Energetics of mesoscale cell turbulence in two-dimensional monolayers

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Investigation of energy mechanisms at the collective cell scale is a challenge for understanding various biological processes, such as embryonic development and tumor metastasis. Here we investigate the energetics of self-sustained mesoscale turbulence in confluent two-dimensional (2D) cell monolayers. We find that the kinetic energy and enstrophy of collective cell flows in both epithelial and non-epithelial cell monolayers collapse to a family of probability density functions, which follow the q -Gaussian distribution rather than the Maxwell-Boltzmann distribution. The enstrophy scales linearly with the kinetic energy as the monolayer matures. The energy spectra exhibit a power-decaying law at large wavenumbers, with a scaling exponent markedly different from that in the classical 2D Kolmogorov-Kraichnan turbulence. These energetic features are demonstrated to be common for all cell types on various substrates with a wide range of stiffness. This study provides unique clues to understand active natures of cell population and tissues.

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Collective cell dynamics plays a critical role in vast morphogenetic processes, such as embryonic development, wound healing, and cancer metastasis^{1–6}. Yet, the energetic mechanisms in living cell systems remain unclear at the collective cell scale. Cell migration primarily results from active motility of individuals energized by intracellular biochemical reactions^{7,8}. In stark contrast to migration of isolated cells, however, collective migration of interacting cells is coordinated at multicellular level through intricate cell–cell interactions, including cadherin-mediated cell–cell adhesion^{5,9–11} and social interactions^{12–15}.

Due to the active motility of cells and complex intercellular interactions, many nonequilibrium dynamic features emerge spontaneously in living cell collectives. For example, migrating cells can self-organize into mesoscale cell turbulence^{16–22}, which is crucial in multicellular organization²³, nutrient mixing²⁴, and thrombopoiesis²⁵. Though the mesoscale cell turbulence apparently resembles the classical turbulent flows, it takes place at an ultralow Reynolds number where the inertial effect can be negligible^{26,27}, profoundly different from the passive turbulence resulting from excessive kinetic energy and inertia. However, the energetics of mesoscale cell turbulence remain unclear. Studying the energetic features in multicellular systems could advance our understanding of tissue morphodynamics, as well as the nonequilibrium statistical properties in active matter systems.

In this article, we combine large-scale experiments and theoretical analysis to investigate the energetic statistics of mesoscale cell turbulence in two-dimensional (2D) multicellular monolayers. By live-cell imaging on diverse cell monolayer systems, we reveal some unique energetic features of mesoscale cell turbulence. The enstrophy scales proportionally to the kinetic energy, which generalizes previous findings^{23,28} to monolayers of diverse cell lines, suggesting a common feature in living multicellular systems. The kinetic energy as well as the enstrophy over time collapse to a family of probability density functions (PDFs), which follow the q -Gaussian statistics. The energy spectra of mesoscale cell turbulence are profoundly different from that in the classical 2D Kolmogorov–Kraichnan turbulence. These features are not only common across different cell types, but also insensitive to the substrate stiffness. An active vertex model is used to reveal the physical mechanisms underlying these energetic statistics.

Results

Experiment. In our experiments, 2D cell monolayers consisting of cell lines including Madin Darby canine kidney (MDCK), human umbilical vein endothelial cells (HUVECs), C2C12 mouse myoblast cells (C2C12), and NIH-3T3 mouse embryo fibroblast cells (NIH-3T3) are investigated. Cells are seeded on 35 mm Petri dishes and cultured for enough time (typically 1 day) to grow into gap-free monolayers with a density of $\sim 1.4 \times 10^5 \text{ cm}^{-2}$. Subsequently, live-cell imaging is performed to obtain consecutive phase contrast images (Fig. 1a; “Methods”). Hereafter, the concept of time in experiments refers to time after live-cell imaging start. The field of view (FOV) has size $1.33 \text{ mm} \times 1.33 \text{ mm}$, which is much larger than the spatial correlation length ($\sim 200\text{--}300 \mu\text{m}$) of mesoscale cell turbulence^{16,18,19}, and thus large enough to access its energetic statistics.

Mesoscale cell turbulence in various cell monolayer systems. To quantitatively investigate the collective cell flows in a confluent cell monolayer, we calculate the velocity field via the particle image velocimetry (PIV) analysis (“Methods”; for the accuracy validation, see Supplementary Note 1 and Supplementary Fig. 1). Figure 1b, c

shows representative velocity fields and corresponding vorticity fields in the four kinds of cell monolayers. Interestingly, turbulent motion patterns are observed for all the four kinds of cell monolayer systems throughout the live-cell imaging experimental duration of 12 h, in spite of the distinct difference of cell motility in these cell lines. This suggests that turbulent flows are ubiquitous in 2D cell monolayer systems.

