Cell Mechanosensors and the Possibilities of Using Magnetic Nanoparticles to Study Them and to Modify Cell Fate

YAJING SHEN, YU CHENG, TARO Q. P. UYEDA, and GUSTAVO R. PLAZA

Abstract—The use of magnetic nanoparticles (MNPs) is a promising technique for future advances in biomedical applications. This idea is supported by the availability of MNPs that can target specific cell components, the variety of shapes of MNPs and the possibility of finely controlling the applied magnetic forces. To examine this opportunity, here we review the current developments in the use of MNPs to mechanically stimulate cells and, specifically, the cell mechanotransduction systems. We analyze the cell components that may act as mechanosensors and their effect on cell fate and we focus on the promising possibilities of controlling stem-cell differentiation, inducing cancer-cell death and treating nervous-system diseases.

Keywords—Mechanotransduction, Signaling cascade, Cytoskeleton, Magnetic nanoparticles, Rotating magnetic field, Functionalization, Organelle targeting, Localized stimulation, Cell differentiation.

INTRODUCTION

It is well established by the biomechanics community that mechanical stimuli are of fundamental importance to dictate cell fate. In particular, mechanical stimuli and also the stiffness of the extracellular matrix have been proven to affect differentiation of stem cells. The influence of mechanical stimuli on cell differentiation is mediated by the mechanotransduction process in the cell, by which mechanical stimuli are translated into chemical signals, through specific mechanosensors and the downstream signaling pathways. Such a translation occurs primarily at mechanosensing proteins whose interaction with other proteins is modified by the mechanical stimuli. There is a network of mechanosensors and the downstream signaling pathways in the cell, as we describe in the following sections, which can trigger different cell responses.

A future technology allowing a precise mechanical control of cell differentiation would require the possibility of activating separately the different mechanosensors and the use of different conditions of mechanical loading. Using magnetic nanoparticles (MNPs) is a promising technique in this field since MNPs can be functionalized to target different components in the cell, and there is a wide range of possible mechanical stimuli to be applied by controlling the magnetic field.

Here we review the advances in the identification of mechanosensors and downstream signaling networks, the effect of mechanical stimuli on cell fate and the possibilities of using MNPs to apply mechanical loading, in particular to stem cells, cancer cells and neurons.

MECHANOTRANSDUCTION

Mechanotransduction may be defined as the ability to influence, through mechanical forces, biological outcomes.[26,48] In our lives, mechanotransduction is of chief importance for our hearing and tactile sensing. Mechanotransduction plays a key role in cell choices in

the cases of cell differentiation, tissue growth and homeostasis, motility and tumor formation and progression.[18,25,51,84] Taking into account the effect of the translated signals, the cells may respond to mechanical stimuli (i) *as a probe*, when mechanotransduction produces signals translated to the exterior of the cell: neuronal signals, like in cochlear hair cells, or signals emitted by the cells to the exterior medium that regulate the development and remodeling of tissues, like osteocytes sensing deformations, which is at the origin of the remodeling and homeostasis of bone[25]; and (ii) *complying* to them, when mechanotransduction produces changes in the cell that affect its particular behavior (migration, differentiation, apoptosis…). In this article we are interested on the second type of response, important for the possibilities of mechanically controlling cell fate.

Mechanosensors

The molecules or structures which may experience a conformational change and whose interaction with other molecules is consequently affected by mechanical forces are known as mechanosensors (see Table 1). In certain cases, the forces exerted on molecules are required to trigger biologically important reactions. Typically the response is of biochemical nature and some authors identify mechanotransduction with the translation of mechanical forces specifically into biochemical signals[70] although there are other possible responses. For instance, the mechanical stimuli are translated into electrical signals (change in the membrane potential due to ion flow) in the case of the ion channels in cochlear hair cells in response to vibrations, resulting in neuronal signaling and sound sensing.[20] In the case of a force translated into a chemical signal, which in some cases regulates gene expression, the term mechanotransduction signaling cascade is commonly used. There is a great variety of mechanosensors, including ion channels and cytoskeletal proteins and assemblies of proteins: ion channel permeability, binding affinity of proteins and dissociation rates of molecular assemblies depend on the applied forces. Yet the sensing mechanisms of numerous mechanosensors are poorly understood and the whole set of participating mechanosensors is not identified in many mechanotransduction-driven phenomena.

Mechanosensitive ion channels exist on the plasma membrane of all cells and their function depends on the stimulus[60]: specialized mechanoreceptors, for instance cochlear hair cells, need specific good matching of the mechanical impedance to couple ion channels and external forces, while in general mechanosensitive ion channels are stimulated by stress in the plasma membrane. The only requirement for the transduction of a mechanical stimulus into an ion current is a change of the probabilities of the two possible states of the channel, e.g., the probability of the open state is increased and the probability of the closed state is reduced, resulting in an increased flow of ions. Piezo proteins, identified in recent years, form mechanosensitive channels[13]: Piezo1 has roles in various physiological processes, including controlling cell differentiation,[50] and Piezo2 participates in gentle touch sensing.[64] Nevertheless, all ion channels, including ligand-gated or voltage-gated channels, are mechanically sensitive.[43] For instance, it was shown, in several cell types, that deformation increases intracellular levels of Ca^{2+},[68] which activates molecules participating in the mechanotransduction signaling cascades.[28] Important examples where Ca^{2+} mechanosensitive ion channels contribute significantly to mechanically induce intracellular Ca^{2+} increase (reviewed in detail by Weinbaum et al.[85]) are kidney epithelial cells and also osteocytes, which serve as mechanical receptors contributing to remodeling and homeostasis of bone.[25]

Focal adhesions (FA) are sites with the strongest interactions between the cell and the extracellular matrix, linking the surrounding environment and the cytoskeleton in the cell. Already in initial studies, FA proteins were proposed to contribute to the mechanotransduction process.[65] In FA, integrins are the heterodimeric proteins connecting both sides of the plasma membrane. Many other proteins participate in focal adhesions,[65] including structural proteins (like talin, paxillin, zyxin and vinculin) and also signaling proteins (kinases like tyrosine kinase Src, integrin-linked kinase (ILK), focal adhesion kinase (FAK), and phosphatases like receptor-like tyrosine phosphatase α (RPTP-α)). As an example, Rho GTP-ases, which control actin-filament organization, can be regulated by activation of Src or FAK.[45] The structural proteins link other FA proteins and the actin cytoskeleton. Mechanotransduction at the FA typically takes place through exposing, by molecular stretching of structural proteins, cryptic sites of target molecules participating in the signaling pathways.[83] For instance, stretching of talin results in the unfolding of the rod domain, exposing cryptic binding sites for vinculin and consequently recruiting vinculin.[14] Vinculin may transmit force at FA, stabilizing the connection between FA and actin cytoskeleton, and affects FA assembly and disassembly.[21] Similarly, stretching p130Cas (a substrate of Src kinase) increases its exposure to Src family kinases.[63] FA may also participate in mechanotransduction in the extracellular matrix; for instance, cellular forces can deform the fibronectin molecule to expose binding sites, triggering fibrillogenesis.[71] In a similar way, in the case of cell–cell adherens junctions,

TABLE 1. Examples of mechanosensing proteins and the effect of their conformational change by mechanical deformation.

