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Membrane permeability during pressure ulcer formation: A computational model of dynamic competition between cytoskeletal damage and repair

N. Suhas Jagannathan [a], Lisa Tucker-Kellogg [a,b,c,*]

[a] Centre for Computational Biology, and Duke-NUS Graduate Medical School Singapore, 8 College Road, Singapore
[b] Program in Cancer and Stem Cell Biology, Duke-NUS Graduate Medical School Singapore, 8 College Road, Singapore
[c] BioSystems and Micromechanics (BioSyM) Singapore-MIT Alliance for Research and Technology, 1 Create Way, Singapore

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ABSTRACT

Pressure ulcers are debilitating wounds that arise frequently in people who have lost mobility. Mechanical stress, oxidative stress and ischemia–reperfusion injury are potential sources of damage during pressure ulcer formation, but cross-talk between these sources has rarely been investigated. In vitro experiments with mechanically-induced cell damage previously demonstrated that non-lethal amounts of static cell deformation could induce myoblast membrane permeabilization. Permeabilization, in turn, has the potential to induce oxidative stress via leakage of calcium, myoglobin or alarmins. In this work, we constructed a hypothetical causal network of cellular-scale effects resulting from deformation and permeabilization, and we investigated the theoretical sensitivity of cell death toward various parameters and pathways of the model. Simulations showed that the survival/death outcome was particularly sensitive to the speed of membrane repair. The outcome was also sensitive to whether oxidative stress could decrease the speed of membrane repair. Finally, using the assumption that apoptosis and necrosis would have opposite effects on membrane leakage in dying cells, we showed that promoting apoptosis might under certain conditions have the paradoxical effect of decreasing, rather than increasing, total cell death. Our work illustrates that apoptosis may have hidden benefits at preventing spatial spread of death. More broadly, our work shows the importance of membrane repair dynamics and highlights the need for experiments to measure the effects of ischemia, apoptosis induction, and other co-occurring sources of cell stress toward the speed of membrane repair.

1. Introduction

Pressure ulcers (PU) are painful, slow-healing wounds that develop over bony prominences during periods of prolonged immobility. Pressure ulcers can devastate the quality of life for people who have poor mobility. Skin is the most common layer for PU formation (Barczak et al., 1997; Vanderwee et al., 2007), and without proper care, pressure ulcers can worsen by expanding inward toward muscle and bone. Another category of pressure ulcer progresses from muscle outward. Deep tissue injury (DTI) occurs when prolonged mechanical force between bone and muscle causes the muscle to necrose near the bone, while the cutaneous layer remains intact. PU of muscle have been studied in vivo using methods such as MRI, ultrasound, and histology (Aoi et al., 2009; Bosboom et al., 2003; Linder-Ganz et al., 2007; Peirce et al., 2000; Ruan et al., 1998; Stekelenburg et al., 2007; Strijkers et al., 2005). We focus primarily on PU of muscle because they are particularly severe and debilitating.

Previous work in the field of myocardial infarction demonstrated that muscle tissue can survive prolonged periods of ischemia but is more vulnerable to ischemia–reperfusion injury (IRI) (Vanden Hoek et al., 1996). Ischemia–reperfusion injury can damage cells through oxidative stress and production of free radicals, particularly mitochondrial superoxide (Liu et al., 1997; Shiva et al., 2007). Oxidative stress contributes to pressure ulcer pathophysiology (Taylor and James, 2005), but the etiology of pressure ulcers is more complex than ischemia or IRI alone. Studies using MRI to examine muscle perfusion and death during combinations of ischemia–reperfusion and mechanical pressure showed that the volume of necrosis induced by IRI alone was much smaller than the volume of necrosis induced by the same amount of IRI combined with mild but prolonged mechanical pressure (Loerakker et al., 2011; Stekelenburg et al., 2007).

* Corresponding author at: Centre for Computational Biology, Duke-NUS Medical School, 8 College Road, Level 5, 169857, Singapore. Tel.: +65 6516 7654.
E-mail address: Lisa.Tucker-kellogg@duke-nus.edu.sg (L. Tucker-Kellogg).

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Therefore, pressure ulcers are not simply due to IRI, and the application of mechanical force is harmful, beyond its ability to induce ischemia/IRI.

Although cultured muscle cells can survive large amounts of mechanical deformation, small amounts of non-lethal deformation (3–12% tensile strain) were sufficient to induce permeability of myoblasts toward fluorescently tagged dextran molecules (Gefen et al., 2008; Leopold and Gefen, 2013; Slomka and Gefen, 2012). Mechanically-induced permeability allows efflux of intracellular factors, and it also allows influx of Calcium, due to the large gradient in muscle cells (0.05–0.1 μM intracellularly, μM–mM extracellularly). Although the sarcoplasmic reticulum (SR) can scavenge excess Ca^{2+}, high levels of Ca^{2+} influx cause SR stress, oxidative stress (Gissel, 2005) and activation of calpains, which are calcium-dependent, death-promoting proteases (Croall and DeMartino, 1991). Little is known about the cell stress and physiological effects caused by non-lethal strain and partial permeability.

