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Cytoskeleton and plasma-membrane damage resulting from exposure to sustained deformations: A review of the mechanobiology of chronic wounds

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ABSTRACT

The purpose of this review paper is to summarize the current knowledge on cell-scale mechanically-inflicted deformation-damage, which is at the frontier of cell mechanobiology and biomechanics science, specifically in the context of chronic wounds. The dynamics of the mechanostructure of cells and particularly, the damage occurring to the cytoskeleton and plasma-membrane when cells are chronically deformed (as in a weight-bearing static posture) is correlated to formation of the most common chronic wounds and injuries, such as pressure ulcers (injuries). The first occurrence is microscopic injury which onsets as damage in individual cells and then progresses macroscopically to the tissue-scale. Here, we specifically focus on sub-catastrophic and catastrophic damage to cells that can result from mechanical loads that are delivered statically or at physiological rates; this results in apoptosis at prolonged times or necrosis, rapidly. We start by providing a basic background of cell mechanics and dynamics, focusing on the plasma-membrane and the cytoskeleton, and discuss approaches to apply and estimate deformations in cells. We then consider the effects of different levels of mechanical loads, i.e. low, high and intermediate, and describe the expected damage in terms of time-scales of application and in terms of cell response, providing experimental examples where available. Finally, we review different theoretical and computational modeling approaches that have been used to describe cell responses to sustained deformation. We highlight the insights that those models provide to explain, for example, experimentally observed variabilities in cell damage and death under loading.

1. Introduction

The dynamics of the mechanostructure of cells and particularly, the occurrence of cytoskeletal and plasma-membrane damage when cells are chronically deformed is correlated to formation of the most common chronic wounds, including pressure injuries (also known as pressure ulcers) and diabetic foot ulcers. Those wounds occur under conditions of a person's deficient neuro-alarm mechanisms or their lack of ability to alleviate localized mechanical loads. Thus, the cells within these tissues are subjected to localized, sustained deformations (strains) and mechanical stresses that eventually cause injury. The first occurrence is microscopic injury that onsets as damage in individual cells and then progresses macroscopically to the tissue-scale.

The purpose of this review is to summarize the current knowledge on cell-scale mechanically inflicted deformation-damage which is at the frontier of cell mechanobiology and biomechanics science. Mechanistic understanding of these phenomena is still incomplete, yet current knowledge already points to parallels between injuries that were thought to have separate and different pathways, such as pressure injuries and diabetic foot ulcers. Here, we focus on mechanical loads that are delivered statically or at physiological rates, e.g. deformation to fat cells during wheelchair sitting or cyclical deformations in a diabetic foot while walking. For completeness, we refer to rapid stretch experiments leading to mechanical damage of neural cells and axons, in the context of traumatic focal and diffuse brain injury. However, our emphasis is on cell-level deformation-inflicted damage in non-traumatic, chronic wounds, and its induction of cytoskeleton (CSK) and plasma-membrane (PM) damage responses at the relevant loading magnitudes and rates.

We begin this paper by providing a basic background of cell mechanics and dynamics, focusing on the PM and the CSK, and discuss approaches to apply and estimate deformations in cells. We then consider the effects of different levels of mechanical loads, i.e.

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low, high and intermediate, and describe the expected damage in terms of time-scales of application and in terms of cell response, providing experimental examples where available. Finally, we review different theoretical and computational modeling approaches that have been used to describe cell responses to sustained deformation. Throughout the paper, we highlight the insights that those models provide to explain, for example, experimentally observed variabilities in cell damage and death under loading.

1.1. Cytoskeletal mechanics and dynamics

Structural stability of cells and integrity of their PMs mostly relies on the dynamics and function of the cytoskeleton and its associated molecular motors. Those allow cells to maintain or adaptably modify their morphology to facilitate cell division, motility, and other biological activities [1–3]. The CSK includes the dynamic actin and microtubules which enable rapid adaptive responses, and intermediate filaments that mostly provide structural support but take longer to structurally modify. The main roles of the CSK are to: (i) spatially organize the cell contents, by maintaining local and global (cell-wide) structure and facilitating intracellular transport; (ii) connect the cell to its external environment, e.g. to neighboring cells or the extracellular matrix (ECM), and mechanically stabilize the PM, and (iii) generate coordinated forces that enable shape changes and movements [4,5]. The Weihs group has previously shown that disruption of specific elements of the CSK reduces cell adhesion and forces that (cancer) cells apply to a soft gel [6], and also affects cell morphology, overall CSK organization and dynamics of intracellular transport [7,8]. The PM permeabilization observed by the Gefen group during mechanical deformation of cells [9,10], is very likely preceded by CSK disruption [1].

