Interaction of a migrating cell monolayer with a flexible fiber

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(Dated: June 5, 2020)

Mechanical forces influence the development and behavior of tissues. This creates the need to model tissue interaction with the surrounding elastic bodies that exert these forces, raising the question: which are the minimum ingredients needed to describe such interactions? We conduct experiments where migrating cell monolayers push on carbon fibers as a model problem. Based on these observations we develop a minimal active-fluid model that reproduces the experiments and predicts quantitatively relevant features of the system. This minimal mechanotransductive model points out the essential ingredients needed to describe tissue-elastic solid interactions: an effective inertia and viscous stresses.

The forces applied by or on a living tissue have a strong impact on its behavior and development. For instance, during embryogenesis, the forces exerted by individual cells influence differentiation, migration and proliferation at the tissue level, which in turn contributes to shape the future organism. Forces also regulate wound healing. In one of the stages of wound repair a cell monolayer migrates until it encounters another tissue that halts the monolayer’s motion by applying a pressure, which is known as contact inhibition. Forces are important not only in the development of healthy tissue, but also in the origin and progression of some diseases, notably cancer. All these situations have in common that the tissue exchanges forces with external constraints whose mechanical or geometrical properties in turn affect its behavior.

The importance of mechanical forces in biological tissues has motivated the development of a large variety of measurement techniques able to work in realistic physiological conditions. Generally speaking, these techniques rely on the deformation of an elastic body of known properties. For instance, Campús et al. proposed to use a drop embedded in a three-dimensional tissue. Different authors have refined this procedure and made it more reproducible by replacing the drop by elastic beads. Moreover, beads can be used not only to probe forces, but also to exert them, which allow a direct quantification of the tissue’s mechanical properties.

Although force measurements are informative in themselves, their physical and biological interpretation benefits from theoretical mechanical models which, in turn, feed on experimental measurements for their validation and tuning. Many of these models describe the tissue as fluid- or solid-like active-matter characterized by fields such as velocity, normal and shear stress, or cell density. These quantities obey conservation laws which are expressed as partial differential equations.

To reduce the complexity of the models, an important question is: what are the essential effects that need to be modeled to describe a certain behavior? For instance, phenomena as complex as the appearance of mechanical waves have been explained by continuum models that take into account the exchange between elastic energy and cell polarity, which can be interpreted in this context as a gradient in the concentration of contractile molecules.

To offer an answer to the question of which are the minimum ingredients needed to model the mechanical interaction of a tissue with a flexible object we propose a relatively simple, and thus easy to control, configuration: a migrating epithelial cell monolayer pushing a slender millimetric-long carbon fiber fixed at one of their ends. Despite the relative simplificy of the system, a migrating cell monolayer is commonly used in biophysics as a model problem for more complicated phenomena taking place in wound healing or embryogenesis. Moreover, besides the fundamental interest of these experiments to aid the development of theoretical active matter models of biological tissues, the configuration we choose has potential applications in the development of an inexpensive technique that can be used to probe the force exerted by a migrating three-dimensional tissue, as will be discussed below.

**Force exerted by the migrating monolayer** – We conduct experiments in which a monolayer of skin epithelial cells migrates to close a wound, which we produce artificially by using a stencil to gently remove part of the monolayer at the start of the experiment. At a fixed distance from the stencil, we glue one of the ends of a carbon...
FIG. 1. Time lapse showing the progress of a migrating monolayer interacting with a flexible carbon fiber. The $x$ axis denotes the direction of migration, whereas the $y$ axis runs parallel to the initial, undeformed, fiber. (a),(b) and (c) denote the three regions used to obtain the kymographs shown in Fig. 3 and Fig. 6 of the Supplemental Material. Scale bar: 1000 µm.

The most salient feature of these curves is the existence of a maximum force, and thus deflection, after which the fiber recoils. This maximum is very prominent in the shorter fibers and is barely noticeable in the longer ones, its value not being very different from the asymptotic deflection reached at long times. The recoil of the fiber and the decay of the force start to take place when the cell monolayer stops, as can be inferred from kymographs like the one shown in Fig. 3. These kymographs display the time evolution of the magnitude of the streamwise velocity, $u(x, t)$, (color scale) at the three different vertical lines indicated in Fig 1 as a, b and c. To correlate the time evolution of the velocity with the fiber deflection, we show the position of the fiber at each location with a thick white line.

