negative pressure. For example, two closely related ferroelectrics and piezoelectrics that are widely used in applications because of their excellent performance, Pb(Zr,Ti)O$_3$ and PbMg$_{1/3}$Nb$_{2/3}$O$_3$-PbTiO$_3$, could be improved further with negative pressure. In their determination of the crystal structure of the PX phase of PbTiO$_3$, Wang et al. previously found that the PX phase can accommodate up to 17% Zr, implying that the same synthesis route may also work for Pb(Zr,Ti)O$_3$. The method could also be generally applicable to completely different compositions of ferroelectric and non-ferroelectric matter formed from a lower density amorphous precursor. As amorphous precursors typically have significantly lower density, such a route might be used to impart negative pressure on the higher density product into which they transform.

The possibility of applying negative pressure to other materials — beyond ferroelectrics — to enhance their performance is also intriguing. For example, superconductors (such as Sr$_2$RuO$_4$) or ferromagnets (such as SrRuO$_3$), where positive pressure lowers their transition temperatures$^{12,13}$, might also benefit from this treatment. Negative pressure is a versatile tuning parameter and, like positive pressure or other forms of strain, offers a way to access hidden ground states. Aided by the ability to now access the negative pressure axis to tweak the properties of sensitive materials, the design space for materials becomes all the more immense. Navigating this space to discover useful materials for tomorrow is best achieved through a combination of theory (materials-specific theory in particular) with synthesis methods that can tailor matter with atomic specificity, including the ability to apply negative pressure. As a new degree of freedom, negative pressure is an important component for the emerging materials-by-design paradigm.

Darrell G. Schlom is in the Department of Materials Science and Engineering, Cornell University, Ithaca, New York 14853-1501, USA. Craig J. Fennie is at the School of Applied & Engineering Physics, Cornell University, Ithaca, New York 14853-3501, USA. e-mail: schlom@cornell.edu

References

Published online: 10 August 2015

TISSUE MECHANICS

Cell jam
Collective cell migration and jamming in the bronchial epithelium helps to understand the pathophysiology underlying asthma.

Melody A. Swartz

Under homeostatic conditions, cells that make up mature tissues — such as blood endothelium, skeletal muscle, and pulmonary epithelium — are thought to exist in optimized configurations. These cells do not generally migrate, unless they are subjected to remodelling (as occurs in wound repair, or in chronic pathologies such as fibrosis and cancer) or perturbed (by mechanical stress or chemical stimuli, for example). Yet when such normally immotile cells wander, they rearrange themselves relative to their surroundings, and are often seen to move in clusters — a phenomenon referred to as collective cell migration$^1$. Such cell clusters can invade surrounding tissue and migrate to distant sites, which occurs in diseases such as cancer, endometriosis and lymphangioleiomyomatosis. For example, in breast cancer, the transition from ductal carcinoma in situ to invasive carcinoma is often associated with finger-like projections of cells invading as a cohesive, synchronized group into surrounding tissue rather than as lots of single cells migrating independently of each other. Confluent cell monolayers (two-dimensional cell sheets) can also undergo collective cell migration; in this case, swirling cellular movements can be observed, with cells again seeming to move in packs rather than as individuals. When isolated and placed in culture, airway epithelial cells are initially highly motile, but their movements diminish over time until the cells seemingly become lodged and stop moving$^2$.

Despite the importance of collective cell migration in numerous physiological and pathophysiological processes, to date the study of the phenomenon has largely remained observational, and its underlying mechanisms are poorly understood. Reporting in Nature Materials, Jin-Ah Park and colleagues now propose a theoretical framework that describes collective cell interactions in airway epithelial cell sheets, and demonstrate that flaws in such behaviour might underlie pathophysiology, at least in airway epithelial cells from asthmatic patients$^3$.

Park and co-authors base their analysis on the physical phenomenon of particle jamming, where, as a result of physical crowding, a dense collection of particles undergoes a transition from a fluid-like state into a solid-like one (as when rice grains clog while flowing through a funnel). Such transition from fluid-like (unjammed) to solid-like ( jammed) is distinct from crystallization; particles in the jammed state remain disordered and can be unjammed (for example, by shaking the funnel to unclog it), and the transition is generally athermal$^{4,5}$. Jamming is typically studied in the context of granular media but can also describe materials such as foams, bubbles and clays, and has been applied to problems as diverse as highway traffic and the flow of people through an emergency exit$^{4,5}$. Particle jamming can, in fact, be described by statistical mechanics$^6$, where a critical value of the packing fraction as well as temperature and stress determine where the fluid–solid transition occurs; just around the critical value, the newly jammed
system exhibits some properties of the glass transition. Such an analysis allows for the prediction of jamming thresholds in a variety of particulate systems.

Unlike inert particles, however, cells are alive and motile, their movements powered by intracellular machinery and tensional forces generated from within. Yet the behaviour of collections of cells going from a seemingly stable configuration into suddenly changing ones, and back to a new equilibrium configuration, is reminiscent of unjamming and re-jamming. Surprisingly, although one might expect the jamming transition to be associated with increased interparticle adhesion (as has been shown for inanimate particles), Park and co-authors found that the bronchial epithelial cells follow the opposite trend — that is, unjamming increases with cell–cell adhesion. Moreover, intercellular adhesion is required for cell monolayers to pull on each other and generate line tension or traction forces within the cellular collective, and intercellular tension is needed for collective cell migration. To understand their observations, the authors used a theoretical model to analyse cell shape (this included cell area and perimeter, which reflect the balance between energies associated with intercellular cell–cell adhesion and intracellular cortical tension). They found that a critical cell-shape index predicts the jamming transition and corresponds well with their experimental data.

Furthermore, by imaging bronchial epithelial cells isolated from normal and asthmatic patients and plated in cell culture, the authors observed that normal cells undergo a phase transition from highly motile (moving as cell clusters) to a more locally stable, non-moving (jammed) state, but that cells from asthmatic patients maintain motility much longer and are more resistant to jamming (Fig. 1). The authors suggest that the unjammed state promotes tissue remodelling and could contribute to the pathology seen in asthma, in which airway–wall remodelling exacerbates the pathology. But in normal cells, excessive mechanical stress, such as that experienced during asthmatic bronchoconstriction, can also promote unjamming. The authors hypothesize that the different baseline levels of cytoskeletal integrity (that is, cortical tension) observed in cells from asthmatic patients (in normal cells, lower levels of cytoskeletal integrity can also be induced by mechanical stress) may translate, in the framework of the theoretical model, into a shift in the energy balance that lowers the barrier to unjamming. In vivo, this would imply that, whereas normal epithelial cells undergo migration and presumably remodelling only under high-stress conditions, asthmatic tissue behaves similarly in both stressed and unstressed states, promoting thickening of the airway epithelia.

Although the analogy between jammed particulate materials and jammed cells has limitations, it is fascinating that the frameworks developed for the simple non-living system can be extended to rationalize behaviour in the living one. The system studied by Park and colleagues, a two-dimensional cell collective, is a useful starting point for such analysis. It will be interesting to determine the extent to which the analogy can be extended into three-dimensional cell clusters, which are particularly relevant in skin fibrosis and cancer invasion.

Melody A. Swartz is in the Institute for Molecular Engineering and the Ben May Department for Cancer Research, University of Chicago, Chicago, Illinois 60637, USA. e-mail: melodyswartz@uchicago.edu

**References**

Unjamming and cell shape in the asthmatic airway epithelium

Jin-Ah Park1*, Jae Hun Kim1†, Dapeng Bi2, Jennifer A. Mitchel1, Nader Taheri Qazvini1,3, Kelan Tantisira4, Chan Young Park1, Maureen McGill1, Sae-Hoon Kim1, Bomi Gweon1, Jacob Notbohm1, Robert Steward Jr1, Stephanie Burger1, Scott H. Randell5, Alvin T. Kho6, Dhananjay T. Tambe1,7, Corey Hardin1, Stephanie A. Shore1, Eliot Israel4, David A. Weitz5, Daniel J. Tschumperlin9, Elizabeth P. Henske4, Scott T. Weiss4, M. Lisa Manning2, James P. Butler1,4, Jeffrey M. Drazen1 and Jeffrey J. Fredberg1

From coffee beans flowing in a chute to cells remodelling in a living tissue, a wide variety of close-packed collective systems—both inert and living—have the potential to jam. The collective can sometimes flow like a fluid or jam and rigidify like a solid. The unjammed-to-jammed transition remains poorly understood, however, and structural properties characterizing these phases remain unknown. Using primary human bronchial epithelial cells, we show that the jamming transition in asthma is linked to cell shape, thus establishing in that system a structural criterion for cell jamming. Surprisingly, the collapse of critical scaling predicts a counter-intuitive relationship between jamming, cell shape and cell-cell adhesive stresses that is borne out by direct experimental observations. Cell shape thus provides a rigorous structural signature for classification and investigation of bronchial epithelial layer jamming in asthma, and potentially in any process in disease or development in which epithelial dynamics play a prominent role.

