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Oscillations in collective cell migration

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The ability of organisms to spontaneously generate order relies on the intricate interplay of mechanical and bio-chemical signals. If the general consensus is that chemical signaling governs the behavior of cells, an increasing amount of evidence points towards the impact of mechanical factors into differentiation, proliferation, motility and cancer progression. In this context, several studies recently highlighted the existence of long-range mechanical excitations (i.e. waves) at the supra-cellular level.

In this review, we discuss all recent observations of mechanical oscillations on *in vitro* monolayers on a planar geometry, and their potential role for the patterning of cellular behavior at large scales. We focus on the importance of externally imposed confinement on the appearance of different phenotypes, namely traveling and standing waves, and how these mechanical stimuli might have a different potential for future research. We tackle the debate of whether oscillations are innate to the cell or only dependent on the boundary conditions, and propose a framework to reconcile these two points of view. We conclude with an outlook on the future of this field, and the directions to be explored in order to give mechanical oscillations a biological role.

I. INTRODUCTION

Every multi-cellular organism begins its life as a single cell, a fertilized egg, and later spontaneously develops into an extremely complex entity, whose existence depends on the precise hierarchy of shapes, structures and functions of its tissues. All these morphological features, which arise almost identically over thousands of generations, can be traced back to moments where cells spontaneously (i.e. without external guidance) alter their behavior, being it growth, differentiation or movement. The reproducibility of this phenomenon is, in fact, resting upon its complexity: thousands of processes happen under strict spatio-temporal control, the nature of which is still subject to debate.

A first explanation was provided by Alan Turing in his seminal paper in 1952 [1], where he explained how, based on the interaction between two molecules with different diffusion constants, stable periodic patterns could spontaneously arise from a homogeneous system. If any of these two molecules were to have a biological function, i.e. being what Turing refers to as a *morphogen*, this theory could provide an explanation for the patterning of cell behavior [2–4]. Since then, the tools available to measure and observe cells have grown, and with them our awareness that cell behavior is inherently correlated to the environment and its mechanical properties. We now know that cells continuously probe the response of the surrounding matrix, and that patterns of its mechanical properties can directly affect proliferation [5, 6], extrusion [7, 8], differentiation [9–11] and migration [12, 13]. If, initially, our explanations were strictly bio-chemical, new theories were formulated, where actors of a different nature, mostly mechanical, could also play a role [14–17]. More recently, practical examples of such influence on organ morphogenesis have been found [18–23].

In this regard, the presence of wave-like mechanical

patterns in the motion of cells, reported in the past decade, could be significant. These oscillations, which concern cell velocity [24], forces cells applied to the substrate [25] and the stresses within the monolayer [26], have been observed *in vitro* within cell sheets. When viewed from the perspective of the self-organization of tissues *in vivo*, the periodic nature of these mechanical phenomena could provide a “reference system”, allowing the specification of different sub-populations of cells and, ultimately, leading to supracellular patterning. Moreover, the propagating nature of these mechanical signals could be relevant for cellular communication at larger scales.

In this review, we briefly describe collective cell motion and the emergent properties arising from the coordination of cell behavior. We will then concentrate on the reports of mechanical oscillations in monolayers. The literature on the matter encompasses different cell types and experimental setups. If, on the one hand, each work seems to highlight a specific aspect of these phenomena, on the other, the similarities, both in terms of typical scales and cellular behavior, seem to be painting a common picture. This review is meant to provide a broad overview of these similarities, with a final outlook towards the future challenges to be tackled to assess the biological significance of such wave-like phenomena.

II. MECHANISM OF COLLECTIVE CELL MOTION

Before describing the various reports of mechanical oscillations, it is useful to outline the basic concepts behind cell migration and their interaction with their environment.

A. Mechanical machinery

The mechanical machinery of single cells (described in [27, 28]) allows them to physically interact with their surroundings and exert forces. The cytoskeleton is responsible for this latter function and the forces are relayed to the environment through two main channels. Focal adhesions are protein complexes that span the membrane to bridge the actin network with the external world, in the form of the extra-cellular matrix [29, 30]. Some of the proteins present at focal adhesions are mechanosensitive: responding to the characteristics of the environment, they can recruit different signaling molecules, reinforcing or weakening the cytoskeleton, and ultimately affecting gene expression. Similarly, cell-cell junctions, which connect the membranes and cytoskeletons of two adjacent cells, can play a role in regulating many aspects of cell activity [31, 32]. This extended mechanical network grants cell layers structural integrity and, together with the bi-directional communication between mechanical and biochemical channels, allows cells to coordinate their behavior at a scale larger than that of the individual. Ultimately, this supra-cellular communication gives rise to emergent behaviors, which are at the core of many processes typical of morphogenesis [21] and tissue homeostasis [33].

B. Collective cell migration

One such process is collective cell migration (CCM): the coordinated movement of multi-cellular ensembles, fundamental for wound healing, tissue shape, and cancer progression [34, 35]. The most evident advantage of supracellular coordination is the higher persistence of migration. If, on the one hand, single cells have a higher instant velocity, their migration tends to be somehow more erratic. Cell-cell contacts increase the overall persistence, creating a more directional and “efficient” system, which can also generate and sense bio-chemical and mechanical gradients on a scale bigger than that of single entities [36]. Although CCM refers to many forms of collective behavior *in vivo*, the vast majority of the research is carried out *in vitro*, on planar geometry, for its convenience in terms of imaging and force quantification. Moreover, 2D migration recapitulates many of the general mechanisms characteristic of *in vivo* conditions [37, 38].

C. The role of density

It is well known that, when seeded on 2D substrates, cells proliferate until all available space is filled (confluent state), and will later reach a quiescent state with little to no cell division, a phenomenon also accompanied by a steady decrease in cell motion. These two effects are concomitant and generally referred to as *contact inhibition*. Interestingly, when confluent cells are

exposed to empty space, e.g. by physically scratching the monolayer, both processes resume, and the resulting invasion of this adhesive area allows for the reliable observation of collective migration. This experiment, historically called wound healing [39], has taught us much of what we know about emerging properties during collective migration (reviewed in [40]).

Density also plays a fundamental role in controlling the cooperativity of the cell migration. This is better expressed as the velocity correlation length, which represents the distance over which cells move in a correlated fashion, i.e. the size of the cell patch moving in the same direction [41, 42] or the radius of a swirl [43, 44]. As cells become more dense and slow down their movement, the correlation length first increases to approximately 10 cells lengths or 150 μm [41, 45], and later drops to the single cell value for the highest packing. This process is referred to as *jamming* because of its similarity with the behavior of amorphous materials [46]. Interestingly, substrate stiffness has also been shown to affect the extent to which directional cues are relayed [43, 47].

A complete picture of the collective phenomena reported in the last decade rests on all these notions: cells feel their environment through focal adhesions, coordinate their behavior thanks to junctions, and this allows collective motion to be influenced by the presence of boundaries and, thus, cell density [48]. Specifically, in this review, we concentrate on mechanical oscillations, a term we use to identify all periodic fluctuations of mechanical parameters, such as cell velocity, the resulting strain, substrate tractions and stresses. These oscillations are typically present in space and time, and can be divided into two major categories: expanding monolayers are characterized by *propagative* waves [26, 49–51] (i.e. signals that are traveling through the system like sound waves through a medium), while in fully confined environments (such as circles or rectangles) cell migration is limited to local cell rearrangements and oscillations resemble *standing* waves [8, 25, 44, 52].

III. PROPAGATIVE WAVES

We will first consider the case of propagative mechanical waves, which have been shown for the first time within freely expanding monolayers [26]. This is a stereotypical example not only because, chronologically, it was the first one reporting the presence of mechanical waves, but also because it highlights the existence of both oscillations in velocity and in forces/stresses.

A. Mechanical forces in expanding monolayers

These experiments are the direct evolution of the more classical wound healing [53]. With the help of a polydimethylsiloxane (PDMS) polymeric membrane, cells are

first confined to a small space, where they are allowed to proliferate until confluence is reached and motion is almost entirely inhibited. The removal of the membrane exposes cells to a free surface, a stimulus sufficient to trigger the global polarization towards the newly available space and its invasion [54, 55]. The major characteristics of the migration of expanding tissues are summarized in [40, 48].

Overall, the edge of the monolayer advances at a speed of approximately $30 \mu\text{m}/\text{h}$ [26, 56], a velocity dependent on the steady increase of density provided by mitosis [26, 51]. Cells at the edge are the first ones to react: they polarize towards the wound and consequently begin migrating and exerting forces onto the substrate. Initially limited to a boundary layer near the edge, this behavior is then relayed to the neighboring cell, propagating backwards towards the bulk and progressively creating a state of global motion [57]. The study of the forces on the substrate, measured through the deformation of the underlying material, reveals that cell-generate tractions follow a similar trend: initially, the largest forces are localized at the front of the monolayer and perpendicular to the its edge, and will eventually propagate towards the bulk. Generally, cells far from the leading edge exert smaller forces on the substrate, although important tractions are generated up to several hundreds of micrometers inside the monolayer, where forces are extremely heterogeneous in magnitude and direction [26, 58, 59].