Localization	Molecular effect of mechanical deformation		
	Different affinity for ligands	Stabilization or dissociation	Other
Extracellular matrix			
Fibronectin[71]: stretching exposes the sites required for its fibrillogenesis	√		
Membrane proteins and complexes			
Talin[14] at focal adhesions: stretching unfolds the rod domain of talin, recruiting vinculin	√		
p130Cas[63] at focal adhesions: stretching increases its exposure to phosphorylation by Src family kinases	√		
Ion channels[60]: open or closed states depend on deformation			√
Cytoskeleton			
Actin filament: stabilized under tension, inhibited cofilin binding[22] and enhanced binding to non-muscle myosin II[81]	√	√	
Non-muscle myosin[58]: sliding speed depends of force			√
Titin,[40,56] filamin,[66]: stretching can reveal hidden protein binding sites	√		
Nucleus and nuclear envelope			
Lamin-A[77]: stabilized by tension, it plays an important role in cell differentiation		√	
Chromosomes[78]: gene expression dependent on nuclear shape	√		

cellular tension facilitates the binding of vinculin to a cryptic site in the protein α-catenin.[87]

The molecular connections between focal adhesions and actin filaments, main component of the cytoskeleton, allows a direct transmission of adhesion forces to the cytoplasmic structural molecules. Actin filaments themselves possess mechanosensing activity. It was demonstrated, *in vivo* and *in vitro*, that tensed actin filaments are more resistant than relaxed filaments to severing by cofilin.[22] Conversely, non-muscle myosin II shows improved binding to actin filaments when the filaments are under tension,[81] and myosin II density is higher in cellular areas of higher tension.[47,81] The integrity of the actin-filaments cytoskeleton is guaranteed by cross-linking proteins, which may also act as mechanosensors through conformation-dependent interactions with other proteins. One example is filamin: various studies have demonstrated that stretching of this cross-linking protein (for instance by actomyosin contractile forces) can expose binding sites.[66] The effect of cross-linking proteins on the mechanical behavior of the cell[54] may be related to protein-expression regulation and cell homeostasis.

The forces generated internally are crucial for the mechanical behavior of the cell and for mechanotransduction. Myosins are motor proteins which contribute predominantly to generate intracellular tension. In particular non-muscle myosin II, already mentioned, arranges in filaments that cross-link actin filaments and may produce contractile forces by pulling on actin filaments with opposite polarities. Mainly due to this contractility, non-muscle myosin II is of paramount importance for the mechanical behavior of the cell. It is downstream of convergent signaling pathways[82] and is vital to control cell adhesion and migration. Additionally non-muscle myosin II activates other proteins as described below. Moreover, myosin itself is a mechanosensor: for individual molecules[58] and for the whole cytoskeleton,[53] it has been shown that the myosin sliding speed on actin filaments depends on the applied force. This dependency contributes to the control of processes like symmetrical cell division.[58]

In muscle fibers, contraction is produced by sliding of muscle myosin motor domains on the surface of myosin filaments along the thin actin filaments in sarcomeres. Myosin filaments are linked to titin molecules, which may be significantly contracted during the muscle contraction. Titin is the largest protein known to date (3–4 MDa),[39] it can bind a multitude of ligands and acts as a mechanosensor, sensing sarcomere strain and regulating protein expression in muscle fibers.[40,56]

Both focal adhesions and internally-generated tension are determinant in cell motility. Focal adhesions are more stable under tension,[51] by molecular mechanisms not totally understood. Besides, cells typically generate higher tractions in stiffer environments and consequently FA become more stable on stiffer substrates,[51] resulting in a natural progressive displacement of cells towards stiffer substrates[41] known as durotaxis. The correlation between the extracellular-

matrix stiffness and the internally-generated tension has been explained by pointing out that in the case of a compliant matrix the shortening of the contractile apparatus is more important, reducing the force that in can be generated, due in part to the internal friction.[48] Furthermore, myosin activation is controlled by tension-dependent signaling cascades that initiate at FA proteins (see below).

The LINC complexes, linking the cytoskeleton and nucleoskeletal proteins (lamins), contribute to the transmission of mechanical signals to the nucleus. Swift et al.[77] showed that lamin-A filaments, present in the nuclear lanima, are mechanosensors: the results suggest that tension inhibits turnover of these filaments. They found that the concentration of lamin-A correlates with the stiffness of the tissue. Previously the same group had revealed that the tension in the cells grows with the stiffness of the extracellular matrix.[18] Lamin-A would contribute to matrix-stiffness-directed differentiation of stem cells: on one hand, the steady-state lamin-A levels co-regulate factors key in development through tissue-specific gene expression, including serum response factor (SRF), which regulates expression of proteins associated to stress fibers, and YAP1, involved in cell proliferation. On the other hand, the steady-state lamin-A level would be the result of two opposed effects: (i) tension stabilizes lamin-A by reducing its phosphorylation and (ii) lamin-A expression is regulated by the retinoic-acid signaling pathway, but lamin-A translocates retinoic acid receptor gamma (RARG) into nuclei, which reduces the expression level of lamin-A. A different study by Philip et al. showed that high shear stress resulted in upregulation and reorganization of lamins on the nuclear boundary.[52]

Forces applied to the nucleus may modify the conformation or the packing density of DNA, thus influencing gene expression, or even compromise DNA integrity. Therefore chromosome may also be seen as a mechanosensing molecule. In fact, gene expression, in particular of collagen I, is dependent on the nuclear shape.[78]

The arising picture, with the previous ideas, is a system of mechanosensors connected both in series and in parallel (see Fig. 1): mechanosensing molecules in the extracellular matrix, in the focal adhesions or in the cell–cell junctions, in the cytoskeleton, in the nuclear lamina and, finally, DNA. Because these molecules are all, more or less rigidly, connected by molecular interactions (being the actomyosin cytoskeleton the main component putting all together), various mechanosensors may be activated by the same external mechanical load, or by the internally generated tension (mainly by the actomyosin cytoskeleton). The ion channels are more compliantly connected to the other mechanosensors by the plasma membrane. Additionally the cytoplasm provides an additional fluidic connection.

Mechanotransduction Signaling Cascades

Several signaling cascades known to be regulated by mechanosensors are represented schematically in Fig. 1. For instance, we mentioned above that focal-adhesion proteins may trigger signaling cascades regulating stability of actin filaments and contractile activity of myosin: focal-adhesion kinase (FAK) is activated and localizes at focal adhesions and induces Rho GTP-ase down-regulation[59]; Rho binds and activates Rho-kinase (ROCK). ROCK is an effector of various proteins,[2] in particular it activates LIMK, which produces cofilin phosphorylation resulting in inactivation of its actin depolymerizing activity. ROCK also phosphorylates, therefore inactivates, MLC-phosphatase and presumably may phosphorylate directly myosin light chain (MLC).[2] Phosphorylation of MLC promotes formation of myosin II filaments and activates its ATP-ase activity. Extracellular signal-regulated kinases (ERKs) may participate in different signaling cascades.

Many of the molecules regulating cell functions have been identified; however, fundamental aspects of the control mechanisms remain incompletely understood. Cell fate decisions, including differentiation and apoptosis, depend on complex, spatiotemporally coordinated, networks of proteins.[35] An important reason for the difficulties identifying the mechanotransduction signaling cascades that are activated by a given mechanosensor may be the difficulty of developing experiments in which forces are applied exclusively on the mechanosensor of interest. For example, an experiment focusing on applying forces on integrin receptors may also produce a deformation on mechanosensitive ion channels.

EFFECTS OF MECHANICAL STIMULI ON CELL FATE

Mechanical stimuli are important for remodeling and homeostasis of the structural tissues in our body. For instance, bone adapts its form to the varying mechanical loads through the mechanosensing activity of osteocytes[25]; chondrocytes, the cells in articular cartilage, sense loading and osteoarthritic chondrocytes display altered mechanosensitivity compared with healthy chondrocytes[30]; skeletal muscle also remodels and several proteins act as mechanosensors, including titin, already mentioned above.

