Strange Dynamics of a Dynamic Cytoskeleton

Trang T. B. Nguyen¹ and Jeffrey J. Fredberg¹

¹Harvard School of Public Health, Boston, Massachusetts

A novel physical perspective of molecular interactions within the cytoskeleton of the airway smooth muscle cell may help to explain why the most efficacious of all known bronchodilatory agencies—a simple deep inspiration—becomes abrogated during the spontaneous asthma attack and leads thereby to excessive airway narrowing. This perspective invites us to think of airway smooth muscle not only biochemically as a nidus of traditional cell signaling and immune modulation or mechanically as a motor for generation of active forces but also physically as a phase of soft condensed matter that can restrict airway stretch and dilation. This is perhaps a risky path and is surely an unconventional one, but it is where the trail of evidence leads. This line of investigation is unlikely by itself to provide an asthma cure but will lead to a new conceptual framework without which novel pathways, unsuspected phase transitions, and unanticipated mechanisms of action of target molecules would almost surely remain hidden. Glassy dynamics of the cytoskeleton are likely to be important in a wide range of biological functions and disease processes, but had it not been for their preeminent role in bronchospasm, they might never have been discovered.

Keywords: glass; stretch; fluidization

Current asthma research emphasizes upstream causal events at the level of immune modulation within the airway microenvironment, but it is the downstream physical consequences of these events—most notably excessive narrowing of the airway—that lead to the morbidity and mortality that are attributable to the disease. Although the cascade of specific causal events remains unclear, the key end-effector of acute airway narrowing is the airway smooth muscle (ASM) cell. To complement advances in understanding at the upstream end of this cascade, here we consider this final pathway that is common to every upstream immune perturbation and its mechanical behavior. It is little appreciated but firmly established, for example, that of all known bronchodilatory agencies or drugs the single most efficacious is a simple sigh or deep inspiration (DI) (1–3), but during the spontaneous asthma attack this potent agency fails (1, 2, 4–9). Although the mechanism remains unexplained, growing evidence suggests that the contracted airway smooth muscle cell becomes refractory to a DI because its cytoskeleton (CSK) fails to fluidize and remains frozen in a stiff, solid-like glassy phase (10–12). Here we use the term CSK in a generalized sense to mean any structures that contribute appreciably and directly to cell mechanical properties and as such include the contractile apparatus and its scaffolding.

The notion of CSK dynamics as falling within the class of glassy systems, as defined below, has generated attention because it is so basic (11–15). If true it would affect, and in some cases rule

out, conventional characterizations of physical interactions between CSK proteins based upon the notions of dilute solution chemistry, Boltzmann statistics, diffusive macromolecular mobility, the generalized Stokes-Einstein relationship, elasticity, viscoelasticity, and even the sliding filament model of muscle contraction and emerging notions of protein interaction maps. If they play out within a glassy phase, molecular motions can become virtually stuck, or trapped, in a glassy phase, and, as a result, each of these theories would need to be rethought within a new framework. If this perspective may bring us one step closer to the truth, then in taking that step we may have raised more questions than we have answered because the dynamics of glassy systems remains one of the greatest unsolved problems in all of science (16).

The hypothesis advanced here is not mutually exclusive with other mechanisms suggested in the literature but rather encompasses those mechanisms and sets them within a new integrative framework. For example, the transition from rapidly cycling cross bridges to slowly cycling latch bridges is not an alternative mechanisms but rather an intrinsic part of the hypothesis, as described by Fredberg and Gunst (17). Similarly, the series-to-parallel transition postulated by Seow and coworkers (18) is perhaps the purely structural counterpart of what is described here in dynamic and functional terms; the glass hypothesis is consistent with plasticity and concerns itself mainly with the rates at which the plastic remodeling can progress. If so, then the hypotheses of plasticity and glassiness would be structural versus dynamic pictures of the same process. Glassy dynamics does not supplant established biochemical mechanisms but rather suggests that the rates at which these biochemical processes progress might be dramatically slowed and that the picture of protein–protein interactions becomes modified as well (12). Finally, unloading and uncoupling of the airway smooth muscle from parenchymal tethering due to remodeling of airway connective tissues have been shown recently to play a minor role in airways hyperresponsiveness (19).