One of the central unresolved mysteries of asthma is how the asthmatic airway remolds itself. This remoulding is often progressive and has long been thought to be the end product of a cascade of cell-signalling events that are initiated in the bronchial epithelium and driven by repetitive inflammatory, allergic, or viral insults. Tissue-remodelling events more broadly, including those underlying morphogenesis, wound repair and cancer invasion, have been linked to collective cellular migration, often in the context of the epithelial-to-mesenchymal transition (EMT; ref. 6). But no clear physical picture has yet emerged that can capture these collective biological processes and their interconnections. Here we provide evidence that the physical process of cell jamming and unjamming, which has been missing from descriptions of tissue remodelling, might tie together at least some of them.

Primary human bronchial epithelial cells (HBECs) were derived from non-asthmatic and asthmatic donors, plated on a porous Transwell insert, and established in air/liquid interface (ALI) culture (Methods). Initial culture in submerged conditions causes basal cells to proliferate, and subsequent culture in ALI conditions inhibits any further proliferation and triggers differentiation of those basal cells into a mature confluent pseudostratified bronchial epithelial layer comprising goblet cells and ciliated cells. Here we show that such a pseudostratified layer derived from non-asthmatic donors is quiescent. Cellular motions are relatively small, cellular rearrangements among neighbouring cells are rare, and each cell remains virtually caged by those immediate neighbours. Statistical analyses of these motions confirm that such a layer is solid-like and jammed. However, application of an apical-to-basal mechanical stress mimicking the compressive effect of bronchospasm (Supplementary Fig. 1) is sufficient to trigger large cellular motions and cooperative cellular rearrangements. Cells move chaotically, but the motions exhibit cooperative packs and swirls; such a layer is fluid-like and unjammed. We then examine the more complex process of progressive layer maturation, where we find an innate tendency of the maturing layer to transition from an immature, fluid-like, unjammed phase in which cells readily rearrange and flow, into a mature, solid-like, jammed phase in which cells become virtually frozen in place. As compared with non-asthmatic donors, however, in the maturing layer derived from asthmatic donors this jamming transition is delayed substantially or disrupted altogether. In all these cases, the transition between unjammed and jammed phases is continuous; as the cell layer approaches the jamming transition, cellular motions become progressively slower, pack sizes become progressively larger, and pack lifetimes become progressively longer.

One might have expected that cell jamming would be caused by increasing mutual cell–cell adhesive stresses such that cells become stuck to immediate neighbours and, as a result, the cellular collective rigidifies, the mutual cellular rearrangements stop.
Figure 1 | In a confluent layer of well-differentiated HBECs, compressive stress mimicking bronchospasm, as in asthmatic bronchospasm, provokes the transition from a solid-like jammed phase to a fluid-like unjammed phase. a, Speed maps (left panels) showed compressive stress at a magnitude of 30 cm H\textsubscript{2}O induced hypermobility of HBECs on ALI day 16. Within any optical field the migration speed was spatially heterogeneous but increased strongly with increasing \( P \). Colour scale is shown at the bottom of the left panels. The size of vectors (right panels) increased with increasing \( P \) and showed large-scale dynamic heterogeneity. Vector scale is shown at the bottom of the right panels. b, As \( P \) was progressively increased to 30 cm H\textsubscript{2}O (red filled circles), the mean square displacement, MSD, and the self-diffusion coefficient \( D_s \) increased (inset; \( D_s = \lim_{\Delta t \to \infty} \frac{\text{MSD}(\Delta t)}{4\Delta t} \)), and the system became strongly super-diffusive. Error bars in the inset represent the standard deviation. c, When \( P \) was less than 20 cm H\textsubscript{2}O, the relative overlap of each cell with its initial position was nearly perfect for time intervals \( (\Delta t) \) of less than 144 min, as quantified by the ensemble average, \( \langle Q(\Delta t) \rangle \), close to 1. When \( P \) was 30 cm H\textsubscript{2}O (red filled circles), the overlap decreased to 0.17. d, The four-point susceptibility \( \chi_4(\Delta t) \) is approximated by \( N(\langle Q(\Delta t)^2 \rangle - \langle Q(\Delta t) \rangle^2) \), where \( N \) is the number of cells. When movements are cooperative, \( \chi_4(\Delta t) \) exhibits a peak whose position corresponds roughly to pack lifetime, and whose magnitude corresponds roughly to pack size. When pressure was 30 cm H\textsubscript{2}O (red filled circles), \( \chi_4(\Delta t) \) showed a peak indicative of cooperative packs of faster-moving cells with a pack lifetime of 45 min and a pack size of approximately 70 cells.

and the constituent cells cannot move\textsuperscript{17}. Much to our surprise, direct measurements defied this expectation—in layers that become jammed, the adhesive stresses between a cell and its neighbours were attenuated, not augmented. To explain this paradox, we turned to the well-known vertex model, wherein a competition between cell–cell adhesive stresses and cell cortical tension control changes of cell shape\textsuperscript{18–20}. Novel analysis of this model, including a critical scaling analysis, predicts that increased adhesion leads to increased fluidity, and that cell jamming occurs as a well-defined index of cell shape approaches a critical value. We show that the shape index acts as a simple structural order parameter that takes on different values on either side of the jamming transition. Using that shape index, we show not only that cell shape in the bronchial epithelial layer differs between cells derived from non-asthmatic versus asthmatic donors, but also that, regardless of cell origin, cell shape at the jamming transition matches theoretical predictions, and thus resolves the paradox. This new physical picture raises questions about the relationship between the epithelial-to-mesenchymal transition and unjamming and, conversely, between the mesenchymal-to-epithelial transition and jamming. Similarly, it suggests new, testable hypotheses concerning asthma aetiology and asthma therapy. The more fundamental significance of these findings, however, may be to broaden notions of jammed matter and generalize understanding of jamming mechanisms.
Figure 2 | In HBECs over the course of ALI culture, a spontaneous phase transition occurs from a hypermobile, unjammed, fluid-like phase into a quiescent, jammed, solid-like phase, which was delayed in cells from asthmatic donors. a–c, Speed maps (left panels) and vector maps (right panels) showed that HBECs from a representative non-asthmatic donor were hypermobile on an early ALI day (a, day 3), but spontaneously became quiescent on later ALI days (b, day 6; and c, day 8). Colour and vector scales are shown at the bottom of c. d–f, Speed maps (left panels) and vector maps (right panels) showed that HBECs from a representative asthmatic donor were hypermobile until later ALI days (d, day 6; and e, day 10) and became quiescent on ALI day 14 (f). Colour and vector scales are shown at the bottom of f. g, Four-point susceptibility $\chi_4(\Delta t)$ for HBECs from a non-asthmatic donor showed peaks indicative of cooperative packs of faster-moving cells with a lifetime of 81 min with a corresponding pack size of approximately 20 cells on ALI day 3 (blue triangles), whereas peak was undetectable either on ALI day 6 (blue circles) or 8 (blue asterisks). Inset: MSD. h, Four-point susceptibility $\chi_4(\Delta t)$ for HBECs from an asthmatic donor showed peaks indicative of cooperative packs of faster-moving cells with lifetimes of 72 and 90 min with corresponding pack sizes of approximately 26 and 12 cells on ALI day 10 (red circles) and 6 (red triangles), respectively, whereas peak was undetectable on ALI day 14 (red asterisks). Inset: MSD.
Compression unjams the jammed HBEC layer

Mechanical compression of the bronchial epithelium occurs during severe bronchospasm (Supplementary Fig. 1) and is sufficient to induce maladaptive airway remodelling even in the complete absence of inflammatory events. Although certain cell-signalling modules have been identified, such as autocrine signalling through the epidermal-growth-factor-receptor family of ligands, the physical mechanism remains unclear. To trigger these events we followed the protocol of ref. 14; on day 16 of ALI culture we exposed the HBEC layer to a compressive apical-to-basal stress, \( P \), spanning the physiologic range (0, 3, 10, 20, or 30 cm H\(_2\)O; Fig. 1 and Supplementary Fig. 1), where 3 cm H\(_2\)O corresponds roughly to the maximal compressive stress expected to be exerted on HBECs by quiet tidal breathing, and 30 cm H\(_2\)O corresponds to that expected during severe bronchospasm.

The manner in which a cell moves provides clues about mechanisms that promote or impede its mobility (Fig. 1a). Therefore, we began by quantifying cellular motions using their mean square displacement (MSD) over a given time interval, \( \Delta t \), averaged over many cells in several optical fields (Methods). In many physical systems, the MSD increases with time as a power law, \( \Delta t^\alpha \), where the exponent \( \alpha \) is determined empirically. When the exponent is unity (\( \alpha = 1 \)), as in uncorrelated random Brownian motion, particle motions are diffusive. When \( \alpha < 1 \), particle motions are sub-diffusive, as when a particle might, in time, escape the cage comprising its immediate neighbours only to become quickly recaged by its new immediate neighbours; this uncaging–recaging process thereupon repeats in a stochastic fashion. When \( \alpha > 1 \), particle motions are super-diffusive, and when \( \alpha = 2 \), as occurs in simple linear translation at constant velocity, motions are ballistic.