1.2. Plasma-membrane mechanics

The cell's plasma-membrane serves as a dynamic, controlled-penetrability barrier, and it is composed of a phospholipid-based bilayer with various embedded functional molecules. The PM physically separates the cell from its surroundings while also facilitating exchange of material and information (e.g. ions, signaling molecules, etc.) between its internal and external microenvironments. Mechanosensitive ion channels, for example, are directly affected by stretch, leading to mechanotransduction of external, mechanical forces into various intracellular signals [11]. Concurrently, the PM includes specific sites for cell-cell and cell-extracellular matrix (ECM) connections, respectively, using cadherins and integrins. Such adhesion molecules connect (typically) to the actin cytoskeleton, which then provides the PM's resistance to shear and deformation by facilitating dynamic membrane-mechanics. Actin dynamics are used to balance the membrane tension, e.g. during endocytosis [12].

2. Applying and estimating dynamic deformations and responses of cells *in vitro*

Deformations have been experimentally applied to single cells and to cell groups, from monolayers to tissue constructs. Appropriately, varied approaches have been developed to facilitate application of deformations from local to global scales. We highlight a few of the current approaches used to induce and measure the dynamic response of the cell CSK and cell-colony capabilities, including cell-cell and cell-substrate interactions.

Much work has been done on the single cell level. Intracellular particle and stained-object tracking have been used to obtain the combined effects of dynamics and structure in single cells, revealing native cellular responses to various disease conditions (e.g. cancer) and to applied treatments [7,13–18]. The baseline

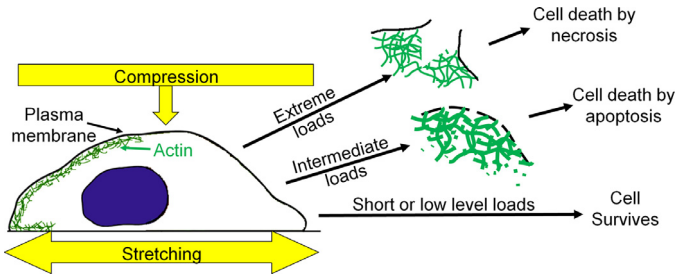

Fig. 1. The mechanisms of possible cell responses under mechanical loads (e.g. external compression or stretching) are cell survival, apoptosis or necrosis for, respectively, short or low-level, intermediate or extreme extents of loads. Qualitatively, the extreme loads will likely lead to immediate rupture and breakdown of the plasma-membrane and cytoskeleton (typically the actin), respectively. The intermediate loads may lead to local failure of the cytoskeleton, consequently causing poration of the plasma-membrane which then becomes leaky; homeostasis is gradually lost and the cell eventually dies by apoptosis. Cells can withstand short or low-level loads, e.g. by self-repair.

dynamics of the cells' PM fluctuations has also been identified through optical and mechanical interferometry [19,20]. To evaluate effects of deformations, disruptions to cell structures were externally induced in many different ways. Extensive internal changes in cell CSKs were for example induced by ultrasound irradiation which caused cell-wide responses of transient CSK breakage, which was reversible under appropriate conditions [21]. External cell measurements have been used to apply deformations and to measure local CSK and PM responses as well as whole cell mechanics. For example, methods such as atomic force microscopy [22,23], magnetic [24,25] and laser tweezers [26,27], have been used to apply forces at specific sites on the PM, inducing direct changes to the underlying actin CSK and revealing local PM dynamics and response to deformations. Whole cell stretching has been applied to adherent cells in two general approaches: those requiring cells to be suspended in solution [28], and those where cells are adhered on a typically deformable substrate [9,10,29–33].

Stretchable, elastic substrates have provided a platform to evaluate effects of cell deformation and mechanical changes on several scales, from single cells, through monolayers, and tissue constructs. Single cells and monolayers have been shown to directly interact with the environment, changing their morphology, applying force and deforming the substrate and neighboring cells [34,35]. Specifically, the Weihs lab have shown that single cancer cells may locally deform soft elastic gels, by modifying their internal structures to facilitate force application [5,36,37], an ability which correlates directly with their tendency to invade adjacent tissue. Mechanical interactions of cells with their substrates have also been shown to affect differentiation, alignment, and migration capabilities [38–40]. In contrast, deformations applied to and by cell monolayers and tissue constructs, have shown the dynamics of mechanical interactions forming between developing cell groups and their substrates [41] as well as larger scale responses. For example, stretching airway smooth muscle cells modifies the mechanical interactions and changes the tissues function [35]. Similarly, stretching has been shown to accelerate differentiation of adipocytes and production of intracellular lipids [30,42], and has also caused transient membrane poration [9,10,30,32,33]. Thus, the effects of locally or globally applied mechanical deformations are extensive and far reaching.

