Rachel Zielinski

Biomedical Engineering Department,
The Ohio State University,
Columbus, OH 43210

Cosmin Mihai

Biomedical Engineering Department,
The Ohio State University,
Columbus, OH 43210;
Department of Internal Medicine,
Division of Pulmonary, Allergy,
Critical Care and Sleep Medicine,
Wexner Medical Center,
The Ohio State University,
Columbus, OH 43210

Douglas Kniss

Biomedical Engineering Department,
The Ohio State University,
Columbus, OH 43210;
Department of Obstetrics and Gynecology,
Wexner Medical Center,
The Ohio State University,
Columbus, OH 43210

Samir N. Ghadiali¹

Biomedical Engineering Department,
The Ohio State University,
Columbus, OH 43210;
Department of Internal Medicine,
Division of Pulmonary, Allergy,
Critical Care and Sleep Medicine,
Wexner Medical Center,
The Ohio State University,
Columbus, OH 43210;
Dorothy M. Davis Heart and
Lung Research Institute,
Wexner Medical Center,
The Ohio State University,
Columbus, OH 43210
e-mail: ghadiali.1@osu.edu

Finite Element Analysis of Traction Force Microscopy: Influence of Cell Mechanics, Adhesion, and Morphology

The interactions between adherent cells and their extracellular matrix (ECM) have been shown to play an important role in many biological processes, such as wound healing, morphogenesis, differentiation, and cell migration. Cells attach to the ECM at focal adhesion sites and transmit contractile forces to the substrate via cytoskeletal actin stress fibers. This contraction results in traction stresses within the substrate/ECM. Traction force microscopy (TFM) is an experimental technique used to quantify the contractile forces generated by adherent cells. In TFM, cells are seeded on a flexible substrate and displacements of the substrate caused by cell contraction are tracked and converted to a traction stress field. The magnitude of these traction stresses are normally used as a surrogate measure of internal cell contractile force or contractility. We hypothesize that in addition to contractile force, other biomechanical properties including cell stiffness, adhesion energy density, and cell morphology may affect the traction stresses measured by TFM. In this study, we developed finite element models of the 2D and 3D TFM techniques to investigate how changes in several biomechanical properties alter the traction stresses measured by TFM. We independently varied cell stiffness, cell-ECM adhesion energy density, cell aspect ratio, and contractility and performed a sensitivity analysis to determine which parameters significantly contribute to the measured maximum traction stress and net contractile moment. Results suggest that changes in cell stiffness and adhesion energy density can significantly alter measured tractions, independent of contractility. Based on a sensitivity analysis, we developed a correction factor to account for changes in cell stiffness and adhesion and successfully applied this correction factor algorithm to experimental TFM measurements in invasive and noninvasive cancer cells. Therefore, application of these types of corrections to TFM measurements can yield more accurate estimates of cell contractility. [DOI: 10.1115/1.4024467]

Keywords: cell contractility, cell mechanics, cell adhesion, strain hardening, cancer cells, epithelial-to-mesenchymal transition

1 Introduction

Several biological processes including embryogenesis, wound healing, angiogenesis, stem cell differentiation, and cell migration depend on interactions between cells and their extracellular matrix [1–4]. Cell-ECM interactions are also important for tissue engineering applications [5–7] and play an essential role in several diseases including cancer metastasis, fibrosis, and inflammation [8–12]. Cells attach to the ECM at specific focal adhesion sites, which contain transmembrane receptors (i.e., integrins) that are associated with actin stress fibers [13]. As a result, contractile forces generated within the cell due to actin-myosin interactions can be transmitted to the ECM, and this force transmission results in traction stresses within the ECM [14]. These traction stresses have been shown to correlate with cell contractility and are important in cell adhesion, spreading, migration, and ECM remodeling [4,14–16]. As a result, the ability to quantitatively measure cell

contractility and the resulting traction stresses in the ECM have become essential to further our understanding of basic physiological and pathophysiological processes [13].

