Endocrinology. First published ahead of print December 16, 2009 as doi:10.1210/en.2009-0932

Minireview: A Tiny Touch: Activation of Cell Signaling Pathways with Magnetic Nanoparticles

Nathan J. Sniadecki

Department of Mechanical Engineering, University of Washington, Seattle, Washington 98195

Magnetic nanoparticles can be coated with specific ligands that enable them to bind to receptors on a cell's surface. When a magnetic field is applied, it pulls on the particles so that they deliver nanoscale forces at the ligand-receptor bond. It has been observed that mechanical stimulation in this manner can activate cellular signaling pathways that are known as mechanotransduction pathways. Integrin receptors, stretch-activated ion channels, focal adhesions, and the cytoskeleton are key players in activating these pathways, but there is still much we do not know about how these mechanosensors work. Current evidence indicates that applied forces at these structures can activate Ca^{2+} signaling, Src family protein kinase, MAPK, and RhoGTPase pathways. The techniques of magnetic twisting and magnetic tweezers, which use magnetic particles to apply forces to cells, afford a fine degree of control over how cells are stimulated and hold much promise in elucidating the fundamentals of mechanotransduction. The particles are generally not harmful to cellular health, and their nanoscale dimensions make them advantageous for probing a cell's molecular-scale sensory structures. This review highlights the basic aspects of magnetic nanoparticles, magnetic particle techniques and the structures and pathways that are involved in mechanotransduction. **(*Endocrinology* 151: 0000–0000, 2010)**

Cells contain mechanically sensitive receptors that activate signaling proteins, allowing them to respond to cues in their local environment. The signaling pathways activated by mechanical stimulation can affect cellular functions like apoptosis, communication, contraction, differentiation, migration, proliferation, and secretion. The process of mechanotransduction, which is how cells convert physical force into a biochemical signal, plays a major role in the development and pathology of human tissue through its influence over cell function. Mechanical stimulation can provide another avenue of control over cells in addition to biochemical ones, so there is strong interest in the fields of tissue engineering (1) and stem cell biology (2) to understand the basics of mechanotransduction and develop ways to harness it. Here the hope is that one can instruct cells to change their function or undergo differentiation through the influence of a scaffold's mechanical properties or a regimen of applied forces. The combination of appropriate physical and chemical cues will make it effective to create a wider range of *de novo* tissue types for repair or replacement. Mechanotransduction also has importance in diseases like atherosclerosis (3) and cancer (4), which have pathophysiological aspects that are physical in nature. It is hypothesized that cells may be steered toward adverse outcomes through physical cues in their microenvironment, *e.g.* shear forces or matrix stiffness. A deeper understanding of mechanotransduction creates opportunities to identify the receptors and pathways that govern mechanotransduction, which may make them viable targets for therapeutic treatments (5).

The research on mechanotransduction holds many promises, but the diminutive nature of cells and their mechanosensitive receptors causes a high degree of ambiguity. With bioanalytical techniques or molecular approaches, one can study the role of a single type of protein or gene by transforming it to be on or off and then examining the changes in cell function or signaling. However, the response of a cell to applied force is not always straightforward because when force is applied at the macroscale, the whole structure of the cell is distorted, making it dif-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2009-0932 Received August 5, 2009. Accepted November 17, 2009.

Abbreviation: FcεRI, High-affinity IgE receptor.

ficult to scrutinize the response of a single type of receptor from the inadvertent activation of others. For this reason, magnetic nanoparticles have been relied on to overcome the experimental difficulties with macroscopic approaches. This review highlights the use of magnetic particle technology to identify the receptors, pathways, and responses involved in mechanotransduction signaling.

