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Thuan Beng Saw

National University of Singapore

Xumei Gao

University of Melbourne

Jianan He

University of Melbourne

Anh Phuong Le

National University of Singapore

Supatra Marsh

National University of Singapore

Keng-hui Lin

Academia Sinica Taiwan

Alexander Ludwig

Nanyang Technological University

Jacques Prost

Institut Curie

Chwee Teck Lim (✉ ctlm@nus.edu.sg)

National University of Singapore <https://orcid.org/0000-0003-4019-9782>

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Transepithelial potential difference governs epithelial homeostasis by electromechanics

Thuan Beng Saw^{†, #, 1, 2}, Xumei Gao^{†, 3}, Jianan He³, Anh Phuong Le², Supatra Marsh², Keng-hui Lin⁴, Alexander Ludwig^{5, 6}, Jacques Prost^{#, 2, 7}, Chwee Teck Lim^{#, 1, 2, 8}

[†]Co-first authors, [#]Co-corresponding authors

1. Department of Biomedical Engineering, National University of Singapore (NUS), 4 Engineering Dr 3, #04-08, 117583, Singapore
2. Mechanobiology Institute, NUS, 5A Engineering Dr 1, 117411, Singapore
3. ARC Centre for Personalised Therapeutics Technologies, University of Melbourne, 08 Medical Building, Melbourne, Australia
4. Institute of Physics, Academia Sinica, Taipei, 115 Taiwan
5. School of Biological Sciences, Nanyang Technological University, 60 Nanyang Dr, 637551, Singapore
6. NTU Institute of Structural Biology, Nanyang Technological University, 59 Nanyang Drive, 636921 Singapore
7. Institut Curie, PSL University, 26 rue d'Ulm F-75248 Paris Cedex 05, France
8. Institute for Health Innovation and Technology (iHealthtech), NUS, MD6, 14 Medical Dr #14-01, 117599, Singapore

Abstract

Studies of electric effects in biological systems, from the historical experiments of Galvani¹ and the ground-breaking work on action potential² to studies on limb regeneration³ or wound healing⁴, share the common feature of being concerned with transitory behavior and not addressing the question of homeostasis. Here using a novel microfluidic device, we study how the homeostasis of confluent epithelial tissues is modified when a trans-epithelial electric potential (TEPD) different from the natural one is imposed on an epithelial layer. We show that epithelial fate is dependent on TEPD of few Volts/cm similar to the endogenous one. When the field direction matches the natural one, we can restore a perfect confluence in an epithelial layer turned defective either by E-cadherin knock-out or by weakening cell-substrate adhesion; additionally, the tissue pushes on the substrate with *kilo-Pascals* stress, inducing active cell response such as death and differentiation. When the field is opposite, homeostasis is destroyed by the perturbation of junctional actin and cell shapes, and the formation of dynamical mounds⁵, while the tissue pulls with similar strengths. Most of these observations can be quantitatively explained by an electro-hydrodynamic theory involving local electro-osmotic flows. We expect this work to motivate further studies on long time effects of electromechanical pathways with important tissue engineering applications.

Main text

Among the many bioelectric field effects observed previously, the closest to our study concerns epithelial wound healing^{4, 6}. During wound healing, electric fields on the order of 1 V/cm can drive cell migration toward gaps, and this process is closely related to a field-induced cell migration mode called galvanotaxis^{7, 8}. In the case of single cell galvanotaxis⁷, the electric field is a symmetry breaking field acting orthogonal to the substrate normal. Intact tissues such as epithelia are strongly polar in the direction normal to the layer with a natural TEPD of 1 – 10 mV^{9–11}. The corresponding electric field is similar in magnitude to those observed around wounds, but its orientation is crucially different, being along the cell apico-basal polarity and substrate normal rather than orthogonal. Such fields are known to drive nutrient or ion transport in organs¹², but how they could play a more direct role in influencing collective cell behavior and epithelial homeostasis is essentially unknown. Although interesting per se, what has been learned with galvanotaxis or wound healing cannot be translated straightforwardly to epithelial tissues homeostasis and new experiments have to be designed.

To study the potential roles of the TEPD in epithelial homeostasis, we used Madin-Darby Canine Kidney (MDCK) cells which are known to be apico-basally polarized in a monolayer, and first characterized their electrical properties on a transwell system (Methods). As the cells reached confluency and grew and matured, the TEPD magnitude steadily increased to a plateau of ~ 1 mV after a few days, with the basal side of the epithelium becoming increasingly electrically positive (fig. S1a), consistent with reported values for MDCK strain II¹³. At the same time, the resistivity of the mature monolayer reached $\sim 10^2 \Omega \cdot \text{cm}^2$ (fig. S1b). We then asked whether the disruption of the natural TEPD would lead to the perturbation of the epithelial monolayer, and conversely whether its reinforcement would maintain normal epithelial characteristics. To answer this question, we fabricated a two-layered microfluidic device with a collagen-I surface-coated polyacrylamide gel. This allowed us to apply an external E-field or ion current perpendicular to confluent monolayers grown on the gel, and to modify their TEPD (Methods, Fig. 1a, fig. S2). An exogenous apical-to-basal field (AtB) opposes the original TEPD direction and disrupts it, while a basal-to-apical field (BtA) reinforces the TEPD (Fig. 1b). We induced an average perturbation of the TEPD on the same order as the natural one measured *in vitro*, that is ~ 1 mV which is physiologically relevant⁹⁻¹¹ (Methods).

With this setup amenable to optical imaging, we examined the effects of the TEPD by comparing cell morphologies between control and field conditions. In no-field controls, cells that had grown for a few days and had reached high cell densities exhibited low contrast cell-cell junctions under phase-contrast imaging (Methods, Fig. 1c - Ctrl, 1d). This corresponded to the flat apical surfaces that were maintained across neighbouring cells (Methods, Fig. 1e - Ctrl). At the same time, the junctions also portrayed high junctional actin intensity indicative of strong cell-cell junctions (Methods, Fig. 1f - Ctrl, 1g). Strikingly, clear changes to these normal characteristics were observed shortly after the AtB field was applied to disrupt the natural TEPD. Cell-cell junctions brightened dramatically under phase-contrast after ~ 10 minutes of the field application (Fig. 1c - AtB, 1d, Movie S1), suggesting the formation of sharp edges between cells, which lasted as long as the field was applied (fig. S3). Indeed, the cells at this stage exhibited convex apical cell shapes that gave rise to sharp intercellular edges reminiscent of mesenchymal cell shapes (Fig. 1e - AtB). This was followed by a significant weakening of junctional actin and thus a possible destabilization of cell-cell junctions¹⁴ within a few hours (Fig. 1f - AtB, 1g). Conversely, BtA fields maintained normal epithelial characteristics similar to controls (Fig. 1c - h, - BtA, Movie S2, Movie S3). These short time-scale observations from minutes to hours provided a first evidence that a TEPD magnitude similar to that of the original one is important in governing cell shapes and junctional properties depending on the field direction.

We wondered what constituted the main underlying mechanism for driving these E-field dependent cellular changes. Since the apical cell shape changes occurred as early as 10 minutes after AtB field application, the observation was unlikely driven by transcriptional or genetic changes¹⁵, but due to more direct effects from the E-field. Motivated by Mechanobiology studies, we hypothesized that the TEPD exerts an electromechanical stress that induces appreciable cell deformations. To understand how an E-field can induce mechanical stress in cells, it is important to note that cells are constituted of charged molecules and can undergo passive electrophoretic movements under the action of a field¹⁶. Since these cells are attached to a bound substrate, we expect relative cell-substrate movement to induce forces in the monolayer depending on the E-field directions. To predict the relation between the E-field and the force, we further note that intracellular mammalian proteins are largely estimated to have a median isoelectric point lower than the pH values of their local subcellular environment¹⁷, which implies net negative charges on the surfaces of subcellular components and the cell^{16,18}. A hypothesis based on simple electrophoresis would then predict that these cells move in the direction opposite to the E-field. This means that AtB fields would pull the epithelium away from the substrate thus inducing tensile stresses, while BtA fields would push the cells toward the substrate leading to compressive forces (Fig. 2a).