Traction force microscopy is an experimental technique developed to quantify the contractility of adherent cells. As described previously [13], one commonly used TFM method involves seeding cells on a polyacrylamide gel and tracking the displacements of embedded microbeads during cell contraction (2D TFM) [14,17,18]. One advantage of using polyacrylamide gels is that their stiffness can be tuned to represent different physiological ranges of ECM stiffness. ECM proteins (i.e., collagen type I) are normally conjugated to the gel surface to facilitate cell adhesion and the resulting cell-induced deformation of the substrate is determined by monitoring bead position before and after cell detachment by trypsinization. The displacement field can be determined by either tracking individual beads [18–20] or by correlation-based particle image velocimetry [21,22]. Traction stresses within the projected cell area are then determined by first approximating the polyacrylamide gel as a semi-infinite elastic half space where the displacement field is related to the traction force field by a Boussinesq–Green function [23]. Obtaining traction stresses requires inversion of the Boussinesq solution, and previous investigators have used a probability-based algorithm [14] or Fourier transforms [21] to accomplish this inversion.

¹Corresponding author.

Present address: Department of Biomedical Engineering, The Ohio State University, 270 Bevis Hall, 1080 Carmack Road, Columbus, OH 43210.

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Alternatively, finite element methods can be used to directly apply the measured bead displacements as boundary conditions and calculate the resulting traction stresses [20]. This finite element approach has significant advantages since it does not require an iterative solution and can be used to accurately measure traction stress in finite thickness gels. In addition to monitoring traction stresses generated at the cell-substrate interface, confocal microscopy images and finite element analysis has been used to investigate the propagation of traction stresses away from the cell-substrate interface (i.e., into the gel) for cells cultured on top of polyacrylamide substrates [24,25]. Investigators have also recently used 3D microscopic imaging and finite element based techniques to calculate the 3D traction stress field generated by cells embedded in either polyethylene glycol hydrogels [26] or collagen gels [9] (3D TFM).

TFM is commonly used to evaluate the role of contractile force generation in numerous biological and pathological processes. For example, Hammer and colleagues have used TFM to investigate how substrate stiffness and chemoattractant concentration gradients influence contractile forces during neutrophil migration [12,27,28]. Marinkovic et al. [11] used a high throughput TFM technique to demonstrate that changes in matrix mechanics can regulate the contractile forces generated by lung fibroblasts. Finally, previous investigators have used TFM to investigate how cellular contractility correlates with metastatic potential in cancer cells [8–10]. Interestingly, results in this area are currently contradictory where Kraning-Rush et al. [10] reported increases in cellular traction with increasing metastatic potential while Indra et al. [8] observed a significant decrease in traction stress with increasing metastatic capacity. In addition, Koch et al. [9] reported that noninvasive cells can have higher contractility as assessed by 3D TFM.

Since it is based on measurements of substrate displacements, traction stresses obtained via TFM are not a direct measure of the internal contractile force generated by the cell. Specifically, internal contractile forces must be transmitted through the actin cytoskeleton to focal adhesion sites before they can cause deformation of the surrounding material. Therefore, changes in cytoskeletal stiffness and/or adhesion energy may independently alter force transmission, substrate deformation, and the traction stresses measured during TFM. In addition to stiffness and adhesion, changes in cell morphology/aspect ratio and compressibility may also alter the traction stresses measured via TFM [29]. The goal of this study was to use computational finite element techniques to investigate how changes in several biomechanical and morphological properties influence the traction stress field measured by TFM. Results indicate that cell-substrate adhesion and cell stiffness can significantly alter the traction stress field even for equivalent internal contractile force conditions. In addition to highlighting this potential source of error in TFM measurements, we have also developed a correction factor for TFM that uses measured changes in cell adhesion and cell stiffness to generate a more accurate estimate of changes in internal contractile forces. Finally, we apply this correction factor to TFM measurements obtained in invasive and noninvasive breast cancer cells to demonstrate the utility of this technique.

2 Materials and Methods

2.1 Finite Element Analysis. Three-dimensional finite element models of both 2D and 3D TFM techniques were developed using the ADINA 8.7 Finite Element Package (ADINA R & D,

