Contractile stresses in cohesive cell layers on finite-thickness substrates

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Using a minimal model of cells or cohesive cell layers as continuum active elastic media, we examine the effect of substrate thickness and stiffness on traction forces exerted by strongly adhering cells. We obtain a simple expression for the length scale controlling the spatial variation of stresses in terms of cell and substrate parameters that describes the crossover between the thin and thick substrate limits. Our model is an important step towards a unified theoretical description of the dependence of traction forces on cell or colony size, acto-myosin contractility, substrate depth and stiffness, and strength of focal adhesions, and makes experimentally testable predictions.

Many cell functions, such as spreading, growth, differentiation and migration, are affected by the elastic and geometric properties of the extracellular matrix \([1]\). Considerable effort has been devoted to the study of cell adhesion to elastic substrates \([2]\). Cells adhere to a substrate via focal adhesion complexes that link the substrate to the actomyosin cytoskeleton, which in turn generates contractile forces that deform soft substrates \([3]\). The traction forces that the cell exerts on the substrate are regulated by the cell itself in a complex feedback loop controlled by cell activity and substrate elasticity.

Two powerful experimental techniques have been developed to measure forces by cells on substrates: traction force microscopy, used to probe cell adhesion to continuous substrates \([4,5]\), and the imaging of cell-induced bending of microfabricated pillar arrays \([6]\). These two techniques have also been recently combined \([7]\). These experiments have yielded new insight on substrate rigidity sensing and have opened up new questions on the physics of individual and collective cell adhesion: What controls the length scale that governs the penetration of traction forces? What is the relative role of active cellular contractility and cell-cell-interaction in controlling the emergent response of cell layers? In this Letter we describe minimal models of individual cells and adhering cell colonies that reproduce qualitatively several experimental findings. The traction stresses exerted by cells on substrates are extracted directly from measurements of micropillar displacements or inferred from the displacements of fiducial markers embedded in a continuum substrate. It is found that traction stresses by isolated fibroblasts and epithelial cells on pillar arrays are localized near the cell edge, while contractile stresses (referred to below as cellular stresses) built-up inside the cellular material is largest near the cell center \([4,8]\), as shown schematically in Fig. 1. This behavior, also observed in adherent cell sheets and in migrating cell colonies \([9,11]\), is predicted by our model. Further, both substrate thickness and stiffness affect cellular and traction stresses \([12]\). The magnitude of the traction stress increases with substrate stiffness, saturating at large stiffness \([8]\), and it decreases sharply with substrate thickness, indicating that

\[
\sigma_{\text{max}} \propto \frac{1}{h_c} \text{ for } h_c \ll L
\]

![FIG. 1. Schematic of a cell layer of lateral extent L and thickness h, adhering to a substrate of thickness h_s. The build-up of contractile stress \(\sigma\) in the cell layer is indicated by the color map, while the traction stresses in the substrate are shown as vectors (blue online). The spatial variation of both traction and cellular stresses in the lateral (x) direction are characterized by the length scale \(\ell_p\), referred to as the penetration length.](image)

Our model builds on recent work \([14,15]\) describing the cell or cell layer as a contractile elastic medium, with local elastic response of the substrate (as appropriate for micropillar arrays or very thin substrates). In contrast, here we consider substrates of finite thickness where the non-locality of the elastic response must be included. While previous studies have analyzed the deformations of finite-thickness substrates due to point traction forces on their surface \([16,17]\), our work considers the inhomogeneous traction due to an extended contractile cell layer. A central result for our work is the expression for the scaling parameter referred to as the lateral penetration length \(\ell_p\) (Fig. 1). This length scale characterizes the in-plane spatial variations of both adhesion-induced traction stresses on the substrate and cellular stresses within the cell layer in terms of cell and substrate elastic and geometrical properties. Our model also quantifies the experimentally-observed role of substrate thickness \(h_s\) in controlling the
Bottom: Cell contraction $\Delta \ell^c$, showing strain stiffening (right). For simplicity we define with respect to an initial state where the cell has length $\ell_0$. The net contraction is then $\Delta \ell = \ell_0 - \Delta x = u_1 - u_2$. The traction force by the cell on the substrate is localized at $x_1$ and $x_2$, yielding a traction force density $f_T(x)$ assuming linear elasticity, the substrate deformation is given as a function of substrate thickness.