To examine this hypothesis, we measured the deformation of the cell substrate when an external E-field (either AtB or BtA) was applied across a confluent monolayer, as any force will be transmitted to the soft hydrogel underneath it and hence deform it (Methods). Indeed, the gel started to deform within a similar timescale as the AtB field-dependent cell shape changes occurred and reached a deformation of $\sim 10 - 20 \mu\text{m}$ in magnitude about an hour after external field application, which is larger than the typical cell height (Fig. 2b). As expected, minimal gel deformations were seen in no-field conditions or when fields were applied on non-confluent epithelia, showing that the E-fields interacted mainly with the cells and not the substrate made of electrically neutral polyacrylamide gels (Fig. 2b). With confluent monolayers, the AtB fields consistently induced an upward gel deformation, while BtA fields induced a downward deformation (Fig. 2b), matching the prediction that AtB fields stretch cells while BtA fields compress them. Such deformation values translate into electromechanical stresses, $\sigma_{nn} \sim K * (\delta L/L) \sim 2 - 4 \text{ kPa}$, knowing that the polyacrylamide gel is elastic, with a stiffness of $K \sim 23 \text{ kPa}$ and a gel thickness of $L \sim 250 \mu\text{m}$ in our setup. Importantly, these stress magnitudes are similar to physiologically relevant mechanical stresses that not only change cell shapes but also induce active cellular responses^{19,20}.

Having established that E-fields generate electromechanical stress, we next examined whether such stress can indeed alter epithelial cell architecture (Fig. 1e - AtB). To examine this idea, we first inferred the pressure difference across the curved cell membrane, $\delta P = (2 * \gamma * \cos(\theta(z)))/r(z)$, using Young-Laplace's law for in-plane isotropic cells with single maximum height (Methods, Fig. 2c). Here, the cell surface tension, γ can be approximated to be uniform based on F-actin staining, with typical cortex-membrane tension values of $\sim 1 \text{ mN/m}^{21}$, $\theta(z)$ is the angle of the membrane tangent with the substrate normal and $r(z)$ the distance of the cell surface to its axis (Fig. 2c). With this relation, the pressure difference across the cell surface appeared to increase linearly with height in cells (Fig. 2d). A linear fit of the apical surface stresses produced theoretical cell heights and whole cell shapes that highly resembled experimental measurements (Fig. 2e, f), providing a first validation that the electromechanical stress transformed cell shapes. Further, the linear fit allowed us to estimate the maximum pressure difference at the tips of cells (Fig. 2g). Using a two-component hydrodynamic theory of the cell body keeping electroosmotic effects⁵, we predicted this apical pressure difference to be essentially half the value of the cell-substrate stress (Methods). This prediction was confirmed by the fit which gave a pressure difference of $\sim 1.4 \text{ kPa}$ on average (Methods). These results confirmed that electromechanical stress induced from electroosmotic effects directly governs cell morphology.

Based on the results at the minutes-to-hours timescale and the subcellular-to-cellular length-scale, we further examined the relevance of this electromechanical stress at influencing the behaviour of the whole epithelium over days. Firstly, it has been reported that upward forces due to tensile stresses of few hundred Pa, created by active processes involving actin or osmotic pumping, can lead to apical cell extrusion^{22,23} or epithelial blisters²⁴. We thus predicted that a normal tensile stress at the cellular level could integrate over the whole MDCK monolayer to produce 3D structures in the epithelium under the action of the AtB field with similar tensile stresses. In contrast, the BtA field and its compressive stress is not expected to produce such structures. To test this prediction, we applied E-fields across the confluent monolayers for 2 - 3 days and examined the epithelial response by quantifying the heterogeneity of the spatial cellular distributions using the Relative Uniformity Deviatory Measure (RUDM), defined based on the spatial correlation functions of the image intensity (Methods). Indeed, AtB fields induced the collective extrusion of live cells throughout the epithelium, which correlated with a significant increase in the RUDM value (Fig. 3a, b, c - AtB, Movie S4). The emergence of these extrusions resembled tumour-like structures previously observed in Src oncoprotein-activated cell collectives within MDCK monolayers²⁵, and were not found in controls (Fig. 3a, b, c - Ctrl). As expected, BtA fields which exert compressive stresses continued to maintain cells within a single layer, similar to controls with low RUDM values (Fig. 3a, b, c - BtA). Similar observations were also made in immortalized N/TERT-1 human keratinocyte cell lines²⁶ (Methods). These keratinocyte multilayers

naturally exhibited weakly heterogeneous spatial cell distributions, with localized cell stratification which can grow slowly in time without E-fields (Fig. 3d, e, f - Ctrl). With an AtB field, these heterogeneities and the RUDM values increased significantly compared to controls and led to the formation of 3D mounds (Fig. 3d, e, f - AtB, Movie S5), reminiscent of active, mechanically-driven structures found in the developing avian skin²⁷. Again, BtA fields produced relatively flat keratinocyte layers with similar characteristics as controls (Fig. 3d, e, f - BtA). These results show that the TEPD-induced stress not only elicits acute and significant transformations in cell morphologies, but also induces pronounced 3D epithelial sculpting when applied for days.

Having shown that the AtB field induces marked morphological changes compared to no-field conditions, we further examined the effects of BtA fields on epithelial behaviour. Compressive stresses of few hundred Pa have been reported to induce programmed cell death or arrest of proliferation in epithelial monolayers and spheroids^{20,28}, and similarly cell differentiation in keratinocyte cells²⁹. We predicted, therefore, that BtA fields that impose similar compressive stresses, but not AtB fields, would exacerbate such events in MDCK and human N/TERT-1 layers. To test this, we quantified the frequency of cell nucleus fragmentation or condensation as an indicator of cell death rates in monolayers of MDCK cells expressing Histone1-GFP (H1GFP)²² (Fig. 4a). In addition, we analysed cell differentiation rates in N/TERT-1 layers stably expressing the FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator) reporter³⁰. The differentiation rates were quantified by the number of nucleus condensation events with the absence of the cell cycle indicators, that is the Cdt1 (red) and Geminin (green) expressions (Fig. 4b, Methods). Consistent with our predictions, cell death and differentiation occurred with higher frequency in the MDCK and N/TERT-1 layers exposed to BtA fields respectively while cells exposed to AtB fields were indistinguishable from control cells in this respect (Fig. 4c, d, Movie S6, Movie S7). Such observations reinforce the idea that electromechanical stress is dependent upon the field direction and induces different active cell responses.

We have shown that AtB fields disrupt flat or homogeneous epithelial layers. We hypothesized, therefore, that BtA fields may rescue intrinsically heterogeneous layers. We searched for conditions that impose high epithelial heterogeneity intrinsically by modulating cell-substrate and cell-cell adhesion strengths, which are important for determining epithelial morphologies^{31,32}. We first sought to weaken cell-substrate adhesions and hence destabilize epithelial structures by altering the type of extracellular matrix (ECM) coating. To this end, the polyacrylamide gel support was coated with fibronectin (instead of collagen-I), which resulted in slower cell spreading after cell seeding, as expected. Although an intact epithelium was found on fibronectin-coated gels when grown for a few days at zero field, the spatial cell distribution became more heterogeneous over time, characterized by a large RUDM value leading to cell overlap and weak stratification (fig. S4a, b - Ctrl). Intriguingly, while the epithelium exhibited similar heterogeneities under AtB fields (fig. S4a, b - AtB), application of external BtA fields caused the formation of a significantly more uniform monolayer (fig. S4a, b - BtA). Of note, BtA fields could also rescue monolayers that had already adopted a highly heterogeneous conformation (quantified by a negative average rate of change of the RUDM), which reinstated the uniform monolayer state (quantified by a positive average rate of change of the RUDM) (Fig. 4e, f, Movie S8). Consistent with the previous results in naturally homogeneous epithelia, the BtA field rescued the uniformity of the epithelium by inducing higher cell death rates at dense cell regions compared to areas with sparse cell numbers (Fig. 4g). This further reinforces the idea that the field-induced cell death is related to compressive stress, as a higher cell packing density naturally possesses larger pressures²² that would favour such events. In contrast, cell division rates were not significantly different between regions of different cell densities (fig. S5). We thus demonstrated that the BtA field can safeguard normal epithelial morphologies under non-favourable adhesive conditions.