3. Deformation-induced cell damage phenomena

Damage induced by mechanical loads applied to living cells can be classified into one of three levels that induce different effects on cell viability and function (Fig. 1):

(a) *LOW LOADS* (physiological-scale loads, the specific magnitudes of which depend on the loading mode and cell type) – Cells will be able to tolerate the loads for a long period on the scale of the duration of the life-cycle of the studied cell type. The cells will function normally under the applied loads throughout the loading period. Examples for this happen daily, in numerous real-life scenarios, e.g. as cells of the skin and subcutaneous tissues survive for days under the weight of a wrist-watch.
(b) *HIGH LOADS* (i.e. above-physiological) – Cells will fail catastrophically e.g. by being squashed, torn or crushed, thus irreversibly and instantaneously disrupting their structural integrity and consequent biological functions. That can occur in traumatic injuries, e.g. as a result of a car crash, which causes immediate, large compressive damage to soft tissues.
(c) *INTERMEDIATE LOADS* (possibly at physiological magnitudes, but delivered continuously and without relief for long times) – Cells will not fail immediately, but will also not survive for a normal cell life-cycle. Instead, the effects of these loads on the structure and function of cells, particularly on the dynamics and the integrity of the CSK and PM, will be such that cells will gradually and slowly die; if loads are removed at a timely manner, cells may still recover.

The latter two damage scenarios, with a focus on the last one which is somewhat more complex, are explained from a mechanobiology perspective in this paper. We define here sub-catastrophic damage mechanism as one resulting from loads that shorten the life-cycle of a cell (typically causing cell death by apoptosis), yet do not cause catastrophic damage leading to immediate destruction of the cell structures and instantaneous cell death (necrosis).

3.1. Catastrophic structural failure of cells

In vitro experiments to determine the tolerance of cells to deformation exposures were performed either on isolated cells or on cells embedded in a model ECM as part of tissue engineered models of deformation-inflicted injury. Peeters and colleagues [43] for example compressed single unconfined C2C12 mouse myoblast cell lines using a specialized, bespoke micro-compression device, and found that the cells were irreversibly damaged at strains of approximately 70–80%. Essentially, cells were squashed and their PMs had buckled when reaching these compressive strain levels; PM buckling was an early marker of structural failure. Later on, Gefen and co-workers confirmed the above findings in tissue-engineered models of skeletal muscles, where cells within the construct were instantaneously and irreversibly damaged and thus destroyed at the same compressive strain levels [44]. Though no equivalent quantitative data has been reported for other cell types as of yet, it is expected that at strains greater than the above values, the CSK will be significantly disrupted and the PM will massively tear (regardless of the cell type), thereby causing immediate, necrotic cell death.

3.2. Sub-catastrophic and reversible damage

Sustained deformations likely cause biological damage to build-up in the cells over time, e.g. by gradual destruction of the CSK and PM [1], and death by apoptosis; that is in contrast to catastrophic damage which is instantaneous. This was indicated through the monotonous and significant increase over 24 h in the percentage of dead cells in cultured myoblasts subjected to sustained compressive deformations, where no such increase was observed in control undeformed cells [45]. One of the early studies of sub-catastrophic, deformation-inflicted damage in tissue-engineered model systems was that of Breuls and co-workers, about a decade ago [46]. By compressing skeletal muscle constructs, they found that under 30%-strain, cell death occurred within 1–2 h, while compression of 50%-strain led to an earlier onset of cell death. This showed that the strain-level threshold for irreversible cell damage depends on the time of cell exposure to the sustained deformations. Importantly, since these tissue-engineered constructs were fully oxygenated and were kept at a normal, physiological pH (7.4), the damage must have been caused directly by the inflicted deformations. That contradicted the conventional thinking at the time. Specifically, it was erroneously believed that in native tissues that are deformed to large extents for prolonged periods or cyclically, so that pressure injury or diabetic foot ulcers form, the damage onsets primarily due to ischemia. However, as will be discussed below, the time-scales of ischemia damage are much longer than the observed, indicating that deformation is the primary cause of the damage.

To substantiate and extend the basic studies, Gefen and colleagues [44] have built-upon the Breuls paper and extended the work considerably, by developing an experimental method to determine the continuous strain/time threshold function for sub-catastrophic cell damage. Using tissue-engineered skeletal muscle constructs under static compressive strains ranging continuously between 0%–80%, they quantified cell death with fluorescent propidium iodide (PI) staining. Thus, they obtained a threshold curve with a clear time-dependence, which, for deformation exposures shorter than 6 h, mathematically fit a single-step sigmoid. Their work determined a 95% likelihood that myofibers could tolerate compressive strains $< 40\%$ and $< 65\%$ for 4-5 h and 1 h, respectively. The decrease in tolerance of the cells to the applied strains hence occurred predominantly 1-3 h post-loading. This suggested a mechanism for gradual decrease in cell tolerance to sustained loading leading to sub-catastrophic cell death.