Overview of Magnetic Particles

There is a great deal of inventiveness in the use of magnetic particles because one can tailor their dimensions, magnetic properties, and surface coatings (6–8). They are unique probes for mechanotransduction because the diameters of the particles are at the same length scale as the biological structures to be interrogated. More importantly, their magnetization is not significantly diminished at the nanoscale. They can be synthesized as small as a few nanometers in diameter but still achieve good dimensional uniformity within a fabrication batch (6). At this size, each particle possesses a single magnetic domain and superparamagnetic properties, in comparison with larger magnetic particles, which have multiple ferromagnetic domains and permanent magnetic properties (6). The force that an external magnetic field exerts on the particle can range from 10^{-12} to 10^{-9} newtons, which are the typical levels that cells experience *in vivo* (9). For mechanotransduction studies, iron oxide particles are used more commonly than other magnetic materials like cobalt or nickel because they are simpler to synthesize by coprecipitation from iron salts (6). In fact, batches of synthesized iron oxide micro- or nanoparticles are available from commercial manufacturers and come prepared with reactive functional groups on the surface (Fig. 1A). By selecting the appropriate surface functional group, it is feasible to chemically attach a ligand to a particle, which enables it to bind to a chosen receptor type on a cell's surface (7). However, a more straightforward manner is to adsorb matrix proteins from solution onto the particle's surface through hydrophobic interactions (10, 11). In general, proteins like collagen or fibronectin keep their native conformation when adsorbed and so cells can bind through their receptors' recognition of the protein's ligand domains that remain intact. Biocompatibility of magnetic materials is a concern, so iron oxide is a more favorable magnetic material than cobalt or nickel because iron homeostasis is tightly controlled by a cell to clear excess iron (8). Over the course of many hours, however, nanoparticles can be internalized through endocytosis, which may contribute to cytotoxicity by overwhelming the mechanisms of iron clearance. For mechanotransduction studies, magnetic particles are predominately used to study receptors on the outside of a cell and are seldom used for internal probing, so issues of cytotoxicity and internalization are often avoided through cautious, short-term studies.

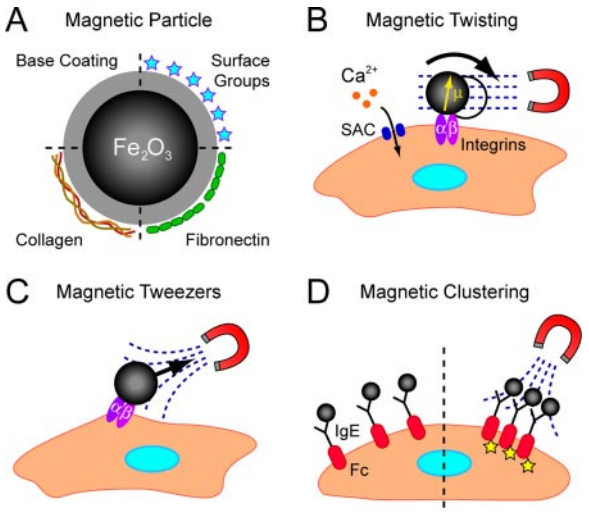

FIG. 1. Magnetic nanoparticles for mechanical activation of cell receptors. A, Nanosynthesized magnetic particles can be coated with a organic or nonorganic base coating that protects the magnetic core and provides a foundation for subsequent conjugation with reactive surface groups or adsorption of matrix proteins like fibronectin or collagen. B, Magnetic twisting cytometry imparts a mechanical torque through an applied magnetic field perpendicular to the particle's magnetic dipole moment (μ). The induced shear stress, which can be as strong as 4 N/m^2, mechanically activates integrin receptors or stretch-activated ion channels (SAC). C, Magnetic tweezers use a gradient field to pull magnetic particles toward the pole tip of an electromagnetic coil or permanent magnetic. With magnetic tweezers, forces up to 10 nN have been reported. D, Magnetic aggregation can be used to activate immunological responses in mast cell using superparamagnetic nanoparticles to induce clustering of IgE-bound FcεRI receptors.

Magnetic Particle Techniques for Cell Signal Activation

In general, the idea that physical force can act as a regulatory signal has parallelism to endocrine signaling. Force, like hormones or growth factors, can guide the development and function of cells through changes in gene expression. The process starts with mechanoreceptors that lead to changes in protein kinase or phosphatase activity inside the cell. In general, the signal is propagated forward to activate transcription factors that regulate the expression of target genes. As with mutations in extracellular signaling, mutations in mechanotransduction pathways can muddle the sensation of force and cause errors in signal interpretation. Dysfunction in mechanotransduction is hypothesized to drive cells to pathological outcomes like cancer, atherosclerosis, or asthma (4, 12). It should be pointed out that mechanotransduction changes do not always need to go through the nucleus; force can activate

pathways that modulate the activation of cell functions like migration, contraction, or secretion. Although many phenomenological observations have been made, the mechanisms of how force initiates biochemical changes are not fully understood (9, 13). It may involve stretch-induced conformational changes in proteins in the vicinity of the receptor (14), force transmission from the receptor to specialized mechanosensors within a cell (15, 16), and/or changes in tensional integrity (tensegrity) of the cytoskeleton (17). However, what is apparent is that there is not a single master switch that initiates cellular mechanotransduction.