Finally, we tested the ability of BtA fields to restore epithelial monolayer phenotypes induced by weakening of cell-cell junctions using an E-cadherin knockout line (E-cad KO). It was previously found that E-cad KO cells exert larger contractile force dipoles than Wild-Type (WT) cells³³, and still possess

cadherin 6 junctional molecules that could maintain a certain level of cell-cell connection. We reasoned that such conditions could lead to disrupted epithelial morphologies. Indeed, even though E-cad KO cells proliferated and initially produced a monolayer on the fibronectin-coated polyacrylamide substrate, these monolayers ruptured globally after some time and exhibited de-wetting transitions from the substrate surface³¹, forming multicellular clumps and compromising epithelial integrity throughout the experimental area (Methods, Fig. 4h, i - first image). We hypothesized that the compressive stresses from BtA fields could prevent such epithelial disintegration or even rescue this process, as the pushing of cells on the substrate would increase cell-substrate contact times and adhesion strength³⁴ and facilitate cell spreading. Indeed, only BtA fields were able to impede the self-disruption of E-cad KO monolayers (Methods, Fig. 4h). Further, when the BtA fields were turned on after the E-cad KO epithelium had already formed multicellular clumps and gaps, some areas regained confluency, and 2/6 of such experiments even achieved close to 100% confluency over large millimetre-size experimental field-of-views (fig. S6). In contrast, no-field and AtB-field conditions exhibited disconnected clumps in all observable areas at all times (fig. S6). It was further confirmed that this BtA-field-induced rescue is dependent on the correct field direction, as all the E-cad KO epithelia which attained a good spreading phenotype globally under BtA fields (> 80% of total areas) quickly formed clumps and gaps in many locations as early as few hours after the switch from BtA to AtB fields (Fig. 4i, j, Movie S9). Overall, we showed that the BtA field induces cell spreading and higher local cell death rates to recover normal monolayer characteristics under conditions which usually disrupt them, as expected from electromechanical stress considerations.

Altogether, our results converge to the conclusion that the TEPD plays an important role in epithelial homeostasis through electromechanics. The natural TEPD is found to be in the same direction as a BtA field which not only reinforces intracellular junctional actin, but also influences cell death and differentiation coupled to a uniform epithelial morphology through compressive stresses. This raises important questions of whether and how the original TEPD could play a role *in vivo* during embryonic development³⁵, and whether its disruption could lead to diseases such as the formation of carcinoma. The possibility of the latter is motivated by the fact that an AtB field with a reverse TEPD and tensile stress build-up disrupts normal epithelial morphologies to induce incomplete transition to mesenchymal-like cell shapes, collective live cell extrusions and 3D mound formation reminiscent of tumor structures *in vitro*. Importantly, *in vivo*, the value of the TEPD is not necessarily a local property, thus local pathologies could result from dysfunction in remote places of the organism. Note that the TEPD and its corresponding electromechanical stress effects are fundamentally different from in-plane E-field effects that are thought to govern wound healing and cell migration due to the electrophoresis of membrane proteins on cell surfaces⁷. Finally, the modulation of the TEPD also demonstrates a strong functional aspect in controlling epithelial integrity and will find potential uses in tissue engineering applications.

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Author Contributions

T.B.S. and J.P. conceived the project. T.B.S., X.G., and J.P. designed research, T.B.S. and X.G. performed experiments, T.B.S. and J.P. performed theoretical calculations and numerical fitting, T.B.S., X.G., J.H., A.P.L., S.M., K.L., and A.L., performed image analysis, or contributed new reagents/cells and computational tools, T.B.S., X.G., A.L., J.P., and C.T.L. provided guidance and input. T.B.S., X.G., and J.P. wrote the manuscript/made the figures, T.B.S., J.P., and C.T.L. supervised the project. All authors read the manuscript and commented on it.

Main Figures

Fig. 1. AtB field induces disruption to cell shape and cell-cell junctions, while BtA field maintains normal epithelial characteristics. **a**, Schematic of two-layered microfluidic setup to apply E-fields perpendicular to epithelial plane. Purple arrows show AtB fields, cell monolayer sits on polyacrylamide gel (cyan) coated with ECM protein (see Methods for details). **b**, Schematic shows natural TEPD direction of MDCK, and how external fields disrupt or reinforce this parameter depending on their direction. **c**, Top-down view of phase-contrast imaging of MDCK layers under control (no-field), AtB and BtA field conditions. **d**, Quantification of corresponding relative junctional intensities in (c). Data are represented as mean $\pm$ s.e.m. Ctrl: $n = 730$ junctions from 3 independent experiments in 2 biological replicates. AtB: $n = 731$ junctions from 3 independent experiments in 3 biological replicates. BtA: $n = 980$ junctions from 3 independent experiments in 3 biological replicates. Two-tailed, two-sample t-test between AtB-BtA, AtB-Ctrl, Ctrl-BtA $*P < 0.001$. **e**, Side-view, confocal images of fixed and phalloidin-stained epithelia under the three field conditions. $0.1 \mu\text{m}$ z step-size. **f**, **g**, **h**, Similar images and quantifications as (c, d, e) but for fluorescent actin signals. Arrow heads in (f) point to junctional actin. (g) is average $\pm$ s.e.m. Ctrl: $n = 170$ junctions from 3 independent experiments in 2 biological replicates. AtB: $n = 232$ junctions from 3 independent experiments in 3 biological replicates. BtA: $n = 199$ junctions from 3 independent experiments in 3 biological replicates. Two-tailed, two-sample t-test between AtB-BtA, AtB-Ctrl. $***P < 0.001$. (h) $1 \mu\text{m}$ z step-size. All scale bars, $10 \mu\text{m}$.

Fig. 2. E-fields exert electromechanical stress which govern cell shape. **a**, Schematic of direction of gel deformation depending on field direction (light cyan – gel, dark cyan – gel deformation, purple arrows - field lines, pink - medium). **b**, Quantification of gel deformation at ~1 hr after field application. BtA-NC means field was applied across non-confluent epithelia. Line is mean, scatter points are individual experiments. Ctrl: n = 2 independent experiments in 2 biological replicates. AtB/BtA: n = 3 independent experiments in 3 biological replicates. BtA-NC: n = 4 independent experiments in 4 biological replicates. Two-tailed (one-tailed), t-test against normal distribution of mean = 0 for Ctrl and BtA-NC (AtB and BtA). *P = 0.01. **c**, Schematic to show how the Young-Laplace's law derived for isotropic case, $\delta P = (2 * \gamma * \cos(\theta(z)))/r(z)$, is a function of z . In an in-plane isotropic case, each point at the apical cell surface (green) is tangent to a sphere (grey) of radius, R . $\theta(z)$ and $r(z)$ are the angle and distance of the cell surface from the centre of the cell which vary with cell height, z , and $R \cos(\theta) = r$. **d**, **e** Four examples of predicted pressure difference along the apical cell surface as a function of cell height, and their predicted cell shape. Scatter points are different points on the cell surface. The maximum internal pressures decrease from Cell 1 to 4. Scale bar, 5 μm. **f**, Comparison of predicted and measured total cell height. Black line is linear fit passing through origin ($y = 1.02x$, R-squared = 0.78). **g**, Predicted pressure difference across cell surface at the tip of cells. Line is mean. (**f**, **g**) scatter points are individual cells (n = 37). Green arrows show the individual values of such pressures for example Cell 1 to 4.