Watertown, MA). Cell geometries were represented as either half of a prolate spheroid (2D TFM) or a full prolate spheroid (3D TFM) and the baseline geometry had equivalent major and minor axis lengths of 20 μm (i.e., spherical). Two-dimensional TFM was simulated by attaching the cell to the surface of a gel block and applying a symmetrical contractile load to internal surfaces at the cell periphery. Three-dimensional TFM was simulated by embedding the cell in the gel block and applying contractile loads to internal surfaces at the cell periphery. The gel was modeled as a 3D block with a thickness of 100 μm and length and width of 200 μm for the 2D TFM model, or length, width, and thickness of 200 μm for the 3D TFM model. Gel dimensions were chosen such that edge effects were negligible. The cell body and gel volume were meshed with four-noded tetrahedral 3D solid elements using a free-form meshing algorithm. A maximum element edge length of 0.5 μm was specified for the cell volume and gel face to ensure convergence and accuracy of the solution. The average number of elements for the 2D and 3D TFM models was 186,745 $\pm$ 5967 and 436,598 $\pm$ 8363, respectively. Cell-gel adhesions were modeled as linear adhesion elements using ADINA's six-degree-of-freedom spring elements. Adhesion energy density was specified by altering the linear force-displacement relationship spring constant k_{adh} . Adhesion energy was calculated according to

$$E_{\text{adh}} = \frac{1}{2} k_{\text{adh}} d_{\text{rup}}^2 n_s \quad (1)$$

where k_{adh} is the spring constant, $d_{\text{rup}} = 10 \text{ nm}$ is the assumed rupture distance [30], and n_s is the number of adhesion elements. Adhesion energy density was calculated by dividing the total adhesion energy by the surface area of the cell. Spring adhesion elements were meshed between adjacent cell and gel nodes using a nearest-neighbor matching algorithm in MATLAB (MathWorks, Natick, MA).

2.2 Governing Equations and Constitutive Models. For all models, both the cell and the gel were initially modeled as linearly elastic isotropic solids,

$$\sigma_{ij} = \frac{E}{1+\nu} \varepsilon_{ij} + \frac{\nu E}{(1+\nu)(1-2\nu)} \varepsilon_{kk} \delta_{ij} \quad (2)$$

where $\varepsilon_{ij} = 1/2(\partial u_i/\partial x_j + \partial u_j/\partial x_i)$, σ is the Cauchy stress tensor, ε is the strain tensor, E is Young's modulus, ν is Poisson's ratio, δ is the Kronecker δ function, x is the nodal position, and u is the nodal displacement. All equations are written in standard indicial notation. Cell and gel deformations are governed by the stress balance relationship,

$$\frac{\partial \sigma_{ij}}{\partial x_j} = 0 \quad (3)$$

The gel was assumed to be nearly incompressible ($\nu = 0.49$) with $E = 1 \text{ kPa}$, and the cell was initially modeled as slightly compressible ($\nu = 0.4$), with a baseline $E = 1 \text{ kPa}$ (see Table 1).

Although we initially modeled the cell as a linear elastic material, previous studies indicate that cell stiffness can vary with cell contractility [34]. To account for this nonlinearity, additional simulations were performed using a strain-stiffening material

Table 1 Parameter values used in simulations

Parameter	Description	Value range	Baseline parameter value
E	cell stiffness	0.5 to 10 kPa [31]	1 kPa
E_{adh}	adhesion energy density	3 E-9 to 1.5 E-4 J/m ² [32]	3 E-5 J/m ²
σ_c	applied contractile stress	1 to 100 Pa [21,32,33]	10 Pa
AR	cell aspect ratio	1:1 to 4:1	1:1

Note: References cited in table are [21,31–33].

Fig. 1 Representative plots of Young's modulus versus absolute value of strain for the strain-stiffening material model parameters including (a) Young's modulus E_o , (b) slope of strain-stiffening curve m , and (c) strain at which material begins to stiffen ε_o . E_o was varied between 0.1 kPa and 10 kPa, m was varied between 0 kPa and 10 kPa, and ε_o was varied between 0 and 1.

property for the cell. Strain-stiffening was implemented in ADINA as a nonlinear material where E was a function of the first principal strain according to

$$\begin{aligned} E(\varepsilon) &= E_o & \text{for } \varepsilon < \varepsilon_o \\ E(\varepsilon) &= E_o + m * (\varepsilon - \varepsilon_o) & \text{for } \varepsilon \geq \varepsilon_o \end{aligned} \quad (4)$$

where E_o is the small strain Young's modulus, ε is the first principle strain, ε_o is the strain at which the material begins to strain stiffen, and m is the slope of the strain-stiffening curve. In this model, cell stiffness is coupled to the contractility through the resultant strains, such that E increases as cell contraction (and strain) increases. The effect of different strain-stiffening models was investigated by varying E_o , ε_o , and m over a wide range as shown in Fig. 2. Note that strain-stiffening was implemented in both compression and tension, and we plot E as a function of $|\varepsilon|$ in Fig. 1.