Magnetic particles can overcome the limits apparent in other techniques used for studying mechanotransduction. Approaches that subject cells to shear or stretch continue to pioneer the field of mechanotransduction, but they apply forces at multiple points on a cell, which obscures the identification of the mechanoreceptors involved in the process. Magnetic particles offer more control at the nanoscale because one can readily manipulate the strength, direction, and location of the magnetic force by the placement of the magnetic fields and the ligand coating on the bead (Fig. 1A). This isolates out the interactions from other receptors and sidesteps the convolution of stimulating multiple receptors types that could activate several pathways in parallel.

The laws of physics allow for different ways to twist, pull, or cluster magnetic particles and so several magnetic technique platforms have been developed (18, 19). Magnetic twisting cytometry generates a mechanical torque at the particle-cell interface by applying a field in a direction perpendicular to the magnetic dipole of the particle (Fig. 1B) (10). The torque imparted by the applied field drives a particle to twist or roll on a cell's surface. Because the particle is physically restrained by the bonds at the receptor-ligand interface, the rolling action produces a shearing force at the cell's receptor. Force can be applied at high frequency by modulating the current passing through the electromagnetic coils that generate the field and thereby impart cyclic loading on the cell. The second main approach with magnetic particles is magnetic tweezers, which are able to pull on particles by gradients in a magnetic field (Fig. 1C) (20). Here one of the pole ends of an electromagnetic coil has a long, sharp tip that is placed using a micropositioner to be a close distance from a particle. The field lines that emanate from the pole tip radiate outward and loop back to the other pole of the electromagnetic coil. If a particle is situated within these field lines, it is pulled toward the tip because in that direction, the field gradient is strongest; they become more concentrated spatially as the distance decreases between the tip and a particle. As one positions the electromagnetic coil so that tip-to-particle distance is smaller, the magnetic pulling force at the particle-cell interface is stronger.

The remaining focus of this review summarizes the findings on mechanotransduction receptors and signaling pathways that have resulted from magnetic twisting and magnetic tweezers. However, it is important to highlight a recent accomplishment of magnetic nanoparticles technology whose feat of control over physical interactions of individual receptors was groundbreaking (21). Magnetic nanoparticles were used to activate mast cells in a manner that replicated the immunological activation of IgE receptors by allergens. Here superparamagnetic particles with 30-nm diameters were coated with dinitrophenyl by conjugating it to surface amine groups with glutaraldehyde. Mast cells were then incubated with antidinitrophenyl IgE antibodies to complex them with their high-affinity IgE receptor (FcεRI). When the particles and cells were combined, each nanoparticle became bound to one IgE-FcεRI receptor complex (Fig. 1D). Initially, the nanoparticles were nonmagnetized and there was no external magnetic field, so they were free to travel with the diffusive movements of the FcεRI receptors in the membrane. When a gradient field was applied, the induced magnetization in the particles caused them to aggregate together and drove the FcεRI receptors into a clustered arrangement. Magnetic clustering in this manner replicates receptor activation that occurs with multivalent allergens. An allergen contains multiple ligand domains that spatially arrange FcεRI receptors to be clustered in close proximity with each other and allows the receptors to autoactivate one another. Nanoparticle clustering of FcεRI receptors activated Ca^{2+} ions release from intracellular stores in mast cells, which is a response that mediates IgE-associated secretion of histamine during allergic reactions (22). The power of nanoparticles was elegantly demonstrated here for immune receptors, but the technique could be extended to physically manipulate other receptor types. It is exciting to anticipate future studies in cellular signaling that would benefit from such a controllable, yet noninvasive technique.