Force transmission through the substrate, if easily measured, is only half of the physical picture of collective cell migration. The other half is the transmission of tensions at cell-cell junctions, and it lies hidden within the monolayer.

One method to indirectly access this information is monolayer stress microscopy (MSM) [59, 60]. The measured local traction exerted by the cells on the substrate is precisely balanced by the internal stress generated within the internal monolayer. By applying Newton's laws, and considering the cell monolayer as a continuous, homogeneous and isotropic thin sheet, the mechanical stress within the monolayer is calculated as the integral of the traction forces over the tissue area. The internal stresses σ_{ij} are those required to balance the measured traction forces, irrespective of whether monolayer material is active or passive, elastic or visco-elastic, linear or nonlinear. With the additional hypothesis that the cell monolayer is elastic and incompressible, one can indirectly compute the planar strain tensor ϵ_{ij} . This mechanical stress accumulates from the edge towards the bulk of the tissue [58], a trend also validated with the use Förster resonance energy transfer (FRET) sensors [61]. Like substrate tractions, stresses are heterogeneous, with areas under compressive and areas under tensile stress. These results describe a situation where single cells engage in a global tug-of-war: a given cell within the monolayer feels the pulling force exerted at the front through its neighbors and, in turn, pulls on the substrate along the same direction, but opposite orientation. The bal-

ance of forces is ultimately satisfied when, through the junctions, the cell pulls on the neighbor immediately behind it, describing a mechanical signal which, traveling backwards through the colony, generates global forward migration. This integration of local forces along the tissue has the net result of a global state of tensile stress [26, 58, 59, 62, 63], believed to be an important ingredient to achieve global polarization in migrating tissues. The assumptions mentioned above to apply MSM are much more restrictive than they seem. In fact, multicellular systems are not homogeneous, because they are made up of cells, extracellular matrix and interstitial fluid, they are anisotropic, because cells possess a natural polarization, and are constantly kept out of equilibrium by an internal energy source. As a consequence, it remains difficult to measure in a reliable manner the stress within a 2D monolayer. To circumvent this limit, Serra-Picamal et al. measured the strain rate $\dot{\epsilon}_{ij}$ in an independent manner, by Particle Imaging Velocimetry [26]. In the following of this chapter, we will mainly focus on experiments based on the direct measurements of the deformation field, which does not require assumptions on the constitutive model of the cell monolayer.

B. Oscillations in expanding monolayers

Superimposed to this general landscape is the presence of marked oscillations. The trends described, velocity and tractions propagating backwards through the tissue, are not only typical of the initial moment, but continue in a fluctuating manner throughout the expansion of the monolayer. By averaging their measurables over y (i.e. to obtain a measurement as a function of the distance from the edge, x), Serra-Picamal et al. obtained the kymographs in Fig. 1, which demonstrate in a clear way the presence of periodic mechanical waves of velocity, stress and tissue strain-rate ($\dot{\epsilon}_{xx}$) [26]. Cells at the edges of the monolayer exert forces pulling inwards (i.e. away from the edge), but as we move further within the tissue we notice nearly periodic oscillations in the direction of the forces (Fig. 1f). The presence of traveling perturbations is more transparent in the graph of the strain rate, calculated from the velocity ($\dot{\epsilon}_{xx} = \partial v_x / \partial x$): wave-like excitations originate from the edge, propagate backwards into the tissue, past the midline and towards the opposite edge at a speed of roughly $60 \mu\text{m}/\text{h}$, describing what the authors call "X-waves" [26]. If, normally, one would expect stress to rise monotonically from the edges towards the midline, X-waves modulate this behavior, so that monolayer stress in the center of the tissue oscillates with a periodicity of ~ 5 hours (see Fig. 1c). Interestingly, these fluctuations are in phase with those of cell area in a trend reminiscent of purely elastic behavior.

The importance of cell-cell junctions for the propagation of waves is confirmed by the authors by disrupting adherens junction with calcium chelation. In these conditions, the speed of the advancing monolayer is unaffected,

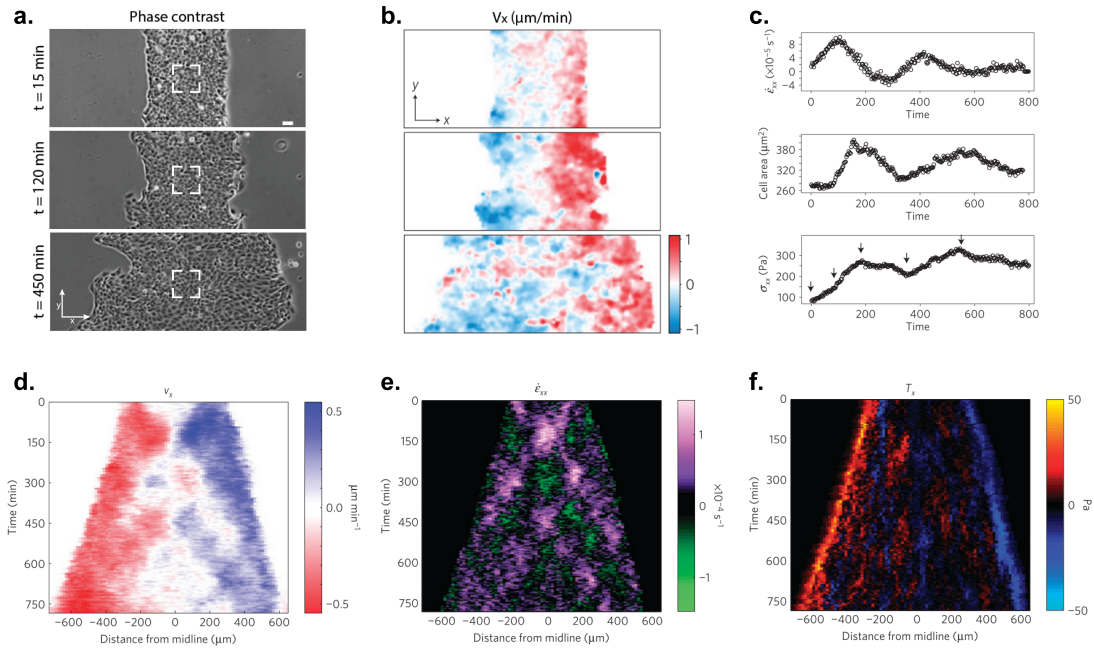


FIG. 1. Mechanical waves in expanding monolayers and at repulsive interfaces. A confluent tissue is grown within a rectangular hole in a PDMS membrane, which is in turn resting on top of a collagen substrate. The membrane is lifted and the tissue starts invading the available space (a.), moving outwards with a velocity measured with PIV (b.). The mechanical behavior of the tissue is described by averaging the components in the x -direction over y (the tissue edge) to generate kymographs of x -velocity (V_x , d.), strain-rate ($\dot{\epsilon}_{xx}$, e.) and x -tractions (T_x , f.). The average values of $\dot{\epsilon}_{xx}$, cell area and monolayer stress component (σ_{xx}) at the monolayer midline (averaged over the white square in a.) are reported in (c.). Adapted from [26].

but no waves are present. Similarly, treatment with blebbistatin, which prevents myosin II activity, demonstrates the importance of contractility for the accumulation and fluctuation of stress [26, 59]. Interestingly, similar velocity oscillations, with comparable periodicity (~ 4 hours, $\sim 700 \mu\text{m}$ traveling at $\sim 170 \mu\text{m/h}$), are also observed at the edges of cancerous cell monolayers, albeit with circular symmetry [64].

C. Colliding tissues

Surprisingly, mechanical waves are not only distinctive of expanding monolayers, but they have also been observed in situations where a migrating tissue meets an obstacle, being it another epithelium or a wall [49, 65]. When two repulsive tissues meet, they create an interface characterized by parallel cell alignment and strong tractions. As time proceeds, cell density increases and the tissue start jamming, a conditions necessary for the appearance of waves at boundaries. Once more, cells at the interface exert forces pulling away from the edge, followed by periodic oscillations in their direction towards the bulk. Rather than homogenizing, this pattern seems to be stable in time, in what is a long-lived supracellular cooperative behavior. The periodicity is approximately $50 \mu\text{m}$, a value comparable with those obtained for traveling oscillations in expanding monolayers [26, 58]. If

these characteristics might have been expected based on previous works, a peculiarity of colliding epithelia is the presence of deformation/velocity waves propagating backwards across the monolayer at a velocity of $\sim 120 \mu\text{m/h}$, without attenuation. Interestingly, similar waves were also observed at the boundary with a repulsive wall and identical tissues, making velocity waves a generic feature of repulsive interfaces [49].