In a variety of ischemic injuries including pressure injury (Stekelenburg et al., 2006; Stekelenburg et al., 2007) and burn (Lanier et al., 2011; Singer et al., 2011a), the immediate death of direct injury may be followed by delayed death of surrounding tissue, a phenomenon of spatial progression or secondary damage that is not yet well understood. Injury progression may be caused in part by loss of perfusion (Hirth et al., 2013), oxidative stress (Singer et al., 2011b), influx of calcium (Duncan, 1978; Gissel, 2005), efflux of alarmins (Hirth et al., 2012) or other disruptions of the environment. Efflux of alarmins means release of factors that indicate injury or trigger inflammation, for example, extracellular ATP (Killeen et al., 2013). Muscle cells have extremely high levels (~5 mg/g of wet weight) of myoglobin (Möller and Sylvén, 1981), a protein that can cause oxidative stress directly through pseudo-peroxidase reactions (Moore et al., 1998; Reeder and Wilson, 2005), or indirectly through release of heme or iron into the environment (Kumar and Bandyopadhyay, 2005; Solar et al., 1991). Pressure ulcers in animals caused significant release of myoglobin (Makhsous et al., 2010). In this work we will use Myoglobin (Mb) to represent any member of the class of diffusible intracellular factors that can cause oxidative stress (potentially via inflammation) if released extracellularly. Likewise, we use Calcium (Ca) to represent the class of extracellular molecules that can enter permeabilized cells to cause oxidative stress. The Ca category is very similar to the Mb category, except they have opposite direction of transport, and only extracellular factors are permitted to affect neighboring cells. In our model, we assumed that internal and external sources of oxidative stress are equivalent, because many ROS molecules (such as H_2O_2) can be transported across the membrane (Fisher, 2009; Miller et al., 2010). Hence, our model differentiates between the Ca and Mb class of molecules in their ability to diffuse but not in the nature of the resulting stress.

While membrane permeability can cause cell stress and cell death, cell death is also able to affect membrane permeability. During apoptotic death, cellular contents are packaged into membrane-bound vesicles called apoptotic bodies, thereby blocking the release of alarmins and minimizing the inflammatory response (Kerr et al., 1995; Kroemer et al., 2009). In contrast, necrotic death causes lysis (rupture) of the cell membrane and release of cellular contents, resulting in inflammation (Edinger and Thompson, 2004; Mevorach et al., 2010). The threshold of injury required for causing apoptosis is lower than for causing necrosis, particularly in the case of oxidative stress (Hampton and Orrenius, 1997; Teramoto et al., 1999). However, apoptosis requires hours to execute (Albeck et al., 2008a, 2008b; Goldstein et al., 2000; Saraste, 1999), whereas necrotic lysis can be instantaneous. In other words, a cell that encounters increasing stress might initiate apoptosis at an early time-point, but before the apoptotic program can complete, the cell might succumb to necrosis. As a result, induction of apoptosis can only prevent necrosis from occurring if the environment is hospitable enough for the cell to remain viable during the apoptotic delay.

When cells are damaged, they generally mount countermeasures, for example, membrane repair in response to membrane disruption. Important work in the lab of Arthur Mak (Duan et al., 2015) performed non-lethal laser ablation, and discovered that oxidative stress decreases the speed of membrane repair. In other words, oxidative stress can impair endogenous countermeasures against mechanical stress. Because such experiments are costly and labor-intensive, there is a need for theoretical work to explore the landscape of potential cross-talk relationships and to prioritize the choice of variables for future experimental studies.

In this work we study multiple stresses (mechanical force, partial permeability, Ca influx, Mb efflux, IRI) in the context of dynamic competition between damage and repair, and we address the following questions – (1) How sensitive is the total death to each of the rate parameters in the model. (2) What are the individual contributions of permeability and oxidative stress to the total death and how does feedback between the two impact the system? (3) How do the apoptosis/necrosis divide and related parameters affect the total death in the system? Our qualitative model captures roughly 7 cellular-scale processes that may contribute to muscle injury during pressure ulcer formation, and interconnects them in a causal network. Causal networks are reviewed by Tenenbaum et al. (2011). Each causal network is repeated in many cellular compartments over a 2-dimensional space. The 2-dimensional space can be subjected to user-defined mechanical deformations, and we have provided a simple finite element mesh with simulated force, to provide mechanical stimulus to the system.

Because very few experiments have yet been performed to quantify the damage and repair processes we study, we performed qualitative computational modeling, repeated many times across a range of variable parameters. This method allows us to explore regions of parameter space (e.g., combinations of stresses and responses) that affect the relevant outcomes, and to identify configurations of the system that may have interesting or non-monotonic behaviors. Understanding why different variables may have different relative influence over cell death may be useful for prioritizing future experiments.

2. Model

The model consists of repeating hexagonal compartments over a 2D plane. The hexagons are simplified abstractions of muscle fiber cross-sections, where each hexagon is a myotube compartment. Each compartment has a set of (scalar) state variables, such as membrane permeability. The dynamic competition between state variables is governed by ordinary differential equations (ODEs) with respect to time. The interdependence between variables and equations creates a causal network, described below. At each step of the ODE simulation, transport processes allow extracellular quantities to diffuse isotropically between adjacent compartments. We call this the biochemical section of the model.

To study the interplay between biochemical effects and mechanical effects, we require a physical strain field. Other labs have performed detailed finite element simulations for the effects of force on different anatomical regions (Cheung et al., 2005; Linder-Ganz et al., 2007; Linder-Ganz and Gefen, 2004; Makhsous et al., 2007). However, in keeping with the highly simplified nature of our model, we performed a finite element simulation with an ellipsoidal region of force applied to a uniform elastic 2D mesh [Supplementary Text 4] to obtain a measure for deformation of each compartment. We call this the mechanical section of the

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