Energetics of mesoscale cell turbulence in MDCK cell monolayers. We first study the energetics of the mesoscale turbulence in a confluent MDCK cell monolayer. Based on the cell velocity and vorticity fields, we calculate the kinetic energy $E(\mathbf{x}, t) = \frac{1}{2} |\mathbf{v}(\mathbf{x}, t)|^2$ and enstrophy $\Omega(\mathbf{x}, t) = \frac{1}{2} |\omega(\mathbf{x}, t)|^2$ per unit mass at space $\mathbf{x}$ and time t , where $\mathbf{v}(\mathbf{x}, t)$ is the velocity vector and $\omega(\mathbf{x}, t)$ is the vorticity^{23,28}. Figure 2a, b shows the representative kinetic energy and the enstrophy field in a MDCK cell monolayer, demonstrating distinctively spatial heterogeneity. We thus examine the PDF of the kinetic energy. $E(\mathbf{x}, t)$ is rescaled as $\tilde{E}(\mathbf{x}, t) = E(\mathbf{x}, t) / \langle E(\mathbf{x}, t) \rangle_{\mathbf{x}, t}$, where $\langle \cdot \rangle_{\mathbf{x}, t}$ stands for an average over the entire FOV throughout 1 h period of time, during which cell motility keeps nearly invariant. The PDFs of the rescaled kinetic energy at different time points collapse to a family of PDFs which vary little over time (Fig. 2c), though the average kinetic energy decreases considerably with time. This suggests time-invariant statistics of collective cell flows during the jamming transition of cellular motions as the monolayer matures^{18,29}. These rescaled PDFs display a fat tail distribution characteristics, which deviates distinctly from the classical Maxwell–Boltzmann distribution of energy, $p_B(\tilde{E}) = \exp(-\tilde{E})$ (Fig. 2c), evidencing that the examined cell monolayer system is nonequilibrium. The nonequilibrium feature of living cell systems stems from the active motility of cells.

In our previous study, we have explored the distribution statistics of the velocity field. It was found that cell velocities satisfy the 2D q -Gaussian distribution³⁰ (Supplementary Fig. 2a),

$$f_{q-G,2D}(\tilde{\mathbf{v}}; q) = A_q \left(1 + B_q |\tilde{\mathbf{v}}|^2 \right)^{-\lambda_q}, \quad (1)$$

where $\lambda_q = 1/(q-1)$, $A_q = (1/\pi) (\lambda_q - 1) B_q$, and $B_q = (\pi/4) [\Gamma(\lambda_q - 3/2) / \Gamma(\lambda_q - 1)]^2$ depend on the Tsallis index q , i.e., the entropic index^{31–33}; $\Gamma(\cdot)$ is the Legendre gamma function. In statistical mechanics, the q -Gaussian distribution of velocities results from optimizing the Tsallis entropy^{31,34}. This indicates that the collective cell dynamics is governed by the Tsallis entropy rather than the classical Boltzmann–Gibbs entropy. Based on the q -Gaussian distribution feature of velocities, we can deduce that the PDF of the kinetic energy should obey the following distribution function (also referred to as the q -Gaussian distribution hereafter for simplicity),

$$p_{ke}(\tilde{E}; q) = \frac{\lambda_q - 1}{\lambda_q - 2} \left(1 + \frac{\tilde{E}}{\lambda_q - 2} \right)^{-\lambda_q}. \quad (2)$$

As expected, our experimental data clearly demonstrate that the PDF of the kinetic energy can be well captured by the q -Gaussian distribution, i.e., Eq. (2), as shown in Fig. 2c. Similarly, we find that cell vorticities satisfy the 1D q -Gaussian distribution³⁰ (Supplementary Fig. 2b),

$$f_{q-G,1D}(\tilde{\omega}; q) = \frac{C_q}{(1 + D_q \tilde{\omega}^2)^{\lambda_q}}, \quad (3)$$

Fig. 1 Turbulent cell dynamics in various cell monolayers. **a** Representative phase contrast images. MDCK for Madin Darby canine kidney cells, HUVEC for human umbilical vein endothelial cells, C2C12 for C2C12 mouse myoblast cells, and NIH-3T3 for NIH-3T3 mouse embryo fibroblast cells. **b, c** Representative fields of **b** the velocity and **c** the vorticity at $t = 6$ h. The color codes refer to the velocity magnitude and the vorticity intensity, respectively, and the black arrows indicate the velocity vectors. Scale bars, 300 μm .

where $\tilde{\omega} = \omega/\omega_{\text{rms}}$ is the rescaled vorticity with $\omega_{\text{rms}} = \sqrt{\langle \omega^2 \rangle}$ being the root-mean-square vorticity; $C_q = \Gamma(\lambda_q)/[\sqrt{\pi(2\lambda_q - 3)\Gamma(\lambda_q - 1/2)}]$ and $D_q = 1/(2\lambda_q - 3)$. Based on the q -Gaussian distribution feature of vorticities, we can obtain the distribution law of the enstrophy Ω (Fig. 2d), which obeys the following q -Gaussian statistics:

$$\tilde{p}_{\text{enstr}}(\tilde{\Omega}; q) = \frac{C_q}{\sqrt{\tilde{\Omega}}(1 + D_q \tilde{\Omega})^{\lambda_q}}, \quad (4)$$

where $\tilde{\Omega} = \Omega/\langle \Omega \rangle$ is the rescaled enstrophy. In addition, to verify whether the landscape of the energetic PDFs is affected by the PIV calculation, we have varied the window size of PIV analysis (from 1.5 cell size to 3 cell size) and found that it has no marked effect on the PDFs of the rescaled kinetic energy and the rescaled enstrophy (Supplementary Fig. 3).

