be retained. For harvesting of total tubulin, cells were rinsed in PBS and MTSB without Triton X-100. All cells were lysed for 5 min in 0.5% SDS lysis buffer with the same antiproteases as used in the previous procedure. Rinses and incubations were done at 37°C. Cells were scraped with a rubber policeman and transferred to a microcentrifuge tube. Lysates were sonicated and pelleted in a microcentrifuge at 12,000 g for 15 min. The supernatant fraction was transferred to a new tube, and β-mercaptoethanol was added to a final volume of 0.1%. Samples were boiled for 3 min and stored at −80°C until used for Western blot analysis.

Immunofluorescence. At the end of an experiment, the medium was removed and the cells were fixed in 2% formaldehyde and permeabilized in 0.25% NP-40. With the use of methods previously described (31), myotubes were visualized with mouse monoclonal anti-α-actinin, which recognizes sarcosomic α-actinin (Sigma). The secondary goat anti-mouse monoclonal IgG was labeled with Alexa Fluor546 (Molecular Probes). Fluorescence was examined by confocal microscopy (Bio-Rad 510).

Atomic force microscopy. The transverse mechanical properties of the cells were measured by using a Bioscope atomic force microscopy (AFM) probe mounted on an inverted microscope. Probes were made of 200-µm oxide-sharpened silicon nitride cantilevers (Digital Instruments, Santa Barbara, CA) with spring constants of 0.03–0.06 N/m and a cone angle of 35°. Before each experiment, the spring constant for each cantilever was calibrated by using the thermal vibration-based software in the Nanoscope 4.23 r3 version. The cantilever was secured in the fluid holder, and a drop of medium was placed on the tip before it was inverted and lowered to the surface. Sensitivity was obtained for each experiment on a glass coverslip with medium and was consistently near 0.01 V/nm. Medium was removed from cells and replaced with medium containing 1 mM EGTA to prevent spontaneous contractions of skeletal muscle cells.

AFM uses a cantilever arm that comes in contact with the cell to induce cellular deformations. The force applied was calculated from Hooke's Law and is the product of the spring constant, k, and the deflection of the cantilever, d. The equal and opposing force with which the cell resists was estimated by using the Hertz model (9, 37). From this known force, the elastic modulus can be calculated. The tip of the AFM is cone shaped, resulting in the force-indentation relation given by (37)

\[ F = \delta^2 \frac{2E}{\pi(1-\nu^2)} \tan \alpha \]  

where F is the loading force, E is the elastic modulus, υ is the Poisson ratio (a value of 0.5 is commonly used for cells; Ref. 25), α is the opening angle of the cone tip, and δ is the indentation depth (22). The indentation is equal to the difference between the piezo height, z, and the cantilever deflection (δ = z − d). To obtain υ and δ values, the contact point was defined as the point at which the slope of the baseline begins to deviate positively and set as the zero reference point. Changing the contact point on the order of nanometers did not affect the calculated value of the apparent elastic modulus (data not shown). Having then determined the indentation at each point, Eq. 1 was used to plot the force vs. the square of the indentation, and an elastic modulus was obtained.

The elastic properties are defined as an “apparent” elastic modulus, because there are viscous contributions within the cellular response and nonlinear elastic behavior is not appar-
ent from the deformations studied (9). Hysteresis was calculated from force vs. extension (or indentation) and retraction curves as follows (see Fig. 1). Each force vs. distance curve was fit to a third-order polynomial equation, which was integrated over the indentation distance, yielding the area under the curves for extension (Aext) or retraction (Aretr). Hysteresis was calculated by subtracting Aretr from Aext and represents the energy dissipated into the cell from the indentation of the AFM tip. The area was normalized by dividing the hysteresis value by the input energy, $A_{\text{ext}}$. This definition of hysteresis is analogous to the definition of hysteresis used by Fredberg et al. (12) to characterize the viscous dissipation during longitudinal stretching of strips of airway smooth muscle cells.