Although cells are often assumed to be nearly incompressible, variations in the cell's compressive stiffness due to changes in cytoskeletal organization may have an effect on traction force.

Therefore, we investigated the effect of cell compressibility by varying the bulk modulus of the cell over a wide range while holding the Young's modulus constant at 1000 Pa.

2.3 Loading and Boundary Conditions. For simulations of 2D TFM, a fixed, zero displacement boundary condition was specified on the bottom and side surfaces of the gel while a free surface condition was used at the top surface of the gel including the surface attached to the cell via linear spring adhesion elements. Simulations of 3D TFM utilized fixed, zero displacement boundary conditions on all outer surfaces of the gel and the gel surface in contact with the cell was unconstrained. Cell contraction was simulated by applying a symmetric pressure at the cell periphery, directed toward the center of the cell and oriented along the long axis of the cell (Fig. 1). The baseline contractility load was 10 Pa for both 2D and 3D TFM models. Application of these internal contractile loads resulted in deformation of the cell, the linear adhesion springs, and the surrounding gel. Although experimental TFM techniques convert the measured gel

Fig. 2 Representative 3D finite element mesh and loading vectors for (a) 2D TFM and (b) 3D TFM models. Representative plots of traction stress fields from (c) 2D TFM model gel surface and (d) 3D TFM model gel tractions projected onto cell surface.

Fig. 3 Maximum resultant traction versus applied contractility at different cell stiffness values in the (a) 2D TFM model and (b) 3D TFM model. Net contractile moment versus applied contractility at different cell stiffness values in the (c) 2D TFM model and (d) 3D TFM model.

displacements into traction stresses at the cell surface, this conversion was not necessary in our simulations since the finite element solutions include the traction stresses at the gel surface as a function of spatial location $T_i(r)$. Figure 1 contains an example output of these traction stress fields for the 2D and 3D cases.

2.4 Parameter Variation and Sensitivity Analysis. A parameter variation study was conducted by varying the cell stiffness, cell-gel adhesion energy density, cell contractility, and cell aspect ratio over a wide range about baseline conditions as indicated in Table 1. Models were queried for the resultant maximum traction stress in the gel $T_{\max} = \max [T_i(r)]$ and net contractile moment M_{net} [21].

$$M_{\text{net}} = M_{xx} + M_{yy} + M_{zz} \quad \text{where} \quad (5)$$

$$M_{ij} = \frac{1}{2} \int d^2r (x_i T_j(r) + x_j T_i(r))$$

where r is the position on the gel surface and x is the distance from the cell center of mass to the gel surface element.

A log-scale derivative-based sensitivity analysis was performed to determine how relative changes in the different biomechanical properties (cell stiffness, cell adhesion, contractility, and aspect ratio) influence the $T_{\max}$ and M_{net} . Sensitivities were calculated by determining the maximum slope of the resultant variable ($T_{\max}$ and M_{net}) versus parameter value curves on a log-log scale.

$$\alpha = \frac{\partial \{\log(y)\}}{\partial \{\log(x)\}} = \frac{x}{y} \frac{\partial y}{\partial x} \quad (6)$$

where α is the sensitivity value, $\partial y / \partial x$ is the local slope of the resultant variable versus parameter curve, y is the resultant variable, and x is the parameter value.

2.5 Traction Force Microscopy Measurements. Computational results indicate that changes in cell stiffness and cell-substrate adhesion can significantly impact TFM measurements (see Figs. 3 and 4), and in Sec. 4 we propose a correction factor technique for