The kinetic energy and the enstrophy of the whole cell monolayer can be quantified by $E(t) = \langle E(\mathbf{x}, t) \rangle_{\mathbf{x}}$ and $\Omega(t) = \langle \Omega(\mathbf{x}, t) \rangle_{\mathbf{x}}$, respectively, where $\langle \cdot \rangle_{\mathbf{x}}$ stands for an average over the entire FOV. Figure 2e plots the kinetic energy $E(t)$ versus the enstrophy $\Omega(t)$ at different time points during the experimental duration. It shows that both $E(t)$ and $\Omega(t)$ reduce over one

order of magnitude within 10 h, indicating a jamming transition of cellular motions. Intriguingly, the enstrophy $\Omega(t)$ scales linearly with the kinetic energy $E(t)$ during the jamming transition. This scaling behavior defines a conserved characteristic length scale $\xi_a = \sqrt{E/\Omega}$, which stems from the force balance between active and passive forces and roughly reflects the size of vortices^{23,28,35}. For the MDCK cell monolayer, our measurement gives $\xi_a \approx 44.1 \mu\text{m}$, approximately corresponding to a length scale of two cells. We note that a similar scaling behavior of E versus Ω has been found in other biological systems, such as human bronchial epithelial cell monolayers²³ and bacterial suspensions²⁸, revealing a potentially conserved energetic property in these active matter systems.

We noted that previous theoretical studies have predicted some universal scaling laws for active nematic turbulence^{36,37}. For example, there exists power scaling laws of the kinetic energy spectrum and the enstrophy spectrum. We thus seek to examine whether these energetic statistics hold for diverse cell monolayer systems. To gain further insights into the energetic landscape of mesoscale cell turbulence, we examine the energy spectrum $E(k)$ of collective cell flows. $E(k)$ is related to the velocity field by $\langle |\mathbf{v}(\mathbf{r}, t)|^2 \rangle_{\mathbf{r}, t} = 2 \int_0^{+\infty} E(k) dk$, and thus reflects the accumulation of kinetic energy over different spatial scales. In 2D space, $E(k)$

Fig. 2 Energetic statistics of mesoscale cell turbulence in Madin Darby canine kidney cell monolayers. **a, b** Representative fields of **a** the kinetic energy and **b** the enstrophy at $t=1$ h with the approximate cell density $1.5 \times 10^5 \text{ cm}^{-2}$. Scale bars, 300 μm . **c, d** Probability density functions (PDFs) of **c** the rescaled kinetic energy and **d** the rescaled enstrophy. Data shown here are from one typical field of view (FOV); different symbols correspond to different time points. The green dashed curves represent the Maxwell-Boltzmann distribution, while the blue solid curves refer to the fittings to q -Gaussian distributions (Eq. (2) for **c** and Eq. (4) for **d**) with the Tsallis index $q = 1.2$. The cell densities corresponding to time 0, 3, 6, 9, and 12 h are manually counted and estimated approximately as $1.4 \times 10^5 \text{ cm}^{-2}$, $1.6 \times 10^5 \text{ cm}^{-2}$, $1.9 \times 10^5 \text{ cm}^{-2}$, $2.1 \times 10^5 \text{ cm}^{-2}$, and $2.3 \times 10^5 \text{ cm}^{-2}$, respectively. **e** The enstrophy Ω versus the kinetic energy E over time. Different symbols represent different data set (eight independent FOVs and four independent samples); the dash line refers to the fitting $\Omega = E/\xi_a^2$ with the characteristic length scale $\xi_a \approx 44.1 \mu\text{m}$ and the correlation coefficient $R^2 = 0.952$. **f, g** The energy spectra $E(k)$ (**f**) and the enstrophy spectrum $\Omega(k)$ (**g**) at different time points. The arrows indicate the plateaus. Here, k_c represents the wavenumber at the scale of one cell.

can be calculated by

$$E(k) = \frac{k}{4\pi} \int \langle \mathbf{v}(\mathbf{r}, t) \cdot \mathbf{v}(\mathbf{r} + \mathbf{R}, t) \rangle_{\mathbf{r}, t} \exp(-i\mathbf{k} \cdot \mathbf{R}) d^2\mathbf{R}, \quad (5)$$

where $k = |\mathbf{k}|$ is the wavenumber.

Figure 2f shows that the energy spectra $E(k)$ at different time points exhibit a similar structure in the wavenumber space, suggesting a high robustness of energetic statistics over time. Our experimental data suggest two regimes that exhibit different behaviors. Let l_c denote the average cell size. Then $k_c = 2\pi/l_c$ represents the wavenumber at the scale of one cell and, thus, sets the upper bound for the spectral range of the cell turbulence. At small wavenumbers, $E(k)$ slightly increases with k . Previous theoretical studies predicted a scaling of $E(k) \sim k^{-1}$ at small wavenumbers^{36,37}. However, it seems it's not the case here. More data points (i.e., larger FOV size) are needed to identify the specific scaling law here. At large wavenumbers $k/k_c > 0.1$, in contrast, $E(k)$ displays an overall decay with increasing k . We observe three energy plateaus for wavenumbers (i) $0.06 < k/k_c < 0.15$, (ii) $0.4 < k/k_c < 0.6$, and (iii) $0.8 < k/k_c < 1.0$ (Fig. 2f). The first plateau (i) occurs at length scales ranging from 10 cells to 17 cells, which corresponds to the scale of swirls^{16,18,19}. The second (ii) and the third plateau (iii) stem from the filtering and averaging effects of PIV calculation (see Supplementary Note 2). When the PIV window size is varied, these two plateaus shift in the wavenumber space, but the scaling behavior of the energy spectrum does not vary significantly (Supplementary Fig. 4). Therefore, the two plateaus will be ignored hereafter. The energy spectra obey a power scaling as $E(k) \sim k^{-\beta}$ for large wavenumbers $0.2 < k/k_c < 0.4$ and $0.6 < k/k_c < 0.8$. These two decay scaling regimes possess the same exponent β and are reminiscent of the “inertial subrange” for classical passive