The mechanism of the damage spiral for sub-catastrophic cell-level failure was revealed by the Gefen group several years later. Using the above-described tissue-engineered model systems [9,10,44,47], under physiological (muscle) tissue deformation levels and using coupled MRI and computational modeling [48-50], clearly indicated that death occurs initially at sites of the most highly distorted cells. In the human body, cells routinely become distorted under sustained deformations, when tissues are deformed by bodyweight and during weight-bearing. This occurs particularly near bony prominences e.g. the ischial tuberosities, sacrum, and posterior aspects of the calcaneal bones when lying. The central hypothesis of the Gefen group was that such sustained tissue deformations, if not relieved in a timely manner, may induce cell-scale structural damage to the CSK and the PM. Specifically, actin fragmentation eliminates the cytoskeletal support of the PM, which then causes appearance of pores in the PM [1]. PM pores unbalance the controlled trafficking of ions, metabolites and waste products in and out of the cell and, thereby, disrupt the intracellular homeostatic conditions. Using fluorescence activated cell sorting (FACS), the Gefen group have shown that myoblasts stretched to substrate tensile strains $\leq 12\%$ exhibit statistically significant rise in uptake of FITC-labeled dextrans [9] where low molecular mass (smaller) dextrans entered readily [10]. This was also shown using mathematical and computational models of trans-membrane transport [32,33,51]. Additional evidence for a deformation-caused PM permeability-increase was later reported with regard to cultured osteoblasts, where elevation in cytosolic free-calcium concentrations (likely fluxing inwards from the extracellular space) were observed when the PM was punctured with an atomic force microscopy tip [52]. Hence, the sustained mechanical distortion of cells causes an intracellular biochemical unbalance which may eventually lead to cell death. Clearly, in both bone and muscle cells, calcium concentrations that are above-critical are cytotoxic as they lead to mitochondrial failure [53].

Thus, uncontrolled Ca^{2+} influxes (i.e. not via calcium channels) may cause cell death shortly after the PM is perforated. Hence, deformation-driven PM poration and increased permeability not only transiently compromise homeostasis, but also, if sustained, could eventually cause cell death at timeframes ranging from tens-of-minutes to several hours. That is substantially faster than the progression of any local ischemia-related damage, which precludes ischemia as the primary cause of chronic wounds caused due to bodyweight loads e.g. diabetic ulcers and pressure ulcers [54-56].

4. Modeling cell deformations

Analytical modeling provides first approximations to cell deformation states that can be correlated with development of transient cytoskeletal damage and PM poration or even catastrophic cell failure. One such simple model was suggested by Slomka and Gefen [9], where a simplified disk-shaped cell, adhered on a planar substrate is radially deformed, hence causing cell stretching. When the substrate is not stretched, the cell is in its undeformed configuration, with the dimensions characterizing the cell being its initial diameter, d_0, and height, H. When the substrate is radially stretched to a stretch ratio of λ, the surface area dilatation Γ (increase in the surface area A of the cell relative to the undeformed surface area, A_0) is given by:

$$\Gamma = \frac{A - A_0}{A_0} = \frac{\left(\lambda^2 - 1\right)(d_0/2) + (1/\lambda - 1)H}{d_0/2 + H} \tag{1}$$

An analogous model for a single cell compressed by a flat substrate was proposed earlier, by Takamatsu and Rubinsky [57,58]:

$$\Gamma = \frac{A - A_0}{A_0} = \left(-\frac{\pi^2}{16} + \frac{2}{3}\right)\left(\frac{h}{d_0}\right)^2 + \frac{1}{3}\left(\frac{d_0}{h}\right) + \frac{\pi}{4}\left(\frac{h}{d_0}\right)^2 \times \sqrt{\frac{\pi^2}{16} + \frac{2}{3}\left\{\left(\frac{d_0}{h}\right)^3 - 1\right\}} - 1 \tag{2}$$

where h is the gap size into which the cell (defined by its diameter d_0) is being squeezed. It is important to note that the values of the surface area dilatation for radially stretched cells and compressed cells are not expected to be identical.