TFM measurements that uses these computational results to more accurately assess changes in contractility. To demonstrate the utility of this technique, we have applied this correction to a set of TFM measurements obtained in invasive and noninvasive cancer cells. As described previously [35], silencing a protein involved in membrane repair (myoferlin) in the highly invasive MDA-MB-231 breast adenocarcinoma cell line results in a significant reduction in invasive capacity. For this study, control and myoferlin-deficient (MYOF-KD) MDA-MB-231 breast adenocarcinoma cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum. Polyacrylamide substrates with a Young's modulus of 5.9 kPa, as measured by indentation atomic force microscopy, were prepared on 25 mm glass coverslips using 40 μL of an 8% acrylamide, 0.04% $\text{N,N}'$ -methylene-bis-acrylamide solution (BioRad, Hercules, CA), and embedded with 0.01% of 0.5 μm -diameter red fluorescent carboxylate-modified beads (Invitrogen, Carlsbad, CA). The surfaces of the gels were functionalized using Sulfo SANPAH (Thermo Scientific, Waltham, MA), and coated with 0.2 mg/mL bovine collagen type I (Advanced BioMatrix Inc., San Diego, CA). Cells were seeded sparsely ($\sim 1000/\text{gel}$) and allowed to adhere overnight. Phase contrast images of cell boundaries were obtained for isolated adhered cells, and fluorescent images of bead positions were obtained before and after cell detachment by trypsinization. Image registration and tracking of bead displacements was completed using correlation-based particle image velocimetry (MathWorks). The traction stress field on the gel surface was calculated using the 3D finite element software COMSOL Multiphysics (Burlington, MA) where the measured bead displacements under the cell were applied as a boundary condition, and the resulting stress field was analyzed for $T_{\max}$ and M_{net} according to Eq. (5). We analyzed 10–15 control and MYOF-KD cells.

3 Results

3.1 Effect of Cell Stiffness, Cell-Substrate Adhesion, and Aspect Ratio on TFM Measures. Simulations of 2D and 3D TFM were performed by independently varying cell contractility,

Fig. 4 Maximum resultant traction versus applied contractility at different cell-gel adhesion energy density levels in the (a) 2D TFM model and (b) 3D TFM model. Net contractile moment versus applied contractility at different cell-gel adhesion energy density levels in the (c) 2D TFM model and (d) 3D TFM model.

cell stiffness, cell-gel adhesion energy density, and cell aspect ratio from baseline conditions (see Table 1), and output traction fields from the simulations were analyzed for $T_{\max}$ and M_{net} according to Eq. (5). $T_{\max}$ and M_{net} were then plotted as a function of contractility for different values of cell stiffness and cell-gel adhesion energy density (see Figs. 3 and 4). As expected, increasing the contractile force generated by the cell resulted in a nearly linear increase in $T_{\max}$ and M_{net} . However, as shown in Fig. 3, for both the 2D and 3D TFM models, increasing the cell's Young's modulus (i.e., stiffness) resulted in a lower $T_{\max}$ and M_{net} even when all other parameters including the contractile force were held constant. Therefore, an independent increase in cell stiffness may result in a decrease in $T_{\max}$ and M_{net} , which could be incorrectly interpreted as a reduction in contractility. Similarly, Fig. 4 demonstrates that an independent increase in adhesion energy density (E_{adh}) can result in a dramatic increase in $T_{\max}$ and M_{net} even when contractile force is held constant. This effect is most significant at low adhesion energy density, while increasing adhesion energy density beyond $3\text{E-}6\text{ J/m}^2$ has little effect on maximum traction or contractile moment. This indicates that at low adhesion energy density, traction stresses calculated by TFM may underestimate the actual cell contractile force. Finally, as shown in Fig. 5, in both the 2D and 3D TFM models increasing the cell aspect ratio from 1:1 to 4:1 has little effect on the calculated $T_{\max}$. However, increasing the aspect ratio did result in a small increase in M_{net} especially in the 2D TFM model. However, this dependence of M_{net} on cell dimensions is expected because as the aspect ratio increases, the distance between the applied contractile loads on the cell periphery increases, and this results in an increased moment.

3.2 Sensitivity Analysis. The data in Figs. 3, 4, and 5 clearly indicate that TFM measures of $T_{\max}$ and M_{net} can be altered by changes in cell stiffness and/or cell-gel adhesion energy density even when the cell is exerting the same contractile force. To further quantify the sensitivity of these TFM measures to system parameters, we first plotted the resultant variable (i.e., $T_{\max}$ or