FROZEN OBJECTS: SMALL AIRWAYS, BIG BREATHS, AND ASTHMA

A Novel View of Airway Homeostasis

A critical homeostatic phenomenon is demonstrated by exposing a healthy individual to a bronchospastic drug. Bronchospasm can be induced readily, but with a single sigh or DI bronchospasm is immediately reversed (1, 5–9). Humans sigh about 10 times each hour (20); therefore, as we are going about our daily business and in doing so inhaling a variety of respiratory irritants and allergens, each one of us partakes once every 6 minutes of a bronchodilating agency that is more efficacious than any known asthma drug (1, 3, 5–9). This mechanism is so automatic and so potent that we rarely give it a thought, except during an asthmatic attack. During the spontaneous asthmatic attack—but not during induced obstruction in the laboratory—this most beneficial of all known bronchodilating agencies becomes ablated (5, 7, 9, 21). It was first noted by Salter (4) in 1859 that during the spontaneous attack the patient with asthma loses the ability to dilate the airways with a deep inspiration, almost as if the airways had narrowed and become frozen in the narrowed state.

(Received in original form May 1, 2007; accepted in final form June 8, 2007)

This work was supported by NIH grants R01 HL065960 and R01 HL084224 to J.J.F.

Correspondence and requests for reprints should be addressed to Jeffrey J. Fredberg, M.D., Professor of Bioengineering and Physiology, Program in Molecular and Integrative Physiological Sciences (MIPS), Department of Environmental Health, Harvard School of Public Health, Room 1-313, 665 Huntington Avenue, Boston, MA 02115. E-mail: jfredber@hsph.harvard.edu

Proc Am Thorac Soc Vol 5, pp 58–61, 2008

DOI: 10.1513/pats.200705-055VS

Internet address: www.atsjournals.org

The spasm may be broken through, and the respiration for the time rendered perfectly free and easy, by taking a long, deep, full inspiration. In severe asthmatic breathing this cannot be done; but in the slight bronchial spasm that characterizes hay asthma I have frequently witnessed it. It seems as if the deep inspiration overcame and broke through the contracted state of the air-tubes, which was not immediately re-established.

It was suggested that the failure of this potent homeostatic phenomenon may be the proximal cause of the excess morbidity and mortality that is attributable to the disease (1, 5–9). This mystery attracted an impressive pedigree of luminaries—Salter (4), Nadel and Tierney (1), Fish and colleagues (5), Green and Mead (2), Ding and colleagues (22), Lim and colleagues (7), Skloot and coworkers (9), Moore and colleagues (8), Crimi and coworkers (23), Dowell and colleagues (24), and Chen and investigators (25)—but the mechanism has remained elusive.

ASM as a Motor Driving Airway Constriction

In the asthma attack, the key end-effector of acute airway narrowing is the ASM cell. For that reason, the ASM cell continues to be studied in the context of being the motor that drives acute airway narrowing, being the end-target of oxidative stressors or cytokines and mediators that are sensed by a variable complement of distinct receptor systems and downstream signaling cascades, being a synthetic cell that lays down extracellular matrix and remodels the airway wall, being a hyperplastic and/or hypertrophic cell that augments muscle mass and uncouples contractile forces of the ASM from parenchymal distending stresses, and being an immunomodulatory cell that can influence the inflammatory cascade and the inflammatory microenvironment (26). Although each of these remains an area of intense current investigation, excessive airway narrowing in asthma remains poorly understood.

ASM as a Material Restricting Airway Dilation?