turbulence. The power-law exponent is fitted to be $\beta = 4.55 \pm 0.17$ (Fig. 2f), which, nevertheless, strikingly differs from the classical $k^{-5/3}$ -decay of 2D Kolmogorov–Kraichnan turbulence. This difference may be attributed to the different mechanisms of energy injection and dissipation. Compared to classical turbulence, mesoscale cell turbulence is powered by energy production of individual cells due to intracellular biochemical reactions and coordinated between neighbors by intercellular mechanical linkages, such as cadherin-mediated cell–cell junctions^{38,39}. Similar energy-scaling laws but with different power-law exponents have been found in other active matter systems. For instance, a $k^{-8/3}$ -decay scaling law was found in dense bacterial suspensions⁴⁰, whereas a k^{-4} -decay scaling law was predicted for active nematics^{36,37}. In addition, we have also examined the enstrophy spectra $\Omega(k)$ and observed similar scaling behavior (Fig. 2g). However, our experiments show that the enstrophy spectrum does not follow the law $\Omega(k) \sim k^2 E(k)$, which was predicted by active nematic theory^{36,37}.

Energetics of mesoscale cell turbulence across diverse cell monolayers. Are these energetic characteristics of mesoscale cell turbulence specified to MDCK cell monolayers? To address this question, we examine the energetic landscape in cell monolayers of HUVECs, C2C12 cells, and NIH-3T3 cells. Across these systems, both the enstrophy Ω and the kinetic energy E decrease over time, and the linear relation $\Omega = E/\xi_a^2$ still holds (Fig. 3a). This results in a characteristic length scale ranging from $\xi_{a, \text{HUVEC}} \approx 30.7 \mu\text{m}$ to $\xi_{a, \text{MDCK}} \approx 44.1 \mu\text{m}$, corresponding to a narrow length range of 1.5–2 cells. Besides, both the PDFs of the kinetic energy (Fig. 3b) and the enstrophy (Fig. 3c) in these different cell monolayer systems collapse to a common q -Gaussian

Fig. 3 Energetic statistics of mesoscale cell turbulence in monolayers of different cell types. **a** The enstrophy scales linearly with the kinetic energy over time. Different symbols refer to different cell types: red squares for Madin Darby canine kidney cells (MDCK); blue circles for human umbilical vein endothelial cells (HUVEC); purple upward-pointing triangles for C2C12 mouse myoblast cells (C2C12); and green downward-pointing triangles for NIH-3T3 mouse embryo fibroblast cells (NIH-3T3). For each cell type, data were collected from eight independent field of views (FOVs) and four independent samples. The dashed lines represent linear fittings to $\Omega = E/\xi_a^2$ with the characteristic length scales $\xi_{a,MDCK} \approx 44.1 \mu\text{m}$ for MDCK and $\xi_{a,HUVEC} \approx 30.7 \mu\text{m}$ for HUVEC. The yellow sector represents the data set area bounded by MDCK and HUVEC. **b, c** Representative probability density functions (PDFs) of **b** the rescaled kinetic energy and **c** the rescaled enstrophy. Values are rescaled by their average. The gray dashed curves represent the Maxwell-Boltzmann distribution, while the black solid curves refer to the fittings to q -Gaussian distributions (Eq. (2) for **b** and Eq. (4) for **c**) with the Tsallis index $q = 1.2$. **d** Representative energy spectra $E(k)$. k_c represents the wavenumber at the scale of one cell. Inset: the scaling exponent β for each cell type (symbols refer to different cell types). Error bars: mean $\pm$ SD (for each cell type, data were collected from eight independent FOVs and four independent samples).

distribution, which deviates remarkably from the classical Maxwell-Boltzmann distribution. Further, Fig. 3d (Supplementary Fig. 5a, respectively) shows the energy spectra (enstrophy spectra, respectively) in these different cell monolayer systems, which demonstrate similar landscapes and scaling behaviors. We linearly fit these curves at large wavenumbers in the log-log plots, and extract the scaling exponent β . For these different cell monolayers, β is bounded in a narrow range from 4.05 to 4.55 (Fig. 3d). These results reveal nearly common statistics of 2D mesoscale cell turbulence.

Insensitivity of the energetics of mesoscale cell turbulence to the substrate stiffness. Since the stiffness of the underlying substrate plays a significant role in regulating cell-matrix adhesion^{41–44} and cell migration^{16,45,46}, we next explore how it affects the energetic statistics of 2D mesoscale cell turbulence. We then culture MDCK cells on various substrates of different Young's moduli, including polyacrylamide (PA) gel (~ 10 kPa), polydimethylsiloxane (PDMS; ~ 2 MPa), plastic (~ 1 GPa), and glass (~ 70 GPa). Mesoscale cell turbulence can be observed in all MDCK cell monolayers adhering to these substrates. Interestingly, the energetic statistics of cell turbulence, including the