In HBECs from non-asthmatic donors in ALI culture, cellular motions were smallest when the compressive stress, \( P \), was zero and increased systematically as \( P \) was increased (Fig. 1b and Supplementary Movie 1). When \( \Delta t \) was small and \( P \) was zero, the MSD increased slowly with time in a sub-diffusive manner as if each cell were caged or jammed by its immediate neighbours. But as \( \Delta t \) exceeded 10 min the MSD grew as \( \Delta t^{1.3} \), indicating that motions at longer times were slightly super-diffusive, and suggesting that each cell was eventually able to escape its cage and move in a persistent direction. But as \( P \) was increased progressively to 3 cm H\(_2\)O, the MSD at the highest \( P \) increased by about two orders of magnitude and varied as \( \Delta t^{1.8} \). Cells in this compressed state were seen to become highly mobile and strongly super-diffusive (Fig. 1b).

These swarming cellular motions and their changes were visually striking (Supplementary Movie 1). To provide further quantitative characterization of these motions, we measured the fractional change of cellular position in a given time increment \( \Delta t \). Much as is done conventionally in studies of cooperative particle motions in jammed or glassy inert systems, here we chose 15% of average particle (that is, cellular) diameter as the reference length scale; if a region moved less than 15% of the average cellular diameter over the time interval \( \Delta t \), we considered this as 100% overlap with the initial position, whereas if a region moved more than 15% of a cellular diameter, we considered this as 0% overlap (Methods).

The average value of this overlap function over the entire optical field was defined as \( Q(\Delta t) \) (ref. 23) and the ensemble average over all sequence images, \( \langle Q(\Delta t) \rangle \), was computed. Uncompressed cells and those compressed with \( P \) less than 20 cm H\(_2\)O showed nearly perfect overlap \((\langle Q(\Delta t) \rangle = 1)\) for as much as 144 min, as if cells were immobile and jammed. However, for cells compressed with \( P \) of 30 cm H\(_2\)O, \( \langle Q(\Delta t) \rangle \) fell to 0.17 at 144 min (Fig. 1c), suggesting appreciable mobility and, potentially, unjamming.

The MSD and the overlap function \( Q \) are useful metrics of cellular motions. However, these metrics by themselves fail to distinguish uncorrelated cellular motions from the cooperative motions that comprise the strings, clusters, swirls or eddies that are the hallmark of the structurally heterogeneous dynamics and typify jammed matter close to a jamming transition.\(^{16,24}\) In such systems, as the jamming transition is approached, the cooperativity of these motions increases and, as a result, the length scale and the timescale of these motions tend to grow sharply.\(^{25,26}\) Therefore, from the overlap function \( Q(\Delta t) \) we computed the four-point susceptibility, \( \chi_4(\Delta t) \), which exhibits a peak whose position corresponds roughly to pack or swirl lifetime and whose magnitude corresponds roughly to pack or swirl size (Methods).\(^{24,27}\) Accordingly, after several pack lifetimes a sufficient degree of structural rearrangements will have occurred such that any individual cell will find itself surrounded by a different set of immediate neighbours, and the original pack will have become shuffled with its neighbours to the extent that it becomes unrecognizable. As in the well-established glass transition,\(^{28}\) there is no objective cutoff that defines a jamming transition; rather, the transition is continuous, and jamming is said to occur when pack lifetime grows to exceed the laboratory measurement window, which we take here as 144 min.

In HBEC layers derived from non-asthmatic donors, \( \chi_4(\Delta t) \) revealed no peak for \( P \) less than 20 cm H\(_2\)O and time intervals less than 144 min (Fig. 1d); if a peak exists at all in these cases, it must be for substantially longer times. However, for \( P = 30 \) cm H\(_2\)O, a well-defined peak in \( \chi_4(\Delta t) \) emerged, corresponding to faster-moving cooperative packs comprising roughly seven cells with an average pack lifetime of 42 min (Fig. 1d). Compressive stress of 30 cm H\(_2\)O pressure was therefore sufficient to unjam the layer comprised of HBECs from non-asthmatic donors, whereas at lower compressive stresses the induced motions were far smaller, and structurally heterogenetic dynamics was not evident, as if the layer were frozen.

In cells from asthmatic donors, jamming is delayed

In submerged conditions, primary HBECs grow until confluence and, subsequently, in ALI culture they differentiate without further proliferation. These events recapitulate repair processes that are known to occur in vivo following epithelial injury or sloughing.\(^{3}\) Roughly by day 3 in ALI culture the early phase of rapid proliferation was completed and the maturation and differentiation of the layer then continued; cell density varied from well-to-well and from donor-to-donor, ranging roughly from 4,700 to 5,300 cells mm\(^{-2}\) and averaging 5,116 ± 233 cells mm\(^{-2}\), but did not increase systematically with ALI day \((p = 0.37)\). The cell layer increasingly exhibited a well-differentiated pseudostratified phenotype that included goblet cells and ciliated cells, apicobasal polarization, tight junctions, and an increasingly tight barrier function, as reflected in progressive increases of the trans-epithelial electrical resistance (TEER; ref. 29). As shown previously in HBECs (refs 29,30), we also found on the same ALI day lower TEER in cells from asthmatic donors compared with non-asthmatic donors (Supplementary Fig. 2a), suggesting defective barrier function in those asthmatic cells. In uncompressed layers, we then examined how these differences in barrier function correspond with changes in cellular jamming. Whether from non-asthmatic or asthmatic donors, the MSD and \( \chi_4(\Delta t) \) showed that cells were highly motile and unjammed on early days in ALI culture (Fig. 2a,d and Supplementary Fig. 2b and Supplementary Movie 2). For example, on day 3 (Fig. 2g, triangles), HBECs from a non-asthmatic donor showed peaks in \( \chi_4(\Delta t) \) indicative of faster-moving cooperative packs comprising roughly 20 cells with an average lifetime of 81 min, but by days 6 (Fig. 2g, circles) and 8 (Fig. 2g, asterisks), \( \chi_4(\Delta t) \) showed no peak, thus indicating that the layer had jammed.

Importantly, in cells from asthmatic donors compared with those from non-asthmatic donors this transition to the jammed phase in ALI culture was substantially delayed. For example, cells from a representative non-asthmatic donor jammed by day 6
(Fig. 2a–cg and Supplementary Movie 2), whereas cells from a representative asthmatic donor became jammed only by day 14 (Fig. 2d–f) and Supplementary Movie 3). On days 10 (Fig. 2h, circles) and 6 (Fig. 2h, triangles), HBECs from an asthmatic donor showed peaks in $\chi_2(\Delta t)$ indicative of faster-moving cooperative packs comprising approximately 26 and 12 cells with lifetimes of 72 and 90 min, respectively, but by day 14 (Fig. 2h, asterisks) showed no peaks in $\chi_2(\Delta t)$, thus indicating that the layer had jammed. This delayed jamming transition correlated well with delayed increases of TEER in cells from asthmatic donors compared with cells from non-asthmatic donors. For example, in cells from a representative non-asthmatic donor (Supplementary Fig. 2 and Supplementary Movie 2), TEER progressively increased to 370 $\Omega$ cm$^2$ by day 6 as cells jammed, and continued to increase to 700 $\Omega$ cm$^2$ until day 14, indicating that the early jamming transition corresponded to a rapid increase of barrier integrity. However, in cells from a representative asthmatic donor, TEER reached only 421 $\Omega$ cm$^2$ even on day 21 (Supplementary Fig. 2 and Supplementary Movie 3). Moreover, this delay in the spontaneous jamming transition was not donor specific, but was consistently observed in cells obtained from multiple asthmatic donors compared with those from non-asthmatic donors acquired from the same source (The University of North Carolina, Methods; Supplementary Fig. 3). Whereas cells from all non-asthmatic donors ($n = 5$) became jammed between day 4 and 10 (Supplementary Fig. 3, blue circles), cells from asthmatic donors ($n = 4$) mostly remained unjammed as late as day 14 (Supplementary Fig. 3, red circles).

Together, these systematic changes in cooperative cellular motions establish that HBEC layers in ALI culture express spatially heterogeneous dynamics of the kind that in inert systems has been taken as being the signature of matter that is close to a jamming transition. As shown below, all the epithelial systems studied here existed in the vicinity of a jamming transition. It is already known that asthmatic HBECs exhibit an aberrant injury response and compromised differentiation, and here we demonstrate, further, that maturation of the airway epithelial layer coincides in time with the transition from an unjammed to a jammed condition, and that this transition is appreciably delayed in layers derived from asthmatic compared with non-asthmatic donors (Fig. 2).