D. Orientation at external boundaries

Collective cell migration has also been studied in the presence of externally imposed boundaries, defining regions of space that cells cannot invade. In general, cells show a tendency to align tangentially to a boundary between adherent and non-adherent substrates [7, 66–68]. Drawing general conclusions is not trivial because the specifics of this alignment depend strongly on cell type and density.

Most of the works on cellular alignment have been carried out on spindle-shaped fibroblasts, which develop weaker cell-cell adhesions and whose interactions rely mainly on steric hindrance [66, 67, 69]. These properties make quantification easier, but mean that the knowledge obtained on these cell types is harder to transfer on epithelial cells, whose strong cell-cell junctions and propensity to assume hexagonal shape make for a slightly dif-

ferent behavior. Madin-Darby Canine Kidney (MDCK) monolayers growing on $230\ \mu\text{m}$ wide rings, all cells, including the ones in the center, align within 15° of the tangential direction [68]. This is unsurprising because we know that cells coordinate their migration velocity over a well-defined correlation distance, $\sim 150\ \mu\text{m}$ for MDCKs, so we expect any forced orientation to propagate within the tissue for approximately the same length. If cells at the edges can only migrate parallelly to the border, then an overall orientation is to be expected. Similar results are obtained for MDCKs confined on strips of different widths, confirming that cell orientation increases with decreasing width, and becomes global below $\sim 100\ \mu\text{m}$ [50] (see Fig. 2a).

E. Collective migration with confining borders

The presence of boundaries, together with the preferential orientation they cause, enabled scientists to isolate and reproduce some of these behaviors.

The tendency of cells to correlate their velocity over a specific distance produces, during CCM on planar geometry, swirling motion. Patches of cells spontaneously begin rotating, a behavior that can be replicated on round patterns (i.e. discs) of different size. The most rudimentary form is 2 cells confined on small circular islands, situation in which the angular symmetry is spontaneously broken and cells are observed to rotate in a coordinated manner around the center of the pattern [70, 71]. On a larger scale, the presence of boundaries and the preferential orientation they cause enhance this phenomenon [44], whose persistence increases in a density-dependent manner [72]. Before reaching confluence, cell clusters move mostly in a disorganized manner, occasionally displaying collective rotation, although with extremely short persistence. Once confluence is reached, the tissues begin rotating in a “solid disc-like” synchronized and collective manner, with stochastic inversions of the rotation direction [8]. As expected, the increase of density associated to cell division produces a slow decrease of the rotational speed, with average velocity values going from $\sim 25\ \mu\text{m}/\text{h}$ to $\sim 8\ \mu\text{m}/\text{h}$, and an eventual jamming of the tissue [44]. There seems to be a critical diameter ($\sim 250\ \mu\text{m}$) of the circular patterns above which global rotation is substituted by smaller vortices and transient coordinated flows. This is reasonable when viewed with the velocity correlation length ($\sim 150\ \mu\text{m}$) in mind. For small circles, the whole area falls within this distance, and cells can coordinate their motion globally. When the diameter approaches twice the correlation length, cells at opposing sides of the colony behave “independently”, and multiple swirls are formed. Since supra-cellular coordination depends on cell-cell junctions, it is unsurprising that MDCKs where cadherins are down-regulated, as well as cancerous cell lines, cannot give rise to collective rotations.

These experiments tell us that increasing the confinement length above the typical velocity correlation distance drives a transition between migration modes, and this is also verified on adhesive strips [50]. By creating a cell reservoir, and then releasing the tissue onto a pre-patterned substrate, Vedula and colleagues confined the wound healing migration of MDCK tissues to strips of different widths (see Fig. 2b). As one might expect, wide channels allow the formation of vortices (diameter $\sim 300\ \mu\text{m}$), a behavior reminiscent of that of unconfined wounds. If under these circumstances the velocity of the advancing edge is approximately constant ($\sim 20\ \mu\text{m}/\text{h}$) [73], the vortices become less prominent when the migration of cells is confined to strips whose width is smaller than the velocity correlation length (100 to $200\ \mu\text{m}$). In this situation, cell velocity becomes, on average, oriented in the direction of the strip, and this causes an increase in the speed of the advancing edge for thinner channels (up to $\sim 40\ \mu\text{m}/\text{h}$) [50]. In this regime, the velocity of cells follows a plug-flow-like distribution perpendicularly to the channel, and the overall flow is well described by a constant drift component and a diffusion-like contribution dependent on local cell density [73]. Vortices disappear altogether under the strongest confinement. In very narrow strips ($\sim 20\ \mu\text{m}$), cells become more elongated, the velocity becomes strongly aligned in the direction of the strip, and the front of the tissue advances more rapidly. Moreover, PIV analysis shows that in highly confined geometries, MDCKs switch to a contraction-relaxation or “caterpillar-like” migration mode, whose cycle spans more or less $100\ \mu\text{m}$ [50] (see Fig. 2b). Interestingly, the advance of monolayers in wide strips is associated to a monotonic increase in the stress component, in line with CCM in wound healing experiments. Cells migrating by contraction-relaxation, on the other hand, exert on the substrate an alternation of positive and negative forces. Even though not specifically reported, the periodicity of these oscillations is approximately 4 hours and $250\ \mu\text{m}$, values in agreement with the mechanical phenomena presented earlier.

It is also worth noting that the motion of cells on strips has been compared with that on external surface of glass wires, where the relationship between radius and the velocity of the advancing tissue-front is somehow conserved, a fact suggesting that the boundary conditions control this effect rather than curvature [74]. On the other hand, under tubular confinement (i.e. cells migrating on the inner surface of a tube), the velocity increases with the radius [75]. Whereas these results are in disagreement, the transition between classical migration and a contraction-relaxation mode is observed for smaller radii in both conditions [74, 75], and is attributed to the stronger relative effect of cell division in smaller spaces by Yevick and colleagues [74]. Interestingly, the experiments on glass wires report an additional phenotype: for radii smaller than $\sim 40\ \mu\text{m}$, cells at the advancing edge escape the monolayer

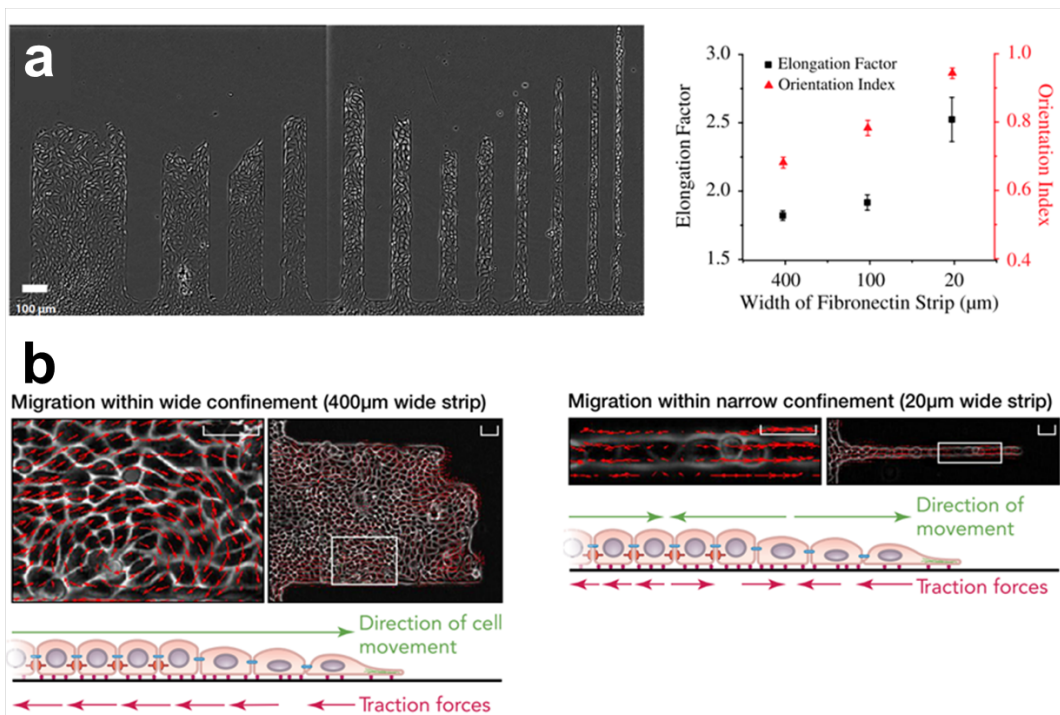


FIG. 2. Cellular orientation in presence of external boundaries. **a)** MDCK monolayer migrating on adhesive strips of different widths (*left*) and the quantification of elongation ratio and orientation for 3 widths (*right*). Adapted from [50]. **b).** PIV measurements of MDCK cells migrating on wide channels (*top*) showing the presence of vortices. On more narrow channels (*bottom*) cells migrate in a contraction-relaxation manner. The schemes summarize the distributions of traction forces for both behaviors. Adapted from [12].

and move back and forth with velocities in the order of $100 \mu\text{m}/\text{h}$, albeit no periodicity is reported by the authors [74].