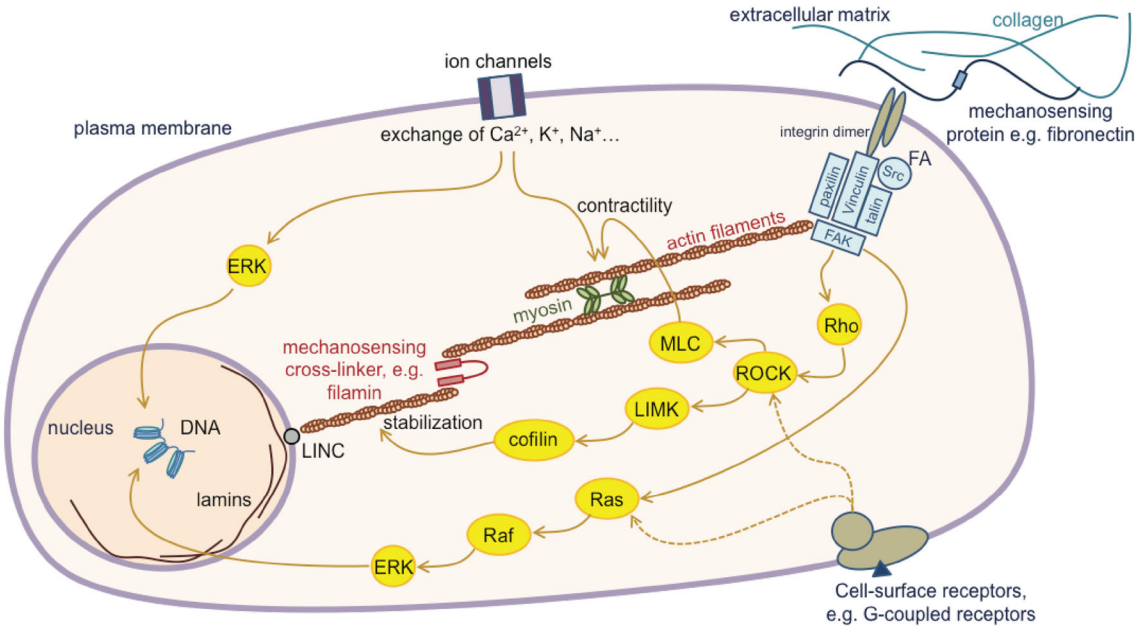

FIGURE 1. Schematic representation of mechanosensors and signaling cascades. The molecular network including DNA, lamins, LINC complex, cytoskeleton and focal adhesions interconnect mechanically the mechanosensors. Ion channels are less rigidly connected to that network by the plasma membrane. The cytoplasm provides an additional connection between mechanosensors, which may be important under certain conditions. The activity of molecules participating in signaling cascades may be regulated by mechanosensors: the figure illustrates schematically cascades with the participation of Rho GTP-ase, the Ras/Raf/ERK signaling pathway and extracellular-regulated kinases (ERKs).

Regarding the influence on cell fate, matrix stiffness and forces acting on a cell may determine gene expression and cell differentiation and can be used for promoting cell death. Therefore mechanotransduction effects are particularly of interest in the fields of controlling stem-cell differentiation and inducing cancer-cell death. Besides, improving our understanding of the role of mechanotransduction in neurons appears as a promising research direction to treat some diseases of the peripheral and the central nervous system. In this frame, two important, transferable to the clinic, ways of applying dynamic loads are ultrasounds and magnetic nanoparticles.

Stem Cells

Adult stem cells, which can, by asymmetric division, renew and differentiate to the specific cells of the tissues they are located in, have been identified in many tissues, including hematopoietic and mesenchymal stem cells (MSCs) in the bone marrow and endothelial stem cells.[79] It is expected that stem cells will be useful in regenerative-medicine therapies. Nevertheless, the capability to control differentiation of these cells is presently limited. It is well established that mechanical stimuli may be as determinant as chemical factors to direct the differentiation process.[17,18] In this direction, the results by Engler et al.,[18] showing the effect of the substrate rigidity on human mesenchymal stem cell differentiation, are remarkable. A simplistic summary is that soft substrates are neurogenic while hard substrates are osteogenic. The mechanosensing mechanisms underlying the effect of the substrate/extracellular matrix rigidity on differentiation are not well understood, and the same group of researchers have proposed that the nuclear envelope, in particular lamin-A, would play a key role,[77] acting as a mechanosensor as explained in the previous paragraphs. They also found that, during the first stages of the differentiation, the anisotropy of the actin stress fibers in MSCs is dependent on the rigidity of the substrates, obtaining the higher anisotropy for cells on substrates of intermediate rigidity.[90] In a different study, it was found that the cytoskeleton structure and the adhesion strength of myoblasts (progenitor cells that differentiate to myocytes) depend on the substrate stiffness, being the adhesion strength higher in stiffer substrates.[88]

Cardiac mesoangioblasts are progenitors that can partially restore the heart tissue after infarction. Low-intensity pulsed ultra sounds (LIPUS, 5 MHz) stimulation of these cells (both from mouse and human heart) produces cellular changes with advantageous effects, including higher deformability and enhanced motility.[5] These changes comprise cytoskeleton reorganization, resulting in more anisotropic stress fibers

distribution. Interestingly, this organization is similar to the effect described above for stem cells on substrates with adequate rigidity. Focal-adhesion proteins were proposed as the main mechanosensors contributing to this effect of ultrasounds.[55] Furthermore, LIPUS may produce also a beneficial effect on osteogenic differentiation of MSCs.[80] As another example of the effect of vibrations, it was found that application of nanometer-scale displacements, with a frequency of 1 kHz (i.e., in the acoustic range), to MSC cells results in osteogenic gene expression.[46] In the study RhoA was found to play a central role. Vibrations also in the acoustic range, with a frequency of 10 kHz, were found to accelerate the differentiation of rat pheochromocytoma cells.[27]

Magnetic beads have been used to apply alternating forces (1 hourly, loading at a frequency of 1 Hz and 1–100 pN/particle during one week) both *in vitro* (human MSCs) and *in vivo* (mice), coating the beads with antibodies to the potassium channel TREK-1 or RGD peptides, and resulting in enhanced production of extracellular matrix molecules, showing the possibility of inducing the differentiation of osteoprogenitor cells toward osteogenic cells.[32]

In vitro, neural stem cells differentiate into neurons, astrocytes or oligodendrocytes depending on soluble factors and on molecular environment and mechanical cues. Substrate rigidity and surface topography are important for neuron differentiation. As mentioned previously, studies by Discher and coworkers demonstrated that the rigidity of substrates directs cell differentiation; in particular compliant matrices are neurogenic.[18] The stiffness of brain tissue is not homogeneous and it varies with age, ranging between 0.1 to 10 kPa.[9] Astrocytic differentiation is improved in stiff substrates (1.5–75 kPa) by activation of RhoA and Cdc42, and inducing higher actomyosin-contraction tension. Contrarily neurogenesis and oligodendrogenesis are favored by soft substrates (0.1–0.7 Pa) or by inhibiting RhoA and Cdc42.[34] Sun et al. showed the acceleration of motor neuron induction of human pluripotent stem cells by using a more flexible substrate when compared with standard culture dishes.[74] In that work the mechanoregulation was found to occur through stiffness-dependent Hippo/YAP and actomyosin contractility. Complementarily to the stiffness of the substrate, mechanical deformations are also important for neural stem cell differentiation. Arulmoli et al. found that an equibiaxial static 10% stretching specifically influences the differentiation of neural stem/progenitor cells into oligodendrocytes, but it does not affect neurons or astrocytes.[3] Additionally, the mechanical environment plays a role through the geometry; Lu et al. showed that the groove pitch of a microgrooving pattern influences the efficiency of differentiation of human embryonic stem cells into neurons.[42]