### Jamming and the adhesion paradox

In these collective cellular systems, what mechanical factors might promote cell jamming? Results from inert particulate matter suggest that plausible mechanisms would include increased mutual crowding and increased mutual adhesion. As regards mutual cellular crowding, we found no corresponding differences in the case of the marked hypermobility in cells from asthmatic versus non-asthmatic donors (Figs 1 and 2 and Supplementary Movies 1, 2 and 3). As regards mutual adhesion, we hypothesized that just as increased particle–particle adhesion promotes jamming in close-packed inert collective systems, so too increased cell–cell adhesion would promote jamming in the confluent living cellular layer. Superficially at least, such a notion would help to explain why maturing HBEC layers derived from non-asthmatic donors tend to jam more rapidly than do those from asthmatic donors (Fig. 2 and Supplementary Fig. 3), and would be consistent with the observations that HBEC layers from asthmatic compared with non-asthmatic donors exhibit decreased TEER, increased permeability and modestly diminished expression of E-cadherin. We rejected this hypothesis, however, based on the experimental evidence described below.

In the confluent intact epithelial layer, tugging (tensile) stress transmitted across the cell–cell junction overwhelmingly dominates compressive stress. As shown below, each cell tugs on immediate neighbours, but rarely pushes. Tugging stress exerted across the cell–cell junction can exist only to the extent that it is supported by cell–cell adhesive stress, there being no other mechanism to transmit tensile stress across a cell–cell junction. As such, we can logically equate tugging stress transmitted across the cell–cell junction to adhesive stress. To measure this adhesive stress within the intact cellular layer, we first used Monolayer Traction Microscopy to map local tractions that each cell exerts on its substrate, and then used Monolayer Stress Microscopy in the same layer to map intercellular stresses that each cell exerts on its immediate neighbours (Methods). We plated primary HBECs obtained from non-asthmatic donors or from asthmatic donors (Fig. 3) on a polycrylamide gel (Young’s modulus = 1.2 kPa, thickness = 100 µm); an important difference here compared with ALI culture conditions in Transwells described above is that plating HBECs on a gel precludes establishing an ALI culture. Hence, we resort to polycrylamide only as a matter of necessity. Nonetheless, these HBECs showed cooperative fluctuations comparable to those described above for cells in ALI culture (Supplementary Fig. 4a,b). On day 3 after seeding, HBECs from three asthmatic donors showed peaks in $\chi_2(\Delta t)$ indicative of cooperative packs comprising approximately 80, 20 and 70 cells with lifetimes of 20, 30 and 40 min, respectively, but HBECs from non-asthmatic donors showed no peaks in $\chi_2(\Delta t)$, thus indicating that the layers had jammed (Supplementary Fig. 4c,d). In a representative layer derived from a non-asthmatic donor, tractions exerted by HBECs on the substrate fluctuated markedly in time and in space (Fig. 3a) on a scale comparable to but somewhat larger than the size of a cell. The intercellular stress was heterogeneous and predominantly tensile (Fig. 3c); in all experimental repetitions, stress averaged over the entire cell field was variable but tensile (average tension: 315 ± 283 Pa; $n = 5$). In the HBEC layers derived from asthmatic versus non-asthmatic donors, r.m.s. tractions exerted on the substrate were not statistically different, but tended to be larger (Fig. 3b; 114 ± 88 Pa versus 24 ± 7 Pa; $p = 0.22$). However, intercellular tensions exerted by each cell on its neighbours were larger by a factor 1.5 to 5 (Fig. 3d; 792 ± 171 Pa versus 257 ± 82 Pa; $p = 0.02$). In both non-asthmatic and asthmatic HBECs, cells moved in packs, with regions of high tension (Fig. 3c,d) that spanned many cells (Fig. 3e,f), indicating that the collective tension was cooperative over distances comparable to many cell diameters. To quantify the spatial extent of this stress cooperativity, we measured the spatial autocorrelation function of tugging stress (tension), $C(R)$, as a function of cell separation distance, $R$ (Methods). In every case the tension correlation $C(R)$ decayed over several hundred micrometres, but with faster spatial decay in cells derived from asthmatic versus non-asthmatic subjects (Fig. 3i; the correlation values $C(R)$ at 14 mm were 0.29 ± 0.02 and 0.48 ± 0.03 in asthmatic and non-asthmatic HBEC layers, respectively; $p = 0.003$). HBECs derived from asthmatic versus non-asthmatic donors bear higher but more localized intercellular tension.

On the basis of these observations we cannot determine if build-up of intercellular adhesive stresses might promote unjamming or, conversely, if unjamming might promote build-up of intercellular adhesive stresses. We can say, however, that unjamming coexists with the amplification of local adhesive intercellular stresses, not with their attenuation (Fig. 3). This finding is counterintuitive, but is in concert nevertheless with comprehensive recent findings of others establishing by direct measurements that cell–cell adhesive stress is not attenuated by reducing the expression of E-cadherin.

### Cell jamming, mutual adhesion and perimeter

To shed light on this paradox we turned to the vertex model of the cellular layer (Supplementary Equations 1 and 2 in the
Figure 3 | In HBECs derived from asthmatic donors compared with those from non-asthmatic donors, tractions and intercellular stresses are greater but the spatial correlation of tension decays faster. a, b. Colour maps of tractions exerted by HBECs derived from a non-asthmatic donor (a; N2 in g–i) and an asthmatic donor (b; A2 in g–i) on their substrates. Colour scale is shown to the right of b. c, d. Colour maps of intercellular stresses exerted across cell–cell junctions for donors N2 (c) and A2 (d) show packs of high tension that span many cell diameters. Colour scale is shown to the right of d. e, f. Phase-contrast maps of HBEC layers on polyacrylamide gels for donors N2 (e) and A2 (f). g. In cells derived from asthmatic donors (red: A1, A2, A3) versus non-asthmatic donors (blue: N1, N2), root mean square (r.m.s.) tractions were not statistically different, but tended to be larger (r.m.s. traction: 792 ± 88 Pa versus 24 ± 7 Pa; variance: 7,821 versus 53; p = 0.22). h. Intercellular tensions were larger by a factor of 1.5 to 5 in cells derived from asthmatic (red) versus non-asthmatic donors (blue; tension: 792 ± 171 Pa versus 257 ± 82 Pa; variance: 29,426 versus 6,787; p = 0.02). i. Spatial autocorrelation function, C(R), of tension as a function of cell separation distance, R, shows that the tension correlation decayed over several hundred micrometres in all cases, but extended to shorter distances in cells derived from asthmatic (red symbols) versus non-asthmatic donors (blue symbols), thus confirming that intercellular stresses were larger in magnitude but more highly localized in the HBECs derived from asthmatic donors compared with non-asthmatic donors (C(R) at 140 µm in asthmatic versus non-asthmatic HBEC layers: 0.29 ± 0.02 versus 0.48 ± 0.03; variance: 0.0004 versus 0.0009; p = 0.003). The r.m.s. tractions, intercellular tensions and spatial autocorrelation in g–i were averaged across three to five experimental repeats for each donor. Error bars in g–h represent the standard error.

Supplementary Information), which represents the projection of each cell in two-dimensions by an irregular curved polygon with a shape index, $p = P/\sqrt{A}$, where $P$ and $A$ are the cell perimeter and projected area, respectively (Supplementary Fig. 7). The model proposes that the mechanical energy associated with each cell is a function of three contributions: an energy associated with cell area and attributable to area compressibility; an energy associated with cell perimeter attributable to the stiffness and contractility of the apical actomyosin ring; and an energy associated with interfaces of cell–cell contact, including the effects of adhesion molecules such as cadherins and associated actomyosin interactions, which, together, are expressible as a net line tension $\Delta \varepsilon$ (Supplementary Information). $\beta$ and $\Delta$ are critical scaling exponents. Importantly, $p^*_\beta \equiv 3.81$ is a critical shape index derived from the analysis of critical scaling behaviour; it is a pure number that rests on no adjustable model parameters or curve fitting to data (Supplementary Information). As the argument of $f_\beta$ becomes small, one branch approaches a finite energy barrier height, thus implying solid-like
Figure 4 | With increasing maturation of HBECs in ALI culture, cell perimeter, as expressed by the non-dimensional parameter $\bar{p}$, decreases systematically towards the critical value $p_c^*$ (3.81) predicted to occur at jamming by the vertex model together with the theory of critical scaling exponents. a. Over the course of maturation in ALI culture, HBECs from a representative non-asthmatic donor (Fig. 2 and Supplementary Movie 2) approached the jammed state, and the median ratio of perimeter to the square root area of cells systematically approached the jamming threshold $p_c^*$. In HBECs from a representative asthmatic donor (Fig. 2 and Supplementary Movie 3), however, the approach of $\bar{p}$ to $p_c^*$ was considerably delayed. Over time, and in both cases, $\bar{p}$ systematically approached the jamming threshold of 3.81. Inset: $\bar{p}$ for representative non-asthmatic and asthmatic donors plotted with the same axis of ALI days to allow comparison of the jamming transition timing. Boxplot shows median and quartiles. Whiskers are maximum and minimum data points. b. Simulated tissues with input parameters of target cell-shape index $p_0 = 4.2$, corresponding to a fluidized state (top panel), and $p_0 = 3.813$, corresponding to a jammed tissue (bottom panel).
be useful for other isotropic tissues, but that it may fail in interesting and potentially predictable ways for anisotropic tissues, such as in the endothelial layer under shear flow.39