Activation of Mechanotransduction Receptors

Nanoparticles used for mechanotransduction have revealed a complex interplay between membrane receptors and the cytoskeleton, which suggests interdependence between membrane surface sensors and the cell's internal structural arrangement. The predominant molecular transducers of force are integrins, which are a family of α and β heterodimer transmembrane glycoproteins that act as extracellular matrix receptors (Fig. 1B) (23). Integrins play an essential role in the signaling and structure of a cell,

so it is a natural expectation that the two roles appear to be intertwined (15, 24). Integrin extracellular portions bind to ligands in the matrix and support cell adhesion, whereas their intracellular domains associate with the cytoskeleton through focal adhesion proteins, which regulate cell survival, differentiation, migration, and mechanotransduction pathways (15, 24). Integrins physically link the mechanical environment outside a cell to its internal cytoskeleton, serving as a conduit for mechanosensation. Because integrin-adhesion complexes can vary in size from nanometers to micrometers, magnetic particles are well suited to probe them at an equal length scale. Studies have pulled on magnetic particles coated with extracellular matrix proteins like fibronectin or collagen (Fig. 1A) and compared the responses with particles with nonintegrin adhesive coatings or in the absence of magnetic fields to show that mechanotransduction is triggered by the specific combination of locally applied forces and integrin-ligand engagement (10, 25). A seminal work with magnetic twisting showed that applied force is able to activate cellular pathways that reinforce the adhesion site and make it more resistant to the particle's twisting (10). Adhesion reinforcement required integrin binding because the effect was seen only if the magnetic beads were coated with anti-β_1 integrin or Arg-Gly-Asp peptide, which is a ligand domain in fibronectin for integrin receptor $\alpha_5\beta_1$. Moreover, their work showed that the cytoskeleton plays an essential role in mechanotransduction because disruption of actin, microtubules, or intermediate filaments led to reduced adhesion reinforcement.

Physical activation of cells is associated with changes in the conformation and/or assembly of focal adhesion proteins on the inside of the cell membrane. Using magnetic tweezers, it has been observed that application of 12 pN of force to talin can stretch its structure about its α-helical domains (26). Talin is a protein that can bind directly to integrins and serves as a scaffold for additional focal adhesion proteins. Its stretched-opened conformation exposes cryptic sites that enhance the binding of vinculin, which is another scaffold protein for focal adhesion assembly. Vinculin can then recruit paxillin, which acts as a docking platform for tyrosine kinases and phosphatases (27, 28). The platform configuration helps stabilize focal adhesion signaling by the close proximity between docked proteins. Congregating signaling proteins together helps their normally short-lived activated states be more efficient in phosphorylating or dephosphorylating their target substrates, allowing their signals to be relayed downstream more efficiently. Vinculin is not the only protein that is recruited to the adhesion site through force-enhanced binding because actin, α-tubulin, filamin, and FilGAP have increase concentration at the adhesion site when integrins are pulled on using magnetic particles (29–32). In addition to integrin receptors, the recruitment responses in other cell-matrix and cell-cell adhesion receptors like urokinase receptor (33), E-selectin (34), E-cadherin (35), and VE-cadherin (36) have been examined with magnetic twisting or magnetic tweezers, but further work is needed to uncover their roles in mechanotransduction signaling. The observations of adhesion reinforcement at these nonintegrin receptors underscores that mechanotransduction is not defined by a single master receptor but that multiple receptors may be involved in the interpretation of mechanical stimulation.

In the membrane, stretch-activated ion channels are primordial mechanosensitive structures found in a variety of bacterial and mammalian cells that play a crucial role in converting mechanical force into electrical and chemical signals. Stretch-activated ion channels act as release valves by alleviating osmotic swelling through rapid regulation in ion concentration before cell lysis occurs. Transport of Ca^{2+}, Na^+, K^+, or Cl^- by stretch-activated ion channels is physically controlled by changes in membrane tension (37). According to the prevalent model of stretch-activated ion channel opening, tension expands the channel's central pore so that it acts as a gate and permits the influx of extracellular ions across the cell membrane and into the cytosol (37). A rapid rise in cytosolic ion concentration can depolarize the membrane, open voltage-dependent channels, or the ions themselves can function as secondary messengers. Mobilization of Ca^{2+} in particular may be responsible for activating signaling pathways associated with mechanotransduction because applied tension through integrin-bound magnetic particles has been observed to cause Ca^{2+} spikes that lead to force-related changes in cell function (11, 38, 39). Interestingly, when stimulated with 2 N/m^2 of stress from magnetic tweezers, cells loaded with calcium-sensitive fluorescence dye showed an initially strong Ca^{2+} spike with force stimulation, but these cells demonstrated progressively lower spikes with each additional pull of the magnetic beads. It was observed that if physical stimulation was applied too frequently (every 6 min), then there was a steady drop in Ca^{2+} spike intensity to almost undetectable levels, but if the force was repeated less frequently (every 10 min), the Ca^{2+} spikes were similar in intensity and could be repeat over longer durations. The lack of repeatability with faster cycles is likely due to a lag in calcium ion pumps, in which storage levels of Ca^{2+} ions are not prepared for the next bout of stimulation, but it also suggests that there may be appropriate frequencies at which mechanical stimulation needs to be applied to elicit the strongest mechanotransduction response.