Together, these results hint at the necessity of considering the role of curvature on the appearance of mechanical waves.

F. Velocity oscillations in confined channels

In a recent article [51], Tlili et al. repeated wound healing experiments while forcing MDCK tissues to migrate along functionalized adhesive strips (Fig. 3a). Even though they do not report a strong effect of the confinement width, when compared to [50], the lack of swirling motion and globally directed quasi-laminar flow place their experiments in the central region, where migration is strongly guided, albeit without the presence of the contraction-relaxation phenotype. The authors inhibit cell division in order to disentangle the contribution of migration and proliferation, and they report faster advance of the leading edge ($\sim 40 \mu\text{m}/\text{h}$) compared to similar examples where mitosis was unaffected. The first observation in such conditions is that, as the tissue spreads and cells in the back progressively join the forward migration, the average velocity decreases from the front towards the bulk.

Interestingly, the velocity is inversely proportional to local cell density, in a trend that does not depend on the distance from the leading edge.

On top of this average velocity profile, the authors observe multiple periods of backwards propagating velocity waves (see Fig. 3b), essentially describing cells that slow down and accelerate with a period of ~ 2 hours and a wavelength of $\sim 1 \text{ mm}$. The absence of cell division guarantees a better contrast and, thanks to the lack of jamming, longer observations ($\sim 20 \text{ h}$). If the nature of the waves is similar to those reported before, their phase velocity is much higher, around $600 \mu\text{m}/\text{h}$. Moreover, the wave crests in Fig. 3b are curved, evidence that the wave slows down near the bulk, where density is higher. There is, in fact, a strong link between velocity oscillations and inhomogeneities in cell numbers. The authors report steady fluctuations of density (period $\sim 200 \mu\text{m}$) parallel to the confining edge (vertical direction in Fig. 3b), which seem to deform with the stretching of the tissue. More interestingly, density oscillations are present in the perpendicular direction, in a fashion that follows velocity waves in phase opposition (Fig. 3c). The authors also demonstrate that lamellipodia inhibition slows or hinders the formation of waves altogether, confirming once more the fundamental role played by actin contractility in these phenomena.

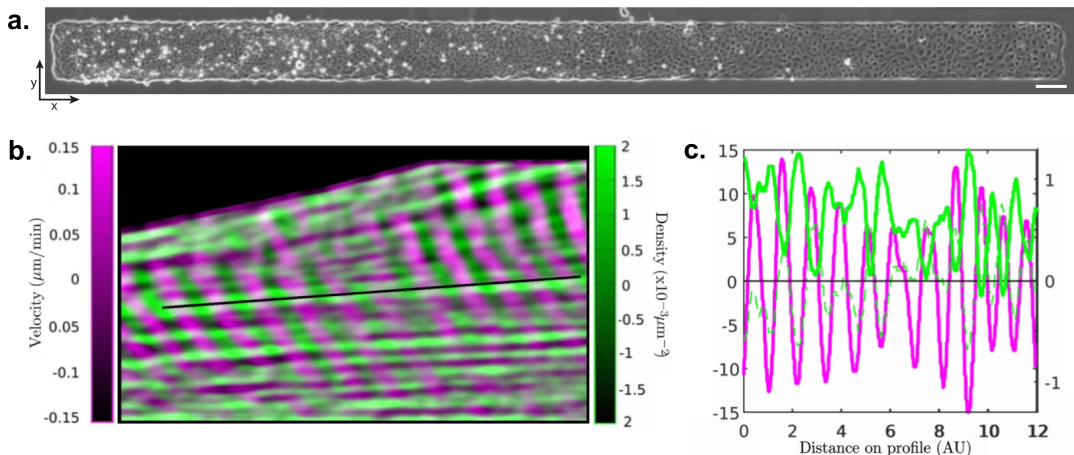


FIG. 3. Mechanical waves in confined expanding monolayers. **a.**) Brightfield image of MDCK cells expanding in confined channels without proliferation (scalebar = $100 \mu\text{m}$). **b.**) Superimposed kymographs of small scale variations in local cell density (*green*) and velocity (*purple*). The space along x is reported on the vertical axis (from 0 at the bottom to 3 mm at the top) and time on the horizontal axis (from 0 on the left to 25 hours on the right). The dark space in the top-left corner represents the area not yet invaded by the tissue. **c.**) Plots of density (*green*) and velocity (*purple*) along the black line in (b). The phase opposition between the quantities plotted is highlighted by inverting the sign of the density plot (*dashed green line*). Adapted from [51].

Overall, these evidences describe backwards propagating mechanical waves as a common feature of all the boundaries of migrating monolayers, being them with free space or repulsive walls. Even though the values vary between reports, there is agreement on the general magnitude for the periodicity of these phenomena, in the order of hours and hundreds of micrometers. An interesting correlation is that with waves of ERK activation, which have been reproduced in three of the situations mentioned in this section: free expansion, swirling motion and contraction-relaxation migration [76]. Strikingly, the periodicity of ERK waves (~ 4 hours and $250 \mu\text{m}$) agrees with the typical time and space of mechanical oscillations, a similarity which begs further experimental work.

IV. STANDING WAVES IN FULLY CONFINED MONOLAYERS

Mechanical waves have also been reported in fully confined environments, where collective motion only happens through neighbor exchange rather than directed expansion. In this situations the available space is finite, which means that continuous proliferation inevitably leads to contact inhibition and the absence of coordinated movement. The presence of oscillatory phenomena is therefore restricted to a narrow time window between confluence and the jammed state [8, 24, 77].

Even though each account concentrates on a different geometry, from thin lines (chosen to go as close to a 1-dimensional system as possible) to wider 2-dimensional monolayers (such as rectangles or circles), they all report the presence of periodic motion resembling that of

a standing wave, with specific periodicity in time and space.

A. Breathing oscillations

Oscillatory movements have been observed in epithelia confined to round patterns of radii smaller than $\sim 300 \mu\text{m}$. Contrarily to the swirling motion presented earlier, these oscillations happen along the radial direction, displaying standing waves of highly coordinated outward and inward cell movement (Fig. 4) [8, 25, 52]. The two phenomena, collective rotation and these “breathing” oscillations, are not mutually exclusive, but are rather superimposed and observed together. With respect to the tangential velocity, radial oscillations are characterized by a lower velocity amplitude, which depends on the account, from $\sim 15 \mu\text{m}/\text{h}$ [52] to less than $1 \mu\text{m}/\text{h}$ [25]. Oscillations are present as fluctuations of this radial velocity, which is generally averaged over the angular coordinate for better visualization (Fig. 4b-right). The cells in the center of the disk and those at the far edge tend to be still, whereas the maximum of the radial velocity is at the mid-point between the two positions [8]. The amplitude of the cell displacements is the order of micrometers, and the period a few hours. Although this latter quantity is in general agreement with those for expanding monolayers, the period of breathing oscillations depends strongly on the radius of the round patch (Fig. 4a-right) [8].

Surprisingly, even though radial motion is very limited, oscillations in cell velocity are accompanied by similar standing-waves in the radial component of the tractions on the substrate, with patterns of inwards and outwards