Could it be that our evolving picture of bronchospasm is highly complex and yet incapable of accounting for the inability of the patient with asthma to dilate the airways with a DI because at the outset we have overlooked a pivotal piece of the puzzle? One realm in which airway smooth muscle has yet to be considered is from the point of view of material science, namely, airway smooth muscle as an engineering material that has the potential to restrict the response of the airway to a DI. In saying this we do not minimize immunologic aspects of the disease but rather shift the focus to a novel gap in our understanding that is far downstream from those initiating events. If immune responses did not cause bronchospasm, then asthma would be a tolerable disease, but asthma is not a tolerable disease mainly because airway narrowing becomes excessive. In this connection, roughly 30 genes have been implicated in asthma, and the number is expected to plateau at about 100 (27). As such, the corresponding number of gene-by-gene and gene-by-environment interactions is likely to be vast, and of the many potential upstream drug targets each could affect only a correspondingly narrow subset of the asthmatic population. Interactions affecting mucus hypersecretion aside, the rest that are of any importance must necessarily converge downstream at the level of excessive airway narrowing. So, what about bronchodilation caused by DIs? We know the airway smooth muscle cell to be the locus of this phenomenon (6, 24, 28–31), but why is this phenomenon so potent? Why during an asthmatic attack does it fail? Does it just happen to fail when we need it most—an epiphenomenon—or does it mark the collapse of a dominant homeostatic mechanism and thus represent the main culprit that is to blame for the excessive nature of the airway narrowing (6,

30, 32)? If so, in our experimental models of asthma have we given insufficient attention to what might be the single most important facet of the phenotype?

THE ASTHMATIC ATTACK AS A CYTOSKELETAL GLASS TRANSITION?

The response of the living cell to mechanical shear triggers upstream response elements, including adhesion molecules and stretch-sensitive ion channels residing within cell membranes (33–36) and stress-dependent cryptic binding sites residing within key stress-bearing molecules that comprise the CSK (37, 38)—the network of biopolymers that confers mechanical stability and integrity of the cell (39). Such response elements have the capacity to sense a local change in physical force and transducer that change into a biochemical signal. Downstream signaling cascades have been shown to cause mechanical stiffening and reinforcement (40–43) and to comprise a negative feedback loop in which application of physical force initiates a sequence of signaling events that maintains localized mechanical stress at a predetermined set point (36, 44). Reinforcement responses such as these have been shown to occur over the course of seconds to tens of minutes (40, 41, 43), and over longer time scales similar mechanisms have been suggested to explain how physical forces act to control apoptosis, proliferation, and gene expression (37, 43, 45).

The response of inert soft materials to mechanical shear seems to be a different matter. It is a familiar experience, for example, that soft glassy materials such as ketchup, shaving foam, and toothpaste tend to fluidize when subjected to shear (46–51), as do granular materials including sugar in a bowl or coffee beans in a chute (52–54) and even certain geophysical strata during an earthquake (55). In response to shear of sufficient magnitude, each of these materials transforms from a solid-like to a fluidlike phase, stiffness falls, and the material flows. Fluidization by shear is understood neither quantitatively nor qualitatively, however; when such a system is subjected to shear, each microscopic stress-bearing element or cluster of elements interacts with neighbors to form a network of force transmission, but how flow is initiated and the nature of energy barriers that must be overcome remain the subject of much current attention (47, 53, 56–58). Fluidization might include depolymerization, breaking weak bonds or cross-links, or overcoming steric constraints (12).

In recent reports we have demonstrated that in response to a transient stretch of duration and magnitude corresponding to the physiologic range, the CSK of the living cell promptly fluidizes and then slowly resolidifies (11, 12), similar to the inert glassy materials described previously. Furthermore, the rate of CSK structural rearrangements on the molecular scale promptly accelerates and then slowly relaxes. These physical responses do not at all contradict the biological mechanisms described previously but rather show that picture to be highly incomplete. Indeed, in experiments spanning wide differences in cellular interventions, cell type, and even integrative scale, we have shown that these physical events conform to common scaling relationships and thereby suggest a universal class of responses (12). Dynamics of a closely similar kind have been reported in inert glassy materials such as those alluded to previously, all of which are soft, structurally disordered, and systematically displaced away from thermodynamic equilibrium (46–55). Indeed, in the recent 125th anniversary issue of *Science* focused on “What we don’t know,” the dynamics of glassy systems was highlighted as being one of the 125 greatest unsolved problems in all of science (16). Our recent discovery of striking phenomenologic similarities between the living CSK and inert glassy