Fig. 3. Only AtB fields induce collective live cell extrusions and three-dimensional mounds in MDCK and N/TERT-1. **a**, Top-down view of nuclei of MDCK (H1-GFP) under the three field conditions, multicellular white patches are collective live extrusions. **b**, Side-view, confocal images of flat monolayer and collective extrusions. **c**, Quantification of endpoint RUDM values. Line is mean, scatter points are individual field-of-views. Ctrl: $n = 11$ independent field-of-views, 6 independent experiments in 3 biological replicates. AtB: $n = 6$ independent field-of-views, 3 independent experiments in 3 biological replicates. BtA: $n = 5$ independent field-of-views, 3 independent experiments in 3 biological replicates. One-tailed, two-sample t-test. $**P = 0.0001$ (Ctrl-AtB), $*P = 0.008$ (BtA-AtB). **d**, **e**, **f**, Similar type of images and quantifications for N/TERT-1 (nucleus is stained by Hoechst 33342). **(f)** Line is mean, scatter points are individual field-of-views. Ctrl/AtB/BtA: $n = 3$ independent experiments in 3 biological replicates. One-tailed, paired t-test. $**P = 0.002$ (Ctrl-AtB), $*P = 0.016$ (BtA-AtB). All scale bars, $50 \mu m$.

Fig. 4. Only BtA fields induce more cell death or differentiation in MDCK or N/TERT-1, and rescue intrinsically heterogeneous/non-intact MDCK through cell density-dependent cell death

and spreading. **a**, Nucleus fragmentation event indicating MDCK cell death, 2 hrs difference from top to bottom images. **b, left column**, Nucleus condensation event indicating N/TERT-1 differentiation, 4 hrs difference from top to bottom images (red nucleus - FUCCI G1 signal, grey nucleus with white halo - condensed nucleus of differentiated cell). **b, right**, Two neighbouring nuclei on the suprabasal layer, small nucleus is condensed nucleus of differentiated cell, large nucleus belongs to non-differentiated cell (blue - Hoechst 33342, grey - involucrin differentiation marker). **c**, Death rates of MDCK cells under different field conditions. Line is mean, scatter points are individual field-of-views. Ctrl: n = 14 independent field-of-views, 3 independent experiments in 3 biological replicates. AtB/BtA: n = 8 independent field-of-views, 3 independent experiments in 3 biological replicates. Two-tailed, two-sample t-test. *P = 0.007 (Ctrl-BtA), *P = 0.018 (AtB-BtA). **d**, Differentiation rates of N/TERT-1 cells under different field conditions. Line is mean, scatter points are individual field-of-views. Ctrl/AtB/BtA: n = 24 independent field-of-views, 3 independent experiments in 3 biological replicates. Two-tailed, two-sample t-test. *P < 0.001. **e, f, g**, Data for MDCK on fibronectin-coated gel. **(e)** Rate of change of RUDM values before (Bef) and after (Aft) BtA fields (positive means increasing heterogeneity, negative means decreasing heterogeneity). Line is mean, scatter points are independent experiments. Bef/Aft: n = 5 independent experiments in 3 biological replicates. One-tailed t-test against normal distribution of mean = 0. *P = 0.01. **(f)** Top-down view of nucleus and cell distributions. AtB field applied till 98 hrs, then switched to BtA field. **(g)** Local death rates comparing dense and sparse regions during BtA field homogeneity rescue. Line is mean, scatter points are independent field-of-views. Dense/Sparse: n = 6 independent field-of-views, 3 independent experiments in 3 biological replicates. Two-tailed, paired t-test. *P = 0.01. **h, i, j**, Data for Ecad KO MDCK on fibronectin-coated gel. **(h)** Measure of tissue integrity comparing no-field/AtB with BtA field conditions (see Methods for details of calculation of this measure). Line is mean, scatter points are mean integrity measures of independent experiments starting from Day 3 when E-field was applied, to Day 5. Ctrl/AtB: n = 8 independent experiments in 2 biological replicates. BtA: n = 4 independent experiments in 2 biological replicates. One-tailed, t-test. *P = 0.03. **(i)** Top-down view of phase contrast images of E-cad KO cells. BtA field applied till 168 hrs, then field was stopped. Yellow stars show gap regions. **(j)** Measure of tissue integrity as a function of time after BtA field was switched off or switched to AtB field. Two-tailed, two-sample t-test between each time point and the time before the switch. *P = 0.06, **P < 0.01. Scale bars, 10 μ m for **(a, b)**, 50 μ m for **(f, i)**.

Methods:

Experimental techniques:

1. Cell culture and reagents:

MDCK strain II was cultured in high glucose, DMEM culture medium (Gibco) with additional 10% heat inactivated FBS (Gibco, USA origin), and 100 Units/mL Penicillin, 100 μ g/mL Streptomycin (Gibco) to prevent bacterial contamination. To study the function of specific proteins, visualize certain parts of the cell, or to monitor cell division, death and cell cycle events, stable cell lines with fluorescent expression or knockout variants of MDCK were used. WT MDCK, and stably transfected GFP-actin MDCK and FUCCI cell cycle reporter MDCK³⁶ (gifts from James W. Nelson), stable GFP-Histone-1 (H1-GFP) MDCK (gift from Sham Tlili) and stable E-cadherin knockout (Ecad KO) MDCK (gift from B. Ladoux, Institut Jacques Monod, Paris) were used. GFP-actin and H1-GFP MDCK were maintained using media supplemented with 0.5 mg/mL geneticin (Gibco) to sustain their gene expression, while WT MDCK had additional 1 mM Sodium Pyruvate. N/TERT-1 keratinocyte FUCCI cell lines stably transfected with Kusabira Orange 2 (mKO2)-Cdt1 and Azami Green 1 (mAG1)-Geminin were cultured in Keratinocyte Serum Free Medium supplemented with human recombinant epidermal growth factor and bovine pituitary extract (Gibco), 100 Units/mL Penicillin and 100 μ g/mL Streptomycin (Gibco). Experiments with MDCK used the same medium as the ones for passaging. For N/TERT-1, the

passaging medium was used for the first day of cell culture on the chip, then later changed to DNMedia (serum free, Denova-Science) for improved cell differentiation. Mycoplasma testing was done every 3 months using the MycoAlert™ PLUS Mycoplasma Detection Kit and Assay Control Set (Lonza).

2. Immunofluorescence staining

For MDCK, cells were fixed with methanol-free 4% PFA at 37°C for 1 hour and permeabilized with 0.1% Triton X-100 for 5 min. Actin filaments were then stained with Alexa Fluor® 568 Phalloidin (Life Technologies, A12380) at 1:100 dilution. Nucleus was labelled with Hoechst 33342 at 1 µg/mL concentration. For N/TERT-1, cells were fixed with methanol-free 4% PFA at room temperature for 3 hours, and permeabilized with 0.1% Triton X-100, then blocked with 2% BSA/0.1% Triton for 1 hour. Incubation with the primary antibody (Involucrin, mouse; Abcam Ab68) diluted at 1:100 in 2% BSA was performed at 4°C overnight. The secondary Alexa Fluor 647 goat anti-mouse antibody (Invitrogen A21235) was added at 1:100 in 2% BSA and maintained at room temperature for 3 hours. 1X PBS was used as the dilution buffer and for the gentle rinsing of samples between every step. Mounting was done with the ProLong™ Glass Antifade Mountant (Invitrogen) at room temperature for a day before the samples were stored at 4°C.

3. Microscopy imaging

Wide-field, phase-contrast and epifluorescence microscopy was done on an Olympus IX81 inverted microscope. Confocal imaging was done either using a spinning disk confocal (CSU-W1 Yokogawa head on a Nikon Eclipse Ti microscope body), or a Nikon A1R MP point scanning microscope. All imaging was done at 37°C and 5% CO₂. For long term experiments from a few days up to a week, the cell medium was refreshed daily on the microscope.

4. Natural Transepithelial Potential Difference (TEPD) and electrical resistance measurement

Cells were seeded at 0.9 million cells/cm² in a 12-well or 24-well Transwell® system (Costar) with 0.4 µm pore size, permeable polyester membrane. Electrical measurements (TEPD and resistance) were done using a Millicell®-ERS (Electrical Resistance System) Voltohmmeter system, one day after the cells were seeded. Based on manufacturer's instructions, the Ag/AgCl electrodes were sanitized with 70% ethanol, calibrated (functional checking) and equilibrated in 1X PBS, then culture medium before the measurements were taken. During this time, the cells that were taken out from the incubator were allowed to rest at room temperature for a few minutes, and the electrode readings were only recorded when these readings stabilized. Several recordings were taken at different positions of the well and averaged. Also, the medium in the inner and outer compartments of the Transwell were fully isolated from the top such that only the electrical properties across the cells/membrane were measured. The samples were returned into the incubator after the measurements were done. The procedure was repeated daily for a few days, during which cells reached confluency at day 2 and continued to grow, and the medium was changed every two days. The monolayer resistance was obtained by subtracting off the resistance of a blank well (without cells).