Cancer Cells

Tumor tissue is generally stiffer than normal tissue,[49] however metastatic cells are typically more deformable than normal cells.[15] In this regard, the increased cancer cell deformability was correlated with the invasiveness for patient-derived ovarian cancer cells.[76] The higher deformability of cancer cells, that could be the result of a process of cell selection based on the ability to intravasate into vessels,[15] is associated to cytoskeletal changes.[75] An elevated cytoskeletal tension and extracellular matrix stiffening by collagen crosslinking, resulting in reinforced focal adhesions, have been shown to produce a significantly high rigidity of breast-cancer tumors.[49] In this case, it was proposed that the Rho/ROCK pathway is activated by mechanosensation of tissue stiffness, and this would raise the tension produced by the actomyosin cytoskeleton.[62]

Among the many efforts undertaken to develop new strategies to induce death of cancer cells, some works have consisted of developing alternative systems for biochemical induction of apoptosis, with the aim of replacing apoptosis-inducing ligands which are easily degraded.[33] Magnetic nanoparticles were used to mechanically cluster death receptor 4 (DR4), building a death-inducing signaling complex (DISC) and inducing apoptosis[11] both in cultured colon-cancer cells and in zebrafish. Furthermore, biofunctionalized magnetic microdiscs were used to apply an oscillatory deformation (in an alternating magnetic field with an amplitude of the order of 10 Oe, with a frequency of tens of Hz, during 10 min) on human glioma cells, producing direct cell death by compromising the integrity of the plasma membrane or inducing apoptosis.[37] Magnetic nanoparticles were also used to disrupt lysosomes in human pancreatic beta cells, by covalently conjugating the nanoparticles with antibodies targeting lysosomal markers and applying a rotating magnetic field (with an amplitude of 30 mT at a frequency of 20 Hz during 20 min), which produced an increased expression of apoptotic markers and impaired cell growth.[91]

We used MNPs functionalized with epidermal growth factor (EGF) for targeting cancer cells. The MNPs were internalized in lysosomes and later they assembled by effect of an external magnetic field, to form elongated aggregates that may produce forces significantly larger than the forces produced by single MNPs[69]: a rotating magnetic field of magnitude 40 mT and a frequency of 15 Hz generated forces of hundreds of pN producing dramatic damages to plasma and

lysosomal membranes. It led to necrosis and programmed cell death.

Neurons

The normal function of the brain includes endogenous forces and mechanotransduction.[23] There is a growing interest in improving our understanding of the mechanotransduction contribution to neural tissue homeostasis, neuron development and brain pathologies.

Axonal growth is related to the chemical-probing activity of growth cones, which are present at the tip of growing axons. The chemical environment regulates the dynamics of actin filaments and microtubules, which determines the forces exerted by the cones and the kinematical evolution of the axon tip.[31] In the case of trauma, during the process of endogenous regeneration in the peripheral nerve system or in the spinal cord, the axons may encounter mechanical barriers for their growth. Particularly, glial scarring at the site of the lesion may constitute simultaneously a physical barrier and a chemical barrier for the axons by the production of axon repellents, and it has been proposed that axonal growth could be enhanced by MNP-enabled stretching of the axons.[36] In this regard, it was shown several decades ago the possibility of accelerating the axonal growth by stretching[7] and, more recently, the combination of magnetic twining and chemical inhibition of specific molecular motors made possible the axonal growth against axonal repellents,[36] with the particularity that smaller magnetic beads resulted in higher elongation rates, which is in agreement with enhanced activation of membrane mechanosensors by smaller beads.

In the case of brain stroke, the lesion cavity is a hostile environment for cell growth, which results in a serious loss of grafted cells that could contribute to recovery.[5] Currently neural stem cells and biomaterials, particularly hydrogels, are studied for possible therapeutic solutions. The abovementioned enhanced axonal growth by magnetic twining suggests that the combination of biomaterials, stem cells and magnetic twining by MNPs, could constitute a promising therapeutic treatment.

Hemphill et al. proposed that pathological mechanotransduction could play an important role in traumatic brain injury, suggesting a research direction to identify mechanosensors that could induce secondary damage following traumatic brain injury.[23] Moreover, it has been proposed that age-related changes may affect the ability of cells for mechanosensing,[86] though their effect on neurodegenerative diseases is not well understood. In this regard, mechanosensitive cation channels are particularly important, as Ca^{2+} flux regulates neurite and axon growth, synapse, synaptic plasticity and gene expression.[86]

Blumenthal et al. discovered that the nanoroughness in the tissue is important for function of neurons and differentiation of neural stem cells, and that neurons require mechanosensitive cation channels to probe nanoroughness.[6] Aging of membrane/lipid rafts, components of the plasma membrane that contribute to mechanotransduction, has been identified as a likely factor in the conversion of amyloid precursor protein to the Aβ peptide,[19] whose accumulation in the extracellular space is related to Alzheimer's disease. The role played by nanoroughness on the normal function of neurons, and the loss of that feature by aging, suggests that nanoparticles with adequate size and specific binding ability could contribute to restore the roughness associated with a healthy brain. These nanoparticles could play complementary roles like magnetomechanical stimulation for axonal growth.

NANOPARTICLE-ENABLED MECHANICAL STIMULI

Magnetic Nanoparticles for Biomedical Applications

Nanoparticles (NPs) are commonly defined as particles with sizes between 1 and 100 nm in two or three dimensions. Their physicochemical properties depend importantly on the large specific surface area and, therefore, the conventional bulk physicochemical rules may not be fully applicable at the nano-scale. Typically the magnetic nanoparticles are combined with the molecules of interest on their surface to obtain specific interactions. Magnetic nanoparticles (MNPs) have received a huge interest in diagnostics, particularly for magnetic resonance imaging (MRI), and therapeutics, including their application in hyperthermia, drug delivery and cellular signaling control. In the case of hyperthermia, magnetic nanoparticles are exposed to an alternating magnetic field and transform magnetic energy into heat by oscillating the magnetic moment of each nanoparticle. The resulting local hyperthermia can disrupt the membrane of the target cancer cells or their intracellular structures.[4] The use of MNPs in guided drug delivery is based on the application of an external magnetic field to force MNPs to accumulate preferentially in a tissue of interest, as a tumor, facilitating the localized drug delivery. In the case of cell-signaling control, it is possible to use the MNPs to carry signaling molecules or to introduce mechanical stimuli. The latter will be described in detail below.

Various materials may be used to produce NPs, including polymers and inorganic materials, in partic-

ular metals and metal oxides.[8] Iron oxide nanoparticles (IONPs) are extensively used in biomedical applications. For example, IONPs are used as MRI contrast agents,[1] for cell tracking on tumor targeting and imaging,[12] for protein separation,[67] for magnetic field-directed drug targeting to tumors across the blood–brain barrier (BBB),[57] and for anticancer treatment based on targeted magnetic hyperthermia.[61] IONPs are considered biodegradable and non-toxic and can be useful in photodynamic cancer treatment. In order to improve the magnetic properties of IONPs, dopants are used to produce spinel ferrites MFe_2O_4 (with M = Mn, Fe, Co, Ni, Zn).[29]

These materials are ferromagnetic, i.e., they may acquire a permanent magnetization. Contrarily, in paramagnetic materials the magnetization is negligible for a null external magnetic field. A particle of ferromagnetic material acquires superparamagnetic behavior when the size is smaller than a transition size. For instance, the transition size is approximately 25 and 30 nm respectively for particles of Fe_3O_4 and Fe_2O_3.[38]

A different way to produce magnetic nanoparticles would be to induce directly mineralization in the interior of the cells, using proteins as a template. In a recent work, Matsumoto el al. performed a genetic screening in yeast cells, selecting variants of the iron-storage ferritin with greater accumulation of iron.[44] New developments in this direction, probably combined with genetic manipulations, could result in a new way to efficiently produce magnetic nanoparticles with various applications.