Fig. 4 Energetic statistics of mesoscale cell turbulence in Madin Darby canine kidney cell monolayers adhering to various substrates of different stiffness. **a** The enstrophy scales linearly with the kinetic energy over time. Different symbols refer to different substrates, including polyacrylamide (PA) gels substrate (red squares), polydimethylsiloxane (PDMS) substrate (blue circles), plastic substrate (upward-pointing triangles), and glass substrate (downward-pointing triangles). The numbers refer to the Young's moduli of these substrates. Data were collected from some independent field of views (FOVs): $n = 10$ for PA gel substrate, $n = 9$ for PDMS substrate, $n = 8$ for plastic substrate, and $n = 8$ for glass substrate. The dashed lines represent linear fittings to $\Omega = E/\xi_a^2$ with the characteristic length scales $\xi_{a,plastic} \approx 44.1 \mu\text{m}$ for plastic substrate and $\xi_{a,PA\text{ gel}} \approx 30.7 \mu\text{m}$ for PA gel substrate. The yellow sector represents the data set area (E, Ω) of $\Omega = E/\xi_a^2$ with $\xi_{a,plastic}$ and $\xi_{a,PA\text{ gel}}$. **b, c** Representative probability density functions (PDFs) of **b** the rescaled kinetic energy and **c** the rescaled enstrophy. Values are rescaled by their average. The gray dashed curves represent the Maxwell-Boltzmann distribution, while the black solid curves refer to the fittings to q -Gaussian distributions (Eq. (2) for **b** and Eq. (4) for **c**) with the Tsallis index $q = 1.2$. **d** Representative energy spectra $E(k)$. k_c represents the wavenumber at the scale of one cell. Inset: the scaling exponent β as a function of the Young's modulus of substrates (symbols refer to different substrates). Error bars: mean $\pm$ SD. Data were collected from n independent FOVs and m independent samples: $n = 10$ and $m = 4$ for PA gel substrate, $n = 9$ and $m = 4$ for PDMS substrate, $n = 8$ and $m = 4$ for plastic substrate, and $n = 8$ and $m = 4$ for glass substrate.

scaling behavior between kinetic energy and enstrophy, energy distribution, and energy spectrum, are all insensitive to substrate stiffness (Fig. 4). Although the substrate stiffness varies over six orders, the characteristic length scale ξ_a just ranges from $38.5 \mu\text{m}$ to $44.1 \mu\text{m}$ (Fig. 4a and Supplementary Fig. 6), which remains ~ 2 cell length. The PDFs of rescaled kinetic energy (Fig. 4b), as well as rescaled enstrophy (Fig. 4c), collapse to a common q -Gaussian distribution. Besides, the scaling exponent β at large wavenumbers also remains within a narrow range of 4.43–4.55 (Fig. 4d and Supplementary Fig. 5b). These findings reveal that the statistics of cell turbulence is almost independent of substrate stiffness.

Numerical simulations. We use an active vertex model⁴⁷ to further probe the energetics of 2D mesoscale cell turbulence. In this model, a cell monolayer is characterized by a polygonal network, and cells are described by interconnected polygons

(Supplementary Fig. 7a). To account for the active motility of cells, each cell is assigned with a self-propelled force^{48,49}. The direction of the self-propelled force, i.e., cell polarity, can be affected by intercellular social interactions and random fluctuations. We consider two typical intercellular social interactions, including local alignment (LA) and contact inhibition of locomotion (CIL). Both LA and CIL are crucial in many morphogenetic processes, such as wound healing and collective chemotaxis^{13,14,50}. LA describes the motion alignment effect among neighboring cells^{51,52}, similar to the Vicsek interaction^{53,54}. In contrast, CIL describes the repulsive interaction among neighboring cells upon contact, as present in many cell types^{13,14,50}, resembling the repulsion effect that occurs in crowded animal groups^{55,56}.

The collective cell dynamics is determined by the evolution of vertex positions, $\mathbf{r}_i(t)$, dictated by

$$\frac{d\mathbf{r}_i}{dt} = \frac{1}{\gamma} \mathbf{f}_i^U + \sum_{J \in C_i} \frac{v_{sp} \mathbf{p}_J}{n_J} + \sum_{J \in C_i} \frac{\varepsilon_T \beta_J^T(t)}{n_J}, \quad (6)$$

where $\mathbf{f}_i^U = -\partial U / \partial \mathbf{r}_i$ stands for the potential force acting on the vertex i , with U being the potential energy of the system; γ is the friction coefficient; v_{sp} denotes the self-propelled velocity, and $\mathbf{p}_J = (\cos \theta_J, \sin \theta_J)$ represents the polarity vector with θ_J being its direction; ε_T is the intensity of translational noises and $\beta_J^T(t)$ are independent unit-variance Gaussian white noise vectors; n_J refers to the number of cells that contact cell J , and $\sum_{J \in C_i}$ computes a summation over all neighboring cells C_i of vertex i . The potential energy U of the system stems from cell area elasticity, cell contractility, and intercellular tension, and can be expressed as^{47,57–60}

$$U = \sum_j \frac{1}{2} K_a (A_j - A_0)^2 + \sum_j \frac{1}{2} K_c L_j^2 + \sum_{(ij)} \Lambda l_{ij}, \quad (7)$$

where K_a represents cell area stiffness; K_c denotes cell contractile modulus; and Λ quantifies the interfacial tension between neighboring cells; A_0 refers to the preferred area and A_j is the current area of the j th cell; L_j is the perimeter of the j th cell; and l_{ij} is the edge length of the cell–cell interface. Considering the LA and the CIL effects among cells, the polarity direction θ_j evolves as⁴⁷