Model formulation – Modelling in full the motion of the cell monolayer is a very complicated task that lies beyond the scope of this work. Instead, we propose here a minimal model that captures the physics of the interaction of the cell monolayer with a flexible fiber that opposes its motions. The one-dimensional model proposed here treats the cell monolayer as a compressible active fluid with velocity field $u(x, t)$. The model incorporates the effect of an effective inertia, Newtonian viscous stresses and a Hookean linear force exerted by the fiber on the tissue. Under these assumptions, momentum conservation reads

$$m \left( \frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} \right) = -k x_f \delta (x - x_f) + \mu \frac{\partial^2 u}{\partial x^2} \quad (2)$$

The left-hand side of the equation, equivalent to an inertia, accounts for the resistance of a cell to change its state of motion \[16\]. The coefficient $m$ regulates the importance of this effect. Note that this effective inertia has a very different origin from the real (i.e. mechanical) one, which is always negligible in cell mechanics \[12\]. We must also point out that we have used the material derivative $D/Dt = \partial/\partial t + u \partial/\partial x$, since it represents the time derivative of a flow property computed following a migrated cell \[19\]. The elastic force exerted by the fiber is proportional to a characteristic deflection, $x_f$, times a proportionality constant $k$, the bending stiffness. This deflection could be, for instance, that at the tip. From equation \[1\] it is possible to infer that $k = \partial f_0/\partial x_f \sim EI/L^4$. Finally, the last term on the right-hand side accounts for viscous stresses. These arise from the friction of the cells with the substrate and between them \[12\].

Equation \[2\] needs two boundary conditions. One is going to be imposed a distance $\ell$ upstream of the initial position of the fiber, i.e. at $x = -\ell$. It is reasonable to assume that, sufficiently far from the fiber, the velocity of the cells does not any longer depend on the position, thus $\partial u/\partial x = 0$ there. The other boundary condition is applied at the front of the monolayer, which coincides with the position of the fiber $x = x_f(t)$. This position needs to be computed as part of the solution. To deal with this moving boundary it is convenient to introduce a scaled spatial coordinate, $\xi = (x - x_f) / (\ell + x_f)$, such...
FIG. 2. (a) Time evolution of the uniform force per unit length $f_0$ for different fiber lengths. The color scale represents the length of the fiber, $L$. The initial time $t = 0$ is that at which the monolayer reaches the fiber. (b) Time evolution of the characteristic deflection of the fiber times stiffness (equivalent to a dimensionless force per unit length) computed numerically for $\hat{\ell} = 5$ and $K = 0.5, 0.3, 0.2, 0.1$ and 0.05. Darker colors correspond in both plots to stiffer fibers.

FIG. 3. Time evolution of the velocity field $u(x,t)$ (colormap) and fiber deflection (white thick line) at three different locations, shown in Fig. 1. Positive velocities are directed downwards, coinciding with the direction of the migration. Length of the fiber: $L = 3$ mm. See Supplemental Material for more kymographs.

that $\xi(x = -\hat{\ell}) = -1$ and $\xi(x = x_f) = 0$. Introducing this new variable turns equation (2) into:

$$\frac{\partial U}{\partial T} + \frac{U - \dot{X}_f}{\ell + X_f} \frac{\partial U}{\partial \xi} = -KX_f \delta[\xi] + \frac{1}{(\ell + X_f)^2} \frac{\partial^2 U}{\partial \xi^2}. \quad (3)$$

We have introduced the following dimensionless notation:

$U = u/u_0$, $T = tu_0/L_c$, $\hat{\ell} = \ell/L_c$, $K = kL_c^3/\mu u_0$ and $X_f = x_f/L_c$. Here $u_0$ is the velocity of the monolayer at the time it touches the fiber—which we assume uniform, at least in a region of size $\ell$—and $L_c = \mu/mu_0$. This length scale $L_c$ measures the size of the region where the effective inertia is of the order of the viscous stresses. As will be discussed later in view of the experimental results, we expect this to be the characteristic size of the flow.