Mechanical Stimulation by Magnetic Nanoparticles

Ferromagnetic nanoparticles can be permanently magnetized by a magnetic field pulse and it is possible to rotate the particles in a controlled manner when the particles are exposed to a rotating magnetic field. The torque required to twist the nanoparticles can be computed, so it is possible to investigate the mechanical stimuli on the cells. For non-homogeneous magnetic fields, it is also possible to produce a force in the direction of the gradient of the magnetic field. In this case, an oscillating magnetic field results in an oscillating force on the nanoparticle. Both kinds of mechanical effects (see Figs. 2a–2b) have been used to mechanically stimulate cells. The relevant magnetic parameter to compute the mechanical effect by a magnetic field B on a magnetic nanoparticle is the magnetic moment $\vec{m}$, proportional to the applied field in linear isotropic paramagnetic materials and equal to the volume times the remanent magnetization for permanently magnetized ferromagnetic nanoparticles. The torque $\vec{\tau}$ produced by the magnetic field is given by[89]

$$\vec{\tau} = \vec{m} \times \vec{B} \tag{1}$$

Additionally, if there is a gradient of the magnetic field, then there is a non-zero magnetic force $\vec{F}$ that may be computed, both for permanently magnetized nanoparticles and linear paramagnetic nanoparticles, as[89]

$$\vec{F} = \left(\vec{m} \cdot \vec{\nabla}\right)\vec{B} \tag{2}$$

MNPs have been used to control cell signaling. Cell responds not only to chemical stimuli, but also to mechanical stimuli, as described previously in this article. Such physical stimuli, by means of magnetic, electrical and optical methods, have been used to control cell signaling. Mechanics are important to pathological and numerous physiological processes, especially for cancer.

In biomedical applications of forced MNP vibration, intensity of MNPs' vibration and thermal damage depend on the geometry of MNPs. Additionally to the therapeutic effect by mechanically stimulating the cells, MNPs may also provide image contrast, serve as drug delivery platforms, and hyperthermia. By oscillation of the magnetic nanoparticles in an alternating magnetic field, it is possible to induce physical destruction of cancer cells and control ion channel or surface receptor cells by transforming energy into mechanical forces or heat, though when nanomaterials link to the cell membrane or are internalized, the heating capability reduces significantly.[16] Cheng et al. produced rod-shaped and spherical Fe_3O_4 MNP. They applied alternating magnetic field to damage the cell membrane or cytoplasm.[10] In that work, due to the relatively low power and low frequency, neither type of MNPs produced a temperature increase.

The most obvious application of MNPs for cell-killing is the direct damage of the integrity of the cell membrane or the cytoplasm, as described previously. Conversely, it is also possible to induce apoptosis by mechanical stimulation of the signaling pathways. Zhang et al. modified iron oxide MNPs with antibodies to target a lysosomal protein marker and demonstrated that the combination of a dynamic magnetic field and lysosome-targeted nanoparticles provides a noninvasive method to induce apoptosis.[91] In that work, a rotating magnetic field produced rotation of the MNPs bound to the lysosomal membrane, thus disrupting the lysosomal membrane and resulting in acidification of the cytoplasm and apoptosis. Cho et al. demonstrated that zinc-iron oxide MNPs ($Zn_{0.4}Fe_{2.6}O_4$), coated with death-receptor-4 antibody could cluster when a permanent magnetic field was

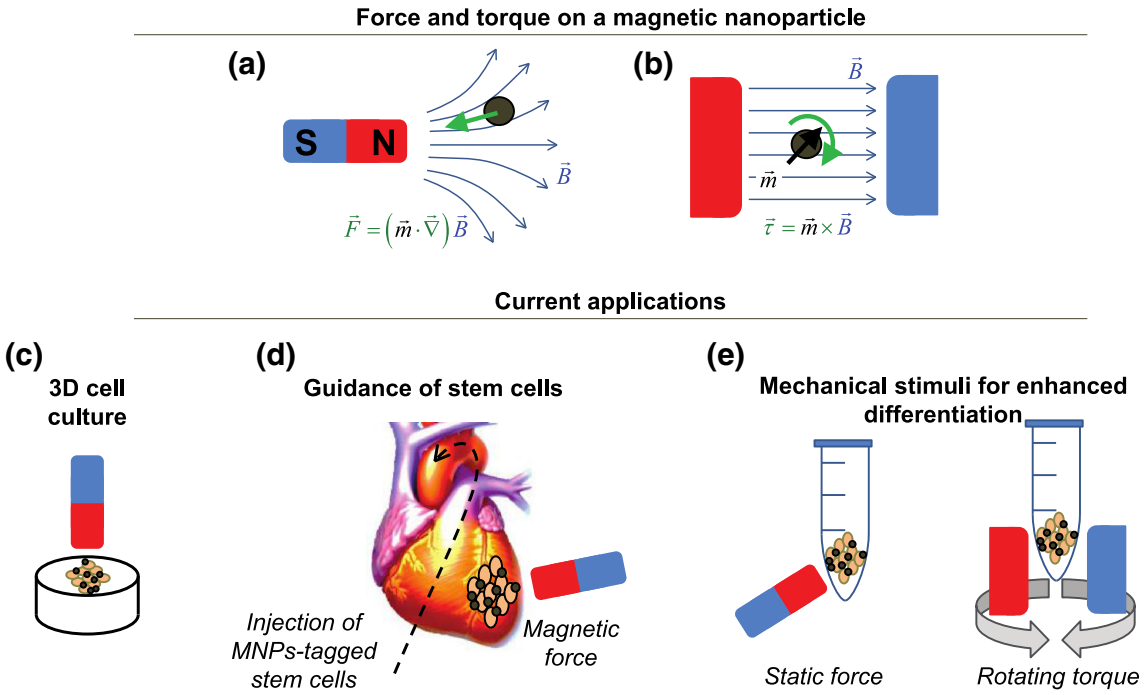

FIGURE 2. Biomedical applications of magnetic forces and torques on magnetic nanoparticles. (a) A force is produced by a non-homogeneous magnetic field, (b) the torque is non-zero if the magnetic dipolar moment is not aligned with the magnetic field. Current applications for stem cells include (c) 3D stem cell cultures, (d) guidance of MNPs-tagged stem cells and (e) enhancement of the differentiation process of MSC, by applying static or dynamic forces.

applied and, by receptor aggregation, induced apoptotic morphological changes in zebrafish.[11]

In the field of stem cells, MNPs have been used to produce 3D cell cultures[73]: the application of a magnetic field results in the aggregation of MNP-tagged cells since the magnetized MNPs tend to aggregate and by controlling the magnetic field it is possible to control the shape of the cell mass. Henstock et al. combined the use of bone morphogenetic protein 2 (BMP2) and MNPs in a culture of human mesenchymal stem cells (hMSCs) on a collagen gel or on a bone tissue model. By applying an oscillating magnetic field with an amplitude of 25 mT that produced a 4-pN force on each NP, an increase in the extension of mineralization was observed.[24] In a recent publication, Son et al. also reported an enhancement of the differentiation process: they used MNPs isolated from *Magnetospirillum* sp. to enhance the differentiation into chondrocytes of bone marrow derived hMSCs,[72] by applying static magnetic fields or magnet-derived shear stresses.

PERSPECTIVE

The ensemble of cell mechanosensors is incompletely known. As described in the previous sections, the use of magnetic nanoparticles and magnetic fields is a potential technique to finely tune the mechanical stimulation of cells, which can be used to study the role of the different mechanosensors and to control the fate of stem cells, cancer cells and homeostasis of tissues, as exemplified in the case of neural tissue.