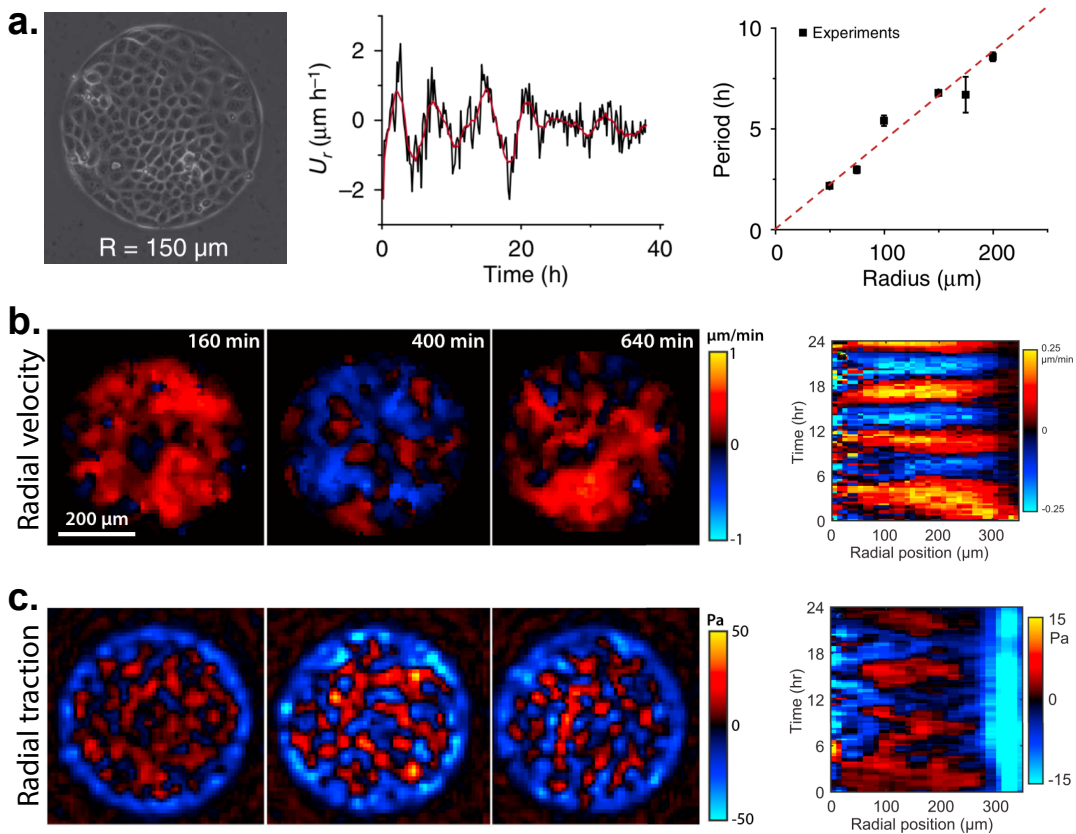


FIG. 4. Breathing oscillations in epithelial tissues confined to round adhesive patterns. **a.**) A phase-contrast image of MDCK cells confined on a disk-like pattern (*left*) and the evolution of the average radial velocity in time (*center*). The typical time period for these oscillations can be quantified as a function of the radius of the tissue (*right*). Adapted from [8]. **b.**) Examples of the distribution of radial velocity over time for MDCK cells on patterns of radius equal to $350 \mu\text{m}$. The velocity is averaged over the angular direction to yield a kymographs (*right*). **c.**) Cell-generated tractions on the substrate (*left*) for the examples in (b) are reported together with a kymograph of the average over the angular direction (*right*). Adapted from [25].

pointing forces over time (Fig. 4c) [25]. These oscillations only interest the central part of the tissue, as cells at the edges constantly pull themselves towards the exterior, in a manner coherent with kenotaxis [78]. One might be tempted to assume that tractions and velocities are interdependent, an assumption carefully disproved by the authors, who demonstrate that no correlation between traction and velocities, nor their relative angles, exists [25]. The study of stresses within the monolayer led to an interesting conclusion: tensions and cell area (i.e. strain) are in phase, implying that the tissue behaves elastically with no viscous contribution.

Given the correlation between the presence of waves and ERK activation advanced recently [76], it is interesting to point out that breathing oscillation were suppressed upon ERK inhibition [25]. This is loosely in contrast with what is reported, admittedly in a very different situation, in [78], indicating that ERK activity has no significant role in kenotaxis.

These breathing oscillations share many similarities with those mechanical waves present in expanding mono-

layers, like the strong dependence on cell-cell junctions and contractility, and might be explained by the same basic principles of force transmission and mechanotransductive coupling with polarization [25]. One big difference lies in that, whereas velocity waves in wound healing are propagating through the tissue, on circular patterns the phase difference between radial positions is insignificant.

B. Multi-nodal standing waves

The discrepancies in the reports presented so far, namely the different spatio-temporal coherence and dependence on confinement, can be seen as evidences that traveling waves and breathing oscillations are independent. On the other hand, the similarities between these phenomena suggest that they might be two faces of the same coin, each emerging for specific geometries and length-scales. Discriminating between these interpretations requires more suitable experiments, specifically tailored to understand whether and how the presence of boundaries plays a role in the emergence of oscillations,

and if it can affect the typical periodicity.

Two simultaneous and independent papers recently tackled this question, both reporting the appearance of extended standing-wave like velocity oscillations in fully confined environments [24, 77]. Contrarily to breathing oscillations, these works extend their observations to larger systems to uncover the presence of nodes, i.e. positions of zero net movement, and study the effect of the confinement on the characteristics of cell motion, albeit from different points of view.

1. Waves on quasi-1-dimensional lines

The first work, published by our research group [24] was carried out trying to understand whether the environment alone dictates the way cells oscillate, or if their behavior is a consequence of some innate feature. This was tackled by searching for the most simplified experimental conditions to reproduce wave-like phenomena, a bottom-up approach resulting in the creation of thin 40 μm wide strips of different lengths (L_X). In accordance with previous reports [50], this geometry forces cell migration along the longitudinal direction, allowing the authors to average over the transverse direction and only study the motion along 1 dimension, thus creating what is referred to as *quasi-1-dimensional* migration.

Wave-like oscillations are reported for MDCK cells on all strip lengths, although two clear phenotypes can be distinguished: *global oscillations*, where all cells move alternatively leftwards and rightwards in phase (see schema in Fig. 6a), and *multi-nodal oscillations*, characterized by multiple iterations of these global movements, in phase opposition and separated by nodes (Fig. 5a). The relative incidence of the two behaviors depends strongly on the length of the line, with a clear transition between modes around a critical length $L_X^c \simeq 400 \mu\text{m}$ (Fig. 5b). We can distinguish three situations: global oscillations are overwhelmingly prevalent for shorter lines; when L_X is comparable with L_X^c tissues display both phenotypes; (iii) above L_X^c multi-nodal oscillations (Fig. 6b) dominate statically. Moreover, a quantification of the typical wavelength and time period confirms the presence of two regimes: for relatively short strips, the time-period scales linearly with the tissue size, resembling the trend reported for breathing oscillations (the wavelength, imposed by the confinement, cannot be calculated), but when multi-nodal waves dominate, both time-period and wavelength are approximately independent of L_X , and can be estimated as $\lambda \sim 380 \mu\text{m}$ and $T \sim 5$ hours. An effective phase velocity is calculated from these values as $u = \lambda/T \sim 80 \mu\text{m}/\text{h}$, which is approximately independent of the tissue length, and is coherent with previous observations.

These results suggest that, when possible, cells spontaneously establish multi-nodal oscillatory modes with predetermined features. When confined to spaces smaller than those characteristic of a full wavelength

(i.e. 400 μm), nodes disappear and the overall behavior changes. The presence of this transition, together with the fact that time and space periods do not adapt to the confinement, leads to the conclusion that velocity oscillations are intrinsically encoded in the biology of the cell.

Interestingly, the presence of these phenomena in confined environments fits well with the physical interpretation of standing waves in as the sum of two identical waves traveling in opposite directions. For the sake of simplicity, this can be compared to what happens in a laser cavity where photons are reflected at the edges and interfere with the following ones. Although a simple reflection does not capture the complexity of the underlying phenomenon, something similar might be happening on thin lines, where standing velocity waves are the superimposition of waves reflected at the shorter edges. Moreover, this interpretation is somehow supported by the observation that traveling velocity waves are typical of all repulsive boundaries [49]. Since biological systems are noisy, cells might be able to oscillate with a wider range of wavelengths, to adapt their motion to the particular length of the line in a resilient manner.

2. Standing waves on extended 2D systems

If the quasi-1-dimensional geometry only allows for “binary” cell polarity and, thus, simplified motion, the presence of standing waves has also been reported on larger systems where cells migration happens along 2 directions in a correlated manner [77]. Human keratinocytes (HaCaT) confined on square patterns (side length 200-1000 μm) display globally coordinated movement along elliptical trajectories (see Fig. 5c), coherent with a polarity vector that rotates in time. When separated into its x and y components, the velocity field displays standing-wave like oscillations in both directions, with a $\pi/2$ -phase shift, whose typical period (in time) and amplitude (in μm) increases linearly with the confinement size (2-10 hours, 20-100 μm) (see Fig. 5d) [77]. This behavior is reminiscent of the *global oscillations* on thin lines, albeit on a much larger scale. The discrepancy between reports can be explained considering the velocity-correlation length of the cell types employed: global oscillations happen for line lengths smaller than the correlation of MDCKs ($\sim 150 \mu\text{m}$), a condition which is also met for HaCaT (correlation length $\sim 800 \mu\text{m}$) on the squares tested by Peyret and colleagues.