The boundary condition at the front can be found by integrating equation (3) along an infinitesimal interval centered at $\xi = 0$. Doing so, we get

$$\frac{\dot{X}_f}{2 (\hat{\ell} + X_f)} + KX_f + \frac{1}{(\hat{\ell} + X_f)^2} \frac{\partial U}{\partial \xi} \bigg|_{\xi=0}. \quad (4)$$

Finally, as in any free-boundary problem, the above equations need to be completed with the kinematic boundary condition imposing that the boundary moves with the local velocity

$$\dot{X}_f = U(\xi = 0). \quad (5)$$

Note that the boundary condition imposed at the front of the migrating monolayer amounts, in fact, to assume that cells do not overpass it. Although in Figs. 1 and 3 we observe that some cells do migrate beyond the fiber, the cell density downstream the fiber is clearly smaller than that right upstream (see Fig. 7 and the associated description in the Supplemental Material). This means that the percentage of cells surpassing the fiber is small and, consequently, that the force with which they may pull from it must be small compared to the push exerted by the bulk monolayer.

**Model results and discussion** — Fig. 2b illustrates the time evolution of the fiber deflection times the stiffness, $KX_f$, predicted by the model for different stiffness. This figure shows that the minimal model proposed here is qualitatively consistent with the behavior of the coupled cell monolayer-fiber system observed in experiments.
At short times, the stiffer the fiber the stronger the force the monolayer exerts.

Besides the agreement with the model’s prediction, the figure also illustrates the good quantitative repeatability of the results, something quite remarkable when working with living cell tissues. The good repeatability of the results and the fact that the model predicts quite well the fiber deflection opens up the door to use this setup as an easy-to-implement and inexpensive tool to measure the force that a migrating tissue is able to exert in real physiological conditions. Although the experiments reported here correspond to a two-dimensional cell monolayer, it is in principle feasible to make other kinds of cells grow and develop in three dimensions embedding the fiber in the process. This is currently ongoing work and we expect to report these results in the future. We remark here that relatively few techniques exist nowadays to measure forces in three-dimensional tissues.

Finally, we expect our model to be useful to understand similar experiments of relevance in biophysics, such as when two cell tissues of different nature meet and oppose each other.

Conclusions – The minimal model presented in this Letter successfully describes the main features of the interaction of an migrating cell monolayer with an elastic slender fiber observed experimentally. Initially, the monolayer deflects the fiber at a nearly constant speed upon coming in touch with it. Later on, the velocity slows down, eventually reaching a maximum deflection after which the fiber recoils and the monolayer comes to a stop. Although the model does not aim at describing the spatio-temporal dynamics of the tissue in its full spatial extension, it illustrates the essential ingredients needed to describe the interaction of a migrating tissue with a slender elastic object. Namely, an effective inertia, viscous stress, and an external elastic force due to the fiber. We believe that our model can be used to enrich other more

(Fig. 2a). In particular, it predicts that the fiber deflects at a nearly constant speed at short times, reaching later a maximum deflection after which it slowly recoils. This behaviour, seen in both model and experiments, is that of a damped harmonic oscillator close to or around the critical damping. In fact, the fiber reaches a single maximum before stopping (for large stiffness) or even exhibits overdamped oscillations (for small stiffness). Establishing an analogy with the flow of a viscous fluid, we could say that the monolayer motion has a Reynolds number, 

\[ Re = \frac{mu_0Lc}{\mu}, \]

of order unity. This justifies the choice of 

\[ Lc = \frac{\mu}{mu_0} \]

as the length scale of the flow, as inertia and viscous stresses are of the same order. More importantly, it supports the choice of the two fluid-like behaviors used to build the minimal model: a) an inertia-like term, as the tissue does not immediately modify its velocity upon touching the fiber; and b) a viscous damping to account for the slow recoil and the fact that at most only one relative maximum deflection is reached. On a longer time scale, associated to the length of tissue affected by the presence of the fiber, \( \ell \), the model predicts that the whole monolayer stops. Note that, in the experiments, it is not possible to observe longer times and thus to confirm that the fiber goes back to its original position, since it was not possible for practical reasons to extend their duration longer than about 120 hours (5 days).