The vertex model is therefore seen to capture essential features of collective cellular behaviour, especially the existence and properties of a jamming transition. Specifically, in some conditions each cell becomes frozen in place relative to immediate neighbours, as if caged, whereas in other conditions each cell readily exchanges places with immediate neighbours and, as a result, the integrated tissue continuously remodels. Concerning rates of cellular motion and rearrangement, across the transition there exist qualitative as well as quantitative differences in rates of cellular motion and rearrangement. The vertex model also predicts precisely how cell rearrangements across this transition—as independently assessed by dynamic metrics of jamming—are marked by changes in a specific index of cell shape (Fig. 4). Nevertheless, interesting theoretical questions remain unanswered. In the vertex model, for example, propulsive stresses are not taken explicitly into account. Theories based directly on self-propelled particles, by contrast, show that propulsive forces help to unjam particulate systems,41,42 but in such systems jamming is driven by mutual crowding rather than by changes in mutual adhesion or line tension. As such, they do not account for the changes of cell shape reported here, and they predict a transition that is therefore of a fundamentally different kind (Supplementary Information).41 For that reason, addition of propulsive stresses to the vertex model would help to resolve the questions of the extent to which propulsive stresses might alter the nature of the transition or the centrality of the critical shape parameter, p_c (refs 20,39,44–46).

The emerging physical picture

It has been argued recently that a wide variety of living collective systems evolve spontaneously towards the neighbourhood of a critical phase transition.43–49. In the particular case of the airway epithelial cellular layer, cellular motions (Figs 1 and 2), cellular forces (Fig. 3) and cellular shapes (Fig. 4) all provide strong evidence that a critical jamming transition occurs in these tissues. Our findings thereby suggest a new physical picture of airway epithelial remodelling, maturation and repair, in which epithelial cells migrate collectively in conditions close to a jamming transition.51. When effective energy barriers are small or non-existent, the tissue is readily able to explore new configurations as the system remodels into new states, some of which might be adaptive and others maladaptive.46,47. But, as energy barriers increase, the tissue becomes locked into a jammed state.

The bronchial epithelium is subject to repeated mechanical perturbations through the action of spontaneous breathing, and is subject to injury through exposure to harmful environmental pollutants, viruses, allergens, reactive oxygen species, or inflammatory mediators. Here we put forward the hypothesis that in non-asthmatic subjects these external factors can cause the cellular collective to unjam, explore various possible configurations, and then re settle into an adaptive quiescent, jammed, solid-like state; by so doing they effect self-repair in that remodelled state. Importantly, we have not yet determined the molecular mechanisms that impact the jamming transition, and we expect that there could be many. Nevertheless, the observations reported here establish a simple and easily measured structural index that quantifies the proximity of the layer to a jamming transition, and suggests that perhaps molecular mechanisms can be investigated and categorized by how they affect jamming.

Bronchial epithelial cells and their mechano-sensitivity are increasingly understood to play a major role in airway remodelling in asthma, but just how these events are linked to asthma pathobiology has remained unclear.12,14,15,21,34,50–52. In cells from asthmatic compared with non-asthmatic donors in ALL culture, we show that the transition from a hypermobile, un jammed, fluid-like phase to a quiescent, jammed, solid-like phase is delayed substantially or disrupted altogether. Whether this delay arises from layer injury, immaturity, or dysmaturity, the prolonged or sustained hypermobile fluid-like phase defines an unanticipated maladaptive phenotype which, to our knowledge, comprises the first known instance—in any disease—where pathobiology is linked to the recent discovery of cellular jamming and unjamming.45,53,54. It remains to be determined if these processes and their downstream effects might be favourably impacted by bronchodilators, corticosteroids, or other therapeutic interventions.

Methods

Methods and any associated references are available in the online version of the paper.

Received 13 February 2015; accepted 18 June 2015; published online 3 August 2015

References


**Acknowledgements**

Authors thank the staff of the UNC CF Center, Tissue Procurement and Cell Culture Core at the University of North Carolina, Chapel Hill. This research was supported by the Francis Family Foundation, the Alfred P. Sloan Foundation, the American Heart Association (13SDG14320004), the National Research Foundation of Korea (NRF-2011H1A2A2035518), the National Science Foundation (BMIB-1334611, DMIR-1352184) and the National Institutes of Health (K25HL091124, P30DK065988, P30ES000002, HL007118, RO1HL102373, RO1HL107561, PO1HL120839).

**Author contributions**


**Additional information**

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.-A.P.

**Competing financial interests**

The authors declare no competing financial interests.
**Methods**

**Culture of primary human bronchial epithelial cells.** We used primary human bronchial epithelial cells (HBECs) derived from ten non-asthmatic and nine asthmatic donors from two distinct sources. Primary HBECs at passage 1 were purchased from Lonza or provided by S. Randell at the Marsico Lung Institute/Cystic Fibrosis Research Center at the University of North Carolina, Chapel Hill. Human lungs unsuitable for transplantation, including three cases of fatal asthma and one with asthma in the medical social history, were obtained under protocol #03-1396 approved by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board. Informed consent was obtained from authorized representatives of all organ donors. All non-asthmatic lungs were from non-smokers with no history of chronic lung disease. HBECs from all donors were cultured as previously described. Passage 2 HBECs were plated at a density of 125,000 cells cm⁻² on 12 Transwell plates with polyester membranes with 0.4 µm pores (Corning) coated with 50 ng ml⁻¹ of type 1 rat tail collagen (BD Biosciences). Cells were cultured for five to seven days under submersed conditions until confluence. Once cells reached confluence, submersed condition was switched to the ALI condition by removing medium from the apical surface. The ALI culture was maintained for another 16–18 days, unless described otherwise. By 10–11 days in ALI culture, cells from all donors produced a substantial amount of mucus at the apical surface.

**Exposure of HBECs to compressive mechanical stress.** On day 16 of ALI culture, when HBECs had matured into a well-differentiated phenotype of confluent bronchial epithelium similar to that existing in vivo, we exposed cells to apical-to-basal compressive stress spanning the physiologic ranges (0, 3, 10, 20, or 20 kPa) and allowed HBECs to grow to confluence for three days. To measure mechanical stresses within the HBEC layer, we seeded HBECs on a polyacrylamide gel (Young’s modulus = 1.2 kPa, thickness = 100 µm). Gel preparation and seeding protocols were similar to published protocols. Briefly, we deposited a polydimethyl siloxane (PDMS) membrane with a rectangular opening (8 × 8 mm) on the gel. After coating the gel with type 1 collagen (BD Biosciences), we seeded HBECs derived from non-asthmatic and asthmatic subjects. We then removed the PDMS membrane and allowed HBECs to grow to confluence for three days. Local gel displacements were quantified from an image of embedded fluorescent markers at each experimental time point and a reference image obtained after trypsinization. Local migration velocities and gel displacements were obtained by the PIV method using a cross-correlation window size of 32 × 32 pixels (pixel size of 0.88 µm). To obtain substrate tractions, we used the numerical procedure from Fourier-Transform Traction Microscopy. To obtain layer stresses, we used the numerical procedure from Monolayer Stress Microscopy. Briefly, we computed a map of the tractions exerted by the cells on their substrate using gel displacements. From these tractions, we obtained the distribution of intercellular stresses within the cellular sheet. At each point in the cellular sheet, we computed the two principal stress components σ_{max} and σ_{min} and their corresponding, mutually perpendicular, principal orientations. We then computed the local tension within the cellular sheet, defined as σ = (σ_{max} + σ_{min})/2. The boundary edge of the patterned cellular sheet was taken to be zero stress. After recovery of a stress map, an area near the edge (~1 mm) was cropped from the map (Fig. 3a–d).