$$\frac{d\theta_j}{dt} = \frac{\mu_{LA}}{n_j} \sum_{K \in C_j} \sin[\theta_K^{(vel)} - \theta_j] + \frac{\mu_{CIL}}{n_j} \sum_{K \in C_j} \sin(\alpha_{j,K} - \theta_j) + \varepsilon_R \eta_j^R(t), \quad (8)$$

where μ_{LA} and μ_{CIL} quantify the intensities of LA and CIL, respectively. $\theta_K^{(vel)}$ is the motion direction of cell K , and $\alpha_{j,K} = \arg(\mathbf{r}_j - \mathbf{r}_K)$ denotes the argument of the cell–cell relative direction pointing from cell K to cell J . C_j represents the neighbor collection of cell J . ε_R denotes the intensity of rotational noise, and $\eta_j^R(t)$ are independent unit-variance Gaussian white noises. In addition, T1 topological transition is involved to account for cell neighbor exchange (Supplementary Fig. 7b), while cell division and apoptosis are omitted for simplicity. We rescale all variables by the length scale $\ell = \sqrt{A_0}$, the time scale $\tau = \gamma / (K_a A_0)$, and the force scale $\sigma = K_a \ell^3 = K_a A_0^{3/2}$ and, then, simulate Eqs. (6) and (8) using periodic boundary conditions and parameters estimated from reported experiments (see Supplementary Note 3).

Our simulations show that the theoretical model can capture the primary energetic characteristics of 2D mesoscale cell turbulence. First, the dynamic swirling pattern can be reproduced, as reflected by cells' trajectories (Fig. 5a), as well as the velocity

Fig. 5 Energetic statistics of mesoscale cell turbulence obtained from simulations. **a** Representative cell trajectories. The color code represents time. Scale bar, 10 cell length. **b** Representative velocity field. Scale bar, 10 cell length. Parameter values: the self-propelled velocity $v_{sp} = 0.1$, the local alignment (LA) intensity $\mu_{LA} = 0.05$, the contact inhibition of locomotion (CIL) intensity $\mu_{CIL} = 1.0$, the translational noise intensity $\varepsilon_T = 0.01$, and the rotational noise intensity $\varepsilon_R = 0.1$. **c** The enstrophy Ω scales linearly with the kinetic energy E when the cell motility v_{sp} decreases gradually over time. The dashed lines are linear fittings to as $\Omega = E/\xi_a^2$. The characteristic length scale ξ_a showed in the plot is rescaled by the cell size ℓ_c . Parameter values: $\varepsilon_T = 0.01$ and $\varepsilon_R = 0.1$. **d, e** Probability density functions (PDFs) of **d** the rescaled kinetic energy and **e** the rescaled enstrophy regulated by cell–cell social interactions. Values are rescaled by their average. The gray dashed curves represent the Maxwell-Boltzmann distribution, while the black solid curves refer to the fittings to q -Gaussian distributions (Eq. (2) for **d** and Eq. (4) for **e**) with the Tsallis index $q = 1.1$. **f** Energy spectrum $E(k)$ regulated by cell–cell social interactions. Here, we keep μ_{CIL} fixed, while only μ_{LA} is changing. k_c represents the wavenumber at the scale of one cell. The peak corresponds to typical vortex size. Parameter values: $v_{sp} = 0.1$, $\mu_{CIL} = 1$, $\varepsilon_T = 0.01$, and $\varepsilon_R = 0.1$.

field (Fig. 5b). Second, to mimic the jamming transition of cellular motions over time, we decrease the motility of cells (i.e., v_{sp}) gradually. Consequently, it predicts a linear relation between the kinetic energy E and the enstrophy Ω (Fig. 5c). Furthermore, our model suggests that the characteristic length scale ξ_a can be regulated by cell–cell social interactions (Fig. 5c). For example, enhancing LA effect tends to enlarge the characteristic length scale ξ_a (Fig. 5c). However, the regulation of cell–cell social interactions leads to a narrow-range variation (~ 2 cell length) of ξ_a , in consistency with our experimental measurements for different cell monolayer systems (Fig. 3a). Our simulations also show that the PDFs of kinetic energy and enstrophy follow the

q -Gaussian distribution rather than the Maxwell–Boltzmann distribution, especially when the CIL effect is strong; enhancing the LA effect could break the q -Gaussian statistics (Fig. 5d, e). As well, the active vertex model reproduces the power decaying law of the energy spectrum at large wavenumbers, with the scaling exponent close to that measured in experiments (Fig. 5f). These results suggest that cell–cell social interactions could play key roles in the emerging energetic statistics of collective cell flows. Remarkably, in the limiting case when the LA effect is too strong, cells will self-organize into a solid-like flocking mode rather than a turbulent motion mode, leading to the energetic landscape strikingly different from our experimental measurements (Supplementary Fig. 8). Therefore, our simulation results point to that the energetic statistics of 2D mesoscale cell turbulence are primarily attributed to active cell motility and cell–cell social interactions, but its experimental validation and underpinning molecular mechanisms await further work.