For a substrate of thickness $h_s$ we use the approximate form:

$$G(x) = \frac{2}{\pi \ell_0 E_s} K_0 \left[ \frac{a + |x|}{h_s (1 + \nu_s)} \right]$$

derived in the Supplemental Material [27], with $a$ the size of adhesion complexes, providing a short-distance cut-off, and $K_0$ denotes the modified Bessel function of the second kind. We obtain $F_T(\Delta \ell) = \frac{1}{2} k_{\text{eff}} (\Delta \ell) \Delta \ell$, with $k_{\text{eff}}^{-1} = k_0^{-1} + [G(0) - G(\ell_0)]$ the effective stiffness of the cell-substrate adhesions. For $\Delta \ell \ll \ell_0$, $k_{\text{eff}}$ is independent of $\Delta \ell$ and $F_T$ scales linearly with $\Delta \ell$. Stiffening sets in for $\Delta \ell > \ell_0 |1 - h_s (1 + \nu_s)/\ell_0|$, as shown in Fig. 2 (top right) with a crossover controlled by the thickness of the substrate $h_s$. Using $F_T = F_A - k_s \Delta \ell$, we solve for both $\Delta \ell$ and $F_T$, shown in Fig. 2 (bottom) as functions of the substrate thickness and stiffness. For very thin ($h_s \to 0$) or infinitely rigid substrates, the substrate elasticity becomes local, $\Delta \ell = F_A/(k_s + k_c/2)$, corresponding to a spring $k_s$ in parallel with a series of two focal adhesions springs $k_c$. In this limit the traction force saturates to $F_T = k_A F_A/(2k_c + k_a)$. Conversely, for a very soft substrate with $E_s \to 0$, the contraction is maximal and given by $F_A/k_c$, and $F_T \to 0$. The substrate thickness above which both cell contraction and traction force saturate is controlled by the cell size and the substrate elasticity, in qualitative agreement with experiments.

**Contractile cell on a soft substrate.** To illustrate the importance of substrate nonlocality, we first analyze a single cell, modeled as a contractile spring of stiffness $k_c$ and rest length $\ell_0$, adhering to a continuum substrate (described as an elastic continuum of Young’s modulus $E_s$ and Poisson’s ratio $\nu_s$) via two focal adhesion bonds (linear springs of stiffness $k_a$) located at $x_1$ and $x_2$ (Fig. 2 top left) [23]. This is motivated by the experimental observation that in adhering cells focal adhesions tend to be localized near the cell periphery [24]. For simplicity we consider a one dimensional model, where the cell lies on the $x$ axis and the substrate lies in the $0 \leq z \leq h_s$ region of the $xz$ plane. Contractile acto-myosin fibers connect the focal adhesions and exert active forces of magnitude $F_A$. Once the cell has fully adhered, the cell-substrate system is in mechanical equilibrium. Force balance at $x_1$ and $x_2$ yields

$$k_a [u_1 - u^a(x_1)] = F_A - k_c (u_1 - u_2),$$

$$k_a [u_2 - u^a(x_2)] = -F_A + k_c (u_1 - u_2),$$

with $u_i$ the displacements of the contact points $x_i$ from their unstretched positions $\Delta x_i = \ell_0$, and $u^a(x_i)$ the displacement of the substrate’s surface at $x_i$. All displacements are defined with respect to an initial state where the cell has length $\ell_0$. The net contraction is then $\Delta \ell = \ell_0 - \Delta x = u_1 - u_2$. The traction force by the cell on the substrate is localized at $x_1$ and $x_2$, yielding a traction force density $f_T(x)$. Assuming linear elasticity, the substrate deformation is given as a function of substrate thickness.

$$G(x) = \frac{2}{\pi \ell_0 E_s} K_0 \left[ \frac{a + |x|}{h_s (1 + \nu_s)} \right]$$

