Rigidity sensing explained by active matter theory

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The magnitude of traction forces exerted by living animal cells on their environment is a monotonically increasing and approximately sigmoidal function of the stiffness of the external medium.

This observation is rationalized using active matter theory: adaptation to substrate rigidity results from an interplay between passive elasticity and active contractility.

MODEL

Active matter theory formulates constitutive equations that take into account viscoelasticity, activity, the polar nature of cytoskeletal biopolymers [13], and respect the principles of linear irreversible thermodynamics. In one spatial dimension, we write the stress of an homogeneous, elastic and contractile material as the sum of an elastic and an active contribution: $\sigma = \sigma^{el} + \sigma_{A}$. The active stress $\sigma_{A} > 0$ is proportional to $\Delta \mu$, the difference of chemical potential between products and reagents of the chemical reaction responsible for mechanochemical transduction (ATP hydrolysis): $\sigma_{A} = \zeta \Delta \mu$, where $\zeta$ is a material parameter of the cytoskeleton [14]. For simplicity, we restrict our description of the cytoskeleton to the linear regime, and include:

(i) elasticity, described by a linear spring of length $l_{C}(t)$ at time $t$, rest length $l_{C}^{0}$ and spring constant $k_{C}$;

(ii) activity, modeled by an active force $F_{A} = \sigma_{A} S$ across a section of area $S$.

In the nonlinear regime, elastic moduli may also depend on activity, as hypothesized in [12]. Assuming, as in [17], that the rest length of elastic cytoskeletal structures is time-dependent due to motor activity is also beyond the scope of the linear regime that we consider here.

The extra-cellular environment is represented by a linear spring of length $l_{ext}(t)$ at time $t$, rest length $l_{ext}^{0}$ and spring constant $k_{ext}$ (see Fig. [1]). The traction force $F(t)$ exerted by the cell on its environment reads:

$$ F(t) = k_{ext} \left( l_{ext}(t) - l_{ext}^{0} \right). \quad (1) $$

The sign is chosen as $F > 0$ for contraction. Under usual experimental conditions, the total length $l_{tot}$ of the system (cell + substrate) is constant: $l_{tot} = l_{C}(t) + l_{ext}(t)$. The force balance equation reads:

$$ -F(t) + k_{C} \left( l_{C}(t) - l_{C}^{0} \right) + F_{A} = 0. \quad (2) $$

In the context of adhesion-dependent mechanosensing, a simple 'two-spring model' was introduced in [1], predicting that stiffer environments lead to stronger traction forces. A 'three-spring model' was later proposed in order to explain the stiffness-dependent orientation of stress fibers in adherent cells [12], where contractility modulates cytoskeletal stiffness via a phenomenological polarizability coefficient. In this Letter, we formulate and solve a simpler model derived from active matter theory, a generic description of living matter where the mechanochemical transduction due to molecular motors (activity) plays a central role [13, 14]. We obtain a (static) force-rigidity relationship that agrees well with experimental data. We give an expression of the (dynamic) loading rate, expected to be valid on time scales too short for cytoskeletal remodeling and protein recruitment to occur ($t \ll 10^2$ s [12, 16]).
The amplitude of the traction force at equilibrium reads:

\[ F_{\text{eq}} = F_{\text{Sat}} \frac{k_{\text{ext}}}{k_{\text{ext}} + k_C}. \]  

(3)

When \( k_{\text{ext}} \gg k_C \), the traction force saturates to \( F_{\text{Sat}} \):

\[ F_{\text{Sat}} = F_A + k_C \left( l_{\text{ext}} - l_C^0 - l_C^0 \right), \]  

(4)

the sum of \( F_A \) and of residual stresses \( k_C(l_{\text{ext}} - l_C^0 - l_C^0) \). We expect the ensemble average of residual stresses to cancel: \( < F_{\text{Sat}} > = F_A \). As long as \( k_{\text{ext}} \ll k_C \), the traction force is a linear function of \( k_{\text{ext}} \): \( F_{\text{eq}} \approx (F_{\text{Sat}}/k_C) k_{\text{ext}} \).

Experimentally, a wide range of rigidities \( k_{\text{ext}} \) can be obtained when the substrate is a dense array of cylindrical elastomer micropillars whose stiffness depends on their radius and height. Depending on the coating protein, traction forces are transmitted through integrin-mediated adhesions (with fibronectin) or through cadherin-mediated adhesions (with N-cadherin). Fig. 2 show that, in both cases, experimental data are well fitted by the force-rigidity relation Eq. (3). Note that the fitted values of saturation forces \( F_{\text{Sat}} \) were not observed: they correspond to values of \( k_{\text{ext}} \) so large that the deflections of pillars would fall below the experimental spatial resolution.