Our model also explains the effect of the fiber elasticity on the dynamics of the monolayer. Since the Young’s modulus and cross-sectional moment of inertia of our fibers cannot be changed, we have carried out experiments with different fiber lengths, \( L \) (see Fig. 2a). Note that, as stated above, the stiffness of the fiber \( k \sim L^{-4} \), so changing \( L \) by a factor of two actually allows us to cover more than an order of magnitude in stiffness. This is supported by the variation in \( K \) needed to replicate qualitatively the experimental results (see Figs. 2a and b).

Let us assume that the cell velocities are of the order of the initial one, \( u_0 \), and that the maximum deflection reached by the fiber is of the order \( x_{fc} \). Since, as discussed above, inertia and viscous forces are of the same order, it is reasonable to assume that the maximum deflections reached by the fiber will take place when the elastic forces can no longer oppose the viscous ones. Thus, balancing the elastic and viscous terms in Equation (2) we get 

\[ x_{fc}^{3} \sim \frac{\mu u_0}{mk}. \]

Using Hooke’s law, 

\[ f_{0,c} \sim kx_{fc}, \]

and recalling that 

\[ k \sim EIL^{-4}, \]

\[ f_{0,c} \sim (EI)^{2/3}(\mu u_0/m)^{1/3}L^{-8/3}. \]  

This prediction is in very good agreement with the dependence of the maximum force measured for fibers of different lengths, shown in Fig. 2. We observe in both model and experiments a feature commonly found in mechanotransduction: the stronger the resistance of the environment, the stronger the force cells are able to exert [20]. In our model, the effective inertia makes cells to push the fiber at a speed that depends weakly on the stiffness, stopping as a result of viscosity. Consequently,
 comprehensive descriptions of migrating cell monolayers \cite{15,17} to allow them to describe the interaction with a compliant external body. Besides the purely fundamental interest of the problem in the fields of biophysics and active matter, we expect our experiments and theoretical model to also pave the way for the development and improvement of experimental techniques to measure the force exerted by a three-dimensional developing tissue in vivo.

\begin{acknowledgments}

We thank Dr. Gustavo Víctor Guinea for providing us with the carbon fibers. Javier Soler and Fernando García collaborated in early stages. We also thank Angélica Corral and Guillermo Vizcaíno for their technical assistance in the experiments. We are indebted to Dr. L. Champaugnay for her valuable comments on the manuscript and results.

We acknowledge the support of the Spanish Ministry of Economy and Competitiveness through grants DPI2014-61887-EXP, DPI2015-68088-P, DPI2017-88201-C3-3-R and DPI2018-102829-REDT, partly funded with European funds. Juan C. Lasheras thanks Universidad Carlos III de Madrid and Banco Santander for financial support from a Chair of Excellence.

\end{acknowledgments}

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SUPPLEMENTAL MATERIAL

**Cell culture:** The human skin keratinocyte cell line (HaCaT) was cultured in Dulbecos Modified Eagles Medium (DMEM, Invitrogen Life Technologies) supplemented with 10% Fetal Bovine Serum (FBS, Thermo Scientific HyClone) and 1% of Antibiotic Antimycotic (ThermoFisher) following standard protocols well established in our laboratory [22].

**Experiments:** A short piece of a carbon fiber is manually positioned near the center of a 35 mm Petri-dish (Corning) and glued at one end with a drop of commercial cyanocrylate glue. The drop has to be as small as possible to minimize its interaction with the cell culture. Then, the fiber is cut to the corresponding length (2 to 5 mm) using a scalpel. The surface of the culture plate and the glued filament, are then functionalized with a solution of bovine skin type I collagen at 0.1 % (w/v) for 2 hours at room temperature under the UV light. Afterwards, a 3D printed polylactic acid (PLA) stencil is placed in the culture plate as shown in Figure 5.

Cells are then seeded at a final density of 1550 cells/mm$^2$ on half of the dish and incubated 24 hours to attach forming a near-confluent monolayer. The day after, the stencil is carefully removed creating the two-dimensional wound. The culture was afterwards washed with PBS, and then 4 ml of fresh culture medium is added. This stencil-based method is more reproducible and causes less harm to the monolayer front than classical pipette tip scratch method to create a wound [23].