A typical result for indentation and retraction at a single location is shown in Fig. 1A. The tip speed was 0.5 µm/s. Hysteresis declined with decreasing tip speed. Below 0.5 µm/s, hysteresis did not decline further (18). Three force indentation curves were collected per cell. Any negative values for hysteresis were due to an artifact of the AFM detector and were discarded. No more than one curve per cell exhibited negative hysteresis, and discarded hysteresis curves represented 5% of the total number of force-indentation curves accumulated.

The apparent elastic modulus was determined by rewriting Eq. 1 as $F = E\delta^2$ where the constant $\Omega$ equals

$$\Omega = \frac{2}{\pi} \tan \alpha \left(1 - \nu^2\right)$$

The extension force was plotted as a function of $\Omega\delta^2$, and the resulting slope is equal to the elastic modulus $E$ (Fig. 1B).

The Hertz model (Eq. 1) describes the case of a rigid tip poking a semi-infinite, isotropic, homogeneous elastic surface. Although the cell is finite, viscoelastic, and anisotropic, these assumptions can be approximately met under the following limiting conditions, and an apparent elastic modulus can be determined. By indenting slowly enough, viscous contributions are small and force measurements are dominated by the elastic behavior (1). Force curves of the cells were obtained in contact mode at a tip velocity (0.5 µm/s) previously determined to be slow enough to minimize the amount of hysteresis (18) yet fast enough to maximize the number of force curves that could be captured in a given experiment. To treat the cell as a semi-infinite material, the indentation must be sufficiently small such that deformations of the cell are not influenced by the cell boundary or the substrate. Indentation depths were <15% of the cell height conditions for which the measured mechanical behavior should not be influenced by the finite cell thickness (9).

*Fig. 1.* A: representative curve of force vs. indentation. From these data, hysteresis is calculated as the difference between the area under the polynomial fit of the extension curve and that of the retraction curve, normalized by the input energy. B: force plotted vs. $\delta^2$ multiplied by the factor $\Omega$ defined in Eq. 2. The apparent elastic modulus is determined from the slope of the linear fit to the curve.

**Treatments to alter transverse mechanical properties.** To assess the role of the microtubules, cells were incubated in either 0.4 µg/ml colchicine (Sigma, St. Louis, MO) or 10 µM taxol (Sigma) in medium for 2 h. After incubation, the treated medium was removed, and cells were either harvested for protein analysis or placed in EGTA medium for AFM analysis. The respective contribution of actin and actin-myosin interactions to the mechanical behavior of the cells was assessed by incubating myoblasts and myotubes with 3 µM cytochalasin D or 50 mM 2,3-butanedione 2-monoxime (BDM; Ref. 28; Sigma) for 5–30 min. For each of these treatments, force-indentation curves were obtained by using AFM before addition of the drug. The drug was added, after which force-indentation curves were obtained from the same cell at the times indicated.

**Statistical analysis.** All values are reported as means ± SE unless otherwise stated. Statistical significance of protein expression and mechanical properties throughout differentiation or after treatment with cytochalasin D or BDM were calculated by using a one-way analysis of variance. Statistical significance ($P < 0.05$) between control and treated groups in taxol and colchicine experiments was calculated by using Student’s t-test.

**RESULTS**

**Effect of replicate indentation in the same location.** To verify that cell behavior did not change with time or with repeated indentations, replicate force curves in the same location were recorded every 10 s. The apparent elastic modulus and hysteresis calculated from these curves were very consistent, yielding average values of 18.0 ± 2.0 and 0.07 ± 0.02 kPa, respectively ($n = 6$), and with no trends with time. This process was repeated in a second experiment at 1- to 5-min intervals over a period of 30 min with similar results. Spontaneous contractions, which produce large amplitude oscillations in the cantilever tip location (26), were not observed.