Discussion

Mesoscale cell turbulence has attracted mounting attention due to its essential significance for many dynamical processes, such as multicellular organization²³, nutrient mixing²⁴, and thrombopoiesis²⁵, but its energetic statistics remain incompletely understood. Using living cell imaging on confluent cell monolayer systems consisting of different cell types and various substrates, we probe the energetics of mesoscale cell turbulence emerging in 2D cell sheets. We find that such active turbulence possesses unique energetic features markedly different from classical turbulence in passive fluids. The PDFs of the kinetic energy and enstrophy of cell flows over time are found to obey the q -Gaussian distributions. Complementary to a recent finding that the distribution of cell geometry during epithelial jamming follows a k -gamma distribution⁶¹, our finding sheds light on cellular self-organization from a perspective of energetics.

Recent study on human bronchial epithelial cell monolayers showed that the enstrophy of collective cell motions is linearly related to the kinetic energy during monolayers' jamming transition²³. Our results suggest that this linear relation is virtually common to vastly different cell lines, both epithelial and non-epithelial. Intriguingly, this linear constraint is found to conserve in coherent cells crawling on substrates with a wide range of stiffness, further emphasizing the generality of collective cell dynamics. It has been known that non-epithelial cells (C2C12 and NIH-3T3 cells) usually display relatively weak intercellular adhesions and substrate stiffness regulates cell dynamics through cell–substrate adhesion primarily^{41,42,62,63}. Therefore, adhesive interactions between cells and between cells and environments may play a trivial role in the emerging common energetics across these epithelial and non-epithelial monolayers. However, our previous study showed that in the existence of free space, the probability distribution of cell velocities can be skewed: at regions close to the free space, cell velocities do not follow the q -Gaussian distribution; whereas away from the free space, the probability distribution of cell velocities recovers to the q -Gaussian³⁰. This is the same case for energetic statistics, suggesting that the unique energetic statistical features of collective cell flows arise from the cell–cell interactions. Nevertheless, what's the specific cell–cell interaction that dictates the unique energetic statistical features of collective cell flows needs to be further explored.

In addition, in all cell lines, the kinetic energy decreases over time as maturation takes place. This is indicative that all cell lines become increasingly more jammed as collective cellular dynamics come to a halt. However, the jamming processes for different cell lines appear to take different dynamical “paths” since the kinetic energy–enstrophy relationship is characterized by a different

parameter ξ_a for different cell lines (Fig. 3a). This could be attributed to the difference of cell–cell social interactions, according to our simulations (Fig. 5c). This suggests that cell jamming^{18,29,61,64} cannot be merely characterized by a dynamical slow down, but rather requires at least one additional dynamical parameter to characterize the degree of cooperativity. These results are in agreement with recent work showing that human airway epithelial cells can also undergo solid-to-fluid transitions via different dynamical and morphological paths⁶⁵.

Our theoretical model and numerical simulations reveal that polarization-based cell–cell social interactions, including CIL and LA effects could contribute crucially to the energetic statistics of collective cell flows. However, the molecular mechanisms of LA and CIL have not been fully understood so far^{13,52}. To grasp a whole picture of mesoscale cell turbulence, a multiscale model that involves molecular events and cellular behaviors simultaneously is highly deserved. Besides, our study never rules out the possibility that other intercellular interactions, e.g., contact following of locomotion¹⁵ and contact enhancement of locomotion⁶⁶, and even individual cellular properties, such as cell memory^{49,67,68}, may regulate the energetic hallmarks of collective cell motions, which merits future investigation.

Taken together, this work could deepen our understanding of multicellular dynamics in diverse physiological and pathological processes, such as tissue morphogenesis, embryo development, and cancer metastasis from the viewpoint of energetics. The combined experimental, theoretical, and numerical results presented here may provide both qualitative and quantitative guidance for future efforts toward identifying the basic principles of dynamic evolution in living cell populations.

Methods

Cell culture and time-lapse imaging. MDCK strain II cells are cultured in a culture medium composed of high glucose DMEM 1× medium (Corning) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% antibiotics solution (100 $\mu\text{g ml}^{-1}$ penicillin + 100 $\mu\text{g ml}^{-1}$ streptomycin; Gibco). HUVECs are cultured in DMEM/F12 (Corning) supplemented with 10% FBS (Gibco) and 1% antibiotics solution (100 $\mu\text{g ml}^{-1}$ penicillin + 100 $\mu\text{g ml}^{-1}$ streptomycin; Gibco). C2C12 myoblasts cells and NIH-3T3 fibroblasts are cultured in Dulbecco's modified Eagle's medium (high glucose + GlutaMAX; Gibco) supplemented with 10% FBS (Gibco) and 1% antibiotics solution (100 $\mu\text{g ml}^{-1}$ penicillin + 100 $\mu\text{g ml}^{-1}$ streptomycin; Gibco). All cells are maintained at 37 °C, 5% CO₂, and 90% humidity.