derived in the Supplemental Material [27], with $a$ the size of adhesion complexes, providing a short-distance cut-off, and $K_0$ denotes the modified Bessel function of the second kind. We obtain $F_T(\Delta \ell) = \frac{1}{2} k_{\text{eff}} (\Delta \ell) \Delta \ell$, with $k_{\text{eff}}^{-1} = k_0^{-1} + [G(0) - G(\ell_0)]$ the effective stiffness of the cell-substrate adhesions. For $\Delta \ell \ll \ell_0$, $k_{\text{eff}}$ is independent of $\Delta \ell$ and $F_T$ scales linearly with $\Delta \ell$. Stiffening sets in for $\Delta \ell > \ell_0 |1 - h_s (1 + \nu_s)/\ell_0|$, as shown in Fig. 2 (top right) with a crossover controlled by the thickness of the substrate $h_s$. Using $F_T = F_A - k_s \Delta \ell$, we solve for both $\Delta \ell$ and $F_T$, shown in Fig. 2 (bottom) as functions of the substrate thickness and stiffness. For very thin ($h_s \to 0$) or infinitely rigid substrates, the substrate elasticity becomes local, $\Delta \ell = F_A/(k_s + k_c/2)$, corresponding to a spring $k_s$ in parallel with a series of two focal adhesions springs $k_c$. In this limit the traction force saturates to $F_T = k_A F_A/(2k_c + k_a)$. Conversely, for a very soft substrate with $E_s \to 0$, the contraction is maximal and given by $F_A/k_c$, and $F_T \to 0$. The substrate thickness above which both cell contraction and traction force saturate is controlled by the cell size and the substrate elasticity, in qualitative agreement with experiments [12].
where $Y_a = k_a/(L\ell_a)$ describes the effective strength of the focal adhesions, $u(x)$ is the displacement field of the cellular medium at $z = h_s$, and $\sigma$ is the thickness-averaged cellular stress tensor, $\sigma(x) = 1/\ell_a h_s^{h_s + \epsilon} \int dz \sigma(x, z)$, given by $\sigma(x) = B_c \partial_x u + \sigma_a$, with $B_c$ the longitudinal elastic modulus of the cell layer.

The one-dimensional model presented here may be relevant to wound healing assays, where the cell layer is a strip with y-translational invariance. Although we have neglected components of the cellular displacements and spatial variations along $z$, the cell elastic constants are those of a three-dimensional cellular medium. The active stress $\sigma_a = F_a/(L h_c)$ arises from acto-myosin contractility [28]. The substrate deformation at the surface is

$$u^s(x) = h_c \int dx' G(x - x') \partial_x \sigma(x'),$$

with $G(x)$ the elastic Green's function of a substrate of infinite extent in $x$, occupying the region $0 \leq z \leq h_s$, evaluated at $z = h_s$. Eqs. (3) can be reduced to integro-differential equations for the cellular stress, as

$$\ell_a^2 \partial^2 \sigma + \sigma_a = -B_c h_s \partial^2_x \int_0^L dx' G(|x - x'|) \sigma(x').$$

The length scale $\ell_a = \sqrt{B_c h_s/Y_a}$ controls spatial variations of cellular stresses induced by the stiffness of the focal adhesions. It is the size of a region where the areal elastic energy density $Y_a \ell_a^2$ associated with focal adhesions is of order of the areal elastic energy density $B_c h_s$ of the cell layer. For a cell monolayer with $B_c = 1$ kPa, $h_c = 0.1 \mu m$, $L = 100 \mu m$, $\ell_a = 10 \mu m$ and $k_a = 2.5 nN/\mu m$ [5], we get $\ell_a \approx 6.3 \mu m$, comparable to traction penetration length seen in experiments on stiff microposts [29] [30]. The second term on the right hand side of Eq. (5) describes spatial variations in the cellular stress due to the (generally nonlocal) coupling to the substrate. In the following we examine solutions to Eq. (5), considering various limiting cases for the substrate thickness and analyze the dependence of traction stresses on cell size, substrate stiffness and substrate depth. The equation governing stress distribution in two dimensional cell layers is derived in the Supplemental Material [27].

