Characterization of anomalous movements of spherical living cells on a silicon dioxide glassy substrate

Myeonggu Son,^{1,a)} Gyudo Lee,^{1,a),b)} Jongsang Son,¹ Seungyeop Choi,¹
 Youngho Kim,¹ Sei-Young Lee,¹ Young-Ro Yoon,¹ Dae Sung Yoon,² and
 Sang Woo Lee^{1,b)}

¹Department of Biomedical Engineering, Yonsei University, Wonju 220-710, South Korea

²School of Biomedical Engineering, Korea University, Seoul 136-703, South Korea

(Received 28 November 2014; accepted 26 December 2014; published online 7 January 2015)

The random walk of spherical living cells on a silicon dioxide glassy substrate was studied experimentally and numerically. This random walk trajectory exhibited erratic dancing, which seemingly obeyed anomalous diffusion (i.e., Lévy-like walk) rather than normal diffusion. Moreover, the angular distribution ($-\pi$ to π) of the cells' trajectory followed a "U-shaped pattern" in comparison to the uniform distribution seen in the movements of negatively charged polystyrene microspheres. These effects could be attributable to the homeostasis-driven structural resilient character of cells and physical interactions derived from temporarily retained nonspecific binding due to weak forces between the cells and substrates. Our results provide new insights into the stochastic behavior of mesoscopic biological particles with respect to structural properties and physical interactions. © 2015 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License.

[<http://dx.doi.org/10.1063/1.4905577>]

I. INTRODUCTION

The stochastic process described by normal diffusion (also known as "Brownian motion") has been studied both experimentally and numerically in a wide range of fields, from quantum physics to ecology, since the concept was first established by Einstein in 1905.^{1,2} Such normal diffusion has been regarded as the baseline for examining the behavior of mesoscopic particles suspended in fluid. However, in recent years, there have been reports of anomalous random walks (including non-Gaussian but still Brownian diffusion) that cannot be explained by normal Brownian diffusion alone.³⁻¹² The motion of micro-ellipsoids in a fluid,³ protein transport in cells,^{5-7,9,12} and the diffusion of colloidal nanospheres on lipid tubes and in entangled fibers^{3,13} are examples of anomalous diffusion. The anomalous movement has been attributed mainly to mesoscopic parameters, such as a particle's structural properties,^{3,11} its context in a crowded material,^{3,8,9,11-13} or the structural anisotropy of a tubular system.¹⁰ Intriguingly, the movements of living organisms (including individual cell migration and bacterial movement) have also been described as random walks¹⁴ and often do not align with the description of normal diffusion.¹⁵⁻²⁰ For example, the movement of T cells is obeyed by Lévy flight, which may be because they have to capture and attack external germs, using a hunting strategy similar to that of marine predators such as sharks.²¹ In addition, migration of invasive cancer cells could be described by super-diffusion rather than normal diffusion because they tend to penetrate between tissues and spread efficiently.¹⁷ These implies that describing the mechanisms of such anomalous random walks is very important to understand

^{a)}M. Son and G. Lee contributed equally to this work.

^{b)}Authors to whom correspondence should be addressed. Electronic addresses: leegyudo@yonsei.ac.kr and yusuklee@yonsei.ac.kr

the behavior of living cells. Furthermore, recent studies have shown that more complex random walks of living cells (e.g., the cell-cell interactions) were associated to such anomalous random movements.^{22,23}

To investigate the underlying mechanisms of the random walks of living cells, we have characterized the movement of spherical living cells (B16F10), which are a mouse melanoma cell model used extensively in both cell migration and invasion studies, on a glassy substrate as a substrate in a simple microfluidic device. This is the first proof-of-concept study for stochastic processes of electrically charged microspheres, including cells, nearby a solid-state substrate. In this study, the mean square displacements (MSD) were extracted from observations of the two-dimensional (2D) random walk trajectories under the same conditions, utilizing two negatively charged microspheres such that the B16F10 living cells or the carboxylated polystyrene microspheres (cPSMS) on a silicon dioxide (SiO_2) surface were negatively charged in water. From the MSD, we observed random walks of B16F10 cells that were distinguished from the classical normal diffusion of the cPSMS. Specifically, the negatively charged cPSMS diffused normally, while the living cells showed a super-diffusive behavior. Moreover, we analyzed the distribution of relative angles (θ) of motion between successive time intervals of the random walks for both microsphere and cells, determining that cells moved in an obvious zigzag pattern in contrast to cPSMS. Such characteristics were diminished in dead cells, but still remained. Collectively, our results indicate that the anomalous movement of B16F10 cells may be due to a rocking motion derived from both the homeostasis-driven structural resilient character of the cells and temporarily retained nonspecific binding due to weak forces between the cells and substrates.