We culture cells on diverse substrates of different stiffness to explore the role of substrate stiffness in the energetic statistics of 2D mesoscale cell turbulence. Substrates including PA gels, PDMS, plastics, and glasses are used. In details, PA gel substrates are prepared according to the standard protocols reported previously^{69,70}. We manufacture PA gels of stiffness ~10 and 40 kPa, using the relative acrylamide (Sigma, 40% w/v) and bis-acrylamide (Sigma, 2% w/v) concentrations, as suggested in previous protocols^{69,70}. The PA gel substrates are functionalized with collagen I (500 $\mu\text{g ml}^{-1}$) before seeding cells for cell attachment. PDMS substrates (Sylgard 184; Dow Corning) are prepared by mixing the base and curing agent in a ratio of 1:10 (wt/wt), which results in a stiffness of ~2 MPa (ref. 71), followed by manually stirring and degassing in a vacuum oven to remove air bubbles. A thin layer of PDMS is poured spin coated over a 35 mm Petri dish, degassed again, and cured at 80 °C for 2 h. The Petri dish coated with a PDMS layer is exposed to UV for 30 min and then functionalized with fibronectins (Corning, 50 $\mu\text{g ml}^{-1}$) before seeding cells for cell attachment. For the plastic substrate, cells are cultured on a 35 mm Petri dish (Corning) of stiffness ~1 GPa. For the glass substrate, cells are cultured on a 35 mm confocal Petri dish (Corning) of stiffness ~70 GPa.

Cells are seeded on a Petri dish (Falcon), or PA gel substrates functionalized with collagen I, or PDMS substrates functionalized with fibronectin, or confocal dish with glass bottom (Nest) for enough time (typically 1 day) to grow into a confluent monolayer of cell density $\sim 1.4 \times 10^5 \text{ cm}^{-2}$. Subsequently, phase contrast images are acquired with a $\times 10$ objective, using an Olympus IX83 inverted fluorescence microscope. Two successive images of the same field are taken at a time interval of 1 min. The FOV is of size $1.33 \text{ mm} \times 1.33 \text{ mm}$, much larger than the spatial correlation length (200–300 μm) of mesoscale cell turbulence^{16,18,19}, thus large enough to access their statistical properties.

Particle image velocimetry analysis. In the PIV analysis, a confluent cell monolayer system is treated as a continuum fluid, and instantaneous cell velocities (average displacement within 1 min) at each grid point are given by cross-

correlation calculation. In details, raw phase contrast images are firstly pre-processed by contrast enhancement followed by high-pass Gaussian filtering to conserve the high-frequency component and remove the background noise, with the kernel size of ~ 1 cell (≈ 20 pixels in length). Velocity field is then computed from these preprocessed images using PIV. In the cross-correlation calculation of PIV, the interrogation window size is taken as 64×64 pixels, which roughly corresponded to a region of 3×3 cell length and is sufficiently large to contain ~ 10 cells, but still small enough to resolve spatial flow field structures on the order of ten cell length⁴⁰. To obtain a more continuous velocity field for calculating the energy spectra and the velocity spatial correlation function, we set a small spacing (four pixels) between two consecutive interrogation windows. Outliers of the obtained velocity vectors are abolished and replaced by fitting values based on the neighboring velocity vectors. Further, we also correct the velocity field for small systematic drift effects by subtracting the mean velocity, as suggested by previous studies^{16,18,40}. We have identified that such subtraction to eliminate the drift-related biases does not affect the statistics of velocity field. Custom PIV software is written in MATLAB. We have verified that our PIV code possesses good accuracy (see Supplementary Note 1 and Supplementary Fig. 1).

Calculation of vorticity field. Based on the velocity field associated at regular grid points, the vorticity field, $\omega(\mathbf{x}) = \partial v_y / \partial x - \partial v_x / \partial y$, is numerically calculated using the standard central differentiation scheme. Specifically, the vorticity at the grid point (i, j) is calculated as

$$\omega^{ij} = \frac{1}{2\Delta x} (v_y^{i+1,j} - v_y^{i-1,j}) - \frac{1}{2\Delta y} (v_x^{i,j+1} - v_x^{i,j-1}), \quad (9)$$

where v_x^{ij} (v_y^{ij}) and ω^{ij} are the velocity and vorticity at the grid point (i, j) , respectively; Δx and Δy are the spacings between the neighboring velocity vectors in the x direction and y direction, respectively. For the simulation results, the velocity field associated at irregular grid points is firstly reconstructed to one associated at regular grid points (see Supplementary Note 4 and Supplementary Fig. 9).

Calculation of energy spectrum. The energy spectrum $E(k)$ is calculated based on Eq. (5), by virtue of the Wiener-Khinchine theorem. In details, we firstly calculate the spatial Fourier transform $\hat{\mathbf{v}}(\mathbf{k}, t)$ of velocity field $\mathbf{v}(\mathbf{r}, t)$; then the Fourier transform of the two-point velocity correlation function is calculated as $S(\mathbf{k}) = \langle \hat{\mathbf{v}}^*(\mathbf{k}, t) \cdot \hat{\mathbf{v}}(\mathbf{k}, t) \rangle_t$, with $\hat{\mathbf{v}}^*(\mathbf{k}, t)$ being the complex conjugate of $\hat{\mathbf{v}}(\mathbf{k}, t)$; finally, we calculate the energy spectrum as $E(k) = kS(k)/(4\pi L^2)$ with L^2 the area of FOV. For the simulation results, the velocity field associated at irregular grid points is firstly reconstructed to one associated at regular grid points (see Supplementary Note 4 and Supplementary Fig. 9).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All experimental data and any related experimental background information not mentioned in the text are available from the authors on reasonable request.

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Author contributions

B.L. and X.-Q.F. conceived the project and designed the research. S.-Z.L. performed experiments and numerical simulations. S.-Z.L., W.-Y.Z., D.B., and B.L. analyzed the data. S.-Z.L., D.B., B.L., and X.-Q.F. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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