Although external force from magnetic tweezers or magnetic twisting acts on the surface and not directly on

the cytoskeleton, its assembled state and structural tension can strongly influence the mechanotransduction response. The architecture of the cytoskeleton is nonuniform and has domains of tightly packed filaments composed of actin, myosin, microtubules, and intermediate filaments that provide structural stiffness to a cell (40). Cytoskeletal filaments span the length of a cell and provide stability to the overall structure but also mechanically connect distant ends of a cell (16). Inhibiting the rate at which these filaments polymerize, motor activity of myosin, or cross-linking of the cytoskeleton by actin-binding proteins can change the mechanosensation ability of integrins and stretch-activated ion channels. For integrins, cytoskeletal tension normally acts as a counterbalance to applied force (10, 41). Without cytoskeletal tension, focal adhesions fail to accumulate in size and many of the associated mechanotransduction signaling pathways are attenuated. For stretch-activated ion channels, the cytoskeleton provides resistance to physical deformations that open the central channel structure. Treating cells with cytochalasin D inhibits actin polymerization, reduced cytoskeletal tension, and causes significantly higher Ca^{2+} spikes when cells are pulled on (38). Here cytoskeletal tension appears to antagonize stretch-activated ion channel signaling. Even though integrins and stretch-activated ion channels respond contrary to the state of the cytoskeleton, both are beholden to its tensional integrity.

Mechanosensitive Signaling Pathways

Studies using magnetic particles have helped to uncover a unique set of pathways that regulate mechanotransduction. These pathways have been corroborated using different techniques other than magnetic particles, so the field has an encouraging degree of consensus in its findings (42). It has been shown that force applied at integrins can activate tyrosine kinases like Src and focal adhesion kinase, which promote downstream signaling and recruitment of proteins to the focal adhesions (25, 43, 44). Activation occurs locally at the particle-membrane interface, but it can propagate to other mechanosensors in a cell through the structure of the cytoskeleton. Actin and microtubule filaments can transmit mechanical force along their lengths to distant focal adhesions, in which the mechanotransduction response is amplified (45, 46). Twisting magnetic beads on the surface of a cell has been shown to cause local activation, but the applied force is also transmittable (45). The actin-at-a-distance response has been shown to cause Src activation at focal adhesions that are distant from the site of force application (46). However, tensional integrity of the cytoskeleton must be maintained so that the applied force can be effectively sent to these other structures.

Other mechanotransduction pathways activated during integrin stimulation include cAMP (47), p38 MAPK (31, 48, 49), and RhoA GTPase (50–52). These pathways cause activation of transcription factors like cAMP-response element binding (47), myocardin-related transcription factor (52), and serum-response factor (51) that lead to changes in gene expression. The dominating cellular response to force stimulation is adaptive changes in the structure-function relationship of the cytoskeleton. It has been observed that cells stimulated by magnetic particles have increased production of proteins like filamin or smooth muscle actin (31, 48–52). Filamin cross-links actin and causes the cytoskeleton to be more resistant to further mechanical distortion. Expression of smooth muscle actin is a hallmark of cellular differentiation into a more contractile state that is accompanied by increased cytoskeletal tension. By opening stretch-activated ion channels with magnetic particles, the resultant Ca^{2+} spikes can activate protein kinase C signaling that leads to increased filamin levels, indicating that there can be overlap in the activation of mechanotransduction pathways by different receptors (30). It also suggests that the cytoskeleton is a common target for mechanically regulated changes. Additionally, alterations in cellular structure-function relationships are not restricted to the individual cell that is under mechanical stimulation. Forces from magnetic particles can cause up-regulation of endothelin-1 in endothelial cells, which is a strong paracrine signal for smooth muscle vasoconstriction (53). In this example, force applied to one cell can affect nearby cells through the release of soluble factors in endocrine signaling.