Thin substrate. If the substrate’s elastic response can be approximated as local, as it is the case for $h_s << L$ or for cells on micropillar arrays, the Green’s function is given by $G(x) = \frac{2 h_s (1+\nu_s) \delta(x)}{L \ell_a}$. Eq. (5) can then be written as $\ell_a^2 \partial^2 \sigma + \sigma_a = \sigma$, where, $\ell_p = \sqrt{B_c h_s / Y_a}$ and $Y_a^{-1} = Y_a^{-1} + 2 h_s (1+\nu_s)/E_s$ describes the combined action of the focal adhesions and the substrate, acting like two linear elastic components in series. Assuming zero external stresses at the boundary, i.e., $\sigma(0) = \sigma(L) = 0$, the internal stress profile is $\sigma(x) = \sigma_a (1 - \cosh [(L-2x)/2\ell_p]/ \cosh [L/2\ell_p])$ [10] [14] [15]. The traction stress $T(x) = Y_a \epsilon_{uu}(u)$, is localized within a length $\ell_p$ from the edge of the cell layer. The penetration length $\ell_p$ can be written as $\ell_p = \sqrt{\ell_a^2 + \ell_s^2}$, with $\ell_s = \sqrt{\frac{2B_c h_s h_c}{E_s (1+\nu_s)}}$ the square root of the ratio of the cell’s elastic energy to the elastic energy density of the substrate. This form highlights the interplay of focal adhesion stiffness and substrate stiffness in controlling spatial variation of stresses in the lateral ($x$) direction. The two act like springs in series, where the weaker spring controls the response. If $Y_a << \frac{E_s}{2(1+\nu_s)h_c}$, then $\ell_p \approx \ell_a$ and the stiff substrate has no effect. Conversely, if the focal adhesions are stiffer than the substrate, then $\ell_p \approx \ell_s$. For an elastic substrate with $h_s = 10 \mu m$, $\nu_s = 0.4$ and $E_s$ in the range 0.01 – 100 kPa, $\ell_s$ lies in the range 0.2 – 17 $\mu m$. This leads to typical values of $\ell_p$ in the range 6.3 – 18 $\mu m$ for a cell layer of length 100 $\mu m$, consistent with experimentally observed traction penetration lengths on thin continuous substrates [10] and on micropillar posts [9].

Infinitely thick substrate. If $h_s >> L$, the substrate Green’s function can be approximated as that of an elastic half plane, $G(x) = \frac{-\gamma L}{\pi} \frac{\log(|x|/L)}{|x|}$, with $\gamma$ the Euler constant [31]. The solution of Eq. (5) with boundary conditions $\sigma(0) = \sigma(L) = 0$ can be obtained by expanding $\sigma(x)$ in a Fourier sine series as, $\sigma(x) = \sum_{n=1}^{\infty} \sigma_n \sin(n \pi x/L)$ and solving the coupled algebraic equations for the Fourier amplitudes $\sigma_n$ given in the Supplementary Material [27]. The effect of the nonlo-
cal elasticity of the substrate is controlled by yet another length scale \( \ell_{s,\infty} = \sqrt{\frac{4B_h h_s L}{\pi E_s}} \) that can be obtained from the length \( \ell_s \) introduced in the case of thin substrate by the replacement \( h_s \rightarrow L \) and \((1 + \nu_s) \rightarrow 2/\pi\). This highlights the known fact that cells or cell layers only “feel” the substrate up to a thickness comparable to their lateral size \( L \). For parameter values quoted in the preceding paragraphs, \( \ell_{s,\infty} \) takes values between \( 0.35 - 35 \mu \text{m} \) for \( E_s \) in the range \( 0.01 - 100 \text{kPa} \), indicating that the thin/thick substrate crossover, although not observable in isolated cells, should be seen experimentally in cohesive cell layers where the lateral extent can exceed \( 100 \mu \text{m} \). The cellular stress and substrate displacement profiles obtained numerically by summing the Fourier series are shown in Fig. 3 (top). The lateral variation of stresses is now controlled by cell layer size, as in the experiments of \[10\]. The model can be extended to incorporate the effects of cell polarization, spatial variations in contractility, heterogeneities in the cell layer or anisotropic elasticity of the substrate.

Discussion. In summary, we have examined the dependence of traction stresses in adhering cell layers on the mechanical and geometrical properties of the substrate. Using a generic non-local model, we provide analytical results for the effect of cell and substrate properties on the stress penetration length, that can be tested in experiments. Although the analysis presented here is restricted to one dimensional layers, isotropic planar cell layers with spherical symmetry can also be considered analytically \[33\], with similar predictions for the dependence of traction fields and their moments on substrate mechanical and geometrical properties. The scaling of traction moments on cell layer size is, however, different in two dimensions \[10\]. The model can be extended to encompass the effects of cell polarization, spatial variations in contractility, heterogeneities in the cell layer or anisotropic elasticity of the substrate.

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To enable a direct comparison between the penetration lengths obtained below and experimentally accessible parameters, $E_s$ is the Young modulus of a three dimensional elastic medium.

See Supplemental Material at [URL will be inserted by publisher] for derivation of substrate Green’s function, Integro-differential equation governing cellular stress distribution, and corresponding equations for two dimensional cell layers.