II. MATERIALS AND METHODS

A. Preparation of cells and negatively charged artificial particles

B16F10 cells (ATCC, USA) used to investigate random walks were detached by treatment with trypsin EDTA (20% (w/w)) for 3 min, resulting in spherical shapes in suspension ($\sim 16 \mu\text{m}$ in diameter). The remaining trypsin EDTA was immediately eliminated by rinsing thoroughly with buffer (8.6% (w/w) sucrose, 0.3% (w/w) glucose, and 1.0 mg/ml bovine serum albumin, pH 5.2).²⁴ The cells were resuspended in buffer for the experiment. For further verification of the stochastic processes of cells, we used dead cell, as confirmed by examination of cells stained with trypan blue on a hemocytometer. We did not stain cells used in the random walk experiments in order to avoid the effect of dye molecules on the stochastic processes. The cPSMS ($15 \mu\text{m}$, Kisker-Biotech, Germany) used for random walk studies were diluted in the same buffer ($\sim 250 \mu\text{g/ml}$) as the cells.

B. Experimental procedures

First, to fabricate a microfluidic device with a negatively charged substrate, a silicon dioxide glassy layer ($\sim 8 \text{ k}\text{\AA}$) was grown on a silicon wafer (100) by the plasma chemical vapor deposition method. The substrate was cleaned by piranha solution and solvent cleaning. A reservoir made of polydimethylsiloxane ($\sim 200 \mu\text{m}$ in thickness) was then placed over the glassy substrate. After introducing a solution containing either microspheres or living cells to the reservoir, a glass slide was used to cover the top of the reservoir to avoid evaporation of the solution. Subsequently, the movement of both particles was recorded using a charge coupled device (CCD) camera at 30 frames/s. More than 100 trajectories of each B16F10 cell and cPSMS were collected to obtain statistically reliable data. The particle tracking was carried out in image analysis software (ImageJ 1.47v, NIH, USA), which allows sub-micron particle tracking.^{25,26} For MSD analysis, we used x -displacements of the trajectories, but found that y -displacements-based calculations yielded the same results (data not shown).

III. RESULTS AND DISCUSSION

A. Tracking of electrically charged microspheres

Two-dimensional random movements of electrically charged microspheres (B16F10 cells and cPSMS) were observed in a microfluidic device (Fig. 1(a)). Cells were $16.2 \pm 1.7 \mu\text{m}$ in diameter, as shown in Figs. 1(b) and S1 (supplementary material, Ref. 27). The behavior of both microspheres appeared similar to classical random walks as they moved dozens of micrometers within a few square micrometers ($\sim 3 \times 3 \mu\text{m}^2$) in an observation time of up to $\sim 33 \text{ s}$ (Figs. 1(b) and 1(c) and Figs. S2 and S3 of the supplementary material²⁷). The largest difference between cells and cPSMS was in whether the pattern of movement overlapped or not: living cells tended to return many times to the same place. The dominant weak force affecting the random walks was electrostatic repulsion between the negatively charged microspheres (including cells) and the glassy surface (Fig. S4).²⁷ In contrast, to validate the effect of electrostatic attraction between the microspheres and the glassy surface, we confirmed that PSMS functionalized with amino groups (positively charged in the given solution) did not exhibit any movement on the substrate because of strong electrostatic attractive forces between the positively charged amino-PSMS and the negatively charged glassy substrate (Fig. S5).²⁷

To verify whether the random movement at the given time ($\sim 33 \text{ s}$) was fluctuation on the spot or not, we performed long-time observation of spherical B16F10 cells for 90 min (Fig. S6).²⁷ Most of cells showed large scale of displacements ($\sim 35 \mu\text{m}$) at the given time, which implies not only that the cellular movement by thermal agitation was not just fluctuation on the spot but diffusion but also that the conventional time scale (i.e., $\sim 33 \text{ s}$) was enough to observe and analyze such cellular movements.