**Effects of differentiation on mechanical properties.** Apparent elastic moduli were calculated by applying the Hertz model to the force vs. indentation curves obtained from AFM. Values were calculated for undifferentiated myoblasts and differentiated myofibers from shift day 3 to shift day 10. Cells at the various time points were from a mixed population of myofibers ( multinucleated cells expressing proteins characteristic of differentiated cells) and postmitotic myoblasts...
(single-nucleated cells that express proteins characteristic of differentiated cells). The average percentage of fibers in these mixed populations increased with time and, by observation, was determined to be ~20% on shift day 3 and 90% on shift days 8 and 10. AFM measurements were always taken on myofibers near the center of the cell.

The average apparent elastic moduli ranged from 11.5 ± 1.3 kPa for myoblasts to 45.3 ± 4.0 kPa for myofibers at shift day 8 (Fig. 2A). Elastic moduli were similar throughout differentiation until shift days 8 and 10, when there was a large and significant increase in the apparent elastic modulus. Normalized hysteresis values ranged between 0.13 ± 0.02 and 0.21 ± 0.03 throughout differentiation (Fig. 2B). No significant differences in hysteresis were detected throughout differentiation (P = 0.47).

Levels of α-actinin and myosin increased steadily throughout differentiation (Figs. 3 and 4). Myoblasts did not stain for α-actinin (not shown). Striations in α-actinin staining were not apparent until day 3. By day 6 many cells exhibited striations, and clear striations were present on all myotubes at day 8 (Fig. 3). Such striations are indicative of sarcomere formation. Beginning on shift day 4 and each day afterward, myofibers expressed significantly higher levels of myosin than undifferentiated myoblasts (Fig. 4). (Because of cell loss from the cultures between days 8 and 10, protein levels were not quantified on day 10.) Levels of total β-tubulin were similar throughout differentiation through shift day 8, with no significant differences detected after the initial day (Fig. 4).

Because of the increase in apparent elastic modulus on days 8 and 10, there was a nonlinear relationship between the apparent elastic modulus and sarcomeric myosin (Fig. 5). No relationship was observed between apparent elastic modulus and β-tubulin protein levels, and no relationship was found between hysteresis and either myosin or β-tubulin protein levels. Also, there was no correlation between the apparent elastic modulus and hysteresis over the course of differentiation.

**Effects of taxol and colchicine on transverse mechanical properties.** Although changes in the apparent elastic modulus were not correlated with tubulin protein levels, myotubes were treated with taxol or colchicine to determine whether alteration of the microtubular network affected the mechanical properties. Results are presented as a ratio of the treated value to the control value (Fig. 6). Shift day 5 myofibers treated with taxol for 2 h had a significantly lower average apparent elastic modulus than respective controls, whereas cells treated with colchicine for 2 h exhibited no significant change in elastic properties. In addition, cells treated with taxol exhibited significant morphological changes that included rounding of edges to form cylindrical tubes with round bulges, whereas cells treated with colchicine had slightly rounded edges but to a lesser degree than those treated with taxol (not shown). The average percentage of viscous contributions in cells treated with either taxol or colchicine was not different from their respective controls (P > 0.05).

To verify the effect of the taxol and colchicine treatment on microtubule polymerization, the amount of polymerized and total β-tubulin was measured. In two separate experiments, the fraction of microtubules (ratio of polymerized β-tubulin to total β-tubulin) in taxol-treated cells was more than three times the fraction of microtubules in the control group, whereas the fraction of microtubules in colchicine-treated cells was undetectable (not shown).

**Effect of cytochalasin D and BDM on the transverse mechanical properties.** To assess whether alterations in actin polymerization or myosin cross-bridge formation affected the transverse mechanical properties, differentiating myotubes were incubated with either 3 μM cytochalasin D or 50 mM BDM, and the elastic modulus and hysteresis were determined. For myoblasts examined 3, 6, 8, and 10 days after shifting of medium, addition of either drug led to a time-dependent decrease in the apparent elastic modulus (Fig. 7) but did not affect hysteresis (Fig. 8). Cytochalasin D produced a significant decrease in the apparent elastic modulus on all days studied (Fig. 7A), whereas BDM led to significant reductions in the modulus only on shift days 6 and 8 (Fig. 7B). The effect of cytochalasin D and BDM was greatest on shift day 8.