Regardless of the mechanism by which the cells are deformed, cells in culture typically exhibit variability in size, and a population of cells can be described statistically as in Eq. (3) [57,58]:

$$f(d_0) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{(d_0 - d_{med})^2}{2\sigma^2}\right] \tag{3}$$

where d_{med} is the median of cell diameters, and σ is the standard deviation assuming a Gaussian distribution. The distribution of cell diameters will result in a distribution of deformations induced on the cells in culture following external loading (either stretching or compression, or a combination of which). If only accounting for instantaneous, catastrophic, structural failure of cells (necrosis) when subjecting the cells in culture to a given external mechanical load, one can assume a Heaviside function for the cell viability. Thus, a viable (even early apoptotic) cell will have a viability of unity for $\Gamma < \Gamma_{crit}$ and a viability of zero for $\Gamma \geq \Gamma_{crit.}$ However, this approach does not account for sub-catastrophic loads that cause PM poration and leakage of ions or molecules, prior to cell death. For example, consider three adjacent cells in a culture with d_0 of 30, 40 and 50 μm and common height of 10 μm. If the culture is stretched to λ=1.2 then, following Eq. (1), their area dilatations Γ are 20%, 24% and 27%, respectively. That is, even though the substrate is stretched uniformly (radially), cell strains will vary, with smaller cells being subjected to less strain, an observation that is also consistent with the compression loading model in Eq. (2). Continuing the same example, if we assume that catastrophic failure occurs at Γ = 25% and PM poration begins developing at Γ > 20% and becomes more severe with increase of Γ within the sub-catastrophic range, then cells can die either instantaneously or gradually over time, through leakage of vital substances and loss of homeostasis. In this case of the 3-cell example, the largest cell will die instantaneously. The mid-sized cell will eventually die as well, yet due to loss of homeostasis, within a time period that will depend on the PM poration state. Only the smallest cell will survive the aforementioned loading exposure.

To simulate these types of complex damage phenomena a different viability function is required. The Heaviside viability function with the binary condition (i.e. dead or alive) is insufficient here. Thus, we can assume a dose-response viability function (e.g. a sigmoid or a Gompertz function [59]) which describes a continuous state between a 'fully viable' and a 'completely dead' cell. To expand the viability function, we consider another aspect of the individual cells, their stiffness and its changes controlled by dynamic remodeling of the CSK. In Eqs. (1) and (2) and the derivation thereafter, cells are considered to be passive and unable to resist the external loads. However, again for the sake of the argument, under the hypothetical assumption that a cell can make itself infinitely stiff (i.e. a rigid body) while the substrate is stretched, it will not deform with the substrate and hence Γ will never exceed sub-catastrophic (CSK damage preceding PM poration) or catastrophic (non-reversible) thresholds. Clearly, cells cannot make themselves that rigid, yet they can remodel their CSK to stiffen their structure if given a sufficient time to respond, which will reduce the effective Γ, and will in turn protect their PM and their viability. This illustrates the likely relation between CSK integrity and function and PM loads, and eventually, homeostasis and viability.

More advanced structural phenomena in mechanically loaded cells can be evaluated, with the same principles, using finite element (FE) modeling; a review of the body of work on computational cell modeling is available in [60]. In the context of the above discussion, however, Slomka and Gefen developed an approach to obtain three-dimensional (3D) cell-specific FE models to simulate experiments involving large cell deformations, based on analysis of confocal (z-stack) images of multiple undifferentiated skeletal muscle cells (C2C12 myoblasts) [61,62]. Using those models, the magnitudes and distributions of strains developing in cells with different structures were evaluated under large-deformation compression and stretching; similar large deformations have been used to experimentally simulate chronic wounds, in our group and others. The large deformations caused localized stretches in the PM and in the nuclear membrane, and the cell-specific computational models [61,62] demonstrated, as in the analytical models above, the considerable variability in cell morphology and architecture; such variability is expected not only across phenotypes, but also within the same phenotype and even within the same culture dish. Thus, although the external loading is applied uniformly (e.g. by substrate stretching or compression), different cells are expected to be exposed to substantial variable strains. These differences in the strains developed in each cell, observed analytically and by computational FE models, explains the variabilities across experiments and within them in rapid necrotic death as well as in times of early apoptosis or loss of viability due to impaired homeostasis; that is since each cell is essentially bearing a different strain level. Considering cells that are also differentiating, the structural organization of the cell changes over time, which affects its stiffness and CSK arrangement, and hence its ability to resist deformation or deform to redistribute the loads. For example, in differentiating adipocytes or myoblasts, respectively, lipid droplets and myotubes form, modifying the cells ability to resist load, which has been demonstrated by the Gefen lab, particularly in adipocytes [63-65].

5. Summary

Various types of chronic wounds and tissue injuries are associated with sustained tissue and cell deformations: pressure injuries (induced by bodyweight loads); diabetic foot ulcers (again bodyweight loads); urinary tract and kidney disorders (urine pressure) etc. In all these examples, the mechanical deformation is counteracted by the dynamic CSK, and if the CSK cannot counteract the external loads transferred from the extracellular environment, the PM may be irreversibly deformed up to local failure, and the cascade then leads to PM leakiness. While other underlying causes and stages may exist, we propose that the main tissue damage originates from this mechanical cascade.