B. Statistical analysis of random walks

To understand basic information about trajectories, we calculated the average track velocity (v) (Fig. 2(a)) from the trajectories of the living B16F10 cells and cPSMS as shown in Figs. 1(b) and 1(c). Specifically, v was calculated from the displacement $r(\tau) = (\Delta x(\tau)^2 + \Delta y(\tau)^2)^{-1/2}$

FIG. 1. (a) Schematic illustration of the stochastic process of a microsphere with electrostatic interaction between the spheres and a glassy substrate (SiO_2). (b) and (c) 1000 typical steps of the two-dimensional random walk of a living cell (d) and cPSMS (e) on the substrate. Inset: top-view optical images of a living B16F10 cell pseudocolored with green (b), and a cPSMS pseudocolored with red (c) on the substrate. Scale bar is $15 \mu\text{m}$.

FIG. 2. (a) The average track velocity (v) calculated from the displacement of each step divided by the time interval (i.e., 0.033 s) was $v_{\text{B16F10}} \approx 4.27 \pm 0.25 \mu\text{m s}^{-1}$ and $v_{\text{cPSMS}} \approx 1.36 \pm 0.07 \mu\text{m s}^{-1}$, respectively. (b) MSD of a living cell (green rhombus) and cPSMS (red circle). (c) From the analysis of 100 trajectories, the PDF of the displacement of the living cells (green) and cPSMS (red) on a glassy substrate are plotted with the best-fit curves by a Gaussian fit (dashed line), respectively.

of each step divided by the time interval (i.e., $v \sim \partial r / \partial \tau$). We note that v was dependent on τ (here, 33 ms), which is attributed to the fact that displacements (r) at the given time interval (τ) were not too large (Fig. S7).²⁷ Interestingly, living cells were approximately 3 times faster than cPSMS (i.e., $v_{\text{B16F10}}/v_{\text{cPSMS}} \sim 3$). Moreover, the histogram of v_{B16F10} was broader than that of cPSMS. Both faster movement and the larger standard deviation of v_{B16F10} imply more complicated interactions between the cells and the substrate compared to cPSMS. For further characterization of the stochastic process, the one-dimensional MSD of the cPSMS and living cells was calculated based on the measured data (Fig. 2(b)). We found that the calculated MSD of cPSMS followed a normal diffusion model, while the random motion of living cells was governed by super-diffusive behavior instead. Specifically, the MSD of cPSMS was well fitted by Einstein's relation $\langle \Delta x^2(t) \rangle = 2Dt$, where D is the diffusion coefficient, implying that $D \approx 0.024 \mu\text{m}^2 \text{s}^{-1}$ for cPSMS. This result is consistent with the value of D ($\approx 0.028 \mu\text{m}^2 \text{s}^{-1}$)

obtained from the Stokes-Einstein relation,¹ $D = k_B T / 6\pi\mu r$, where k_B is the Boltzmann constant, T is the temperature, μ is the viscosity coefficient (assumed to water), and r is the radius of the microsphere. In the case of the living cells, however, the MSD was fitted by the Lévy walk model, which is described as $\langle \Delta x^2(t) \rangle \sim t^\gamma$, where γ is a parameter to decide the characteristics of a random walk.²⁸ The MSD of B16F10 living cells was proportional to t^γ , $\gamma \approx 1.5$ (i.e., super-diffusion). We also examined the probability density function (PDF) for x -displacement of cPSMS and living cells (Fig. 2(c)). The PDF for both microspheres (i.e., B16F10 and cPSMS) could be fitted to a Gaussian function that describes microscopic fluctuations $f(x, t) \sim \exp(-x^2/4Dt)$, although the PDF for living cells had a heavy tail compared to cPSMS. This difference implies that the random walk of B16F10 living cells is also a super-diffusion process, which is consistent with the MSD tendency (Fig. 2(b)). Therefore, according to MSD and PDF analysis, the random walk of cPSMS follows normal diffusion, while B16F10 living cells follow a super-diffusion process. However, we observed no long steps corresponding to a Lévy walk in the random walk trajectories of cells (Fig. 1(b)). This contradictory result suggests that a conventional analytic approach (e.g., v , MSD, or PDF) is insufficient to fully comprehend the anomalous movements of living cells.

To further characterize the random walks of B16F10 living cells, we analyzed the distribution of relative angles (θ) of motion between successive time intervals of random walk trajectories (Fig. 3(a)). This parameter has recently been used to further understand anomalous random walks of proteins or biological fibrils.⁶ The directions of both microspheres moving on the substrate can be altered from $-\pi$ to π by their own movement. As expected, the distribution of θ from the trajectories of cPSMS was uniform from $-\pi$ to π , whereas living cells followed a U-shaped pattern (Fig. 3(b)). Obviously, there was not a chemical gradient because of no chemicals (e.g., cytokines) in our experiments. The anisotropy of angle distribution is attributed to the presence of physical field (i.e., weak binding force or cell rheological features), which will be discussed below. The symmetric regime is a statistical trend that implicates trajectories of a hundred of cells (Fig. S8).²⁷ Specifically, a trajectory of single cell is not symmetry but anisotropy. The symmetry appeared as a growing number of cell trajectories are considered.