The data encompasses three cell types, Madin-Darby Canine Kidney (MDCK) cells, 3T3 fibroblasts, and C2 mouse myogenic cells. Cytoskeletal organisation and adhesive properties of the cells vary substantially according to the type of adhesions and the range of substrate rigidity. More diffuse cortical actin dominates when the environment is softer, whereas actomyosin bundles are preferentially formed at higher rigidities. In all cases, our simple model captures the essence of the force-rigidity dependence, and sums up biological variation into two quantitative parameters, the asymptotic traction force \( F_{\text{Sat}} \) and the cytoskeletal stiffness \( k_C \). The order of magnitude of the saturation force \( F_{\text{Sat}} \sim 10 \text{nN} \) corresponds to an active stress of the order of \( 10^4 \text{ Pa} \). We obtain this value upon neglecting possible residual stresses, and using \( \sigma_A \sim F_{\text{Sat}}/S \), where the section \( S \) of a micropillar is of order \( S \sim 1 \mu \text{m}^2 \). The cytoskeletal rigidity is of the order of \( 10^2 \text{nN/\mu m} \), corresponding to elastic moduli of the order of \( 10^2 \text{ Pa} \), a value intermediate between moduli typical of cortical actin and of stress fibers (we used \( E \sim k_C \) for \( d/S \) with \( d \sim 1 \mu \text{m} \)).

When cells are treated with blebbistatin, an inhibitor of contractility, the value of \( F_{\text{Sat}} \) is halved (Fig. 2B). Inspection of Eq. (4) suggests that traction forces remain non-zero due to residual activity of myosin motors, as proposed in 9, or to non-zero residual stresses, or to a combination of both effects. We note that the value of \( k_c \) is almost unchanged. However, assuming as in 12 that activity modulates cytoskeletal stiffness via a polarizability coefficient \( \alpha \) that leads to a ratio \( F_{\text{eq}}/F_{\text{Sat}} = k_{\text{ext}}/(k_{\text{ext}} + (1 + \alpha) k_C) \) that depends through \( \alpha \) on cytoskeletal contractility. Experimental data 9 suggests that \( F_{\text{eq}}/F_{\text{Sat}} \) is independent of the level of contractility, in agreement with our prediction, Eq. (4).

**DYNAMICS**

The loading rate exerted by T cells immediately after receptor engagement with a model antigen-presenting cell was measured thanks to a biomembrane force set-up where micropipette aspiration controls the external rigidity, and found to be linear in \( k_{\text{ext}} \). Motivated by this result, we turn to the dynamics of traction forces, and modify Eq. (4) by taking into account internal protein friction in a linear force-velocity relationship:

\[ F_A(t) = F_S + \xi \frac{dC}{dt} \]

(5)

where \( F_S \) is the stall force, and \( \xi \) is a friction coefficient. Eliminating other variables in Eq. (4), we obtain a differential equation for the traction force:

\[ \frac{dF}{dt} + \frac{F}{\tau} = \frac{F_{\text{eq}}}{\tau} \]

(6)

with a viscoelastic time \( \tau = \xi/(k_{\text{ext}} + k_C) \). Integration from an initial time \( t_0 \) with initial force \( F(t = t_0) = F_0 \) gives

\[ F(t) = F_{\text{eq}}(F_0 - F_{\text{eq}}) e^{-(t-t_0)/\tau} \]  

For zero initial
force, we find that the initial loading rate is proportional to the substrate rigidity:

\[
\frac{dF}{dt} \mid_{t=t_0} \approx \frac{F_{\text{Sat}}}{\xi} k_{\text{ext}},
\]

in agreement with [20], where the initial time \( t_0 \) is set when pulling starts so that \( F^0 = 0 \). We checked that Eq. (7) still holds if we replace the linear force-velocity equation (5) by Hill’s equation [22]. Since \( F_{\text{Sat}} \) is a function of activity, we predict that the loading-rate-rigidity data will be modified upon treatment with contractility agonists and antagonists.

CONCLUSION AND OUTLOOK

At low external rigidity, cell traction forces increase linearly with the stiffness of the substrate. Their constant ratio was first interpreted as a displacement regulated by the cell [3]. We show that regulation is not necessary to explain the force-rigidity relationship. Within the framework of linear irreversible thermodynamics, we propose a minimal model whose consequences are consistent with available experimental data. We predict that both the saturation force \( F_{\text{Sat}} \), exerted for large stiffness, and the constant displacement \( F_{\text{Sat}}/kC \), observed at low stiffness, depend upon the contractility level. Our description is relevant for several types of adhesive structures and cytoskeletal organisation. Unlike [11], we ignore the dynamics of adhesive contacts through which force is transmitted to the substrate. Other monotonically increasing functions of stiffness that depend on two parameters also fit the same experimental data: it is our hope that this work will foster further quantitative experiments to confirm – or disprove – our model.

To treat the dynamics of traction forces, we include internal friction, and obtain an initial loading rate proportional to external stiffness, as observed experimentally [20]. In accord with single-cell rheology assays [6,7], the loading rate \( dF/dt \) responds instantaneously to variations of \( k_{\text{ext}} \).

The merit of our analysis is to show that the simplest equations of active matter dictated by symmetry and conservation laws are sufficient to describe a behavior which might seem to require a more elaborate regulation at first sight. This suggests that other features such as stress fiber diameter and equilibrium with the rest of the actin-myosin system could be described within the general framework of active gels. We hope that extensions of our model will allow to understand quantitatively how more complex cell processes depend on extracellular rigidity. Including membrane elasticity and cortical tension in an appropriate geometry may explain why the initial loading rate exerted by T cells upon receptor engagement saturates for stiffer environments [20]. The dynamics of wetting of the microplate by the cell must be taken into account to describe traction forces exerted during cell spreading [2,6]. Finally, biochemical signaling, protein recruitment and remodeling of adhesive and cytoskeletal structures act over longer time scales [12,10] and may enhance the mechanical effects described here.

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