Interestingly, this length-scale is also associated to the amplitude of the oscillations in velocity, which increases linearly as long as the square edge is smaller than the cell velocity correlation length, after which it becomes approximately constant. This behavior hints at the interplay between pattern size and velocity-correlation as an important regulator of 2-dimensional oscillatory motion. The role of the environment becomes that of prompting cell polarity to rotate in time, a feat that is best achieved

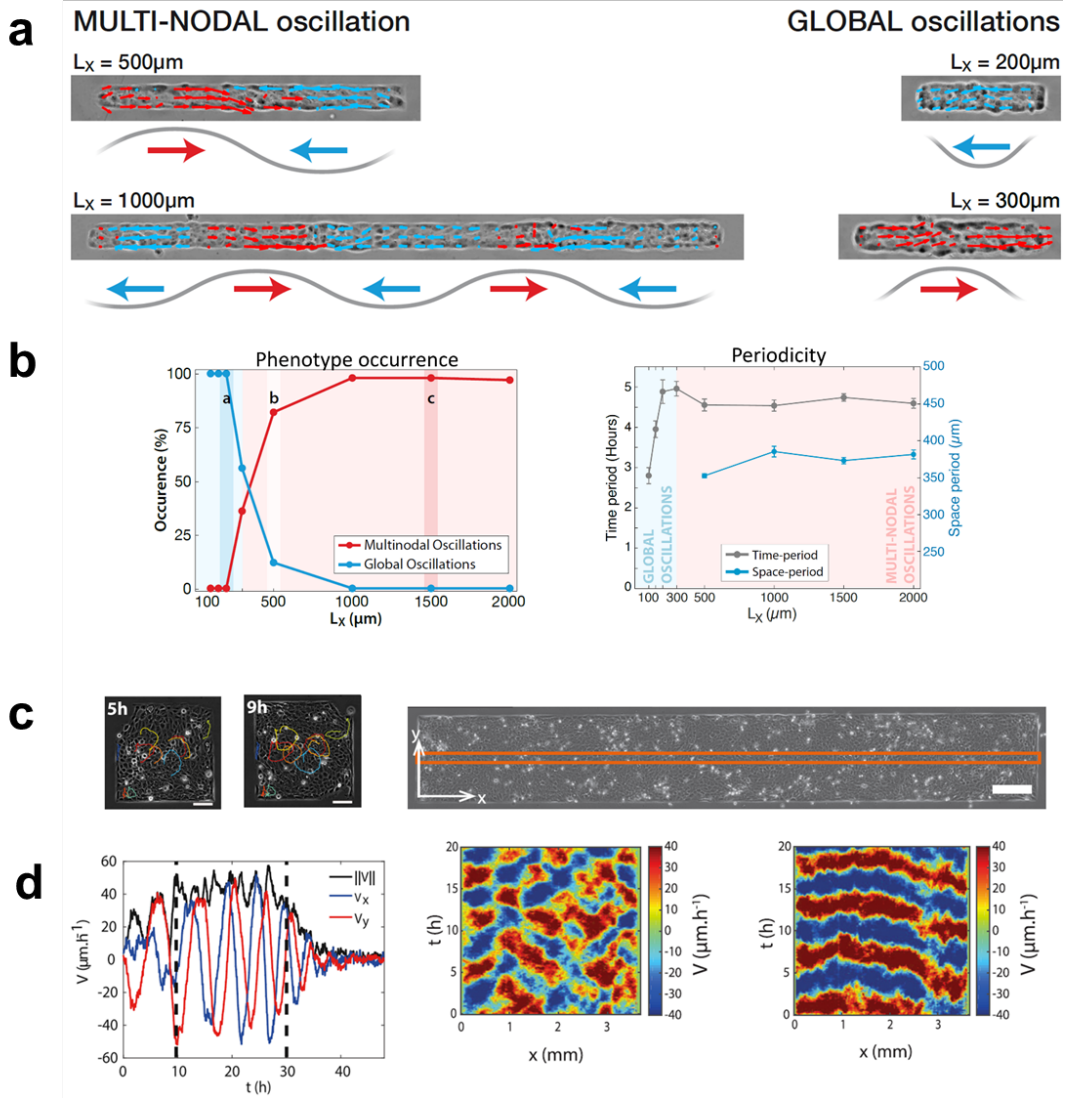


FIG. 5. Wave-like oscillations in epithelial layers. **a.**) Velocity field measured by PIV. Velocities pointing in the positive (negative) x -axis direction are shown in red (blue), in agreement with the arrows reported under the image. Lines longer than $500 \mu\text{m}$ display multinodal oscillations (*left*). Stripes shorter than $300 \mu\text{m}$ exhibit global oscillations (*right*). **b.**) Frequency of multinodal and global oscillations (*left*), and the characteristic time and space periodicity (*right*), for different stripes length. Adapted from [24] **c.**) Snapshots of a confluent HaCaT layer on square (*left*) and rectangular patterns (*right*). **d.**) Temporal evolution of the velocity field on the square pattern (*left*), and the kymograph of the velocity field on the rectangular pattern (*right*) respectively along the long (x) and the short (y) axis. Adapted from [77]

when the two length scales match. On the contrary, when confinement is too large or too small, motion is hindered and oscillations are less pronounced. Moreover, the same behavior is reported for HaCaTs on circular patterns.

From this perspective, the symmetry of the confining geometry directly causes a symmetry in oscillatory motion in x and y . To investigate how the two directions are coupled, the authors confine cells on long rectangles (width $200\text{--}500 \mu\text{m}$, length $2000\text{--}3000 \mu\text{m}$), where the rotatory nature of velocity and cell polarity is confirmed. In this case, they report the presence of global oscillations along the short direction, and a standing wave with mul-

tiples nodes along x , describing a phenomenon reminiscent of multi-nodal oscillations. Interestingly, the phase coherence length (i.e. $1/2$ the wavelength) along both directions approximately matches the tissue width, leading to the conclusion that the characteristics of the oscillations only depend on the smallest confinement size [77]. The authors also confirm this observation on expanding monolayers (i.e. rectangles with a free edge), where the scale of the velocity oscillation along the long axis is determined by the size of the shorter axis.

This might, at first, appear that this statement is in contrast with the previous reports of the wavelength be-

ing independent on the boundary conditions [24]. However, the authors report a linear behavior, albeit with an intercept that is far from zero. This might represent the intrinsic value of the phase coherence length, which is then tailored by the properties of the environment on large systems. Interestingly, a quick estimations of such value is in the range of 100-200 μm , surprisingly coherent with previous reports on very different cell types.

To assess the possible downstream effect of these oscillations, the authors calculate the divergence of the velocity field to prove that these oscillations are accompanied by fluctuations in density and, thus, phases of compression and extensions, although a direct measurement of the correlation between nuclear density and waves is lacking. Further experiments also revealed that areas of low cell density are characterized by high YAP nuclear localization and thicker cellular layers, hinting at the possible role of these oscillations in controlling cell behavior from a bio-chemical point of view. On a side note, related to the possible alignment between cell velocity and traction [25], the authors state that, only on a local scale, the velocity of each cell aligns with the traction forces with a delay of ~ 30 minutes.

V. MECHANICAL CONSIDERATIONS

All the phenomena described represent different aspects of collective migration, and as such, are strongly dependent on cell communication and, more generally, force production [48], a consideration common to all the works presented. On top of actin contractility and the formation of lamellipodia, other processes are responsible for mechanical stresses, such as cell division [79, 80], extrusion [7, 52] and volume changes [81]. The effect of some of these factors on mechanical oscillations has already been tackled, the effect of others remain to be assessed in the future.

A. Affecting the correlation length

Inhibition of myosin II activity, generally carried out with blebbistatin, leads to the the strong decrease of tractions and monolayer tensions [78], and, even though the integrity of cell-cell junctions is unaffected, a drop in correlation length [47]. If, on the one hand, during wound healing this has no effect on the migration velocity and the front of the monolayer advances at the same pace [25, 26], on the other, inhibition of contractility causes a loss of all emerging properties. Monolayers treated with blebbistatin lose the dependence of velocity on confinement geometry [50, 74, 75], the contraction-relaxation phenotype, swirling motion and mechanical oscillations in general [8, 25, 26, 49]. These reports agree that cell contractility is essential for oscillations, the only exception being the paper of Peyret et al. [77]. After treatment with a high dosage of blebbistatin, the authors observe a

sharp drop in cell velocity and the persistence of oscillatory motion, albeit with a larger period and smaller amplitude. These observation leads to the conclusion that cytoskeletal activity only promotes cell motion and their coordination, without having a fundamental role for the presence of oscillations [77]. The discrepancy might be attributable to the different cell type used.

Disruption of adherens junctions has a similar effect, preventing coordinated motion [47, 50] and stress buildup [49, 59, 82], ultimately hindering the propagation of mechanical waves [26, 51] and oscillations as a whole [8, 77]. Interestingly, desmosomes knockdown does not seem to have a similar effect [77].