Concluding Remarks

Magnetic particles provide a means to remotely manipulate the mechanical sensory structures in a cell. In contrast to diffusive signals, actuation of magnetic forces is rapid and controllable in direction, magnitude, and duration. However, by tailoring the ligand coating on the particles, it may be feasible to isolate the responses from one endocrine receptor at a time. Magnetic particles coated in this manner could activate signaling pathways in a synchronized manner so that the dynamics of the signal transduction process can be examined. In considering of the efforts underway in systems biology (54), it could be advantageous to use magnetically activate receptors with synchronization to map out cellular signaling in a systematic way and across many cells. Moreover, through the use of protein or genetic interference techniques, it will be feasible to

study the cellular errors that occur in interpreting chemical or mechanical signals.

Mechanical stimuli are ubiquitous in living systems, and mechanotransduction could represent one of the oldest transduction mechanisms that arose in living organisms. In its simplest form, unicellular organisms like bacteria use stretch-activated ion channels to self-regulate the mechanotransduction response. For multicellular organisms, adhesion molecules like integrins interpret the mechanical signals in the surrounding tissue to induce changes in cytoskeletal structure or gene activity. Whether stretch-activated ion channels are more archaic structures than integrins is beyond the scope of this review, but the fact that mechanoreceptors are found in a range of organisms indicates that it is nearly ubiquitous for living systems to respond to their physical environment.

Cells live in a social context where they are mechanically connected to each other by cell-cell and cell-matrix adhesions and communicate through the release of soluble factors. Together, these mechanical and biochemical connections form a unique hierarchy of communication. It is plausible that mechanosensation activity in one cell may induce changes in its neighbors through endocrine activity brought on by mechanotransduction. As magnetic nanoparticle technology matures and becomes more commonplace, we can expect substantial advancements in deciphering the molecular mechanisms and signaling pathways that are involved. The final goal will be to bring consensus among the different relationships that mechanical forces have on cellular function.

Acknowledgments

I acknowledge the National Science Foundation CAREER award for financial support.

Address all correspondence and requests for reprints to: Nathan J. Sniadecki, Department of Mechanical Engineering, Box 352600 University of Washington, Seattle, Washington 98195. E-mail: nsniadec@u.washington.edu.

Disclosure Summary: The author has nothing to disclose.