**Measurement of mechanical stresses within the HBEC layer.** To measure mechanical stresses within the layer plane, we seeded HBECs on a polyacrylamide gel (Young’s modulus = 1.2 kPa, thickness = 100 µm). Gel preparation and seeding protocols were similar to published protocols. Briefly, we deposited a polydimethyl siloxane (PDMS) membrane with a rectangular opening (8 × 8 mm) on the gel. After coating the gel with type 1 collagen (BD Biosciences), we seeded HBECs derived from non-asthmatic and asthmatic subjects. We then removed the PDMS membrane and allowed HBECs to grow to confluence for three days. Fluorescence and phase-contrast images were acquired at 10-min intervals for 2 h. Local gel displacements were quantified from an image of embedded fluorescent markers at each experimental time point and a reference image obtained after trypsinization. Local migration velocities and gel displacements were obtained by the PIV method using a cross-correlation window size of 32 × 32 pixels (pixel size of 0.88 µm). To obtain substrate tractions, we used the numerical procedure from Fourier-Transform Traction Microscopy. To obtain layer stresses, we used the numerical procedure from Monolayer Stress Microscopy. Briefly, we computed a map of the tractions exerted by the cells on their substrate using gel displacements. From these tractions, we obtained the distribution of intercellular stresses within the cellular sheet. At each point in the cellular sheet, we computed the two principal stress components σ_{max} and σ_{min} and their corresponding, mutually perpendicular, principal orientations. We then computed the local tension within the cellular sheet, defined as σ = (σ_{max} + σ_{min})/2. The boundary edge of the patterned cellular sheet was taken to be zero stress. After recovery of a stress map, an area near the edge (~1 mm) was cropped from the map (Fig. 3a–d).

**Calculation of the spatial autocorrelation function of tension.** To quantify the spatial extent of stress cooperativity, we computed the spatial autocorrelation function of intercellular tension:

\[
C(R) = \frac{1}{N \text{var}(\sigma)} \sum_{i=1}^{N} \sum_{|R_i - R_j| = R} \delta_{\sigma_i} \delta_{\sigma_j}
\]

where \(\sigma_i\) is the local departure of the tension at position \(R_i\) from its spatial mean \(\bar{\sigma}\), \(\text{var}(\sigma)\) is the variance of those departures, and \(|R_i - R_j| = R\) denotes equality within a uniform bin width of 20 µm, within which there are \(N\) points.

**Quantification of cell shapes in ALI culture.** Time-lapse phase-contrast images of HBECs in ALI culture were acquired as described above. For each condition and donor, 130 cells were manually traced using ImageJ software (National Institutes of Health) from four different fields of view, and the shape index \(\delta = P/\sqrt{A}\), where \(P\) and \(A\) are the cell perimeter and projected area, was computed for each traced cell. The median of this shape parameter \(\bar{\delta}\) was computed. With a ‘bootstrap’ method, the data were uniformly randomly sampled with replacement 10⁸ times, from which the distribution of the resulting medians was used to determine confidence intervals and \(\bar{\delta}\) values.

**References**


Unjamming and cell shape in the asthmatic airway epithelium

Authors: Jin-Ah Park1*, Jae Hun Kim1*, Dapeng Bi2, Jennifer A. Mitchel1, Nader Taheri Qazvini1,8, Kelan Tantisira3, Chan Young Park1, Maureen McGill1, Sae-Hoon Kim1, Bomi Gweon1, Jacob Notbohm1, Robert Steward, Jr.1, Stephanie Burger1, Scott H. Randell4, Alvin T. Kho5, Dhananjay T. Tambe1,9, Corey Hardin1, Stephanie A. Shore1, Elliot Israel3, David A. Weitz6, Daniel J. Tschumperlin7, Elizabeth P. Henske3, Scott T. Weiss3, M. Lisa Manning2, James P. Butler1,3, Jeffrey M. Drazen1, Jeffrey J. Fredberg1

1Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
2Syracuse University, Syracuse, NY 13244, USA
3Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115, USA
4The University of North Carolina at Chapel Hill, NC 27514, USA
5Children’s Hospital, Boston, MA 02215, USA
6Harvard University, Cambridge, MA 02138, USA
7Mayo Clinic College of Medicine, Rochester, MN 55905, USA
8School of Chemistry, College of Science, University of Tehran, Tehran, Iran
9Department of Mechanical Engineering, University of South Alabama, Mobile, AL 36688, USA
*These authors contributed equally.
Correspondence to: jpark@hsph.harvard.edu

List of Contents

1. Supplementary Figures 1- 6
2. Supplementary Table 1
3. Supplementary Movie Legends
4. Supplementary Text
5. References
Fig. S1. From the bronchoconstriction in vivo to a simulated experimental system in vitro. During an asthma exacerbation, the airway become narrowed (a), in which bucking of epithelium occurs. The buckled epithelium is exposed to compressive stress (b). This condition is simulated in an in vitro compressive system (c). The permission for the reuse of figures has been requested to the publishers. Reprinted with permission from Elsevier for a, from American College of Chest Physicians for b, and Annual Reviews for c.
Fig. S2. Over the course of ALI culture, a delayed increase of Trans-epithelial electrical resistance (TEER) (a) was well correlated with a delayed decrease of average cell speed (b) in HBECs from a representative asthmatic donor (•, A4), compared to a representative normal donor (△, N3).
Fig. S3. The spontaneous jamming transition was systematically delayed in primary HBECs obtained from multiple asthmatic donors compared with those from non-asthmatic donors. HBEC layers in ALI culture were considered jammed when four-point susceptibility $\chi_4(\Delta t)$ revealed no peak for time intervals less than 144 minutes. Whereas cells from non-asthmatic donors completely jammed between day 4 and 10 (O), cells from asthmatic donors mostly remained unjammed as late as day 14 (O). The jamming transition in HBECs from asthmatic donors was significantly delayed compared with those from non-asthmatic donors. (p=0.0317)
SUPPLEMENTARY INFORMATION

DOI: 10.1038/NMAT4357

Fig. S3. The spontaneous jamming transition was systematically delayed in primary HBECs obtained from multiple asthmatic donors compared with those from non-asthmatic donors. HBEC layers in ALI culture were considered jammed when four-point susceptibility $\chi_4(\Delta t)$ revealed no peak for time intervals less than 144 minutes. Whereas cells from non-asthmatic donors completely jammed between day 4 and 10, cells from asthmatic donors mostly remained unjammed as late as day 14. The jamming transition in HBECs from asthmatic donors was significantly delayed compared with those from non-asthmatic donors. (p=0.0317)

Fig. S4. On day 3 after seeding on polyacrylamide gels, HBECs derived from non-asthmatic donors were jammed, whereas those from asthmatic donors remained unjammed. a-b. Cellular speed maps and corresponding velocity vectors of HBECs derived from a representative non-asthmatic donor (Fig. 3e, N2 in Fig. 3g,h,i) and a representative asthmatic donor (Fig. 3f, A2 in Fig. 3g,h,i). Scale bar: 200µm. c-d. The overlap function $Q(\Delta t)$ and the four-point susceptibility $\chi_4(\Delta t)$ in the layers of HBECs from donors corresponding to those in Fig 3g,h,i. In HBECs from asthmatic donors (red) $\chi_4(\Delta t)$ displayed peaks at 20, 30, and 40 minutes, respectively, while in HBECs from non-asthmatic donors (blue) $\chi_4(\Delta t)$ showed no peaks thus indicating that the layers had jammed.
Fig. S5. With layer maturation, cell shape approaches the critical jamming value. The cell shape parameter, $p$, was measured for maturing HBECs on days 3, 6, and 8 of ALI culture in layers from a representative non-asthmatic donor (O) and on days 6, 10, an 14 of ALI culture in cells from a representative asthmatic donor (■). Solid lines show $\bar{p}$ for the representative donors. Dotted lines show upper and lower bounds of the 95% confidence interval (CI) for the median of the distribution at each day of ALI culture (Supplementary table 1). The long dashed line shows the critical value $p_0^*=3.81$, for which cells in a monolayer are predicted to be jammed. Data are indicated to be statistically different from $\bar{p}=3.81$ with asterisks (**$p<0.0001$, *$p<0.001$; see Supplementary table 1). Together, these data show that as ALI day progressed and HBECs became increasingly jammed, as indicated by decreased mobility and an increase in pack lifetime, $\bar{p}$ approached the jamming threshold $p_0^*$. 

Fig. S6. Unjamming, induced by mechanical compression mimicking bronchospasm, correlates with elevated values of the shape parameter $p$. The cell shape parameter, $p$, was measured for well-differentiated HBECs with or without an application of compressive stress. After the application of mechanical compression the HBECs monolayer became unjammed and $p$ substantially exceeded $p_0^*$. By comparison with Fig. S5, these data suggest that mechanical compression creates and sustains an immature or dysmature layer phenotype.
Fig. S6. Unjamming, induced by mechanical compression mimicking bronchospasm, correlates with elevated values of the shape parameter $p$. The cell shape parameter, $p$, was measured for well-differentiated HBECs with or without an application of compressive stress. After the application of mechanical compression the HBECs monolayer became un jammed and $p$ substantially exceeded $p_0^*$. By comparison with Fig. S5, these data suggest that mechanical compression creates and sustains an immature or dysmature layer phenotype.
Supplementary table 1. The cell shape parameter, $\bar{p}$, was measured for maturing HBECs.

