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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

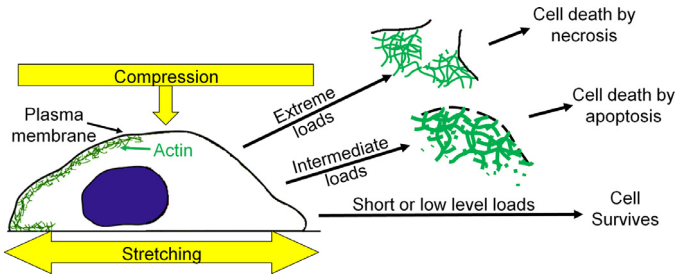

Fig. 1. The mechanisms of possible cell responses under mechanical loads (e.g. external compression or stretching) are cell survival, apoptosis or necrosis for, respectively, short or low-level, intermediate or extreme extents of loads. Qualitatively, the extreme loads will likely lead to immediate rupture 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):