systems leads to the suggestion that CSK dynamics might conform to a common integrative framework—or phenomenologic law—when considered at some coarse-grained level (59) and thus may require considerations that are considerably more generic and substantially less detailed compared with specific molecular signaling cascades. In the case of inert soft glassy matter, the mechanistic basis of shear fluidization is not understood but is usually attributed to the presence of physical interactions with energy barriers that are large enough that thermal energies by themselves are insufficient to drive microconfigurations to thermodynamic equilibrium (47, 60).

What Governs Structural Rearrangements within a Glassy CSK?

A discrete molecular remodeling event is a molecular rearrangement in which the CSK goes from one network microconfiguration to another. To achieve such a structural rearrangement the system must overcome an energy barrier, perhaps associated with the energy required to break a discrete network bond, to resolve a steric constraint, or to drive unfolding or conformational change of a protein. We imagine an energy landscape that describes all possible molecular configurations. If energy barriers are large compared with thermal energies ($k_B T$), then thermal molecular collisions by themselves would be insufficient to push the system over an energy barrier into a different microconfiguration, and near-equilibrium theories would fail. Instead, the rate of molecular rearrangements would become limited by long-lived microconfigurations in which the system becomes trapped. If these microconfigurations were metastable, then their longevity could depend upon a temperature-like source of molecular agitation, but one of distinctly nonthermal origin. One such source of nonthermal agitation is the ATP-dependent conformational change of a neighboring protein, which releases energy of $25 k_B T$ per event at a rate of 10^4 to 10^7 events per cubic micron of cytoplasm per second. Another is energy injected into the system by the action of mechanical stretch.

Stretch in Relation to CSK Dynamics

We consider the response of the airway smooth muscle cell to stretches that occur during spontaneous breathing (12). Stretch triggers specific molecular events and a cascade of downstream intracellular chemical signaling, and we have shown in a recent article that these events can play out in parallel with and become trumped by the nonspecific actions of a slowly evolving network of intracellular physical forces (12). Whether specific or nonspecific, such events cannot occur in the isometric preparation and therefore could never be activated or detected in such a preparation. Although a quantitative understanding of shear fluidization is not at hand, the qualitative physical picture predicts not only fluidization by shear but also scale-free dissipation processes, approach to kinetic arrest, intermittency of structural rearrangements, and breakdown of the generalized Stokes-Einstein relationship, all of which we have established on the molecular scale in the CSK of the living cell (11, 61). We have demonstrated that in response to stretch, the CSK lattice fluidizes and does so in a universal fashion (12). Although living and inert systems clearly differ, the constellation of nonequilibrium features displayed by the CSK of the living cell is seen to be rich, nontrivial, and unexplained and seems to describe a glassy matrix close to a glass transition.

CONCLUSIONS

In the airways of patients with asthma, bronchoconstriction cannot be overcome by DI, as if the airways had narrowed and

then become frozen in the narrowed state. Although this phenomenon remains somewhat mysterious, we suggest here that it may reflect that the cytoskeleton of the ASM cell has become stuck in a stiff glassy phase. More broadly, with every beat of the heart, inflation of the lung, or peristalsis of the gut, cell types of diverse function are subjected to substantial stretch, and, accordingly, the perspective proposed here is likely to be fundamental to a wide variety of higher integrative cell functions, including cell contraction, adhesion, spreading, crawling, invasion, wound healing, and division, and have been implicated in mechanotransduction, regulation of protein and DNA synthesis, and programmed cell death. The potential to change our understanding of a wide range of cell systems and disease processes in addition to asthma is foreseeable, and if these same glassy dynamics govern the nucleus, as is suggested by recent evidence, then they might control the kinetics of gene expression (62).