<table>
<thead>
<tr>
<th>Day</th>
<th>$\bar{p}$</th>
<th>95% CI</th>
<th>p-value</th>
<th>$Q_{150}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.96</td>
<td>[3.91, 4.01]</td>
<td>p&lt;0.0001</td>
<td>0.69</td>
</tr>
<tr>
<td>6</td>
<td>3.88</td>
<td>[3.58, 3.93]</td>
<td>p&lt;0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>8</td>
<td>3.83</td>
<td>[3.81, 3.86]</td>
<td>P=0.152</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>4.11</td>
<td>[4.04, 4.16]</td>
<td>p&lt;0.0001</td>
<td>0.68</td>
</tr>
<tr>
<td>10</td>
<td>3.89</td>
<td>[3.86, 3.95]</td>
<td>p&lt;0.0001</td>
<td>0.59</td>
</tr>
<tr>
<td>14</td>
<td>3.86</td>
<td>[3.83, 3.91]</td>
<td>p&lt;0.001</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Values for $\bar{p}$ and 95% confidence intervals (CI) for each donor and day, corresponding to data in Fig. 4 and Supplementary Fig. S5. Confidence intervals were constructed using a non-parametric bootstrap method (Methods). P-values were obtained against the null hypothesis that the critical value of the shape parameter $p_0^*=3.81$ is the true median of the distribution. If the CI does not contain the critical shape parameter $p_0^*$ (p<0.05) then the structural analysis predicts an unjammed system. If the CI does contain $p_0^*$, then the system is predicted to be jammed. We found that $\bar{p}$ decreases with ALI day, and is not significantly different from $p_0^*$ by day 8 for a representative normal donor. For a representative asthmatic donor, $\bar{p}$ is significantly higher than $p_0^*$, even until day 14. The self-overlap parameter $Q$ is shown for each donor and day at $\Delta t=150$ minutes, indicating the fraction of the cells which no longer overlapped with their original position after 150 minutes. This metric of jamming decreases with increasing ALI, in parallel with the shape parameter $\bar{p}$.
Supplementary Movie Legends

**Movie 1.** Application of compressive stress with 30cm H$_2$O pressure provokes the unjamming transition of well-differentiated primary human bronchial epithelial cells (HBECs) on day 16 of air-liquid interface (ALI) culture.

- Non-asthmatic cells without compression (Fig. 1)
- Non-asthmatic cells after compression (Fig. 1)

**Movie 2.** Movies show the jamming transitions of HBECs from a representative non-asthmatic donor on ALI day 3, 6, and 8.

- Non-asthmatic cells on ALI day 3 (Fig. 2a)
- Non-asthmatic cells on ALI day 6 (Fig. 2b)
- Non-asthmatic cells on ALI day 8 (Fig. 2c)

**Movie 3.** Movies show the jamming transitions of HBECs from a representative asthmatic donor on ALI day 6, 10, and 14.

- Asthmatic cells on ALI day 6 (Fig. 2d)
- Asthmatic cells on ALI day 10 (Fig. 2e)
- Asthmatic cells on ALI day 14 (Fig. 2f)

**Movie 4.** Simulations of unjammed and jammed tissues.
Supplementary Text

1 The vertex model for confluent tissues

We use the vertex model\textsuperscript{18–20,62–67} to predict cell shapes and how those shapes evolve as cells migrate in confluent tissues containing no empty spaces between cells. The vertex model describes a collection of three-dimensional cells that pack in a columnar structure to form a monolayer. In a 2D cross-section through the monolayer, each cell appears as an irregular, curved polygon with a well-defined perimeter and cross-sectional area. Cells shapes often form regular patterns\textsuperscript{66}, and fluctuations due to cellular motile forces generally lead to small or short-lived perturbations to cell shapes\textsuperscript{64}. Based on these observations, the vertex model assumes that cytoskeletal and adhesion forces act together to generate a preferred cell shape, and that a significant amount of work is required to change this preferred shape. Agreement between observed and predicted cell shapes in many tissues validates this assumption\textsuperscript{18,63,66–68}.

The vertex model describes the work required to change a cell’s shape in terms of an effective mechanical energy per cell:

$$E_i = \beta_i (A_i - A) + \xi_i P_i^2 + \gamma_i P_i,$$  \hspace{1cm} (S1)

where the subscript $i$ labels each cell and $A_i$ and $P_i$ are the associated area and perimeter of that cell, respectively. We can also associate a dimensionless shape index $p_i = P_i / \sqrt{A_i}$ with each cell. The first term is quadratic in cell area and captures the bulk elasticity of the cell as described by the modulus $\beta_i$\textsuperscript{18,63}. The second term is quadratic in the perimeter and captures the active contractility due to the apical actin-myosin ring as described by the modulus $\xi_i$\textsuperscript{18}. Finally, the third term is linear in perimeter and captures a line tension as described by the modulus $\gamma_i$. This line tension has two components, one of which is positive and the other of which is negative. The positive component captures the energy associated with cortical tension of the active actomyosin layer near the cortex that tends to minimize the area of cell-cell contact. The negative component captures the energy associated with cell-cell adhesion and tending to maximize the area of cell-cell contact\textsuperscript{64,69}. Clearly, these two effects compete. When $\gamma_i < 0$, cell-cell adhesion makes it more favorable for cells to share interfaces, but when $\gamma_i > 0$, contractile line tension dominates and makes it more favorable for cells to minimize interfaces.

For a tissue made up of cells of the same type (common values for $\beta_i$, $\xi_i$, $\gamma_i$ and $A_0$), Eq. (S1) can be written, apart from an additive constant, in a rescaled dimensionless form

$$\varepsilon = \sum_{i=1}^{N} \left[ (a_i - 1)^2 + \frac{(\tilde{p}_i - p_0)^2}{r} \right],$$  \hspace{1cm} (S2)

with units of energy given by $\beta A_0^2$, and units of length given by $\sqrt{A_0}$, so that $a_i = A_i / A_0$ and $\tilde{p}_i = P_i / \sqrt{A_0}$. In Eq. (S2), $r = \beta A_0^2 / \xi$ is the inverse perimeter modulus and $p_0 = -\gamma / (2\xi \sqrt{A_0})$.\textsuperscript{69}

2 Analysis of critical behavior

3 Vertex model with active cellular motile forces
is the dimensionless target perimeter-to-square root area ratio, and we can re-write the observed perimeter to-square-root ratio \( p_i = P_i/\sqrt{A_i} = \hat{p}_i/\sqrt{a_i} \).

A cellular structure can be simulated starting from a random point pattern\(^{19,20}\). A Voronoi tessellation results in \( N \) cells where each cell is specified by its vertices, edges and how those vertices and edges are shared with other cells in the tissue. Using “Surface Evolver”\(^{70}\), the total energy of the system (Eq. (S2)) is minimized using gradient descent with respect to the vertices of cells. All structures and states were minimized such that the average energy of a cell changes by less than one part in \( 10^{10} \) between consecutive minimization steps.

Figure S7: Behavior of the tissue mechanical energy (Eq. S2) during a T-1 topological swap.

2 Analysis of critical behavior

In confluent tissue monolayers without cell division or apoptosis, T-1 topological swaps are the primary mode for cells to make rearrangements (Fig. S7). Initially the two red cells (Fig. S7) are separated by an edge of length \( \ell_{\text{initial}} \) and two green cells are neighbors. When cells migrate due to cellular motile forces and active cell shape fluctuations, the edge length \( \ell \) decreases. These deformations typically perturb the cells away from their homeostatically preferred perimeter and area, causing an increase in their mechanical energy as \( \ell \) decreases. At \( \ell = 0 \) all four cells share one common vertex and a swap of neighbors takes place, resulting in connected red cells and disjoint green cells. The mechanical energy difference between the point of T-1 and the initial state \( \Delta \varepsilon = \varepsilon_{T1} - \varepsilon_{\text{initial}} \) is an effective energy barrier that reflects the local mechanical response of the tissue with respect to cell shape changes.
Bi and coworkers\textsuperscript{19,20} probed these effective energy barriers and found that there is a rigidity transition controlled by the preferred cell perimeter $p_0$. When $p_0$ is less than a critical threshold $p_0^* = 3.81$, the tissue behaves like a solid and average energy barriers in a tissue is finite, i.e. $\Delta \varepsilon > 0$. At $p_0 > p_0^*$, the energy barriers vanish or $\Delta \varepsilon \to 0$ and the tissue becomes mechanically fluid-like. These energy barrier statistics as a function of $p_0$ and $r$ demonstrate a beautiful scaling collapse, with the solid branch described by a function $f_-$ and the fluid branch described by a different function $f_+$\textsuperscript{19}. The precise value of the critical $p_0^*$ has been determined by identifying the parameters that lead to the best scaling collapse of the critical barrier heights, and matches well with a simple mean-field calculation for the transition point\textsuperscript{19}.