![Fig. 2. A: apparent elastic modulus as a function of differentiation of skeletal myotubes. *P < 0.05; **P < 0.01, significantly different from myoblasts. B: viscous contributions as a function of differentiation. Normalized hysteresis is the ratio of energy dissipated to input energy. No significant differences were detected between normalized hysteresis values throughout the course of differentiation. For both A and B, values are the average of 8–26 cells for each condition, and error bars represent SE.](http://ajpcell.physiology.org/ by Oct 22, 2017 3:31 on August 17, 2017)
DISCUSSION

The objective of this study was to test the hypothesis that the transverse elastic and viscous properties of skeletal muscle cells change throughout the time course of differentiation from myoblast to myofiber.

The most dramatic change in the passive mechanical behavior of myocytes was a significant increase in the apparent elastic modulus with differentiation, which was associated with an increase in myosin after 8 days of differentiation. The elastic modulus of myotubes decreased with the addition of cytochalasin D or BDM,
and this effect was greatest 6 and 8 days after the onset of differentiation. There was no effect of differentiation on hysteresis, nor was there any correlation between the changes in transverse mechanical properties and the expression of β-tubulin. To our knowledge, this is the first report of mechanical properties of skeletal myocytes changing during the course of differentiation.

The elastic modulus is indicative of the extent to which the skeletal muscles deform after application of a stress. The increase in the apparent elastic modulus 8 and 10 days after onset of differentiation indicates that differentiated myotubes exhibit reduced passive deformation in the transverse direction after application of a load. Because muscles maintain a constant volume during contraction, the increase in transverse elastic modulus, in the absence of any change in the longitudinal modulus, could reduce the amount of work done during contraction. Furthermore, the increased apparent transverse elastic modulus may stabilize cell interactions with the extracellular matrix.

The true behavior of most cells is viscoelastic; that is, the cells exhibit time-dependent behavior after imposition of a force or a deformation. The hysteresis area is a measure of the relative contribution of viscous energy losses. Such viscous losses are dependent upon the rate of loading. The particular tip speed chosen for these experiments minimized the viscous losses. The normalized amount of hysteresis did not change significantly throughout muscle differentiation. Because the elastic contributions increased and the relative amount of hysteresis remained unchanged, the absolute viscous energy losses experienced by the cells may have increased during differentiation.

The condition of low tip speed minimized viscous losses so that the apparent elastic modulus could be accurately determined. Normalized viscous dissipation was consistently around 15% of the total energy added. This level of viscous dissipation is similar to that found for human airway smooth muscle (12) but is greater than the hysteresis of endothelial cells and less than that of rabbit cardiac cells (18).

The apparent transverse elastic moduli measured in skeletal myofibers are similar to those reported in other studies of differentiated skeletal muscle cells using AFM (41). Skeletal muscle cells have a higher...
apparent transverse elastic modulus than endothelial cells (18) and chondrocytes (0.65 ± 0.63 kPa, by micropipette technique; Ref. 17) and a lower apparent elastic modulus than cardiac cells (15, 18). Furthermore, the transverse elastic modulus measured in this study for deformations of a few hundred nanometers is similar to values obtained during transverse compression of adult rat tibialis anterior muscle (5).

To assess whether the AFM measurements were sensitive to perturbations in the cytoskeletal structure, we used taxol and colchicine to alter the microtubular network, cytochalasin D to depolymerize actin, and BDM to disrupt myosin cross-bridge formation. Although we did not observe any changes in mechanical properties with the administration of colchicine, we detected a difference in apparent elastic modulus with the treatment of taxol. The decrease observed in the apparent elastic moduli of taxol-treated cells may be due the mechanism of action of taxol. Taxol stabilizes microtubules and induced morphological changes to the cells as also observed by others (10). The resulting change in the apparent elastic modulus may represent a response to the shape change and not a simple response to polymerization of tubulin.