One conclusion to be drawn from these evidences is that any treatment affecting mechanical communication between cells and, thus, the correlation length of their motion, should have an effect on the typical periodicity of mechanical waves. It has been previously reported that communication through the substrate, dependent on its elastic modulus, can affect the transmission of directional cues [47] and the extent of the velocity correlation [43]. In the future, it might be interesting to verify whether this parameter has an effect on mechanical waves. Dissipation processes, such as friction within the monolayer or with the substrate, could also introduce a viscous screening length [67]. Both internal and external frictions should be modified, possibly by changing the concentration of adhesive proteins on the surface and by tuning the strength of cell-cell junctions concomitantly. A precise characterization of dissipation processes, and a better understanding of their role in cell migration, would probably helps us to explain the the formation and disruption of collective patterns [83].

A different approach is to utilize biochemical treatments to change cell behavior. Good candidates are RAB5, shown to restore highly coordinated collective motion even after jamming [84], and various growth factors. EGF, for example, is involved in the inhibition of cell motion during jamming [85], and it has been associated to a change in the elastic modulus of monolayers and an increase in the period of breathing oscillations [25].

B. The role of mitosis

As discussed earlier, cell density is also a very important determinant for CCM (reviewed in [48]). It plays a fundamental role in contact inhibition and jamming, and contributes to the invasion of space during wound healing [26] and the presence of mechanical oscillations [49, 51]. Interestingly, although a transient arrest of mitosis does not alter oscillations in expanding tissues, where cell division is regarded as a perturbation in cell flow and a disturbance for the appearance of waves [51], proliferation has been associated with the contraction-relaxation motility on thin strips [74], two phenomena which share many similarities. Even though there have been con-

trasting reports, intuitively, mitosis should play a role in controlling CCM, and we already know it to be a fundamental factor for the growth of all living organisms. If periodic velocity waves do play a role in controlling cell behavior *in vivo* at the supracellular scale, possibly the most attractive perspective for this field, the role of mitosis should be specifically regarded in more detail in future experiments.

C. Elastic behavior of expanding tissues

Cell layers are generally considered as viscoelastic materials. Over relatively short timescales the response is elastic, and its origin is thought to be related to the passive spring-like behavior of the cytoskeleton and inter-cellular adhesions [86]. On longer timescales (\sim hours), tissues behave as active materials, accommodating deformations by remodeling cell-cell junctions, cell-cell rearrangements [87] or divisions [88], processes that dissipate stress in a manner reminiscent of viscous materials.

A peculiarity of the mechanical waves observed by Serra-Picamal and colleagues is that stress and strain are in phase, describing what is effectively an elastic behavior, on timescales in the order of hours [26]. If the viscoelastic description captures the monolayer expansion, it fails to account for the generation of propagative waves in an overdamped environment. The authors suggest that the introduction of two additional assumptions explains this effective long-term elasticity. First, cell motility increases when the surrounding cells create space. Second, above a given threshold of strain, the cytoskeleton first reinforces then fluidizes over different timescales [25, 26, 82].

Even though the assumption of an effective elastic behavior is far from being universally accepted, other works seem to be pushing the idea forward. Similar mechanical oscillations also emerge in round discs, where Notbohm et al. observed, once again, that cell velocity and monolayer tension are in phase. Moreover, phases of outwards motion were associated with an average cell stretching and, contrarily, inward motion with compression, further implying an elastic relationship with a modulus of 113 ± 28 Pa. An inhibition of actin contractility leads to a decrease of this value, demonstrating that elasticity is of an active nature [25]. Another measurement of this quantity was obtained by stretching a tissue between flexible poles, technique that enabled Harris and colleagues to estimate the elastic modulus at 20 ± 2 kPa for a MDCK cell monolayer [86]. A linear relationship between strain rate and stress was also observed in expanding tissues [82]. Interestingly, the stress buildup is independent of the substrate rigidity, therefore highlighting the role of cell-cell junctions. Without confinement, the traction forces exerted by cell colonies scale with their size, a linear relationship that agrees with the interpretation of the tissue as a (contractile) elastic layer coupled to the substrate [89].

In specific situations, such as the migration of monolayers over thin strips, tissues can extend over non adherent areas, called *epithelial bridges* [90]. As cell-substrate interactions are inhibited outside of the strips, the structural integrity of such bridges is ensured thanks to adherens junctions and actin contractility, which self assemble into a supra-cellular actin-cable under strong tension. The behavior of epithelial bridges in response to geometrical and mechanical perturbations is coherent with that of an elastic material. Interestingly, this is true for primary human keratinocytes, but not for MDCK cells, a discrepancy attributed to the more fluid nature of MDCK tissues [90].

Another peculiar instance of the mechanical response of tissues was reported by Latorre and colleagues. While studying the formation of 3D epithelial domes, they noticed that epithelial sheets could sustain massive deformations under constant tension thanks to the coexistence of super-stretched cells with barely stretched ones, in a behavior reminiscent of the super-elasticity of some alloys [91].

D. Effective inertia at the cellular level

The propagation of mechanical waves is, from a physical point of view, not of any particular complexity. It can generally be explained as a continuous inter-conversion between potential and kinetic energy, damped by friction forces. In this scenario, the presence of oscillations critically depends on the ratio between friction forces (“fighting” the oscillation) and the inertia (i.e. the mass, defining a timescale for the oscillation). When friction is negligible, the system is underdamped and waves can propagate indefinitely. Within tissues, on the other hand, being the mass of the cell extremely small, inertia is negligible compared to friction [63], an overdamped situation where wave propagation is, in principle, impossible. In this case, to explain the presence of the mechanical waves we have just reviewed, an additional phenomenon must be present to play the role of “effective inertia” [49] and restore oscillatory behaviors. Even though the biological nature of this feedback is not clear yet, merlin seems like the most obvious candidate, theoretical works have taken this factor into account by introducing an additional term. Even though a full review of the models utilized to explain oscillations in tissues is beyond the scope of this work (see [92–94]), it is nonetheless interesting to quickly glance at the different solutions embraced in literature.

One example is that of Vicsek models [95], where the velocity of each cell is assumed to align to that of its neighbors [8]. This alignment happens on a typical timescale, a fundamental quantity for the appearance of coordinated motion [96], and is affected by rotational noise. The group of Marchetti introduces a feedback between the local strain and the rate of change in contractile tension. Simply put, larger cells will exert stronger

contractile forces. This, together with the assumption of tissue elastic behavior, explains the presence of oscillations [25, 97]. Other possibilities are a dynamic response from the cytoskeleton, first reinforcing and then fluidizing in response to stretch [26], a slow realignment of cell polarity to the local strain [51] or to the resultant forces on the cell surface [77].

VI. DISCUSSION

Since the first reports, mechanical waves have been observed in a variety of situations, both in confined and unconfined environments. Even though the most studied model is MDCK cells, other types have been employed, and despite the intrinsic variability between them, all reports seem to agree on the typical times and space scale: mechanical oscillations happen with a periodicity of ~ 0.5 -1 mm and ~ 5 hours. Moreover, the effective velocity of transmission of mechanical signals, being them traveling or standing waves, is between 60 and 120 $\mu\text{m}/\text{h}$.

In all situations, the characteristics of the phenomena observed are associated to the degree of cooperativity of cell motion, quantified as the velocity correlation length. On freely expanding monolayers, this cooperation manifests itself as the presence of swirls [44], patches of cells moving parallelly [40] and finger-like protrusions [98]. When contractility or cell-cell junctions are disrupted, the expansion of the monolayer is driven solely by a density gradient, and these peculiar motion patterns are not present. Similarly, the presence of mechanical waves depends on the active communication between cells. Peculiar motion patterns, such as the contraction-relaxation phenotype, only happen when the width of the channel is below the correlation length [50] and the spacing between velocity waves launched the front of the monolayer is approximately twice this quantity [26]. This interpretation is also coherent with the presence of a transition between oscillatory regimes for values approximately equal to double the velocity correlation length (Figure Fig. 6a-b) [24]. As a gross simplification, having cells moving in opposite directions requires two groups of coordinated cells, which, in a quasi one-dimensional geometry, requires twice the correlation length. Interestingly, this change in cell behavior coincides with the transition between global coordinated rotation and the presence of multiple swirls for discs of diameter $\sim 300 \mu\text{m}$ [44].

There seem to be two contrasting points of view on the role of the confinement: the presence of waves in expanding monolayers [26, 51, 64], together with the observation of steady time and space-periods on lines of different length [24], suggests that oscillations might stem from a quantity intrinsic to the cell, which does not depend on the properties of the surroundings. On other hand, the period of breathing oscillations [8, 25] and collective elliptical movements [77] depends directly on the size of the colony (Fig. 6c). Viewed with the correlation length in mind, these two conclusions might be coherent.