Recently, MNP stimulation has been shown to be useful to enhance MSC differentiation into chondrocytes or into bone cells. In both cases the mechanical stimulus was combined with other conditions that would promote differentiation by themselves. Therefore the combination of the MNPs approach and other differentiation-inducing techniques is taking the first steps to develop optimized therapeutic pathways. The value of this approach to enhance the differentiation process is complemented by the possibility of regulating the internal healing process in the clinic, simply through the use of external magnetic fields. The future possibilities of applying the MNPs approach to control the differentiation process of stem cells are beyond the enhancement of current biophysicochemical approaches, because of the possibility of targeting specific components of the cell and accurately controlling the magnetic forces. Therefore the approach is potentially applicable to accurately guiding the stem cell differentiation process independently of other techniques.

The possibilities of inducing cancer-cell death of even producing mechanical destruction by means of MNPs open new ways to treat cancers with unknown effective drugs. The less studied possibilities of treating

neural diseases by MNP-enabled mechanical actuation could require extensive studies to reveal the poorly understood mechanical effects in neurons. A vast and exciting field of research is waiting ahead.

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REFERENCES

[1]Ahamed, M., M. S. AlSalhi, and M. K. J. Siddiqui. Silver nanoparticle applications and human health. *Clin. Chim. Acta* 411:1841–1848, 2010.

[2]Amano, M., M. Nakayama, and K. Kaibuchi. Rho-kinase/ROCK: a key regulator of the cytoskeleton and cell polarity. *Cytoskeleton* 67:545–554, 2010.

[3]Arulmoli, J., M. M. Pathak, L. P. McDonnell, J. L. Nourse, F. Tombola, J. C. Earthman, and L. A. Flanagan. Static stretch affects neural stem cell differentiation in an extracellular matrix-dependent manner. *Sci. Rep.* 5:8499, 2015.

[4]Asin, L., M. R. Ibarra, A. Tres, and G. F. Goya. Controlled cell death by magnetic hyperthermia: effects of exposure time, field amplitude, and nanoparticle concentration. *Pharm. Res.* 29:1319–1327, 2012.

[5]Bernal, A., L. M. Perez, B. De Lucas, N. S. Martin, A. Kadow-Romacker, G. Plaza, K. Raum, and B. G. Galvez. Low-intensity pulsed ultrasound improves the functional properties of cardiac mesoangioblasts. *Stem Cell Rev.* 11:852–865, 2015.

[6]Blumenthal, N. R., O. Hermanson, B. Heimrich, and V. P. Shastri. Stochastic nanoroughness modulates neuron-astrocyte interactions and function via mechanosensing cation channels. *Proc. Natl. Acad. Sci. USA* 111:16124–16129, 2014.

[7]Bray, D. Axonal growth in response to experimentally applied nechanical tension. *Dev. Biol.* 102:379–389, 1984.

[8]Chakraborty, M., S. Jain, and V. Rani. Nanotechnology: emerging tool for diagnostics and therapeutics. *Appl. Biochem. Biotechnol.* 165:1178–1187, 2011.

[9]Chatelin, S., A. Constantinesco, and R. Willinger. Fifty years of brain tissue mechanical testing: from in vitro to in vivo investigations. *Biorheology* 47:255–276, 2010.

[10]Cheng, D., X. Li, G. Zhang, and H. Shi. Morphological effect of oscillating magnetic nanoparticles in killing tumor cells. *Nanoscale Res. Lett.* 9:195, 2014.

[11]Cho, M. H., E. J. Lee, M. Son, J. Lee, D. Yoo, J. Kim, S. W. Park, J. Shin, and J. Cheon. A magnetic switch for the control of cell death signalling in in vitro and in vivo systems. *Nat. Mater.* 11:1038–1043, 2012.

[12]Choi, W. I., J. Kim, S. U. Heo, Y. Y. Jeong, Y. H. Kim, and G. Tae. The effect of mechanical properties of iron oxide nanoparticle-loaded functional nano-carrier on tumor targeting and imaging. *J. Controlled Release* 162:267–275, 2012.

[13]Coste, B., J. Mathur, M. Schmidt, T. J. Earley, S. Ranade, M. J. Petrus, A. E. Dubin, and A. Patapoutian. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* 330:55–60, 2010.

[14]del Rio, A., R. Perez-Jimenez, R. Liu, P. Roca-Cusachs, J. M. Fernandez, and M. P. Sheetz. Stretching single talin rod molecules activates vinculin binding. *Science* 323:638–641, 2009.

[15]Di Carlo, D. A mechanical biomarker of cell state in medicine. *J. Lab. Autom.* 17:32–42, 2012.

[16]Di Corato, R., A. Espinosa, L. Lartigue, M. Tharaud, S. Chat, T. Pellegrino, C. Menager, F. Gazeau, and C. Wilhelm. Magnetic hyperthermia efficiency in the cellular environment for different nanoparticle designs. *Biomaterials* 35:6400–6411, 2014.

[17]Discher, D. E., P. Janmey, and Y. L. Wang. Tissue cells feel and respond to the stiffness of their substrate. *Science* 310:1139–1143, 2005.

[18]Engler, A. J., S. Sen, H. L. Sweeney, and D. E. Discher. Matrix elasticity directs stem cell lineage specification. *Cell* 126:677–689, 2006.

[19]Evangelisti, E., D. Wright, M. Zampagni, R. Cascella, C. Fiorillo, S. Bagnoli, A. Relini, D. Nichino, T. Scartabelli, and B. Nacmias. Lipid rafts mediate amyloid-induced calcium dyshomeostasis and oxidative stress in Alzheimer's disease. *Curr. Alzheimer Res.* 10:143–153, 2013.

[20]Fettiplace, R., and C. M. Hackney. The sensory and motor roles of auditory hair cells. *Nat. Rev. Neurosci.* 7:19–29, 2006.

[21]Grashoff, C., B. D. Hoffman, M. D. Brenner, R. Zhou, M. Parsons, M. T. Yang, M. A. McLean, S. G. Sligar, C. S. Chen, T. Ha, and M. A. Schwartz. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature* 466:263–266, 2010.

[22]Hayakawa, K., H. Tatsumi, and M. Sokabe. Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament. *J. Cell Biol.* 195:721–727, 2011.

[23]Hemphill, M. A., S. Dauth, C. J. Yu, B. E. Dabiri, and K. K. Parker. Traumatic brain injury and the neuronal microenvironment: a potential role for neuropathological mechanotransduction. *Neuron* 85:1177–1192, 2015.

[24]Henstock, J. R., M. Rotherham, H. Rashidi, K. M. Shakesheff, and A. J. El Haj. Remotely activated mechanotransduction via magnetic nanoparticles promotes mineralization synergistically with bone morphogenetic protein 2: applications for injectable cell therapy. *Stem Cells Transl. Med.* 3:1363–1374, 2014.

[25]Huiskes, R., R. Ruimerman, G. H. van Lenthe, and J. D. Janssen. Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. *Nature* 405:704–706, 2000.

[26]Ingber, D. E. Cellular basis of mechanotransduction. *Biol. Bull.* 194:323–325, 1998.

[27]Ito, Y., T. Kimura, K. Nam, A. Katoh, T. Masuzawa, and A. Kishida. Effects of vibration on differentiation of cultured PC12 Cells. *Biotechnol. Bioeng.* 108:592–599, 2011.