Could it be that CSK of the end-effector cell behaves as a phase of soft condensed matter, and contraction as the approach to a glass transition (17, 63)? That is to say, does the cell modulate its mechanical properties and remodel its internal structure in much the same way that a glassblower must heat the object, shape it, then cool it down in order to model a piece of glass (17)? Could it be that the ASM cell becomes refractory to a DI because its cytoskeleton fails to fluidize and instead remains frozen in a stiff, solid-like, glassy phase? If true, the perspective of the asthmatic attack as the approach of a cytoskeletal phase transition would overturn prevailing paradigms of cellular biophysics in asthma and in biology more generally and therefore would have far-reaching implications.

Conflict of Interest Statement: Neither author has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Nadel JA, Tierney DF. Effect of a previous deep inspiration on airway resistance in man. *J Appl Physiol* 1961;16:717–719.
2. Green M, Mead J. Time dependence of flow-volume curves. *J Appl Physiol* 1974;37:793–797.
3. Gump A, Haughney L, Fredberg J. Relaxation of activated airway smooth muscle: relative potency of isoproterenol vs. tidal stretch. *J Appl Physiol* 2001;90:2306–2310.
4. Salter HH. On asthma: its pathology and treatment. New York: William Wood and Company; 1859.
5. Fish JE, Ankin MG, Kelly JF, Peterman VI. Regulation of bronchomotor tone by lung inflation in asthmatic and nonasthmatic subjects. *J Appl Physiol* 1981;50:1079–1086.
6. Fredberg JJ. Frozen objects: small airways, big breaths, and asthma. *J Allergy Clin Immunol* 2000;106:615–624.
7. Lim TK, Pride NB, Ingram RH Jr. Effects of volume history during spontaneous and acutely induced air-flow obstruction in asthma. *Am Rev Respir Dis* 1987;135:591–596.
8. Moore BJ, Verburg LM, King GG, Pare PD. The effect of deep inspiration on methacholine dose-response curves in normal subjects. *Am J Respir Crit Care Med* 1997;156:1278–1281.
9. Skloot G, Permutt S, Togias A. Airway hyperresponsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 1995;96:2393–2403.
10. Bursac P, Fabry B, Trepas X, Lenormand G, Butler JP, Wang N, Fredberg JJ, An SS. Cytoskeleton dynamics: fluctuations within the network. *Biochem Biophys Res Commun* 2007;355:324–330.
11. Bursac P, Lenormand G, Fabry B, Oliver M, Weitz DA, Viasnoff V, Butler JP, Fredberg JJ. Cytoskeletal remodeling and slow dynamics in the living cell. *Nat Mater* 2005;4:557–571.
12. Trepas X, Deng L, An SS, Navajas D, Tschumperlin DJ, Gerthoffer WT, Butler JP, Fredberg JJ. Universal physical responses to stretch in the living cell. *Nature* 2007;447:592–595.
13. Deng L, Trepas X, Butler JP, Millet E, Morgan KG, Weitz DA, Fredberg JJ. Fast and slow dynamics of the cytoskeleton. *Nat Mater* 2006;5:636–640.