References

1. **Guldberg R, Butler DL, Goldstein S, Guo XE, Kamm R, Laurencin CT, McIntire LV, Mow VC, Nerem R, Sah RL, Soslowsky L, Spilker RL, Tranquillo RT** 7 July 2009 The impact of biomechanics in tissue engineering and regenerative medicine. Tissue Eng Part B Rev 10.1089/ten.teb.2009.0340
2. **Discher DE, Mooney DJ, Zandstra PW** 2009 Growth factors, matrices, and forces combine and control stem cells. Science 324:1673–1677
3. **Hahn C, Schwartz MA** 2009 Mechanotransduction in vascular physiology and atherogenesis. Nat Rev Mol Cell Biol 10:53–62
4. **Butcher DT, Alliston T, Weaver VM** 2009 A tense situation: forcing tumour progression. Nat Rev Cancer 9:108–122
5. **Tavi P, Laine M, Weckström M, Ruskoaho H** 2001 Cardiac mechanotransduction: from sensing to disease and treatment. Trends Pharmacol Sci 22:254–260
6. **Lu AH, Salabas EL, Schüth F** 2007 Magnetic nanoparticles: synthesis, protection, functionalization, and application. Angew Chem Int Ed Engl 46:1222–1244
7. **Corchero JL, Villaverde A** 2009 Biomedical applications of distally controlled magnetic nanoparticles. Trends Biotechnol 27:468–476
8. **Ferreira L, Karp JM, Nobre L, Langer R** 2008 New opportunities: the use of nanotechnologies to manipulate and track stem cells. Cell Stem Cell 3:136–146
9. **Orr AW, Helmke BP, Blackman BR, Schwartz MA** 2006 Mechanisms of mechanotransduction. Dev Cell 10:11–20
10. **Wang N, Butler JP, Ingber DE** 1993 Mechanotransduction across the cell surface and through the cytoskeleton. Science 260:1124–1127
11. **Glogauer M, Ferrier J, McCulloch CA** 1995 Magnetic fields applied to collagen-coated ferric oxide beads induce stretch-activated Ca^{2+} flux in fibroblasts. Am J Physiol 269:C1093–C1104
12. **Jaalouk DE, Lammerding J** 2009 Mechanotransduction gone awry. Nat Rev Mol Cell Bio 10:63–73
13. **Vogel V, Sheetz M** 2006 Local force and geometry sensing regulate cell functions. Nat Rev Mol Cell Biol 7:265–275
14. **Vogel V** 2006 Mechanotransduction involving multimodular proteins: converting force into biochemical signals. Annu Rev Biophys Biomol Struct 35:459–488
15. **Geiger B, Bershadsky A, Pankov R, Yamada KM** 2001 Transmembrane extracellular matrix— cytoskeleton crosstalk. Nat Rev Mol Cell Biol 2:793–805
16. **Wang N, Tytell JD, Ingber DE** 2009 Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat Rev Mol Cell Biol 10:75–82
17. **Ingber DE** 1993 Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. J Cell Sci 104(Pt 3):613–627
18. **Sniadecki NJ, Desai RA, Ruiz SA, Chen CS** 2006 Nanotechnology for cell-substrate interactions. Ann Biomed Eng 34:59–74
19. **Hughes S, El Haj AJ, Dobson J** 2005 Magnetic micro- and nanoparticle mediated activation of mechanosensitive ion channels. Med Eng Phys 27:754–762
20. **Bausch AR, Ziemann F, Boulbitch AA, Jacobson K, Sackmann E** 1998 Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. Biophys J 75:2038–2049
21. **Mannix RJ, Kumar S, Cassiola F, Montoya-Zavala M, Feinstein E, Prentiss M, Ingber DE** 2008 Nanomagnetic actuation of receptor-mediated signal transduction. Nat Nanotechnol 3:36–40
22. **MacGlashan Jr D** 2008 IgE receptor and signal transduction in mast cells and basophils. Curr Opin Immunol 20:717–723
23. **Askari JA, Buckley PA, Mould AP, Humphries MJ** 2009 Linking integrin conformation to function. J Cell Sci 122:165–170
24. **Wang YL** 2007 Flux at focal adhesions: slippage clutch, mechanical gauge, or signal depot. Sci STKE 2007:pe10
25. **Glogauer M, Arora P, Yao G, Sokholov I, Ferrier J, McCulloch CA** 1997 Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective responses to stretching. J Cell Sci 110(Pt 1):11–21
26. **del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, Sheetz MP** 2009 Stretching single talin rod molecules activates vinculin binding. Science 323:638–641
27. **Turner CE** 2000 Paxillin interactions. J Cell Sci 113(Pt 23):4139–4140
28. **Turner CE, Glenney Jr JR, Burridge K** 1990 Paxillin: a new vinculin-binding protein present in focal adhesions. J Cell Biol 111:1059–1068
29. **Matthews BD, Overby DR, Alenghat FJ, Karavitis J, Numaguchi Y, Allen PG, Ingber DE** 2004 Mechanical properties of individual focal adhesions probed with a magnetic microneedle. Biochem Biophys Res Commun 313:758–764
30. **Glogauer M, Arora P, Chou D, Janmey PA, Downey GP, McCulloch**