[28]Iwasaki, H., S. Eguchi, H. Ueno, F. Marumo, and Y. Hirata. Mechanical stretch stimulates growth of vascular

smooth muscle cells via epidermal growth factor receptor. *Am. J. Physiol. Heart Circ. Physiol.* 278:H521–H529, 2000.
[29]Jun, Y., J. Lee, and J. Cheon. Chemical design of nanoparticle probes for high-performance magnetic resonance imaging. *Angew. Chem. Int. Ed.* 47:5122–5135, 2008.
[30]Jutila, A. A., D. L. Zignego, B. K. Hwang, J. K. Hilmer, T. Hamerly, C. A. Minor, S. T. Walk, and R. K. June. Candidate mediators of chondrocyte mechanotransduction via targeted and untargeted metabolomic measurements. *Arch. Biochem. Biophys.* 545:116–123, 2014.
[31]Kalil, K., and E. Dent. Touch and go: guidance cues signal to the growth cone cytoskelleton. *Curr. Opin. Neurobiol.* 15:521–526, 2005.
[32]Kanczler, J. M., H. S. Sura, J. Magnay, D. Green, R. O. C. Oreffo, J. P. Dobson, and A. J. El Haj. Controlled differentiation of human bone marrow stromal cells using magnetic nanoparticle technology. *Tissue Eng. Part A* 16:3241–3250, 2010.
[33]Kelley, S. K., and A. Ashkenazi. Targeting death receptors in cancer with Apo2/TRAIL. *Curr. Opin. Pharmacol.* 4:333–339, 2004.
[34]Keung, A. J., E. M. de Juan-Pardo, D. V. Schaffer, and S. Kumar. Rho GTPases mediate the mechanosensitive lineage commitment of neural stem cells. *Stem Cells* 29:1886–1897, 2011.
[35]Kholodenko, B. N. Cell-signalling dynamics in time and space. *Nat. Rev. Mol. Cell Biol.* 7:165–176, 2006.
[36]Kilinc, D., A. Blasiak, J. J. O'Mahony, and G. U. Lee. Low piconewton towing of CNS axons against diffusing and surface-bound repellents requires the inhibition of motor protein-associated pathways. *Sci. Rep.* 4:7128, 2014.
[37]Kim, D., E. A. Rozhkova, I. V. Ulasov, S. D. Bader, T. Rajh, M. S. Lesniak, and V. Novosad. Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction. *Nat. Mater.* 9:165–171, 2010.
[38]Kolhatkar, A. G., A. C. Jamison, D. Litvinov, R. C. Willson, and T. R. Lee. Tuning the magnetic properties of nanoparticles. *Int. J. Mol. Sci.* 14:15977–16009, 2013.
[39]Labeit, S., B. Kolmerer, and W. Linke. The giant protein titin: emerging roles in physiology and pathophysiology. *Circ. Res.* 80:290–294, 1997.
[40]Lange, S., F. Xiang, A. Yakovenko, A. Vihola, P. Hackman, E. Rostkova, J. Kristensen, B. Brandmeier, G. Franzen, B. Hedberg, L. Gunnarsson, S. Hughes, S. Marchand, T. Sejersen, I. Richard, L. Edstrom, E. Ehler, B. Udd, and M. Gautel. The kinase domain of titin controls muscle gene expression and protein turnover. *Science* 308:1599–1603, 2005.
[41]Lo, C. M., H. B. Wang, M. Dembo, and Y. L. Wang. Cell movement is guided by the rigidity of the substrate. *Biophys. J* . 79:144–152, 2000.
[42]Lu, D., C. Chen, C. Lai, S. Soni, T. Lam, C. Le, E. Y. Chen, T. Nguyen, and W. Chin. Microgrooved surface modulates neuron differentiation in human embryonic stem cells. *Hum. Embryonic Stem Cell Protoc.*, 2016. doi: 10.1007/7651_2014_81.
[43]Markin, V. S., and B. Martinac. Mechanosensitive ion channels as reporters of bilayer expansion: a theoretical-model. *Biophys. J* . 60:1120–1127, 1991.
[44]Matsumoto, Y., R. Chen, P. Anikeeva, and A. Jasanoff. Engineering intracellular biomineralization and biosensing by a magnetic protein. *Nat. Commun.* 6:8721, 2015.
[45]Mitra, S. K., and D. D. Schlaepfer. Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr. Opin. Cell Biol.* 18:516–523, 2006.
[46]Nikukar, H., S. Reid, P. M. Tsimbouri, M. O. Riehle, A. S. G. Curtis, and M. J. Dalby. Osteogenesis of mesenchymal stem cells by nanoscale mechanotransduction. *ACS Nano* 7:2758–2767, 2013.
[47]Nunnally, M. H., J. M. Dangelo, and S. W. Craig. Filamin concentration in cleavage furrow and midbody region: frequency of occurrence compared with that of alpha-actinin and myosin. *J. Cell Biol.* 87:219–226, 1980.
[48]Paluch, E. K., C. M. Nelson, N. Biais, B. Fabry, J. Moeller, B. L. Pruitt, C. Wollnik, G. Kudryasheva, F. Rehfeldt, and W. Federle. Mechanotransduction: use the force(s). *BMC Biol.* 13:47, 2015.
[49]Paszek, M. J., N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, D. A. Hammer, and V. M. Weaver. Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8:241–254, 2005.
[50]Pathak, M. M., J. L. Nourse, T. Tran, J. Hwe, J. Arulmoli, T. L. Dai Trang, E. Bernardis, L. A. Flanagan, and F. Tombola. Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. *Proc. Natl. Acad. Sci. USA* 111:16148–16153, 2014.
[51]Pelham, R., and Y. Wang. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* 94:13661–13665, 1997.
[52]Philip, J. T., and K. N. Dahl. Nuclear mechanotransduction: response of the lamina to extracellular stress with implications in aging. *J. Biomech.* 41:3164–3170, 2008.
[53]Plaza, G. R., and T. Q. P. Uyeda. Contraction speed of the actomyosin cytoskeleton in the absence of the cell membrane. *Soft Matter* 9:4390–4400, 2013.
[54]Plaza, G. R., T. Q. P. Uyeda, Z. Mirzaei, and C. A. Simmons. Study of the influence of actin-binding proteins using linear analyses of cell deformability. *Soft Matter* 11:5435–5446, 2015.
[55]Pounder, N. M., and A. J. Harrison. Low intensity pulsed ultrasound for fracture healing: a review of the clinical evidence and the associated biological mechanism of action. *Ultrasonics* 48:330–338, 2008.
[56]Puchner, E. M., A. Alexandrovich, A. L. Kho, U. Hensen, L. V. Schaefer, B. Brandmeier, F. Graeter, H. Grubmueller, H. E. Gaub, and M. Gautel. Mechanoenzymatics of titin kinase. *Proc. Natl. Acad. Sci. USA* 105:13385–13390, 2008.
[57]Qiao, R., Q. Jia, S. Huewel, R. Xia, T. Liu, F. Gao, H. Galla, and M. Gao. Receptor-mediated delivery of magnetic nanoparticles across the blood-brain barrier. *ACS Nano* 6:3304–3310, 2012.
[58]Ren, Y., J. C. Effler, M. Norstrom, T. Luo, R. A. Firtel, P. A. Iglesias, R. S. Rock, and D. N. Robinson. Mechanosensing through cooperative interactions between myosin II and the actin crosslinker cortexillin I. *Curr. Biol.* 19:1421–1428, 2009.
[59]Ren, X. D., W. B. Kiosses, D. J. Sieg, C. A. Otey, D. D. Schlaepfer, and M. A. Schwartz. Focal adhesion kinase suppresses Rho activity to promote focal adhesion turnover. *J. Cell Sci.* 113(Pt 20):3673–3678, 2000.
[60]Sachs, F. Stretch-activated ion channels: what are they? *Physiology* 25:50–56, 2010.
[61]Sadhukha, T., T. S. Wiedmann, and J. Panyam. Inhalable magnetic nanoparticles for targeted hyperthermia in lung cancer therapy. *Biomaterials* 34:5163–5171, 2013.
[62]Samuel, M. S., J. I. Lopez, E. J. McGhee, D. R. Croft, D. Strachan, P. Timpson, J. Munro, E. Schroeder, J. Zhou, V. G. Brunton, N. Barker, H. Clevers, O. J. Sansom, K. I.