M_{net}) as a function of a given system parameter (i.e., contractility, adhesion energy density, cell stiffness, or aspect ratio) on a log-log scale (see Fig. 6(e) for an example). We then used Eq. (6) to calculate the maximum slope of these relationships to determine a sensitivity value (α) for each variable-parameter combination. As shown in Fig. 6, the sensitivity of $T_{\max}$ and M_{net} to contractile force was $\alpha \sim 1$ in both the 2D and 3D TFM models. This is an expected result and simply indicates that $T_{\max}$ and M_{net} scale linearly with the contractile force generated by the cell. Interestingly, the sensitivity of $T_{\max}$ and M_{net} to adhesion energy density ($\alpha \sim 0.9$) was very high and indicates that these TFM measures are just as sensitive to changes in adhesion energy density as they are to changes in cell generated contractile force. Similarly, in the 2D TFM models the sensitivity of $T_{\max}$ and M_{net} to cell stiffness was $\alpha \sim 0.5$ while in the 3D TFM models the sensitivity of these parameters to cell stiffness was $\alpha \sim 0.7$. Therefore, changes in cell stiffness can also significantly influence TFM measures albeit with a slightly lower sensitivity. Interestingly, the sensitivity of $T_{\max}$ to aspect ratio was very low in both the 2D and 3D TFM models (i.e., $\alpha \sim 0.1$) indicating that changes in cell morphology alone do not significantly influence this TFM measurement.

3.3 Effect of Coupling Cell Stiffness and Contractility. In addition to linear elastic material models for the cell, we also conducted a series of simulations where the cell was modeled as a strain-stiffening material according to Eq. (4). For these studies, 2D TFM was simulated as before, and a parameter variation study was conducted by varying the initial cell stiffness E_0 , the strain at which the material begins to strain-stiffen ε_0 , and the rate of strain stiffening m . As shown in Fig. 7, consistent with the previous linear elastic simulations, increasing E_0 results in a decrease in both $T_{\max}$ and M_{net} . Interestingly, changing m or ε_0 had very little effect on $T_{\max}$ and M_{net} . Additional analysis indicates that this is due to negligible changes in cell deformation when m or ε_0 are varied. Therefore, in this system, potential increases in cell deformation are apparently offset by concurrent increases in stiffness, resulting

Fig. 5 Maximum resultant traction versus applied contractility at different cell aspect ratios in the (a) 2D TFM model and (b) 3D TFM model. Net contractile moment versus applied contractility at different cell aspect ratios in the (c) 2D TFM model and (d) 3D TFM model.

Fig. 6 Sensitivity values for maximum resultant traction in the (a) 2D TFM model and (b) 3D TFM model. Sensitivity values for net contractile moment in the (c) 2D TFM model and (d) 3D TFM model. (e) Representative result-ant variable versus parameter curve for sensitivity calculation.

in little change in the $T_{\max}$ and M_{net} measures. Taken together, the data in Fig. 7 indicate that although the TFM measures of $T_{\max}$ and M_{net} depend on the intrinsic stiffness of the cell, changing the non-linear properties of the cell has a minimal impact on $T_{\max}$ and M_{net} .

3.4 Effect of Cell Compressibility on Measured Traction Stress. As shown in Figs. 8(a) and 8(b), increasing the bulk modulus (decreased cell compressibility) results in a smaller $T_{\max}$. This data is consistent with the expectation that a lack of

Fig. 7 Effect of (a) initial cell stiffness, (b) slope of strain stiffening curve, and (c) strain at which stiffening begins on maximum resultant traction in strain-stiffening models. Effect of (d) initial cell stiffness, (e) slope of strain stiffening curve, and (f) strain at which stiffening begins on net contractile moment in strain-stiffening models. (All data is from 2D TFM simulations).

Fig. 8 (a) Maximum resultant traction versus applied contractility at different cell bulk modulus values. (b) Effect of bulk modulus on maximum resultant traction. Shaded area represents the range of Poisson's ratio for biological cells typically reported in the literature. (All data is from 3D TFM simulations).

compressibility of the cell would inhibit load transfer to the gel. However, the range of bulk moduli shown in Fig. 8(a) is much larger than the range of compressibility one would estimate based on the range of Poisson's ratio ($\nu = 0.3-0.45$) typically reported in the literature for biological cells [36–38]. Considering data only in this range (shaded region in Fig. 8(b)) leads to the conclusion that although very low bulk moduli can lead to increased traction stress, changes in Poisson's ratio across the range typical for biological cells does not significantly influence the T_{max} measurement.