5. Microfluidic chip and device design/fabrication, and E-field experiment

The custom electric-cell assay involved a two-layered microfluidic chip made of a UV-curable material NOA73, that was embedded with a neutral polyacrylamide (PA) gel which had a surface coating of extracellular matrix (ECM) of choice, and was connected to cartridges that held the cell medium (fig. S2a). The whole setup was electrically linked to two platinum electrodes (Latech) through 60 cm long Tygon® tubings (Formula 2375, ID 1.6 mm, OD 3.2 mm), and a DC electric field or ion current was applied perpendicularly across the monolayer (Fig. 1a) using a DC source meter (Kiethley, models 2400, 2450 or 2657A).

The experiments were done under constant current, as we could estimate the fixed average current density flowing through the epithelium because total current is conserved. To achieve a field distribution that was spread more uniformly throughout the epithelium, the target gel height and the design of the geometry of the 2nd NOA73 layer were guided by simulations and different experiments (see later sections). Further, the channels within the chip were designed such that their electrical resistance was $\sim 10^6 \Omega$ (fig. S2b), which is orders of magnitude larger than the resistance of the epithelium on the chip ($\sim 10^2 \Omega$). The sample's resistance was thus fully determined by those of the channel, which was fixed and allowed multiple samples to be connected in parallel while a fixed amount of current still passed through each sample (fig. S2a). The use of long tubing that separated the cell area from the electrode holder, the use of inert platinum electrodes, and the refreshing of the medium at the cell region and within the electrode holder during the experiment, allowed the DC field experiment to be performed for durations up to a week without compromising the health of the cells. We also confirmed that the experimental results were solely due to the effects of the fields through different controls. Specifically, we measured that the pH of the cell medium at the cell region was similar between no-field and field experiments at the end of experiments, and that a field-conditioned medium (cell medium that was first passed with current without cells) did not induce cell behaviors related to E-fields.

The preparation of the electric assay involved the following processes in sequence, that is the assembly of the microfluidic chip (fig. S2b), the integration of the PA gel, the coating of the PA gel surface with ECM proteins, the assembly of the cartridges and the connection to the electrodes, then finally cell seeding and E-field application. Briefly, to fabricate the microfluidic chip from a UV-curable adhesive NOA73 (Norland Products), 1 mm-thick glass slides (Marienfeld) that will serve as the base of the chip were pretreated with 0.3% acetic acid and 0.5% 3-(Trimethoxysilyl)propyl methacrylate (Sigma) dissolved in 100% EtOH for good bonding with NOA73. The features of the first NOA73 layer, including channels, were molded onto the pre-treated glass using a 1:10 Polydimethylsiloxane (PDMS) mold and lightly cured with 10 s, 365 nm UV treatment at 75 mW/cm² to produce a solidified structure. The 2nd NOA73 layer that covers the channels and leaves small openings for the passage of ion current was further molded on a PDMS substrate then transferred onto the first NOA73 layer. A 6 s UV treatment (75 mW/cm²) was done to allow the two layers to bond.

To fabricate the cell substrate (fig. S2b), a liquid PA mixture of 40% acrylamide solution (Bio-Rad), 2% bis-acrylamide solution (Bio-Rad), and water at a ratio of 0.25:0.11:0.64, with additional 1 μ L crosslinker TEMED (Bio-Rad) and 10 μ L 10% Ammonium Persulfate (Bio-Rad) added to a 1 mL PA mixture for gelation. This typical ratio gave a final PA gel elasticity of ~ 20 kPa³⁷. 10 μ L of the mixture was added onto the opening of the 2nd NOA73 layer which also flowed into the bottom layer, and was covered with a round coverslip of 12 mm diameter for polymerization to occur. Simultaneously, the sample was quickly treated with 365 nm UV for 8 min at 75 mW/cm² that will fully cure the chip and allow the gel to bond to it. The sample was then left for a more complete gelation for another 30 min. After that, the coverslip was peeled off, exposing the gel, and a pH 7.4, 0.1 M HEPES buffer was used to soak the gel for 2 days such that the unpolymerized polyacrylamide that is toxic for cells can be fully rinsed off.

Next, to coat the PA gel with extracellular matrix (ECM) protein, a protocol using the Sulfo-SANPAH (SS, PierceTM) cross-linker was followed³⁷. Stock SS which was dissolved in anhydrous DMSO (1 mg/50 μ L), was diluted with cold HEPES buffer (pH 7.4, 0.1 M) at 1:40 dilution. This SS solution was pipetted onto the gel surface and treated with 365 nm UV for 5 min at 24.5 mW/cm² for the activation of the SS, and this activation procedure was repeated once more with new SS solution after cold HEPES was used to rinse the sample. Finally, un-attached SS was rinsed off with 1X PBS. For ECM protein coating, we used either Collagen-I (Corning, 50 μ g/mL) or fibronectin (Roche, 50 μ g/mL). Collagen-1, dissolved in cold 1X PBS to prevent initial gelation, or fibronectin, dissolved in 1X PBS at room temperature, was pipetted onto the substrate and incubated for 3 hours at room temperature for the attachment of the ECM protein. As a variant to this protocol, we used fibronectin (Roche, 50 μ g/mL)

at room temperature to coat the PA gel surface for the rescue experiments and cells were seeded at $\sim 0.4 - 0.5$ million cells/cm² on this substrate.

Further, to integrate the microfluidic chip with the cartridges, tubes and electrodes (fig. S2a), the chip (with Collagen-I coated gel) was first dried, and O-rings were placed on it. The sample was then sandwiched between a custom-designed PMMA cartridge and an aluminium base and screwed down to tightly seal and isolate the different compartments from the top using pressure. This ensured that the ion current will only flow through the channels in the bottom NOA layer and the opening of the 2nd NOA layer. The gel was then quickly rehydrated with cell medium, while the Tygon® tubes were plugged into the cartridges and connected to 50 mL Falcon tubes with electrodes. Cell medium was then used to fill the whole setup. To determine whether a sample was well prepared, a total amount of current, I , with certain value was checked to pass through the sample when a fixed voltage, V , was applied across the whole setup, such that the relation $V \sim I \cdot R$ was satisfied. R ($\sim 10^6 \Omega$), the resistance of the chip was determined by the channel geometries and the conductivity of the medium which is ~ 1 S/m^{38,39}.

To do the E-field experiment, MDCK or N/TERT-1 cells were seeded at ~ 0.2 million cells/cm² in each well and left in the incubator for 3 days for the cells to reach confluency. The setup was brought to the microscope for imaging and the E-field was applied then (fig. S2a). After each experiment, the cartridges and accessories were cleaned and sonicated sequentially in detergent, water, and 70% Isopropyl alcohol (IPA). Finally, the materials were rinsed with 100% IPA and dried in a vacuum chamber before storing in a dry cabinet for the next experiment.

Also, to do live-cell experiments which were compatible with high-resolution imaging using the 40X, 1.15 N.A., ~ 0.6 W.D., CFI Apo LWD Lambda S 40XC water-lens objective, we replaced the 1 mm thick glass slides with No. 1.5 precision slides (Marienfeld) which measured $\sim 170 \mu\text{m}$ thick. In this case, the O-rings and the pressure method could not be used to seal the compartments, so a two-part dental silicone (TwinSil, PicoDent) was used instead.