The purpose of this review paper was hence to summarize the current knowledge on cell-scale mechanically-inflicted deformation-damage which is at the frontier of modern cell mechanobiology and biomechanics science, specifically in the context of chronic wounds. The dynamics of the mechanostructure of cells and particularly, the damage occurring to the CSK and PM when cells are chronically deformed (as in a weight-bearing static posture leading to pressure injuries) is correlated to formation of the most common chronic wounds. The first occurrence is microscopic injury which onsets as damage in individual cells that then progresses macroscopically to the tissue-scale. Here, we specifically focused on sub-catastrophic and catastrophic damage to cells that can result from mechanical loads that are delivered statically or at physiological rates; this results in apoptosis at prolonged times or necrosis, rapidly. Theoretical as well as computational (FE) modeling has indicated that the individual cell geometry strongly affects strains that develop in the cells and on them, in the PM, and adequate dynamic function of the CSK is necessary for allowing cells to adapt to external loads so that their PM deformation will be minimal (and so will be the risk for PM poration which necessarily leads to loss of homeostasis). In this paper, we highlighted the insights that the aforementioned theoretical and computational models provide to explain the above phenomena, for example, experimentally observed variabilities in cell damage and death under loading. Better understanding of variabilities in mechanical performances of cells, either of the same type or of different types, is essential for interpreting any experimental data involving application of mechanical loads to cells.

Conflict of interest

No conflict of interest.