Peyret and colleagues report that the period and amplitude of oscillations scale linearly with tissue size, a trend similar to that followed in a regime of global oscillations on quasi-1-dimensional lines [24]. The discrepancy stems from the fact that, whereas thin lines are designed to span a wide range of lengths, both considerably smaller and larger than the correlation length, the squares in [77] remain in the former regime, a fact that could hide the presence of a transition for larger system. From this point of view, the experiments in [77] remain in the “2D version” of the global oscillation regime, controlled by the smaller of two confining lengths. The presence of a transition in both directions might be assessed in the future. Moreover, the dependence of the space periodicity on confinement size (see Fig. 6a-b) describes a linear behavior with a threshold (i.e. the intercept is around 200 μm).

It seems plausible that some intrinsic property of cells is at the root of the periodic motion, which manifests itself whenever cell density is at levels preceding jamming. Above a certain threshold, and when the size of the confinement is of the same order of the correlation length, the cues relative to the presence of edges can be transmitted through the colony, and can actively tune the periodicity. The edges might act as cues for the synchronization of cell motion in time, as after the release of a border, or in space.

Furthermore, breathing oscillations fit in this be interpreted as global oscillations, with an additional cylindrical symmetry. By only considering the radial direction, the system can be reduced to one dimension, where the radius plays the role of the length. The two phenotypes might thus represent similar phenomena, an assumption confirmed by the fact that global oscillations are measured for lengths smaller than 400 μm [24], and all works reporting breathing oscillation are carried out on discs whose radius is smaller than 350 μm [8, 25, 52]. Whether different migration modes, characteristic of larger discs, is not clear.

In this perspective, all reports of mechanical oscillation represent different manifestations of the same phenomenon, guided by an intrinsic quantity and tuned by the symmetry and characteristic size of the available space, which highlight specific aspects of the underlying oscillations. Nonetheless, if breathing oscillations and standing waves describe periodic inversions of velocity and, thus, cell polarity, a fundamental difference with elliptical movement remains to be assessed. When seeded onto round patterns in fact, HeCaT cells do not display swirling motion [77], a discrepancy that sets them apart from other reports.

VII. CONCLUSIONS AND PERSPECTIVE

The presence of mechanical waves acquires new importance when viewed in light of the intricate relationship

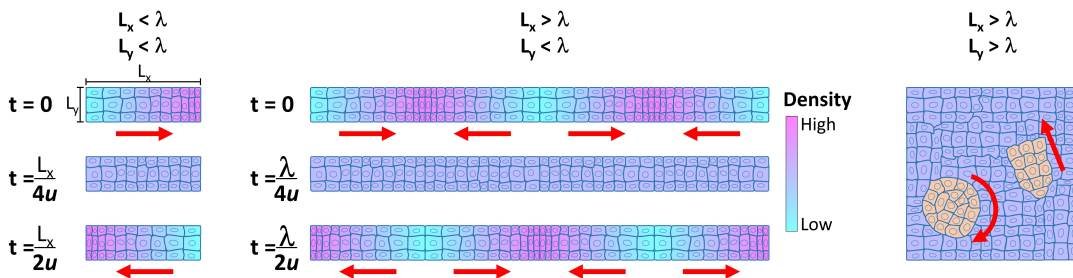


FIG. 6. Schematic view of velocity waves in different geometries. **a.)** Global oscillations appear when cells are confined in both directions below the coherence length λ , with a time period scaling linearly with the confinement size L_x . $u = \lambda/T$ ($u \simeq 80\mu\text{m}/h$) is the effective phase velocity of the wave. **b.)** Multinodal oscillations are established in quasi-1D geometry, along the direction where the confinement length is larger than λ . For any $L_x > \lambda$, the time period of the oscillations is T . **c.)** Swirling motion and collective migration appear in unconfined geometries (both L_x and L_y are both larger than the coherence length λ)

between bio-chemical signaling and mechanosensitive pathways, and their role in understanding the process of morphogenesis. All events leading to complex structures and shapes require cells in different positions to behave differently, in terms of movement, proliferation, differentiation and death [21]. For this to happen, those same cells need to somehow “be aware” of the state of the tissue and their particular location. The concept is summarized as *positional sensing* [62], and, as we now understand, happens through a bidirectional relationship between genetic and epigenetic factors. Mechanical signals are a major player in this latter category [23], their effects on cell behavior are well documented in 2D *in vitro* systems [5, 7–9] and are being understood on more complex environments [13, 20, 22]. In many of these cases, the specific setup prompts the emergence of a long-range mechanical landscape, used as a reference system. An example is that of cells plated on square patterns, where the edges are under stronger stress compared to the interior [5]. Every single cell then feels the local state, and acts accordingly, in this particular case enhancing or reducing proliferation rate. The coordination offered by the mechanical reference system leads to the emergence of a global phenomenon, at a scale much larger than that of the single cell sphere of influence.

In this context, the presence of traveling waves make sense as a mean to tune the local guidance, and exchange information over distances bigger than the immediate surroundings. In fact, the typical velocity of mechanical waves is in the order of $\sim 100 \mu\text{m}/h$, a value approximately tenfold larger than the average velocity of cells within a monolayer (between 4 and $12 \mu\text{m}/h$, depending on cell density [41, 45] and the properties of the environment [57, 99]). Even though the two phenomena might be somehow reconciled considering reflection at the edges and a “laser cavity” interference, there is a fundamental difference between traveling and standing waves,

The mechanical stimulus of a traveling wave is only

transient, and affects, at different times, all cells equally. Considering the velocity of propagation and the size of a cell, the time scale of the stimulus (\sim minutes) is in the range of cytoskeletal rearrangements, but it is much shorter than that needed for a change in gene expression (\sim hours) and, thus, a downstream bio-chemical response. If it is true that some degree of periodicity is generally present [26, 49, 51], this phenomenon is observed in expanding monolayers, associated with a deformation of the system. This makes the assessment of an eventual long timescale effect much more challenging.

Standing waves, on the other hand, are intrinsically periodic, and are observed in fully confined systems, where cells at a certain location are exposed to a regular mechanical stimulus over a longer timescales (\sim days [24, 25, 77]). These factors make standing waves a promising candidate for the exploration of possible downstream effects, a hypothesis which seems to be common to most of these reports. This idea stems from two basic considerations: standing waves are characterized by nodes and anti-nodes in cell velocity, the spacing of which seems to be controlled by an intrinsic quantity; and cells located at the anti-nodes appear to be alternatively stretched and compressed. This selective mechanical stimulus could somehow distinguish those cells, creating two sub-populations which undergo a different fate, and ultimately lead to supra-cellular organization. The report of a transition between migratory modes [24] suggests that standing waves might also provide a “time-zero” for the synchronization of cell behavior. If we consider a growing system, e.g. an embryo, below a certain size-threshold, in a regime of global oscillations, no node is present. As the embryo grows, such threshold is eventually reached, defining a precise moment (dependent of the system size) for the shift in cell behavior. Even though this is nothing but a hypothesis, it is interesting to notice that this intrinsic metric ($\sim 400 \mu\text{m}$) matches the spacing between hair follicles in the mouse embryo [2] and feather buds during chicken embryogenesis [22], both organs whose correct

development depends on coordinated cell migration.

This interpretation, even though extremely enticing, is still suffering from a general lack of documented biological consequences. If mechanical oscillations have been characterized on a number of different systems, their effects on the behavior of cells is still unclear. The obvious candidates for such role are those molecules involved in mechanotransduction, chief among which is YAP/TAZ [100]. This possibility has been recently explored [77], although a direct causal link between cell velocity and YAP translocation is still absent. Other promising molecules are β -catenin and merlin, for their mechanotransductive role in morphogenesis [101, 102] and in collective orientation during CCM [103], respectively. The activity of ERK, whose wave-like activation has been shown to be concomitant with mechanical waves [76], might also be significant.

If the presence of a downstream biological effector could shed light on the possible function of these oscillations, understanding their origin remains an important perspective. Specifically, two issues seem to have the biggest relevance: the source of the active elasticity which seems to characterize the response of monolayers

to stresses and the nature of the feedback mechanism allowing for the presence of sustained oscillations in biological (overdamped) systems. Regarding this second matter, most models rely on an interaction between cell polarity and another quantity, generally of a mechanical nature, making a direct measurement of stresses at cell-cell junctions [83] and a univocal way to assess cell polarity compelling future directions. Moreover, neither of these factors can be regarded at without considering the underlying architecture of the cytoskeleton, which remains an extremely important player for supracellular signal propagation and behavioral control in general.

In conclusion, mechanical oscillations have been characterized on a variety of geometries and cell types, and their presence is now widely accepted. Even if differences between reports remain to be assessed, the many similarities seem to paint a common picture, each observation representing a single point of view, selected by the experimental perspective, rather than being a phenomenon in its own right. In the future, a full understanding of waves requires a better characterization of the underlying biological processes, both in terms of the components that make oscillations possible, and a potential downstream signaling.

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