Because effective energy barrier height within a tissue is difficult to measure experimentally, here we use vertex model simulations to identify an experimentally-accessible shape-based observable that acts as an order parameter for tissue mechanical behavior. We define $\bar{p}$ as the median of the perimeter-to-square root area ratio distribution $\{p_i\}$ and numerically calculated $\bar{p}$ for tissues at various parameters: $r$ between $10^{-3}$ and $10^3$ in decade increments and $p_0$ between 3.3 and 4.3 in 0.01 increments. We observed that when tissues have finite effective energy barriers for local rearrangements, $\bar{p} \approx 3.81$. However, when tissues have zero effective energy barriers for local rearrangements, $\bar{p}$ is proportional to $p_0$ (Fig. S8).

Unfortunately, distributions for $p$ can have very large variance due to simultaneous fluctuations in the area and perimeter, which makes a scaling analysis difficult. To perform such an analysis on the clean simulation data, we define another order parameter $q$, that focuses on perimeter

Figure S8: Dependence of the shape-based $\bar{p}_0$ (measured) on $p_0$ (control parameter) at $r = 1$. 

Because effective energy barrier height within a tissue is difficult to measure experimentally, here we use vertex model simulations to identify an experimentally-accessible shape-based observable that acts as an order parameter for tissue mechanical behavior. We define $\bar{p}$ as the median of the perimeter-to-square root area ratio distribution $\{p_i\}$ and numerically calculated $\bar{p}$ for tissues at various parameters: $r$ between $10^{-3}$ and $10^3$ in decade increments and $p_0$ between 3.3 and 4.3 in 0.01 increments. We observed that when tissues have finite effective energy barriers for local rearrangements, $\bar{p} \approx 3.81$. However, when tissues have zero effective energy barriers for local rearrangements, $\bar{p}$ is proportional to $p_0$ (Fig. S8).

Unfortunately, distributions for $p$ can have very large variance due to simultaneous fluctuations in the area and perimeter, which makes a scaling analysis difficult. To perform such an analysis on the clean simulation data, we define another order parameter $q$, that focuses on perimeter
fluid-like. These energy barrier statistics as a function of $p_{\text{sis}}$ on the clean simulation data, we define another order parameter $\Delta\varepsilon$ for rearrangements, $\bar{\Delta}$ in 0.01 increments. We observed that when tissues have finite effective energy barriers for local collapse, with the solid branch described by a function $r/|p_0 - p_0^*|^\Delta$. The precise value of the critical parameter that lead to the best scaling collapse of the critical barrier heights, and matches well different function $p_{\text{0}} = g^{-}$. The solid branch is denoted $g_{-}$, while the fluid branch is denoted $g_{+}$. We find the same critical scaling exponents as in $^{19}$: $\Delta = 4$ and $\beta = 1$.

fluctuations:

$$q = \frac{\langle P_i \rangle}{\sqrt{\langle i^2 \rangle}},$$

(S3)

We find that $q$ also distinguishes between fluid-like ($q > 3.81$) and solid-like behavior ($q < 3.81$). Moreover, we find that $q$ exhibits a critical scaling collapse and obeys the relation

$$q - p_0^* = |p_0 - p_0^*|^\beta g_\pm \left( \frac{r}{|p_0 - p_0^*|^\Delta} \right).$$

(S4)

The $g_\pm$ are scaling functions for $q$, which are distinct from the scaling functions $f_\pm$ for the energy barriers, although they share the same critical exponents. The $g_\pm$ scaling functions can be characterized by their asymptotic behavior in three regimes (Fig. S9):

- For $p_0 > p_0^*$, as $x = \frac{r}{|p_0 - p_0^*|^\Delta} \to 0$, $g_{+}(x) \to 1$, which means $q \to p_0$.

- For $p_0 < p_0^*$, $g_{-}(x) \to 0$ rapidly as $x \to 0$, which means $q \to p_0^*$.
SUPPLEMENTARY INFORMATION

DOI: 10.1038/NMAT4357

3 Vertex model with active cellular motile forces

In order to understand the effect of cellular motile forces on the rigidity transition, we can add cell motility to the vertex model. We numerically integrate the following overdamped equation of motion for all vertices

\[ b \frac{d\vec{r}_v}{dt} = -\frac{\partial E}{\partial \vec{r}_v} + b v_0 \sum_{i \leftarrow v} \hat{n}_i \]  

(S5)

where \( \vec{r}_v \) is the position of the vertex “\( v \)”, and \( b \) is the damping coefficient. The first term on the R.H.S. in Eq. (S5) is the force on the vertex due to the gradient of the mechanical energy (Eq. (S2)). The last term in Eq. (S5) models inherent cell motility with a motility strength \( v_0 \). It is important to note that although an individual vertex in isolation would move at a velocity \( v_0 \), interactions between cells can prevent the cell from moving that quickly.

![Graph showing the relationship between \( p_0 \) and \( v_0 \).](image)

**Figure S10:** Glass transition and shape index variability in the dynamic vertex model. The dashed lines indicate the value of \( p_0 \) at which the mean shape index \( \bar{p} = 3.81 \); different colors correspond to different active velocities \( v_0 \). Solid lines are the inverse standard deviation in the shape index \( p \) as a function of the model parameter \( p_0 \). Both \( \bar{p} = 3.813 \) and the peak in inverse standard deviation occur at the same point, which is also identical to the point at which cells in the simulation stop diffusing and the tissue transitions from liquid to solid.
Since each vertex is shared by more than one cell, the motility force experienced by each vertex is a sum of contributions from the neighboring cells. The sum is over all cells (labeled “i”) that are adjacent to vertex “v”. To make contact with recent work on self-propelled particles, we assume that the motile force generated by cell i is proportional to a polarization vector of unit length, $\vec{n}_i$. The polarization vector is given by the angle $\psi_i$:

$$\vec{n}_i = (\cos \psi_i, \sin \psi_i)$$  \hspace{1cm} (S6)

and evolves with its own overdamped dynamics, again taken from existing literature$^3$:

$$\frac{d\psi_i}{dt} = -\frac{\psi_i - \phi_i}{\tau} + \eta_i, \quad \langle \eta_i \eta_j \rangle = \sigma^2 \delta_{ij} \delta(t - t').$$ \hspace{1cm} (S7)

The dynamics of the polarization is controlled by a torque proportional to the angle between $\vec{n}_i$ and the instantaneous velocity of a cell’s center of mass,

$$\vec{v}_i = (\cos \phi_i, \sin \phi_i).$$ \hspace{1cm} (S8)

In other words, a cell’s polar axis aligns with the actual velocity, with lag time $\tau$ and a Gaussian random noise $\eta_i$ with zero mean and variance $\sigma^2$.

The dynamics of a tissue can be characterized by two parameters: cell mobility $v_0$ and $p_0$. When cell motility is negligible ($v_0 = 0$), the rigidity transition occurs at $p_0 = p_0^*$. At finite cell motility, there is a line in $v_0 - p_0$ space that separates glassy behavior and fluid behavior originating from ($v_0 = 0, p_0 = p_0^*$)$^{19}$. The dashed lines in Fig. S10 identify the values of $p_0$ at which cells stop diffusing and the tissue transitions from liquid to solid. Interestingly, these values are identical (within our numerical precision) to where the median shape index $\bar{p}$ equals the critical value 3.81.

Our simulations suggest that fluctuations in cell shapes are also controlled by the rigidity transition at ($v_0 = 0, p_0 = p_0^*$). The solid lines in Fig. (S10) show the inverse standard deviation of $p$ as function of $p_0$ at various values of $v_0$. For each value of $v_0$, the inverse standard deviation peaks at value of $p_0$ corresponding to the glass transition point, where the observable $\bar{p} \sim 3.81$. In other words, fluctuations in $p$ decrease near the critical point, which is different from what is typically found in other critical phenomena. In fact, as $v_0$ decreases towards zero, the inverse standard deviation appears to diverge in the vicinity of the rigidity transition.

Although performing a systematic finite size scaling to understand the nature of the divergence is beyond the scope of this work, our simulations clearly indicate that fluctuations should become negligible near the critical point in the $v_0 \to 0$ limit. In contrast, fluctuations remain rather large in the vicinity of the glass transition when the active forces $v_0$, are large. Therefore, this numerical model suggests that large variability in $p$ seen in our experimental cultures is driven by active fluctuations, and future analysis of these fluctuations may be able to constrain the magnitudes of the active forces.

The results presented here are not model specific. We fully expect that Cellular Potts Models (CPM)$^{45,71}$ will have the same type of phase transition as described in this model, because CPM account for cell shapes and the energy functional associated with each cell is nearly identical to Eq. (S1). Although a critical phase transition of the kind we describe has not been reported in such models, we expect it to be there.

In contrast, we expect that particle based modeling of cells such as$^{44,72}$, will not exhibit the same transition. This is because self-propelled particle models only contain isotropic interactions.
between particles, and they do not account for the shape degrees of freedom/anisotropies which are necessary for the transition we describe in this manuscript. For example in 72, it was found that the jamming transition in a tissue is controlled by changes to density, which is not possible in a confluent monolayer without cell division, as in the system described in this manuscript.
References