4 Discussion

Traction force microscopy has emerged as a powerful way to evaluate the contractile properties of different cell types in health and disease. The major output of TFM is the traction stresses generated in a deformable substrate/gel as a result of contractile forces generated by actomyosin machinery within the cell. In addition to standard TFM techniques [14,21], several investigators have recently developed advanced TFM techniques that can monitor traction stresses in a high throughput manner [11], during the collective migration of cells in an intact monolayer [4] and in 3D

systems [9,26]. However, the traction stresses obtained by these TFM techniques are an indirect measure of the contractile forces generated within the cell. In this study, we used computational techniques to demonstrate that several biomechanical properties other than contractile force generation, including the cell stiffness and cell-substrate adhesion strength, can significantly alter the traction stresses obtained via TFM. The fact that multiple parameters other than contractility can influence TFM measurements may lead to inappropriate interpretation of experimental results. Therefore, in addition to a biophysical discussion of model results, in this section we also describe the development and application of a correction factor that can be used to more accurately extract changes in cell contractility from TFM measurements.

Nonmuscle mammalian cells contain a network of actin filaments, and ATP driven actomyosin cross-bridges within these structures generate tensile or contractile forces. These contractile forces are transmitted through the cytoskeleton to focal adhesion sites on the cell membrane, which link the cell to the extracellular matrix. Therefore, modulation of contractile force transmission within the cell may alter the amount of traction stresses that are generated in the ECM. Specifically, our results indicate that stiffer

Fig. 9 (a) Measurement of maximum traction in wild-type MD-AMB-231 control cells (WT) and myoferlin-deficient MD-AMB-231 cells. (b) Application of correction factor to $T_{\max}$ data results in an estimated contractile stress that is dramatically lower in the noninvasive MYOF-KD as compared to the invasive WT cells. * is with respect to WT conditions.

cells undergo less deformation for a given amount of contractile force. As shown in Figs. 3, 7(a), and 7(d), this decrease in cell deformation results in less force being transmitted to the surrounding gel and a decrease in the amount of traction stress even for equivalent contractile loading conditions. Similarly, the strength of integrin-collagen binding at focal adhesion sites may alter the amount of force transmitted to the underlying gel substrate. Our results (Fig. 4) confirm that altering the strength of these cell-substrate adhesions can dramatically alter the amount of force transmitted to the gel, where the reduction in measured traction stress at low adhesion energy density is due to greater deformation of the adhesions, resulting in less deformation of the gel and lower measured traction stress. Finally, although a correlation between cell aspect ratio and traction stress has been reported [29], our simulations indicate that changes in cell morphology alone do not significantly alter traction stresses. In summary, our computational models indicate that experimentally observed changes in traction stresses during TFM may not be directly related to changes in contractile force generation. Therefore, accurately assessing changes in contractile force may require evaluation of how other biomechanical properties (i.e., cell stiffness and adhesion strength) change during an experiment.

In support of the concept that other biomechanical properties can influence TFM measurements, consider the recent study by Indra et al. [8]. These authors used TFM to document a decrease in traction stress for cells with increasing metastatic capacity. This is surprising since metastatic cells have increased migration and invasion capacity and would, therefore, be expected to generate larger contractile forces. Importantly, these authors also demonstrated that cell-substrate adhesion strength was lower in the more metastatic cells. Based on our computational results (Fig. 4), this decrease in adhesion strength may have contributed to the lower traction stresses measured in the metastatic cells. Recently, our lab reported that silencing a protein involved in membrane repair, myoferlin, in the highly invasive MDA-MB-231 breast cancer cells resulted in an epithelial phenotype with significantly reduced invasive capacity. We were, therefore, initially surprised that the maximum traction ($T_{\max}$) measured via TFM was only modestly lower in myoferlin-deficient cells as compared to wild-type controls and that this difference in $T_{\max}$ did not achieve statistical significance (Fig. 9(a)). However, additional experiments in our lab indicate that the MYOF-KD cells have increased adhesion strength and decreased cell stiffness compared to wild-type control cells (data not shown). Specifically, a trypsin release assay performed on the xCELLigence system (Roche Applied Science) indicates that MYOF-KD cells have an adhesion strength that is ~ 2 times larger than wild type controls while an atomic force microscopy cell indentation technique [39] indicates that MYOF-KD cells have an average Young's modulus that is ~ 2 times smaller than wild-type controls. As a result, the $T_{\max}$ measured in MYOF-KD cells may be larger than expected due to these increases/decreases in adhesion/stiffness. Below, we seek to develop a simple, albeit quanti-

tative, correction factor that uses information about relative changes in adhesion/stiffness to more accurately determine relative changes in contractile force from the $T_{\max}$ measurement.