Anderson, V. M. Weaver, and M. F. Olson. Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth. *Cancer Cell* 19:776–791, 2011.
[63]Sawada, Y., M. Tamada, B. J. Dubin-Thaler, O. Cherniavskaya, R. Sakai, S. Tanaka, and M. P. Sheetz. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. *Cell* 127:1015–1026, 2006.
[64]Schrenk-Siemens, K., H. Wende, V. Prato, K. Song, C. Rostock, A. Loewer, J. Utikal, G. R. Lewin, S. G. Lechner, and J. Siemens. PIEZO2 is required for mechanotransduction in human stem cell-derived touch receptors. *Nat. Neurosci*. 18:10–16, 2015.
[65]Seong, J., N. Wang, and Y. Wang. Mechanotransduction at focal adhesions: from physiology to cancer development. *J. Cell Mol. Med*. 17:597–604, 2013.
[66]Seppala, J., H. Tossavainen, N. Rodic, P. Permi, U. Pentikainen, and J. Ylanne. Flexible structure of peptide-bound filamin a mechanosensor domain pair 20-21. *PLoS ONE* 10:e0136969, 2015.
[67]Shao, M., F. Ning, J. Zhao, M. Wei, D. G. Evans, and X. Duan. Preparation of Fe_3O_4@SiO_2@layered double hydroxide core-shell microspheres for magnetic separation of proteins. *J. Am. Chem. Soc*. 134:1071–1077, 2012.
[68]Shen, J., F. W. Luscinskas, A. Connolly, C. F. Dewey, and M. A. Gimbrone. Fluid shear-stress modulates cytosolic free calcium in vascular endothelial-cells. *Am. J. Physiol*. 262:C384–C390, 1992.
[69]Shen, Y., C. Wu, T. Q. Uyeda, G. R. Plaza, B. Liu, Y. Han, M. S. Lesniak, and Y. Cheng. Elongated nanoparticle aggregates in cancer cells for mechanical destruction with low frequency rotating magnetic field. *Theranostics* 7:1735–1748, 2017.
[70]Simi, A. K., A. S. Piotrowski, and C. M. Nelson. Mechanotransduction, metastasis and genomic instability: genomic instability and cancer metastasis. In: Mechanisms, emerging themes, and novel therapeutic strategies 20, edited by C. Maxwell, and C. Roskelley. Switzerland: Springer, 2015, pp. 139–158.
[71]Smith, M. L., D. Gourdon, W. C. Little, K. E. Kubow, R. A. Eguiluz, S. Luna-Morris, and V. Vogel. Force-induced unfolding of fibronectin in the extracellular matrix of living cells. *PLoS Biol*. 5:e268, 2007.
[72]Son, B., H. D. Kim, M. Kim, J. A. Kim, J. Lee, H. Shin, N. S. Hwang, and T. H. Park. Physical stimuli-induced chondrogenic differentiation of mesenchymal stem cells using magnetic nanoparticles. *Adv. Healthc. Mater*. 4:1339–1347, 2015.
[73]Souza, G. R., J. R. Molina, R. M. Raphael, M. G. Ozawa, D. J. Stark, C. S. Levin, L. F. Bronk, J. S. Ananta, J. Mandelin, M. Georgescu, J. A. Bankson, J. G. Gelovani, T. C. Killian, W. Arap, and R. Pasqualini. Three-dimensional tissue culture based on magnetic cell levitation. *Nat. Nanotechnol*. 5:291–296, 2010.
[74]Sun, Y., K. M. A. Yong, L. G. Villa-Diaz, X. Zhang, W. Chen, R. Philson, S. Weng, H. Xu, P. H. Krebsbach, and J. Fu. Hippo/YAP-mediated rigidity-dependent motor neuron differentiation of human pluripotent stem cells. *Nat. Mater*. 13:599–604, 2014.
[75]Suresh, S. Biomechanics and biophysics of cancer cells. *Acta Biomater*. 3:413–438, 2007.
[76]Swaminathan, V., K. Mythreye, E. T. O'Brien, A. Berchuck, G. C. Blobe, and R. Superfine. Mechanical stiffness grades metastatic potential in patient tumor cells and in cancer cell lines. *Cancer Res*. 71:5075–5080, 2011.
[77]Swift, J., I. L. Ivanovska, A. Buxboim, T. Harada, P. C. D. P. Dingal, J. Pinter, J. D. Pajerowski, K. R. Spinler, J. Shin, M. Tewari, F. Rehfeldt, D. W. Speicher, and D. E. Discher. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* 341:1240104, 2013.
[78]Thomas, C. H., J. H. Collier, C. S. Sfeir, and K. E. Healy. Engineering gene expression and protein synthesis by modulation of nuclear shape. *Proc. Natl. Acad. Sci. USA* 99:1972–1977, 2002.
[79]Tsimbouri, P. M. Adult stem cell responses to nanostimuli. *J. Funct. Biomater*. 6:598–622, 2015.
[80]Uddin, S. M. Z., and Y. Qin. Enhancement of osteogenic differentiation and proliferation in human mesenchymal stem cells by a modified low intensity ultrasound stimulation under simulated microgravity. *PLoS ONE* 8:e73914, 2013.
[81]Uyeda, T. Q. P., Y. Iwadate, N. Umeki, A. Nagasaki, and S. Yumura. Stretching actin filaments within cells enhances their affinity for the myosin II motor domain. *PLoS ONE* 6:e26200, 2011.
[82]Vicente-Manzanares, M., X. Ma, R. S. Adelstein, and A. R. Horwitz. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat. Rev. Mol. Cell Biol*. 10:778–790, 2009.
[83]Vogel, V. Mechanotransduction involving multimodular proteins: converting force into biochemical signals. *Annu. Rev. Biophys. Biomol. Struct*. 35:459–488, 2006.
[84]Weaver, V., O. Petersen, F. Wang, C. Larabell, P. Briand, C. Damsky, and M. Bissell. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J. Cell Biol*. 137:231–245, 1997.
[85]Weinbaum, S., Y. Duan, M. M. Thi, and L. You. An integrative review of mechanotransduction in endothelial, epithelial (renal) and dendritic cells (osteocytes). *Cell. Mol. Bioeng*. 4:510–537, 2011.
[86]Wu, M., J. Fannin, K. M. Rice, B. Wang, and E. R. Blough. Effect of aging on cellular mechanotransduction. *Ageing Res. Rev*. 10:1–15, 2011.
[87]Yonemura, S., Y. Wada, T. Watanabe, A. Nagafuchi, and M. Shibata. alpha-Catenin as a tension transducer that induces adherens junction development. *Nat. Cell Biol*. 12:533–542, 2010.
[88]Yoshikawa, H. Y., T. Kawano, T. Matsuda, S. Kidoaki, and M. Tanaka. Morphology and adhesion strength of myoblast cells on photocurable gelatin under native and non-native micromechanical environments. *J. Phys. Chem. B* 117:4081–4088, 2013.
[89]Zangwill, A. Modern electrodynamics. Cambridge: Cambridge Unicersity Press, 2013.
[90]Zemel, A., F. Rehfeldt, A. E. X. Brown, D. E. Discher, and S. A. Safran. Optimal matrix rigidity for stress-fibre polarization in stem cells. *Nat. Phys*. 6:468–473, 2010.
[91]Zhang, E., M. F. Kircher, M. Koch, L. Eliasson, S. N. Goldberg, and E. Renstrom. Dynamic magnetic fields remote-control apoptosis via nanoparticle rotation. *ACS Nano* 8:3192–3201, 2014.