![FIG. 5. Sketch of the experimental set-up (not to scale). A 3D-printed stencil is used to make a repeatable wound which is always placed at a similar initial distance from the fiber.](image)

**Time-lapse experiments:** Experiments are performed in an automated inverted microscope Leica Dmi8 equipped with an OKOLab incubator. A four-Petri-dish adaptor allows to control the correct temperature, air/CO2 and humidity control during the experiment [24]. Time-step between frames is 15 minutes and the total duration of each experiment was 5 days (120 hours). Images are acquired in phase contrast with a 5X magnification objective and a Hamamatsu sCMOS Orca Flash 4.0 LT camera by means of LASX Navigator acquisition software from Leica Microsystems.

**Image Analysis:** Custom-made image analysis programs for fiber detection were developed in Matlab software for fiber detection using segmentation algorithms described in [25] with an spatial resolution of 1.29 $\mu m$.

**Summary of force and fiber deflection data from experiments:** $L =$ Length of the fiber; $f_{0,max}$ = maximum force per unit length, corresponding to the maximum deflection; $x_{tip,max}$ = maximum deflection of the fiber tip; $x_{tip,end}$ = final displacement of the tip at the end of the experiment (120 hours).
| $L$ (µm) | $f_{0,max}$ (nN/µm) | $x_{tip,max}$ (µm) | $x_{tip,end}$ (µm) |
|---------|---------------------|---------------------|---------------------|
| 2006    | 10.94               | 456                 | 343                 |
| 1945    | 9.21                | 369                 | 162                 |
| 1984    | 6.98                | 271                 | 168                 |
| 2063    | 8.61                | 403                 | 264                 |
| 3018    | 3.92                | 837                 | 591                 |
| 3068    | 3.01                | 705                 | 552                 |
| 3007    | 4.25                | 904                 | 796                 |
| 2951    | 4.06                | 770                 | 674                 |
| 3928    | 2.31                | 1446                | 1345                |
| 4049    | 2.10                | 1419                | 1302                |
| 4096    | 2.28                | 1728                | 1630                |
| 4982    | 0.76                | 992                 | 921                 |
| 4988    | 0.60                | 675                 | -                   |
| 4957    | 1.76                | 1204                | -                   |

Images from two of the experiments for the longest fibers ($L = 5$ mm) were not accurately analyzed until the end, but it was checked manually that their final length was very close to the maximum one.

**Velocity measurements:**

Velocity fields were computed in Matlab, using the open-source toolbox PIVLab (Time-Resolved Digital Image Velocimetry Tool for MATLAB) [26]. We set the interrogation window to 128 pixels with an overlap of 64 pixels and a second pass of 32 pixels, leading to a 32-pixel step between vectors. This corresponds to a spatial resolution of approximately 42 µm between velocity vectors. The correlation algorithm chosen for the calculations was Fast Fourier Transform with multiple passes and allowing window deformation. The toolbox includes data validation section to filter noisy vectors by interpolating between neighboring ones.

Kymographs are spacetime plots which display intensity values of a third variable (in our case the streamwise velocity, $u$) thus reducing by projection three-dimensional data ($x$, $t$, $u$) to two dimensions [27]. For each region (denoted by arrows $a$, $b$ and $c$ in Fig. 1) three columns of PIV velocity boxes of streamwise velocity ($u$) are averaged over the spanwise ($y$) direction in a band with a width of 126 µm. Each one of these averages is represented as a function of time with the color showing the value of $u$.

In Fig. 6 we show two additional kymographs for experiments with $L = 2$ mm and $L = 4$ mm respectively.

**Cell density:** Fig. 7 presents cell nuclei stained with fluorescent DAPI to show the cell distribution near the fiber. Cells are fixed with 4 % paraformaldehyde in Phosphate-buffered saline (PBS) at room temperature for 20
minutes directly on the culture plate. Then, they are incubated with DAPI at 1 µg/ml in PBS at room temperature for 5 minutes in the dark. The sample is washed with PBS and observed under the fluorescence microscope at Excitation/Emission of 358/461 nm.

FIG. 7. Nuclear staining with fluorescent DAPI dye. Blue spots are cell nuclei while the carbon fiber is shown in red. Scale bar: 100 µm