There are mixed reports on the effects of microtubules on mechanical and functional properties, especially in the pressure- and volume-overloaded cardiac hypertrophy models. Some investigators have observed an increase in microtubule density and an increase in viscosity that are eliminated when colchicine is added to the isolated cells (29, 32). However, others have not observed changes in levels of tubulin (8), microtubule density (30), or any effect of colchicine on contraction dynamics (2, 8). Likewise, under nonstimulated conditions, there is no effect of colchicine on mechanical properties and contraction dynamics in isolated adult rat cardiocytes (40). Our results are consistent with the latter studies. No effect was seen on either apparent elastic modulus or hysteresis after treatment with colchicine. The reasons the results in these reports vary may be due to several factors such as differences in cell type and species. In addition, some studies employ hypertrophy models, whereas other studies are carried out under steady-state conditions.

Because of the absence of an effect of colchicine on mechanical properties of these cells and the absence of an association between mechanical properties and tubulin protein levels, we conclude that microtubules are
not a major contributor to the transverse mechanical properties of differentiating mammalian skeletal muscle cells. It is possible that the measured mechanical properties are different for conditions that promote microtubule polymerization or on time scales much different than those used in this study. For example, in response to a stretch stimulus, the maximum change in microtubule polymerization in smooth muscle cells occurred 15 min after the beginning of stretch (21), shorter than the timeframe over which AFM measurements are made. Several weeks are required to observe an effect of microtubules on mechanical behavior with in vivo hypertrophy models.

In contrast, the association of myosin protein levels with the transverse elastic modulus and the effect of BDM and cytochalasin D on the elastic modulus suggest that myosin interactions with actin influence the elastic modulus but not the relative viscous contribution. Decreases in the apparent elastic modulus with BDM treatment observed in this study are consistent with a decreased transverse stiffness measured by AFM when rabbit myofibers are transferred from a rigor to a relaxing solution (41). These results are consistent with a model in which resistance to deformation during indentation is due to two components: deformation of the cytoskeleton and breaking of cross bridges. The results with cytochalasin D suggest that actin provides some of the resistance to deformation in the transverse dimension. Other proteins such as desmin, dystrophin, and focal contact proteins may play a role. The correlation between the elastic modulus and myosin protein levels and the effect of BDM would indicate that some actin-myosin cross bridges are broken during indentation. This view is consistent with the observations that the radial stiffness (i.e., the slope of the force vs. fiber diameter curve) of single-skinned rabbit psoas fibers decreased as the density of actin-myosin cross bridges was reduced (6, 38).

By increasing its stiffness, the muscle cell may develop a greater ability to resist stress. It is also possible there is some threshold level of expression of multiple proteins that contributes to this change. Because the increase in myosin expression begins at shift day 4 and continues through shift day 8, but the increase in modulus does not occur until shift day 8, myosin may partially contribute to this effect; however, the myosin contribution is not possible to conclude at this point.

Limitations to this study include the use of a C2C12 mouse cell line. For the application of tissue replacements, these results should be extended to primary cultures and human cells. In addition, we were unable to test the mechanical properties after the tenth day in culture because of myofiber detachment from the substrate, possibly resulting from spontaneous contraction of the myofibers.

In summary, we found that the apparent elastic modulus of mammalian skeletal muscle cells grown in culture increases after differentiation, whereas the viscous contributions remain relatively constant. Myosin and actin, but not tubulin, were found to be associated with a change in the elastic modulus after 8 days of differentiation. These findings may aid studies designed to investigate the use of skeletal myocytes for cardiac tissue replacement, where functional mechanical properties of the donor cells need to be matched to the recipient tissue to optimize contractile activity and functional integration of the transplanted tissue into the repaired myocardium.

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