Simulations/Order-of-magnitude estimates:

1. COMSOL simulation of E-field distribution

To estimate the normal E-field distribution on the level of the whole epithelium, the “AC/DC” module and the “Electric Currents” interface in COMSOL was used to simulate the experiments. We performed two-dimensional simulations because the experimental geometry of the slit ($3 \text{ mm} \times 200 \mu\text{m}$), where the E-field emerged from the second microfluidic chip layer, has a dimension in the plane of the epithelium much larger than the other dimension (fig. S2b). The simulation geometries followed the cross-section of the setup, and some other general specificities of the simulation include constant potential boundary conditions at the inlet/outlet, and electrical insulation at the boundaries representing the walls of the microfluidic chip (fig. S7a). We simulated four different conditions, that is one without an epithelium, one with a fully confluent epithelium, one with an epithelium that has a $200 \mu\text{m}$ gap on top of the slit opening, and one where the gap is at the extremities of the epithelium. The epithelium was simulated with a thin layer approximation due to its small thickness compared to the other length-scales of the system, with current conservation at its interface with the medium, and a conductivity value of $\sigma = 10^{-3} \text{ S/m}$ as inferred from the Volt ohmmeter measurements (conductivity = cell height / (resistance $\times$ area)). The results show that the low epithelial conductivity reduces the normal E-field magnitude at the epithelium and cause the field to spread more uniformly along the epithelial plane compared to when there is no epithelium (fig. S7b - bare surface, confluent epithelium). Further a multicellular gap in the epithelium close to the slit draws a huge amount of current through it, but such a gap has much lesser influence on the epithelial field the further away it is from the slit (fig. S7b - epithelia with central hole, epithelia with far-away holes).

2. Inducing a perturbation of the natural TEPD

Since we have shown in simulations that the E-field spreads across the epithelium, and that we applied a total ion current of 10 μA over a circular epithelial area of 12 mm diameter, this gives an average ion current density of $\sim 10 \mu\text{A}/\text{cm}^2$. Further, the Voltohmmeter-measured MDCK resistance (scaled by area) is $\sim 10^2 \Omega \cdot \text{cm}^2$ (fig. S1b). Thus, the average TEPD induced by the external field on-chip is $V = IR = (I/A) * (RA) \sim (10 \mu\text{A}/\text{cm}^2) * (10^2 \Omega \cdot \text{cm}^2) = 1 \text{ mV}$, which is similar to the intrinsic TEPD measured for the MDCK.

Image analysis:

1. Junctional intensity

To determine the junctional intensity of phase contrast images and fluorescent actin signals, cut-lines were manually drawn using ImageJ perpendicular to bi-cellular junctions, with the midpoint of the cutlines co-localizing with the center of the junctions. The latter was found between two tri-cellular junctions that flanked the two ends of the bi-cellular junctions. The one-dimensional intensity along the cutline was normalized by the intensity of the endpoints of the cutline, which fell in the region of the cell body of the two neighboring cells, and the process was repeated for different junctions. The averaging of all the junction intensities were done by aligning the centers of the cutlines in MATLAB. Whenever junction signals were fuzzy, such as in the phase contrast images under no-field and BtA field conditions (Fig. 1c), or in the fluorescent actin images under AtB field conditions (Fig. 1f), the identification of junctions were aided by the superimposing of the fluorescent images of cell nucleuses and by the scrutinizing of the dynamics of regions around the junctions.

2. Determination of different cell events

Cell death in MDCK was determined by the identification of nucleus condensation, fragmentation, or disappearance in H1-GFP monolayers (Fig. 4a), the disappearance of Cdt1 (red) signal without the appearance of Geminin (green) signal in FUCCI lines, or the sequential appearance and disappearance of a bright signal in GFP-actin cells. Cell division in MDCK was further identified by the appearance of two daughter cells replacing a mother cell in H1-GFP lines, or the disappearance of the Geminin (green) signal in FUCCI cells. Finally, cell differentiation in FUCCI N/TERT-1 cells were determined by nucleus condensation under phase contrast imaging, and the simultaneous disappearance of Cdt1 (red) and absence of Geminin (green) expressions in the fluorescent images (Fig. 4b). Such events were found to generally correlate with high Involucrin expression.

3. Measure of homogeneity of epithelial cell distribution

First, the spatial correlation for coarse-grained image intensities, $I(\vec{r})$ is defined as:

$$C(|\vec{r}|) = \frac{\langle I(\vec{r}' + \vec{r}) \times I(\vec{r}') \rangle_{\vec{r}'}}{\sqrt{\langle I(\vec{r}' + \vec{r})^2 \rangle \langle I(\vec{r}')^2 \rangle}} \quad (1)$$

with $|\vec{r}|$ being the distance. $I(\vec{r})$ is the average measure of local cell density and is coarse-grained over a window size of 50 μm , with $\sim 5 \times 5$ cells in each window. The step size between two neighbouring windows is 25 μm .

The Uniformity Deviatory Measure (UDM) of each image is further defined as:

$$\text{UDM} = 1 - \langle C(|\vec{r}|) \rangle_{|\vec{r}|} \quad (2)$$

which measures the deviation of the average spatial correlation from a unity measure signifying the fully uniform situation. The larger the UDM, the more heterogenous is the epithelium. The correlation function was averaged up to distances of $\sim 150 \mu\text{m}$, which is the typical correlation length of MDCK⁴⁰ and N/TERT-1 epithelia in these conditions. Since $C(|\vec{r}|)$ is calculated based on image intensities and would be sensitive to the imaging apparatus used, it may be difficult to compare the UDM of different samples directly. This difficulty is circumvented by calculating the Relative Uniformity Deviatory Measure (RUDM) for each image:

$$\text{RUDM}(t) = \text{UDM}(t)/\text{UDM}(t = 1), \quad (3)$$

which measures the relative change in the heterogeneity of the sample as a function of time, with reference to the first image. Provided that different samples start at the same biological/physical conditions, this allows the objective comparison of different sample dynamics even when the imaging conditions may be slightly different.

4. Measure of tissue integrity for Ecad KO MDCK experiments

The whole epithelium is partitioned into field-of-views of $\sim (460 \mu\text{m} \times 460 \mu\text{m})$ squares. Each square is classified as 0 if it is determined to have a non-intact part of the epithelium, and 1 for the opposite. The measure of tissue integrity is the percentage of all squares valued as 1. The higher this value, the higher the overall integrity of the layer. To determine whether a square has an intact part of the epithelium, we used Image J's variance filter with radius < 1 pix (typical cell size is ~ 5 pix) to identify gaps within the epithelium as such areas had smoother local intensities. A smaller gap area shows an epithelial region with higher integrity. A gap area ratio threshold of ~ 0.0002 was used to classify each square, where a square was considered intact if the detected gap area ratio was below this threshold.

Calculation of physical quantities from experiments:

1. E-field spatial distribution inference

The first step to estimate the E-field spatial distribution was to inject fluorescent beads (carboxylated, 100 nm, Invitrogen) at $\sim 1:100$ dilution in the cell medium on the chip with cells while the E-field is applied. Since the beads are charged and will undergo electrophoresis along the field lines the visualization of the accumulation of beads and their distribution throughout the epithelium gives us a rough estimate of the E-field distribution. An E-field magnitude that was five times larger than the usual one used for studying the cell behavior was applied to increase the electrophoresis of beads and to minimize the effects of bead diffusion. In particular, beads were uniformly distributed when the epithelium was confluent (fig. S8a, b), while the beads accumulated largely within the multicellular gap region when there was a non-intact epithelium (fig. S8c, d).

The second method to gauge the spatial distribution of the E-field was from inferring the cellular stress induced by the AtB field (Fig. 2d - g). It was found that the average stress decreased from ~ 0.8 kPa at the locations closest to the opening of the 2nd NOA73 layer, where the E-field initially emerged, to ~ 0.6 kPa at locations ~ 2 mm away (fig. S9). This spatial change in the average electromechanical stress (one quarter-fold difference) is small compared to the variation in cell stress from cell to cell, which varied from 0.2 kPa to 1.2 kPa (that is up to 6-fold difference). This showed that the E-field can be considered to be uniform at the whole epithelia level on a first approximation.

Both methods gave the same conclusion, which was that the E-field distribution was much more homogeneous on the whole epithelia scale when there was a confluent epithelium. Interestingly, the E-field distributions obtained from COMSOL simulations seemed to have underestimated the uniformity

of the E-field compared to the experimental estimations. This may be because the epithelial conductivity used in the simulations (10^{-3} S/m) was inferred from the Millicell®-ERS Voltohmmeter system, which uses a 12.5 Hz AC field instead of a DC field for the measurement. This may have caused the simulations to use an underestimated epithelial resistivity, as it is known that AC fields experience a reduced epithelial resistance⁴¹, and thus could cause it to predict a less spread-out/uniform E-field distribution.