14. Seow C. Fashionable glass. *Nature* 2005;435:1172–1173.
15. Stamenovic D. Two regimes, maybe three? *Nat Mater* 2006;5:597–598.
16. Kennedy D, Norman C. What we don't know? *Science* 2005;309:82–83; 75–102.
17. Gunst SJ, Fredberg JJ. The first three minutes: smooth muscle contraction, cytoskeletal events, and soft glasses. *J Appl Physiol* 2003;95:413–425.
18. Seow CY, Pratusевич VR, Ford LE. Series-to-parallel transition in the filament lattice of airway smooth muscle. *J Appl Physiol* 2000;89:869–876.
19. Oliver MN, Fabry B, Marinkovic A, Mijailovich SM, Butler JP, Fredberg JJ. Airway hyperresponsiveness, remodeling, and smooth muscle mass: right answer, wrong reason? *Am J Respir Cell Mol Biol* 2007;37:264–272.
20. Bendixen HH, Smith GM, Mead J. Pattern of ventilation in young adults. *J Appl Physiol* 1964;19:195–198.
21. Ingram RH Jr. Relationships among airway-parenchymal interactions, lung responsiveness, and inflammation in asthma. Giles F. Filley lecture. *Chest* 1995;107(Suppl):148S–152S.
22. Ding DJ, Martin JG, Macklem PT. Effects of lung volume on maximal methacholine-induced bronchoconstriction in normal humans. *J Appl Physiol* 1987;62:1324–1330.
23. Crimi E, Pellegrino R, Milanese M, Brusasco V. Deep breaths, methacholine, and airway narrowing in healthy and mild asthmatic subjects. *J Appl Physiol* 2002;93:1384–1390.
24. Dowell ML, Lakser OJ, Gerthoffer WT, Fredberg JJ, Stelmack GL, Halayko AJ, Solway J, Mitchell RW. Latrunculin b increases force fluctuation-induced relengthening of ach-contracted, isotonically shortened canine tracheal smooth muscle. *J Appl Physiol* 2005;98: 489–497.
25. Chen B, Liu G, Shardonofsky F, Dowell M, Lakser O, Mitchell RW, Fredberg JJ, Pinto LH, Solway J. Tidal breathing pattern differentially antagonizes bronchoconstriction in c57bl/6j vs. A/j mice. *J Appl Physiol* 2006;101:249–255.
26. Seow CY, Fredberg JJ. Historical perspective on airway smooth muscle: the saga of a frustrated cell. *J Appl Physiol* 2001;91:938–952.
27. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006;7:95–100.
28. Fredberg JJ. Airway smooth muscle in asthma: flirting with disaster. *Eur Respir J* 1998;12:1252–1256.
29. Fredberg JJ, Inouye DS, Mijailovich SM, Butler JP. Perturbed equilibrium of myosin binding in airway smooth muscle and its implications in bronchospasm. *Am J Respir Crit Care Med* 1999;159:959–967.
30. Fredberg JJ, Shore SA. The unbearable lightness of breathing. *J Appl Physiol* 1999;86:3–4.
31. Lakser OJ, Lindeman RP, Fredberg JJ. Inhibition of the p38 map kinase pathway destabilizes smooth muscle length during physiological loading. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L1117–L1121.
32. Fredberg JJ. Bronchospasm and its biophysical basis in airway smooth muscle. *Respir Res* 2004;5:2.
33. Martinac B. Mechanosensitive ion channels: molecules of mechanotransduction. *J Cell Sci* 2004;117:2449–2460.
34. Kung C. A possible unifying principle for mechanosensation. *Nature* 2005;436:647–654.
35. Ingber DE. Mechanobiology and diseases of mechanotransduction. *Ann Med* 2003;35:564–577.
36. Bershadsky AD, Balaban NQ, Geiger B. Adhesion-dependent cell mechanosensitivity. *Annu Rev Cell Dev Biol* 2003;19:677–695.
37. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol* 2006;7:265–275.
38. Wang Y, Botvinick EL, Zhao Y, Berns MW, Usami S, Tsien RY, Chien S. Visualizing the mechanical activation of src. *Nature* 2005;434:1040–1045.
39. Bausch AR, Kroy K. A bottom-up approach to cell mechanics. *Nature Physics* 2006;2:231–238.