CA 1998 The role of actin-binding protein 280 in integrin-dependent mechanoprotection. J Biol Chem 273:1689–1698
31. **D'Addario M, Arora PD, Ellen RP, McCulloch CA** 2003 Regulation of tension-induced mechanotranscriptional signals by the microtubule network in fibroblasts. J Biol Chem 278:53090–53097
32. **Shifrin Y, Arora PD, Ohta Y, Calderwood DA, McCulloch CA** 2009 The role of FilGAP-filamin A interactions in mechanoprotection. Mol Biol Cell 20:1269–1279
33. **Planus E, Barlovatz-Meimon G, Rogers RA, Bonavaud S, Ingber DE, Wang N** 1997 Binding of urokinase to plasminogen activator inhibitor type-1 mediates cell adhesion and spreading. J Cell Sci 110(Pt 9):1091–1098
34. **Yoshida M, Westlin WF, Wang N, Ingber DE, Rosenzweig A, Resnick N, Gimbrone Jr MA** 1996 Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. J Cell Biol 133:445–455
35. **Potard US, Butler JP, Wang N** 1997 Cytoskeletal mechanics in confluent epithelial cells probed through integrins and E-cadherins. Am J Physiol 272:C1654–C1663
36. **Kris AS, Kamm RD, Sieminski AL** 2008 VASP involvement in force-mediated adherens junction strengthening. Biochem Biophys Res Commun 375:134–138
37. **Martinac B** 2004 Mechanosensitive ion channels: molecules of mechanotransduction. J Cell Sci 117:2449–2460
38. **Wu Z, Wong K, Glogauer M, Ellen RP, McCulloch CA** 1999 Regulation of stretch-activated intracellular calcium transients by actin filaments. Biochem Biophys Res Commun 261:419–425
39. **Balasubramanian L, Ahmed A, Lo CM, Sham JS, Yip KP** 2007 Integrin-mediated mechanotransduction in renal vascular smooth muscle cells: activation of calcium sparks. Am J Physiol Regul Integr Comp Physiol 293:R1586–R1594
40. **Janmey PA, McCulloch CA** 2007 Cell mechanics: integrating cell responses to mechanical stimuli. Annu Rev Biomed Eng 9:1–34
41. **Giannone G, Sheetz MP** 2006 Substrate rigidity and force define form through tyrosine phosphatase and kinase pathways. Trends Cell Biol 16:213–223
42. **Chen CS** 2008 Mechanotransduction—a field pulling together? J Cell Sci 121:3285–3292
43. **Browe DM, Baumgarten CM** 2003 Stretch of β1 integrin activates an outwardly rectifying chloride current via FAK and Src in rabbit ventricular myocytes. J Gen Physiol 122:689–702
44. **Mack PJ, Kaazempur-Mofrad MR, Karcher H, Lee RT, Kamm RD** 2004 Force-induced focal adhesion translocation: effects of force amplitude and frequency. Am J Physiol Cell Physiol 287:C954–C962
45. **Hu S, Chen J, Fabry B, Numaguchi Y, Gouldstone A, Ingber DE, Fredberg JJ, Butler JP, Wang N** 2003 Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. Am J Physiol Cell Physiol 285:C1082–C1090
46. **Na S, Collin O, Chowdhury F, Tay B, Ouyang M, Wang Y, Wang N** 2008 Rapid signal transduction in living cells is a unique feature of mechanotransduction. Proc Natl Acad Sci USA 105:6626–6631
47. **Meyer CJ, Alenghat FJ, Rim P, Fong JH, Fabry B, Ingber DE** 2000 Mechanical control of cyclic AMP signalling and gene transcription through integrins. Nat Cell Biol 2:666–668
48. **D'Addario M, Arora PD, Fan J, Ganss B, Ellen RP, McCulloch CA** 2001 Cytoprotection against mechanical forces delivered through β1 integrins requires induction of filamin A. J Biol Chem 276:31969–31977
49. **D'Addario M, Arora PD, Ellen RP, McCulloch CA** 2002 Interaction of p38 and Sp1 in a mechanical force-induced, β1 integrin-mediated transcriptional circuit that regulates the actin-binding protein filamin-A. J Biol Chem 277:47541–47550
50. **Wang J, Fan J, Laschinger C, Arora PD, Kapus A, Seth A, McCulloch CA** 2005 Smooth muscle actin determines mechanical force-induced p38 activation. J Biol Chem 280:7273–7284
51. **Wang J, Zohar R, McCulloch CA** 2006 Multiple roles of α-smooth muscle actin in mechanotransduction. Exp Cell Res 312:205–214
52. **Zhao XH, Laschinger C, Arora P, Szászi K, Kapus A, McCulloch CA** 2007 Force activates smooth muscle α-actin promoter activity through the Rho signaling pathway. J Cell Sci 120:1801–1809
53. **Chen J, Fabry B, Schiffrin EL, Wang N** 2001 Twisting integrin receptors increases endothelin-1 gene expression in endothelial cells. Am J Physiol Cell Physiol 280:C1475–C1484
54. **Kitano H** 2002 Systems biology: a brief overview. Science 295:1662–1664