2. Electromechanical stress inference from gel deformation

The normal stress with respect to the monolayer plane in theory is given by $\sigma_{nn} = K * (\delta L/L)^{42}$, where K is the polyacrylamide gel stiffness determined by the gel component mixture ratios³⁷, L is the gel thickness in our setup at the opening of the second NOA73 layer, and δL is the gel deformation which can be negative (downward deformation) or positive (upward deformation). The typical gel thickness was visualized by infusing fluorescent beads (100 nm, Invitrogen) into the PA gel and imaging it on a confocal microscope. Manual re-focusing of the microscope images were done to track the z -positions of the cells in real-time under E-fields, and the deformation of the gel surface was measured by subtracting the reference z -position (height before the E-field was turned on) from these real-time measurements.

3. Electromechanical stress inference and cell shape calculation

To examine whether a stress hypothesis can explain the observed cell shape under the AtB field, we needed to find relations between the cytoplasmic pressure P^c , the fluid external pressure P_a^{ext} and geometrical parameters of the cell such as the apical cell shape and the total cell height. Such a relation is given by the Young-Laplace's law, which expresses the normal force balance at an interface, at low Reynolds number, dictating the pressure drop discontinuity across a curved interface. The law states that $\delta P = \gamma * (1/R_1 + 1/R_2)$, where γ is the surface tension, and R_1 and R_2 are the principal radii of curvature, and δP is the pressure drop across the interface (interior pressure minus the exterior pressure). In general, a larger interior pressure will cause the surface to bulge outward. Further assuming an in-plane isotropic cell with a single maximum height (Fig. 2c), one can express the pressure difference $\delta P = P^c - P_a^{ext}$ as a first integral of Laplace's law^{43,44}:

$$\delta P = \frac{2\gamma \cos(\theta)}{r}, \quad (4)$$

where cylindrical coordinates have been used, in which r is the distance of the considered point to the symmetry axis z , and θ the angle between the tangent and the z axis. Equation (4) thus allowed us to infer the electrically induced pressure difference at the cell surface purely from the apical cell geometry. Also, assuming γ is constant (based on phalloidin staining of F-actin) while θ and r change with z on a surface with variable curvature, δP has to be a function of the vertical position of the cell surface, z .

Importantly, inference results from equation (4) showed that many cells exhibited a pressure difference δP that decreases linearly with increasing z (or increases linearly with cell height) at the cell surface (Fig. 2d). It is thus reasonable to assume the linear relation to hold throughout the cell such that:

$$\delta P = \delta P_0 \left(1 - \frac{z}{H}\right), \quad (5)$$

with H being the total cell height, and δP_0 is the maximum pressure at the tip of the cell. By fitting equation (5) to equation (4), one can obtain the values of the parameters δP_0 and H .

With the expression relating θ , r and z in this geometry (fig. S10a):

$$\cos(\theta) = \frac{dz/dr}{\sqrt{(dz/dr)^2 + 1}}, \quad (6)$$

and combining equations (4), (5), and (6), we arrive at:

$$dz = \frac{dr}{\sqrt{\left(\frac{r_0}{r(1-z/H)}\right)^2 - 1}}, \quad (7)$$

where $r_0 = 2\gamma/\delta P_0$. Using equation (7) and a surface tension ~ 1 mN/m based on cortex-membrane tension measurements²¹, we managed to have a prediction of the apical cell surface by starting at the ($r = 0.1 \mu\text{m}$, $z = 0.1 \mu\text{m}$) point and tracing out the curve in MATLAB by adding dz increments using a fixed dr step size of $0.05 \mu\text{m}$.

We next examined the extent to which the whole experimental/theoretical inference pipeline worked well. Cells are inherently non-isotropic, and the real pressure drop at each point on the membrane is more accurately described by the equation without symmetry assumption as mentioned previously:

$$\delta P = \gamma * (1/R_1 + 1/R_2), \quad (8)$$

where R_1 and R_2 are the two different curvature radii. Thus, the use of equation (4) may underestimate or overestimate the pressure. We thus only used data for single-peaked cells that were weakly anisotropic, where the two different axes of such cells exhibited a difference in length of less than $\sim 30\%$. We confirmed that the isotropic theoretical framework used here worked for such cells. Indeed, within this range of anisotropy, the difference between the predicted cell heights of the two different cross-sections of the same cell did not increase with anisotropy and were largely $< 50\%$ in their difference (fig. S10b). To further understand how much proportion of the cell surface was best described by the theory, we repeated the fitting procedure for each cell using different lengths of the cell surface and checked the best fit solution among these different trials. We found that some of the best fit solutions were based on lengths up to $\sim 90\%$ of the full apical cell length, showing that large parts of the cells fitted well to the theory. We also found that close to 50% of cells had best fits which fitted at least $> 40\%$ of the full length and used the latter as a benchmark to filter the data to reduce unwanted noise. Finally, the whole pipeline converged to predicted total cell heights or cell shapes and electromechanical stresses that were consistent with experimental cell images (Fig. 2e, f, g) and the deformation of the gel (Fig. 2b). This confirmed that the cytoplasm-electromechanical stress hypothesis explains well the observed cell shapes.

4. Stress exerted on the substrate and pressure difference across the apical surface

In the following, we describe the cell cytoplasm as a two-component system composed of a fluid phase, essentially water, which is able to permeate through the cell membranes, and of a stationary phase, essentially proteins and nucleic acids, which is trapped inside the cell. Since all charges are globally balanced at a scale larger than the Debye screening length, which is smaller than a nanometer in a cell, the local force balance simply reads:

$$\partial_j \sigma_{ji}^{tot} = 0, \quad (9)$$

where σ_{ji}^{tot} is the total stress in the cytoplasm. To keep the algebra as simple as possible, we consider a steady state in which the protein distribution is essentially at rest. Under these conditions,

$$\sigma_{ji}^{tot} = \sigma_{ji}^f + \sigma_{ji}^p, \quad (10)$$

with $\sigma_{ji}^f = -P^f \delta_{ji}$ and $\sigma_{ji}^p = -P^p \delta_{ji}$, where σ_{ji}^f, P^f are respectively the fluid stress and fluid pressure, and σ_{ji}^p, P^p are the stationary phase stress and pressure. By construction, the cytoplasmic pressure $P^c = P^f + P^p$. The global incompressibility of the two phases can be expressed in the relation:

$$\phi^f + \phi^p = 1, \quad (11)$$

where ϕ^f, ϕ^p are the volume fraction of the fluid and stationary phase respectively.

For small enough density changes, the stationary phase pressure may be expressed at linear order:

$$P^p = C(\phi^p - \phi_0^p), \quad (12)$$

in which C is the compression modulus of the phase, ϕ_0^p is the volume fraction in the absence of external field, where P^p is defined in such a way that it vanishes in the absence of external E-field. The fact that the stationary phase components does not cross the cell membranes imply:

$$\int_V \phi^p dV = \int_V \phi_0^p dV. \quad (13)$$

Even though the electric field does not contribute to an external body force in equation (9), it does generate an electroosmotic flow of the fluid with respect to the stationary phase. In the following, since the E-field is essentially normal to the substrate, and for the sake of simplicity, we consider a one-dimensional situation, in which all quantities depend on the distance to the substrate, z . This is clearly an approximation, but it will provide the essential results. The electroosmotic effect, coupled to permeation of the fluid through the stationary network, can be expressed in the same spirit as⁵ by:

$$\partial_z P^f + \lambda_p v_z^f + \lambda_e E_z = 0, \quad (14)$$

where λ_p is the cytoplasm fluid permeation coefficient, while λ_e is the cytoplasm electroosmotic coefficient.