To estimate the degree of the U-shaped distribution, we extracted the quadratic polynomial coefficient (α) with a polynomial fitting curve given by $P(\theta) \sim \alpha\theta^2$ ($\alpha_{\text{B16F10}} \approx 1.5 \times 10^{-6}$) (Fig. S9).²⁷ This propensity means that, unlike cPSMS, most living cells had drastic changes in their route within our time interval of observation.⁶ In particular, they may move in a zigzag pattern like a roly-poly chime ball in a balancing act (i.e., a rocking motion). This motion is

FIG. 3. (a) Schematic diagram showing how to extract angle values (θ) from the random walk trajectories of microspheres in the elapsed time (t). (b) The angular distribution of the random walk trajectory of the live B16F10 cells (upper) and cPSMS (lower).

thought to be closely related to the anomalous movement of B16F10 living cells described above. If cells move randomly in response to thermal agitation and also display a rocking motion, the total displacements of their movement would be increased, leading to a different diffusion regime. The described rocking motion could result from structural differences between the cells and cPSMS. The cPSMS is a solid sphere made of homogeneous matter, while cells have soft and resilient structural properties of cytoskeletons with a few kPa.²⁹ Even if B16F10 cells appear to be spherical when detached from the substrate, they could be minutely deformed like water-jelly because of external and/or internal energies needed to maintain homeostasis of the cell.³⁰ In addition, complex ion exchanges involving membrane constituents such as membrane proteins may lead to heterogeneity of the surface charge of the living cell.^{31,32} This heterogeneity could induce a nonspecific weak binding force (e.g., van der Waals force)³³ between the cell membrane and glass substrate, resulting in the different movement characteristics.

C. Observation and analysis of dead cell movement

To better understand why cells behave differently than cPSMS, we created dead cells through starvation (Figs. 4(a) and 4(b)) and characterized their movements. Notably, most dead cells created by starvation maintained their shape (i.e., sphere) (Fig. 4(a)). The parameter values

FIG. 4. (a) Optical image of cells showing dead cells stained by trypan blue. (b) Cell viability test with a hemocytometer after feeding of cells was stopped for 17 h. (c)–(f) Random walk trajectory (c), average velocity (d), MSD (e), and angular distribution (f) of dead cells.

characterizing the random walk of dead cells shifted from the living cell to cPSMS ($v_{\text{dead cell}} \approx 1.95 \pm 0.13 \mu\text{m}\cdot\text{s}^{-1}$ and $\gamma_{\text{dead cell}} \approx 1.3$) (Figs. 4(c)–4(e)). In general, dead cells have less mass,³⁴ which should give them a higher velocity (v) according to the law of momentum conservation. However, the average velocity of the dead cells was 54% less than that of the living cells, suggesting that at least the mass of the particles was not a critical factor in reducing the stochastic parameters of the dead cells' random walk. Moreover, the decreased mass of cells would probably be associated with a stiffening of their mechanical properties.³⁵ Because of their lower water content, dead cells are expected to be less physically resilient and to act more like the solid matter of cPSMS, which implies that it may be relevant to the glassy-like dynamics of cells (i.e., glass transition of cytoskeleton)^{36–38} or cellular tensegrity model.³⁰ In addition, ion exchange does not occur in dead cells, and so surface charge heterogeneity should be lower than in living cells. These differences forecast less interaction between the dead cell and the substrate. The angular distribution for dead cells, however, also displayed a U-shaped pattern, although there were subtle differences in the angular distribution between living and dead cells. Both peaks at the nearby $-\pi$ and π of the U-shaped pattern were smaller for dead cells, but did not disappear ($\alpha_{\text{dead cell}} \approx 7.6 \times 10^{-7}$) (Fig. S9).²⁷ This finding indicates the presence of relatively less cellular rocking motion, which was also shown in the trajectory of the dead cells (Fig. 4(c)).