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References

[1] Gefen A, Weihs D. Mechanical cytoprotection: A review of cytoskeleton-protection approaches for cells. J Biomech 2016;49:1321–9.
[2] Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. Fifth edition. Garland Science; 2008.
[3] Cooper GM, Hausman RE. The cell: a molecular approach. 4th ed. Sinauer Associates; 2007.
[4] Fletcher DA, Mullins D. Cell mechanics and the cytoskeleton. Nature 2010;463:485–92.
[5] Kristal-Muscal R, Dvir L, Ma Schvartzer, Weihs D. Mechanical interaction of metastatic cancer cells with a soft gel. Procedia IUTAM 2015;12:211–19.
[6] Alvarez-Elizondo MB, Weihs D. Metastatic potential and invasion forces in breast cancer cells. Garmisch-Partenkirchen, Germany: 7th German-Israeli Cancer Research School; 2015.
[7] Goldstein D, Elhanan T, Aronovitch M, Weihs D. Origin of active transport in breast-cancer cells. Soft Matter 2013;9:7167–73.
[8] Weihs D, Mason TG, Teitell MA. Effects of cytoskeletal disruption on transport, structure, and rheology within mammalian cells. Phys Fluids 2007;19:103102.
[9] Slomka N, Gefen A. Relationship between strain levels and permeability of the plasma membrane in statically stretched myoblasts. Ann Biomed Eng 2012;40:606–18.
[10] Leopold E, Gefen A. Changes in permeability of the plasma membrane of myoblasts to fluorescent dyes with different molecular masses under sustained uniaxial stretching. Med Eng Phys 2013;35:601–7.
[11] Martinac B. Mechanosensitive ion channels: molecules of mechanotransduction. J Cell Sci 2004;117:2449–60.
[12] Boulant S, Kural C, Zeeh JC, Ubelmann F, Kirchhausen T. Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. Nat Cell Biol 2011;13:1124–31.
[13] Gal N, Lechtman-Goldstein D, Weihs D. Particle tracking in living cells: a review of the mean square displacement method and beyond. Rheol Acta 2013;52:425–43.
[14] Gal N, Weihs D. Experimental evidence of strong anomalous diffusion in living cells. Phys Rev E: Stat Phys Plasmas Fluids Relat Interdiscip Top 2010;81:020903(R).
[15] Gal N, Weihs D. Intracellular mechanics and activity of breast cancer cells correlate with metastatic potential. Cell Biochem Biophys 2012;63:199–209.
[16] Brangwynne CP, MacKintosh FC, Weitz DA. Force fluctuations and polymerization dynamics of intracellular microtubules. Proc Natl Acad Sci U S A 2007;104:16128–33.
[17] Caspi A, Granek R, Elbaum M. Enhanced diffusion in active intracellular transport. Phys Rev Lett 2000;85:5655–8.
[18] Yamada S, Wirtz D, Kuo SC. Mechanics of living cells measured by laser tracking microrheology. Biophys J 2000;78:1736–47.
[19] Reed J, Troke JJ, Schmit J, Han S, Teitell MA, Gimzewski JK. Live cell interferometry reveals cellular dynamism during force propagation. Acs Nano 2008;2:841–6.
[20] Roitshtain D, Sharabani-Yosef O, Gefen A, Shaked NT. Analyzing the texture changes in the quantitative phase maps of adipocytes. 2016. p. 97180R-R-6.
[21] Mizrahi N, Zhou EHH, Lenormand G, Krishnan R, Weihs D, Butler JP, et al. Low intensity ultrasound perturbs cytoskeleton dynamics. Soft Matter 2012;8:2438–43.
[22] Cross SE, Jin YS, Rao J, Gimzewski JK. Nanomechanical analysis of cells from cancer patients. Nat Nanotechnol 2007;2:780–3.
[23] Pelling AE, Dawson DW, Carreon DM, Christiansen JJ, Shen RR, Teitell MA, et al. Distinct contributions of microtubule subtypes to cell membrane shape and stability. Nanomed Nanotechnol 2007;3:43–52.
[24] Fabry B, Maksym GN, Hubmayr RD, Butler JP, Fredberg JJ. Implications of heterogeneous bead behavior on cell mechanical properties measured with magnetic twisting cytometry. J Magn Magn Mater 1999;194:120–5.
[25] Robert D, Nguyen TH, Gallet F, Wilhelm C. In vivo determination of fluctuating forces during endosome trafficking using a combination of active and passive microrheology. PLoS One 2010;5:e10046.
[26] Laurent VM, Henon S, Planus E, Fodil R, Balland M, Isabey D, et al. Assessment of mechanical properties of adherent living cells by bead micromanipulation: comparison of magnetic twisting cytometry vs optical tweezers. J Biomech Eng-T Asme 2002;124:408–21.
[27] Kuo SC, Sheetz MP. Force of single kinesin molecules measured with optical tweezers. Science 1993;260:232–4.
[28] Guck J, Schinkinger S, Lincoln B, Wottawah F, Ebert S, Romeyke M, et al. Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence. Biophys J 2005;88:3689–98.
[29] Desprat N, Richert A, Simeon J, Asnacios A. Creep function of a single living cell. Biophys J 2005;88:2224–33.
[30] Shoham N, Gottlieb R, Sharabani-Yosef O, Zaretsky U, Benayahu D, Gefen A. Static mechanical stretching accelerates lipid production in 3T3-L1 adipocytes by activating the MEK signaling pathway. Am J Physiol Cell Physiol 2012;302 C429-C41.
[31] Toume S, Gefen A, Weihs D. Printable low-cost, sustained and dynamic cell stretching apparatus. J Biomech 2016;49:1336–9.
[32] Leopold E, Gefen A. Stretching affects intracellular oxygen levels: three-dimensional multiphysics studies. J Biomech Eng 2012;134:064501.
[33] Slomka N, Or-Tzadikario S, Sassun D, Gefen A. Membrane-stretch-induced cell death in deep tissue injury: computer model studies. Cell Mol Bioeng 2009;2:118–32.
[34] Butler JP, Tolic-Norrelykke IM, Fabry B, Fredberg JJ. Traction fields, moments, and strain energy that cells exert on their surroundings. Am J Physiol Cell Physiol 2002;282:C595–605.
[35] Krishnan R, Canovic EP, Iordan AL, Rajendran K, Manomohan G, Pirentis AP, et al. Fluidization, resolidification, and reorientation of the endothelial cell in response to slow tidal stretches. Am J Physiol Cell Physiol 2012;303:C368–75.
[36] Dvir L, Nissim R, Alvarez-Elizondo MB, Weihs D. Quantitative measures to reveal coordinated cytoskeleton-nucleus reorganization during in vitro invasion of cancer cells. New J Phys 2015;17:043010.
[37] Kristal-Muscal R, Dvir L, Weihs D. Metastatic cancer cells tenaciously indent impenetrable, soft substrates. New J Phys 2013;15:035022.
[38] Abuhattum S, Gefen A, Weihs D. Ratio of total traction force to projected cell area is preserved in differentiating adipocytes. Integr Biol 2015;7:1212–17.
[39] Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006;126:677–89.
[40] Zemel A, Rehfeldt F, Brown AEX, Discher DE, Safran SA. Optimal matrix rigidity for stress-fibre polarization in stem cells. Nat Phys 2010;6:468–73.