At the apical and basal surfaces, the fluid can flow through the membranes according to standard permeation relations:

$$\Lambda_a v_z^f = P_a^{ext} - P^f(z=0), \quad (15a)$$

$$\Lambda_b v_z^f = P^f(z=h) - P_b^{ext}, \quad (15b)$$

where the suffixes a, b stand for apical and basal respectively. In relation (15), we have assumed osmotic pressure balance in view of the fact that in the absence of field, the cell apical membrane is essentially flat. Λ_a, Λ_b are the membrane permeation coefficients. P_a^{ext}, P_b^{ext} are the fluid pressure at the outside of the cell on the apical and basal side.

Making use of (14) and (15a, b), we find:

$$v_z^f = \frac{(P_a^{ext} - P_b^{ext}) - \lambda_e E_z h}{\Lambda_a + \Lambda_b + \lambda_p h}. \quad (16)$$

where h is epithelial height.

Similarly, making use of (9), (12) and (14), we find:

$$C(\phi^p - \phi_0^p) = \left(\frac{\lambda_p(P_a^{ext} - P_b^{ext})}{\Lambda_a + \Lambda_b + \lambda_p h} + \frac{\lambda_e E_z (\Lambda_a + \Lambda_b)}{\Lambda_a + \Lambda_b + \lambda_p h} \right) \left(z - \frac{h}{2} \right), \quad (17)$$

where we further imposed $\int_0^h \phi^p(z) dz = \int_0^h \phi_0^p(z) dz$, which satisfies (13).

The stress balance on the basal side reads: $P^c(z = h) = P^f(z = h) + P^p(z = h) = P_b^{ext} - \sigma_b^s$, where σ_b^s is the stress in the substrate resulting from the cell cytoplasm transmitted by the basal adhesion proteins.

Using equations (15a) and (17), we obtain:

$$\sigma_b^s = \frac{(P_b^{ext} - P_a^{ext})}{2} \frac{(2\Lambda_b + \lambda_p h)}{\Lambda_a + \Lambda_b + \lambda_p h} + \frac{\lambda_e E_z h}{2} \frac{(\Lambda_b - \Lambda_a)}{\Lambda_a + \Lambda_b + \lambda_p h}. \quad (18)$$

Now, consider the leak pathways giving rise to a flow through the hydrogel, v_z^g and a back-flow through the cell-cell junctions, v_z^j . We have:

$$v_z^g = -(P_a^{ext} - P_b^{ext})/(\lambda_{gel} L), \quad (19)$$

and

$$v_z^j = \epsilon \zeta E_z / \eta - (P_a^{ext} - P_b^{ext})/(\lambda_j L), \quad (20)$$

where λ_{gel} and λ_j being the liquid permeation constants of the hydrogel and cell-cell junction respectively under a pressure difference, η viscosity of water, ϵ permittivity of water and ζ zeta-potential of cell surfaces. Note that there is no electro-osmotic effect through the hydrogel due to its neutrality, but this effect exists at the junctions.

Together with fluid flux conservation, $v_z^f = v_z^g + (w/l)v_z^j$, where w is junctional width and l cell width, equations (19) and (20) give:

$$(P_a^{ext} - P_b^{ext}) \left(1 + \frac{\Lambda_a + \Lambda_b + \lambda_p h}{\left(\frac{1}{\lambda_{gel} L} + \frac{w}{lh\lambda_j} \right)^{-1}} \right) = \lambda_e E_z h \left(1 + \frac{(\Lambda_a + \Lambda_b + \lambda_p h)(w\epsilon\zeta)}{lh\lambda_e \eta} \right). \quad (21)$$

The typical expressions and values are: Λ_a and $\Lambda_b = \eta/w_f$, with $\eta \sim 10^{-3} Pa.s$ and w_f a length scale $\sim 10^{-14} m$ ⁴⁵. $\lambda_p = \eta/a^2$, $\lambda_{gel} = \eta/a_{gel}^2$, and $\lambda_j = 12\eta/w^2$, with cytoskeletal, hydrogel⁴⁶ and junctional pore sizes a and a_{gel} being $\sim 1 nm$ respectively. The expression for λ_j considers a Poiseuille flow through the cell-cell junctions. Additionally, $\lambda_e = \epsilon\zeta/a^2$, $w \sim 5 nm$, l and $h \sim 10 \mu m$ respectively, $L \sim 3 mm$ from our experiments, $\epsilon \sim 10^{-9} F.m^{-1}$, and $\zeta \sim 10 mV$ ⁵. With these values, the terms in the parentheses of equation (21) are much smaller than 1, and this equation simplifies to $P_a^{ext} - P_b^{ext} \sim \lambda_e E_z h$, while equation (16) becomes $v_z^f \sim 0$.

With these relations,

$$\sigma_b^s \sim -\frac{\lambda_e E_z h}{2}. \quad (22)$$

On the apical side, a similar argument and taking advantage of the fact that the pressure is isotropic, we get for the stress exerted by the membrane on the surrounding:

$$\sigma_a = \frac{-(P_b^{ext} - P_a^{ext})}{2} \frac{(2\Lambda_a + \lambda_p h)}{\Lambda_a + \Lambda_b + \lambda_p h} + \frac{\lambda_e E_z h}{2} \frac{(\Lambda_b - \Lambda_a)}{\Lambda_a + \Lambda_b + \lambda_p h}. \quad (23)$$

Note that this stress must be equal to the pressure difference, δP across the membrane as used in section 3.

Again, with $P_a^{ext} - P_b^{ext} \sim \lambda_e E_z h$,

$$\sigma_a \sim \frac{\lambda_e E_z h}{2}. \quad (24)$$

Or in the notations of equation (5) with $h = H - z$, $\delta P_0 = -(\lambda_e E_z H)/2$ such that

$$\delta P = \delta P_0 \left(1 - \frac{z}{H}\right). \quad (25)$$

This is a central result which as detailed in supplementary section 3, provides a good description of cell shape despite several simplifying assumptions such as neglect of the back flow through the junctions and the absence of transverse flow.

It is important to note that the total stress induced in the substrate is not simply given by equation (22), which again only takes into account the stress transmitted through the cytoplasm and basal substrate proteins. Indeed, the stress acting on the apical membrane is entirely transmitted through the vertical cell-cell junctions which is in turn transmitted to the substrate. Adding this contribution, we obtain the overall average substrate stress:

$$\langle \sigma_b \rangle = \langle \sigma_b^S \rangle - \langle \sigma_a \rangle = -\lambda_e E_z \langle h \rangle. \quad (26)$$

From this equation and the gel deformation data that substrate stress is tensile and of order $\sim 2 - 4 \text{ kPa}$, we obtain with $\langle h \rangle = 15 \text{ } \mu\text{m}$, $-\lambda_e E_z \sim (1.3 - 2.6) * 10^8 \text{ Pa/m}$.

From equation (25) or $\delta P_0 = -(\lambda_e E_z H)/2$, and the cell shape estimation of peak pressure difference of $\sim 0.6 - 1.2 \text{ kPa}$, we obtain with $H = 20 \text{ } \mu\text{m}$, $-\lambda_e E_z \sim (0.6 - 1.2) * 10^8 \text{ Pa/m}$. If instead of using $\gamma = 1 \text{ mN/m}$, we use $\gamma = 2 \text{ mN/m}$, which is equally reasonable, we get a perfect agreement between the values extracted from the gel deformation and those extracted from the cell shape. This translates to the stress values given in the main text. Eventually, with $E_z \sim 100 \text{ V/m}$, we find $\lambda_e \sim -10^6 \text{ Pa/V}$, which signals a negative average zeta potential of the stationary phase as expected. This value also gives an estimation of cytoskeletal pore size of $\sim 3 \text{ nm}$ (from the relation: $\lambda_e = \epsilon \zeta / a^2$), which is very reasonable and self-consistent with our previous assumptions.

Statistical analysis:

No statistical methods were used to predetermine sample size. In the case where no statistical significance was observed, the sample size chosen was at least as big as those where differences were observed. Blinding was achieved for cell death rate determination as the data sets were analyzed independently at two different institutes. One-tailed t-test was used to compare distributions when the hypothesis was to test whether one of the distributions was larger. All relevant statistics are reported in the corresponding legends.

Data and code availability:

Source data and custom codes for image analysis are available upon reasonable request.

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