Such variation in angular distribution supports our hypothesis that the anomalous movements of cells, including their rocking motion, can be attributed not only to cellular mechanical properties (i.e., resilience) but also to surface charge heterogeneity. We propose a schematic model to describe this phenomenon (Fig. 5). The heterogeneity of surface charges derived from membrane components (i.e., charged molecules) such as proteins, and thereby ion exchange, as

FIG. 5. Schematic of the rocking motion of a cell with electrostatic interactions between the cell and a glassy substrate. The net surface charge of the cell is negative, resulting in an electrostatic repulsive force, but an attractive force can occur locally by the van der Waals-London forces.³³ Moreover, some molecules (e.g., membrane proteins, glycolipids, and cations through ion channels) on cell membranes can move by membrane fluidity (also known as the fluid mosaic model), which may affect the stochastic processes of the cells. Mechanical resilience of cells may also be a factor. The structural properties of cells tend to pull back the membrane to maintain shape when an external force (thermal agitation) causes cell deformation. These movements may make cells mimic a drunkard's walk with a rocking motion like a roly-poly chime ball made of water-jelly.

well as glycoproteins, could be a critical factor in the local, temporal confinement that induces anomalous movement of cells.^{31,32,39} It is fairly safe to say that these heterogeneities affect the nonspecific binding between living cells and the substrate, resulting in anomalous random walks.³¹ Further study is needed to more clearly understand (i) how the correlation between mechanical properties and surface heterogeneity of cells affects anomalous cellular movements, (ii) the relationship between cells as a soft glassy material and different glassy substrates, and (iii) effect of membrane proteins with a treatment of different chemical moieties on the random movements of cells.

IV. CONCLUSION

In this study, we investigated the random walks of spherical living cells. We found that cPSMS follow normal diffusion, although they move on the substrate (i.e., a two-dimensional area but not three-dimensional space of solution), implying that charged microspheres can follow Brownian motion when they experience electrostatic repulsion between them and the substrate. Moreover, spherical living cells exhibited a super-diffusive behavior based on the calculation of MSD. We revealed that this anomalous movement of cells was attributed to a rocking motion of the cells in random walks, which may be due not only to cellular mechanical properties (i.e., resilience) but also to physical interactions by surface charge heterogeneity of the cells. This deduction was verified and supported by our random walk analysis of dead cells. We observed that in cells (i.e., resilient and flexible spherical matter), random movements can be affected by a subtle deformation due to temporal and weak confinement of the cell membrane as well as non-specific binding between the cells and the substrate induced by cell surface charge heterogeneity. Our findings provide insights into the behavior of cells in the absence of external forces, such as chemical gradient, pressure, or electrical stimuli, which would be helpful in the future applications toward fast and robust discrimination between living cells and dead cells without any cumbersome process like cell staining.

ACKNOWLEDGMENTS

This research was supported by grants from the National Research Foundation (NRF) funded by the Ministry of Education, Science, and Technology (Grant Nos. NRF-2010-0013619, NRF-2013R1A2A2A03005767, and NRF-2013R1A1A2053613) and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare (HI12C1837), Republic of Korea.

- ¹A. Einstein, *Ann. Phys.* **322**, 549 (1905).
- ²G. Parisi, *Nature* **433**, 221 (2005).
- ³B. Wang, S. M. Anthony, S. C. Bae, and S. Granick, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 15160 (2009).
- ⁴Y. Han, A. M. Alsayed, M. Nobili, J. Zhang, T. C. Lubensky, and A. G. Yodh, *Science* **314**, 626 (2006).
- ⁵S. M. A. Tabei, S. Burov, H. Y. Kim, A. Kuznetsov, T. Huynh, J. Jureller, L. H. Philipson, A. R. Dinner, and N. F. Scherer, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 4911 (2013).
- ⁶S. Burov, S. M. A. Tabei, T. Huynh, M. P. Murrell, L. H. Philipson, S. A. Rice, M. L. Gardel, N. F. Scherer, and A. R. Dinner, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 19689 (2013).
- ⁷I. Bronstein, Y. Israel, E. Kepten, S. Mai, Y. Shav-Tal, E. Barkai, and Y. Garini, *Phys. Rev. Lett.* **103**, 018102 (2009).
- ⁸B. Wang, J. Kuo, S. C. Bae, and S. Granick, *Nat. Mater.* **11**, 481 (2012).
- ⁹Y. Tseng, T. P. Kole, and D. Wirtz, *Biophys. J.* **83**, 3162 (2002).
- ¹⁰F. Santamaria, S. Wils, E. De Schutter, and G. J. Augustine, *Neuron* **52**, 635 (2006).
- ¹¹A. Caspi, R. Granek, and M. Elbaum, *Phys. Rev. E* **66**, 011916 (2002).
- ¹²D. S. Banks and C. Fradin, *Biophys. J.* **89**, 2960 (2005).
- ¹³S. S. Lee and L. P. Lee, *Nanoscale* **6**, 3561 (2014).
- ¹⁴H. C. Berg, *Random Walks in Biology* (Princeton University Press, 1993).
- ¹⁵T.-W. Su, L. Xue, and A. Ozcan, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 16018 (2012).
- ¹⁶L. Liang, C. C. Edward, and F. Henrik, *Phys. Biol.* **8**, 046006 (2011).
- ¹⁷P.-H. Wu, A. Giri, S. X. Sun, and D. Wirtz, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 3949 (2014).
- ¹⁸X. Liu, B. Ma, A. B. Malik, H. Tang, T. Yang, B. Sun, G. Wang, R. D. Minshall, Y. Li, Y. Zhao, R. D. Ye, and J. Xu, *Nat. Immunol.* **13**, 457 (2012).
- ¹⁹H. E. Jeong, I. Kim, P. Karam, H.-J. Choi, and P. Yang, *Nano Lett.* **13**, 2864 (2013).
- ²⁰A. A. Potdar, J. Jeon, A. M. Weaver, V. Quaranta, and P. T. Cummings, *PLoS ONE* **5**, e9636 (2010).
- ²¹T. H. Harris, E. J. Banigan, D. A. Christian, C. Konradt, E. D. Tait Wojno, K. Norose, E. H. Wilson, B. John, W. Weninger, A. D. Luster, A. J. Liu, and C. A. Hunter, *Nature* **486**, 545 (2012).