Our computational models clearly indicate that $T_{\max}$ is a function of contractile stress σ_c , adhesion energy density E_{adh} , and Young's modulus E (see Figs. 3(a), 3(b), 4(a), and 4(b)). Furthermore, our log-scale sensitivity analyses (Eq. (6) and Fig. 6) indicate that $T_{\max}$ is related to these parameters via

$$T_{\max} = A * \sigma_c * E_{\text{adh}}^{\beta} * E^{-\gamma} \quad (7)$$

where A is a proportionality constant, β is the sensitivity value for adhesion energy density (i.e., $\beta = 0.9$), and γ is the sensitivity for cell stiffness (i.e., $\gamma = 0.5$). Assuming Eq. (7) holds for the two experimental conditions of interest (i.e., control and MYOF-KD), one can derive the following relationship:

$$\sigma_c(2) = \frac{\sigma_c(1)}{T_{\max}(2)} * \left(\frac{E_{\text{adh}}(1)}{E_{\text{adh}}(2)} \right)^{\beta} * \left(\frac{E(1)}{E(2)} \right)^{-\gamma} * T_{\max}(2) \quad (8)$$

where 1 and 2 indicate the values under control and MYOF-KD conditions, respectively. The experiments described above indicate that $E_{\text{adh}}(1)/E_{\text{adh}}(2) = 0.5$ and $E(1)/E(2) = 2$. Since we are interested in monitoring relative changes, we can assume $\sigma_c(1) = T_{\max}(1)$ and then calculate $\sigma_c(2) = 0.38 * T_{\max}(2)$ where $T_{\max}(1)$ and $T_{\max}(2)$ represents the TFM measurements under control and MYOF-KD conditions as shown in Fig. 9(a). As shown in Fig. 9(b), applying this correction factor results in a different interpretation of the original data. Specifically, by correcting for the effect of adhesion and stiffness, the data indicate that the MYOF-KD cells do exhibit a significantly lower contractile stress σ_c than wild-type controls. Given that the MYOF-KD are less migratory/invasive, this data with respect to σ_c is more consistent with our original expectation that less-invasive cancer cells exert smaller contractile forces as compared to highly-invasive cells.

As with all computational simulations of complex biological systems, the current models contain limitations that could be addressed in future studies. First, we have assumed idealized prolate spheroid cell geometries in this study, and thus, the role of complex cell morphologies on TFM measures was not investigated. Future studies could utilize imaged-based models of cell morphology [40,41] to address this limitation. Second, it is now well recognized that cells are complex viscoelastic structures that exhibit soft-glassy or power-law rheology over many time scales [42]. In addition, previous investigators have implemented time-dependent TFM to monitor the temporal evolution of traction stresses [22]. Therefore, future studies could implement power-law constitutive relationships in the finite element simulations [43] to investigate the effect of cell-substrate adhesion and cell stiffness on the temporal nature of traction stresses. Finally,

although we developed a simple correction factor to extract relative changes in contractile force from TFM measurements, this correction requires separate experimental measurements of changes in adhesion strength and cell stiffness. Typically, these other biomechanical assays for adhesion and stiffness are performed on different microscopy platforms. However, the development of an integrated microscopy platform that is able to obtain TFM, atomic force microscopy stiffness measurements, and cell adhesion measurements on the same cell in one platform would lead to a more accurate assessment of cell biomechanical properties.

In conclusion, we have used computational modeling techniques to demonstrate that factors other than contractile force generation can influence the measurement of traction stresses during TFM. Specifically, models indicate that increases in cell stiffness can independently lower the maximum traction $T_{\max}$ and net contractile moment M_{net} obtained during TFM. In addition, increases in cell-substrate adhesion can dramatically increase the $T_{\max}$ and M_{net} measures. As a result, changes in these biomechanical parameters may lead to inappropriate interpretation of TFM results. We also used our computational results and results from a sensitivity analysis to develop a simple, quantitative correction factor that used experimentally measured changes in adhesion and stiffness to “correct” the TFM measures and provide a more accurate assessment of relative changes in contractile stress generation. This correction factor was applied to TFM measurements in invasive and noninvasive breast cancer cells to demonstrate the utility of this correction technique. This study has, therefore, provided the cell mechanics community with an effective way to more accurately interpret TFM measurements.

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