40. Glogauer M, Arora P, Yao G, Sokholov I, Ferrier J, McCulloch CA. Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective responses to stretching. *J Cell Sci* 1997;110:11–21.
41. Choquet D, Felsenfeld DP, Sheetz MP. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell* 1997;88: 39–48.
42. Giannone G, Jiang G, Sutton DH, Critchley DR, Sheetz MP. Talin1 is critical for force-dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase activation. *J Cell Biol* 2003; 163:409–419.
43. Matthews BD, Overby DR, Mannix R, Ingber DE. Cellular adaptation to mechanical stress: role of integrins, rho, cytoskeletal tension and mechanosensitive ion channels. *J Cell Sci* 2006;119:508–518.
44. Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, Sabanay I, Mahalu D, Safran S, Bershadsky A, Addadi L, et al. Force and focal adhesion assembly: a close relationship studied using elastic micro-patterned substrates. *Nat Cell Biol* 2001;3:466–472.
45. Huang S, Ingber DE. The structural and mechanical complexity of cell-growth control. *Nat Cell Biol* 1999;1:E131–E138.
46. Mason TG, Weitz DA. Linear viscoelasticity of colloidal hard sphere suspensions near the glass transition. *Phys Rev Lett* 1995;75:2770–2773.
47. Sollich P, Lequeux F, Hebraud P, Cates ME. Rheology of soft glassy materials. *Phys Rev Lett* 1997;78:2020–2023.
48. Cloitre M, Borrega R, Leibler L. Rheological aging and rejuvenation in microgel pastes. *Phys Rev Lett* 2000;85:4819–4822.
49. Ramos L, Cipelletti L. Ultraslow dynamics and stress relaxation in the aging of a soft glassy system. *Phys Rev Lett* 2001;87:245503.
50. Viasnoff V, Lequeux F. Rejuvenation and overaging in a colloidal glass under shear. *Phys Rev Lett* 2002;89:065701.
51. Derec C, Ducouret G, Ajdari A, Lequeux F. Aging and nonlinear rheology in suspensions of polyethylene oxide-protected silica particles. *Phys Rev E Stat Nonlin Soft Matter Phys* 2003;67:061403–061409.
52. Liu C, Nagel SR. Sound in a granular material: disorder and non-linearity. *Phys Rev B Condens Matter* 1993;48:15646–15650.
53. O'Hern CS, Langer SA, Liu AJ, Nagel SR. Force distributions near jamming and glass transitions. *Phys Rev Lett* 2001;86:111–114.
54. Corwin EI, Jaeger HM, Nagel SR. Structural signature of jamming in granular media. *Nature* 2005;435:1075–1078.
55. Johnson PA, Jia X. Nonlinear dynamics, granular media and dynamic earthquake triggering. *Nature* 2005;437:871–874.
56. Ostojic J, Somfai E, Nienhuis B. Scale invariance and universality of force networks in static granular matter. *Nature* 2006;439:828–830.
57. Silbert LE, Liu AJ, Nagel SR. Structural signatures of the unjamming transition at zero temperature. *Phys Rev E Stat Nonlin Soft Matter Phys* 2006;73:041304.
58. Zhou J, Long S, Wang Q, Dinsmore AD. Measurement of forces inside a three-dimensional pile of frictionless droplets. *Science* 2006;312: 1631–1633.
59. Poon WCK. Coarse graining in biological soft matter. In: Poon WCK, Adelman D, editors. *Soft condensed matter physics in molecular and cell biology*. London: Taylor & Francis; 2006. pp. 1–15.
60. Fielding SM, Sollich P, Cates ME. Aging and rheology in soft materials. *J Rheol* 2000;44:323–369.
61. Fabry B, Maksym GN, Butler JP, Glogauer M, Navajas D, Fredberg JJ. Scaling the microrheology of living cells. *Phys Rev Lett* 2001;87: 148102.
62. Dahl KN, Engler AJ, Pajerowski JD, Discher DE. Power-law rheology of isolated nuclei with deformation mapping of nuclear sub-structures. *Biophys J* 2005;89:2855–2864.
63. Fabry B, Fredberg JJ. Remodeling of the airway smooth muscle cell: are we built of glass? *Respir Physiol Neurobiol* 2003;137:109–124.