- ²²S. Vedel, S. Tay, D. M. Johnston, H. Bruus, and S. R. Quake, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 129 (2013).
- ²³E. Agliari, E. Biselli, A. De Ninno, G. Schiavoni, L. Gabriele, A. Gerardino, F. Mattei, A. Barra, and L. Businaro, *Sci. Rep.* **4**, 6639 (2014).
- ²⁴A. C. Sabuncu, J. A. Liu, S. J. Beebe, and A. Beskok, *Biomicrofluidics* **4**, 021101 (2010).
- ²⁵C. A. Schneider, W. S. Rasband, and K. W. Eliceiri, *Nat. Methods* **9**, 671 (2012).
- ²⁶Y. Feng, J. Goree, and B. Liu, *Rev. Sci. Instrum.* **78**, 053704 (2007).
- ²⁷See supplementary material at <http://dx.doi.org/10.1063/1.4905577> for size distribution and other examples of random walk trajectories of microspheres and further descriptions of Zeta potential measurements and analyzed parameters.
- ²⁸J.-P. Bouchaud and A. Georges, *Phys. Rep.* **195**, 127 (1990).
- ²⁹T. Watanabe, H. Kuramochi, A. Takahashi, K. Imai, N. Katsuta, T. Nakayama, H. Fujiki, and M. Suganuma, *J. Cancer Res. Clin. Oncol.* **138**, 859 (2012).
- ³⁰D. E. Ingber, *J. Cell Sci.* **116**, 1157 (2003).
- ³¹C. McNamee, Y. Aso, S. Yamamoto, Y. Fukumori, H. Ichikawa, and K. Higashitani, *Pharm. Res.* **24**, 2370 (2007).
- ³²F. Ahimou, F. A. Denis, A. Touhami, and Y. F. Dufrêne, *Langmuir* **18**, 9937 (2002).
- ³³A. S. G. Curtis, *J. Cell Biol.* **20**, 199 (1964).
- ³⁴C. L. Lewis, C. C. Craig, and A. G. Senecal, *Appl. Environ. Microbiol.* **80**, 3622 (2014).
- ³⁵W. A. Lam, M. J. Rosenbluth, and D. A. Fletcher, *Blood* **109**, 3505 (2007).
- ³⁶B. Fabry, G. Maksym, J. Butler, M. Glogauer, D. Navajas, and J. Fredberg, *Phys. Rev. Lett.* **87**, 148102 (2001).
- ³⁷X. Treppe, L. Deng, S. S. An, D. Navajas, D. J. Tschumperlin, W. T. Gerthoffer, J. P. Butler, and J. J. Fredberg, *Nature* **447**, 592 (2007).
- ³⁸C. Semmrich, T. Storz, J. Glaser, R. Merkel, A. R. Bausch, and K. Kroy, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 20199 (2007).
- ³⁹M. Yu, S. Stott, M. Toner, S. Maheswaran, and D. A. Haber, *J. Cell Biol.* **192**, 373 (2011).