[41] Teo A, Lim M, Weihs D. Embryonic stem cells growing in 3-dimensions shift from reliance on the substrate to each other for mechanical support. J Biomech 2015;48:1777–81.
[42] Shoham N, Gefen A. Mechanotransduction in adipocytes. J Biomech 2012;45:1–8.
[43] Peeters EAG, Oomens CWJ, Bouten CVC, Bader DL, Baaijens FPT. Mechanical and failure properties of single attached cells under compression. J Biomech 2005;38:1685–93.
[44] Gefen A, van Nierop B, Bader DL, Oomens CW. Strain-time cell-death threshold for skeletal muscle in a tissue-engineered model system for deep tissue injury. J Biomech 2008;41:2003–12.
[45] Bouten CVC, Knight MM, Lee DA, Bader DL. Compressive deformation and damage of muscle cell subpopulations in a model system. Ann Biomed Eng 2001;29:153–63.
[46] Breuls RGM, Bouten CVC, Oomens CWJ, Bader DL, Baaijens FPT. Compression induced cell damage in engineered muscle tissue: an in vitro model to study pressure ulcer aetiology. Ann Biomed Eng 2003;31:1357–64.
[47] Gefen A, Cornelissen LH, Gawlitta D, Bader DL, Oomens CW. The free diffusion of macromolecules in tissue-engineered skeletal muscle subjected to large compression strains. J Biomech 2008;41:845–53.
[48] Linder-Ganz E, Shabshin N, Itzchak Y, Gefen A. Assessment of mechanical conditions in sub-dermal tissues during sitting: a combined experimental-MRI and finite element approach. J Biomech 2007;40:1443–54.
[49] Linder-Ganz E, Shabshin N, Itzchak Y, Yizhar Z, Siev-Ner I, Gefen A. Strains and stresses in sub-dermal tissues of the buttocks are greater in paraplegics than in healthy during sitting. J Biomech 2008;41:567–80.
[50] Linder-Ganz E, Gefen A. Stress analyses coupled with damage laws to determine biomechanical risk factors for deep tissue injury during sitting. J Biomech Eng 2009;131:011003.
[51] Leopold E, Gefen A. A simple stochastic model to explain the sigmoid nature of the strain-time cellular tolerance curve. J Tissue Viability 2012;21:27–36.
[52] Lopez-Ayon GM, Liu HY, Xing S, Maria OM, LeDue JM, Bourque H, et al. Local membrane deformation and micro-injury lead to qualitatively different responses in osteoblasts. F1000Res 2014;3:162.
[53] Zoeteweij JP, Vandewater B, Debont HJGM, Mulder GJ, Nagelkerke JF. Calcium-induced cytotoxicity in hepatocytes after exposure to extracellular atp is dependent on inorganic-phosphate - effects on mitochondrial Calcium. J Biol Chem 1993;268:3384–8.
[54] Gefen A. How much time does it take to get a pressure ulcer? Integrated evidence from human, animal, and in vitro studies. Ostomy Wound Manag 2008;54(26-8):30–5.
[55] Gefen A, Farid KJ, Shaywitz I. A review of deep tissue injury development, detection, and prevention: shear savvy. Ostomy Wound Manag 2013;59:26–35.
[56] Gefen A. Tissue changes in patients following spinal cord injury and implications for wheelchair cushions and tissue loading: a literature review. Ostomy Wound Manag 2014;60:34–45.
[57] Takamatsu H, Takeya R, Naito S, Sumimoto H. On the mechanism of cell lysis by deformation. J Biomech 2005;38:117–24.
[58] Takamatsu H, Rubinsky B. Viability of deformed cells. Cryobiology 1999;39:243–51.
[59] Gefen A. Mathematical functions and their properties as relevant to the biomechanical modeling of cell and tissue damage. J Appl Biomech 2010;26:93–103.
[60] Slomka N, Gefen A. Finite Element Modeling of Cellular Mechanics Experiments. Cellular and Biomolecular Mechanics and Mechanobiology, Studies in Mechanobiology, Tissue Engineering and Biomaterials. Gefen A, editor. Berlin Heidelberg: Springer; 2011.
[61] Slomka N, Gefen A. Confocal microscopy-based three-dimensional cell-specific modeling for large deformation analyses in cellular mechanics. J Biomech 2010;43:1806–16.
[62] Slomka N, Gefen A. Cell-to-cell variability in deformations across compressed myoblasts. J Biomech Eng 2011;133:081007.
[63] Or-Tzadikario S, Gefen A. Confocal-based cell-specific finite element modeling extended to study variable cell shapes and intracellular structures: the example of the adipocyte. J Biomech 2011;44:567–73.
[64] Katzengold R, Shoham N, Benayahu D, Gefen A. Simulating single cell experiments in mechanical testing of adipocytes. Biomech Model Mechanobiol 2015;14:537–47.
[65] Shoham N, Girshovitz P, Katzengold R, Shaked NT, Benayahu D, Gefen A. Adipocyte stiffness increases with accumulation of lipid droplets. Biophys J 2014;106:1421–31.