# Mini-Review

# Loss, Restoration, and Maintenance of Plasma Membrane Integrity

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A break in the integrity of the plasma membrane immediately compromises this structure's essential role as a barrier, and this can kill the affected cell. Yet animal cell plasma membranes, unprotected by a cell wall, are highly vulnerable to mechanically induced disruption. Moreover, many tissue environments generate and receive "physiological" levels of mechanical force that impose shear, tensile, and compressive stresses on constituent cells. We examine here the tissue conditions that lead to membrane disruptions and the mechanisms that cells use for preventing disruption-induced death and for rapidly restoring membrane integrity.

Mechanical stress also induces an adaptive response by cells, which must sense and respond to this stimulus. A skeletal muscle, for example, experiences during its lifetime a highly variable degree of mechanical load. Its individual myofibers must adapt to this changing mechanical environment, hypertrophying in response to increased load and atrophying in response to decreased load. Such changes in tissue architecture are important because they improve mechanical functioning, make economical use of valuable resources, repair or replace injured components, and/or prevent future injury. We here review briefly what is known about the role of plasma membrane disruption in transducing cell responses to mechanical load.

## Occurrence of Mechanically Initiated Plasma Membrane Disruptions

Plasma membrane disruption has been detected and quantitated in a variety of normal rodent tissues (Table I) (26). Levels of "cell wounding" are highest in muscle (5–30% of cardiac and skeletal myocyte populations under physiological conditions [11, 29]) and lower in epithelia, such as gut epithelium (27), the epidermis of skin (28), and aortic endothelium (46).

The cell wounding frequency observed in tissues (26) and in vitro (25) correlates with the level of mechanical stress imposed, implicating mechanical stress as the disruptive agent. This correlation is evident both in comparisons of different tissues—muscle tissues have the highest measured levels, as already mentioned—and also in comparisons within a single tissue differentially challenged with mechanical stress. For example, in eccentric exercise, a muscle repeatedly contracts while lengthening, exerting high levels of mechanical force on itself. This immediately increases myocyte wounding fivefold over resting levels (29). Finally, pathological levels of mechanical force leave a striking imprint of wounded cells in tissues. For example, the track of a syringe needle puncture of skin (28) or muscle (29) is lined with cells heavily labeled with peroxidase or dextran reporter molecules, or with cells transfected with "naked" DNA (45), when these various reagents are injected through the penetrating needle.

These disruptions are probably survived by cells. First, the morphology of the cells detected is normal; it is neither necrotic or apoptotic (27). Second, when an exogenous "wound" reporter (fluorescein dextran or peroxidase) is used instead of endogenous albumin, it can be delivered as a pulse by injecting it into the blood vascular system. Excretion then eliminates it over a period of $\sim$24 h. Skeletal muscle (9), gut (27), skin (28), and aortic endothelium (46) tissues have been thus assessed, and all contained cells labeled in their cytosol with dextran or peroxidase. These must be surviving cells since dead cells do not retain these freely diffusible tracers. Moreover, during a 60-min interval in an isoproterenol-stimulated animal, when 60% of the cardiac myocyte population becomes wounded, heart function (rate and force of beat) actually increases (11). If all or even a significant proportion of these wounded myocytes were in fact dead, this would have been evident as compromised heart functioning.

A final important issue is the size of the disruptions incurred. At a minimum, they must be $\sim$1 nm, the approximate radius of gyration of albumin, but it is clear that mammalian and other cells can survive far larger ($\geqslant$1-$\mu$m diameter) wounds, as discussed below. So egress or entry of macromolecular aggregates or even organelles might sometimes occur in tissues.

## Surviving/Resealing Plasma Membrane Disruptions

Since disruptions are common in vivo, cells would die in great numbers if they did not rapidly reseal. Until recently, resealing was thought to occur passively; it removed hydrophobic domains from the aqueous environment and

*Table I. Cell Wounding under Physiological Conditions*

| Organ (ref.) | Cell types investigated | Percentage wounded |
|---|---|---|
| Skeletal muscle (29) | Skeletal muscle cells | 5–30 |
| Skin (29) | Epidermal cells | 3–6 |
| G.I. tract (27) | Epithelial cells | not measured |
| Cardiac muscle (11) | Cardiac myocytes | 20 |
| Aorta (46) | Endothelial cells | 6.5 |
| Inner ear* | Auditory hair cells | not measured |

*Unpublished evidence of Dr. M. Mulroy (Medical College of Georgia)

would therefore be energetically favored. Early work on the fusion of liposome vesicles supported this notion. An apparent requirement for $Ca^{2+}$ was explained by its action as a divalent to screen charges on phospholipids and by its ability to induce transient phase separations (35). Erythrocyte ghosts resealed under the right ionic conditions (3, 6, 19), so it was easily accepted that living cell membrane bilayers spontaneously resealed, given millimolar concentrations of divalents. In fact, rapid freeze-fracture electron microscopy of RBC revealed that small electroporation pores, 20–120 nm in diameter, could reseal within 10–20 s even in the nominal absence of divalents (6). This was also true of large liposomes, which could spontaneously reseal electroporation pores up to 1 μm in diameter on a millisecond to second time scale depending on membrane tension (47).

However, there were many reasons to doubt that the thermodynamic argument provided an adequate explanation for the amazing ability of living cells to survive breaks in their plasma membranes, as large in some cases as when an embryonic or skeletal muscle cell is cut in half. There is clearly a gradient of degree of possible membrane disruptions, and so the mechanisms used to reseal could very reasonably depend on how large a hole is created. Resealing in living cells has an absolute requirement for extracellular $Ca^{2+}$ (13, 14) and is antagonized by $Mg^{2+}$ (41), whereas in RBC ghosts it happens without $Ca^{2+}$ and can be facilitated by $Mg^{2+}$ (3). RBC ghosts reseal ruptures spontaneously (3, 6, 19), but slowly (time constant range from 30 min to hours [2] unless the lesion is extremely small [6]). In contrast, living cells normally reseal within seconds to 1 min (14, 41), and this rapid response is probably required for survival.

The requirement of external $Ca^{2+}$ for living cells to reseal suggested that $Ca^{2+}$ had one or more specific targets. In fact, block of multifunctional $Ca^{2+}$/calmodulin kinase inhibited resealing of microneedle punctures in both sea urchin embryos and 3T3 fibroblasts (41). $Ca^{2+}$/calmodulin kinase Type II associates with synaptic vesicles and may regulate exocytotic vesicle availability at synapses by phosphorylation of synapsin I and release from an actin-bound pool (4, 18). Functional block of kinesin, postulated to drive outward-directed transport of vesicles on microtubules, also inhibited membrane resealing (41). Microinjection of botulinum neurotoxin B or tetanus toxin blocked plasma membrane resealing after a delay expected for activation of its proteolytic action against synaptobrevin. Microinjection of botulinum neurotoxin A blocked resealing after a similar delay expected for its action in cleaving synaptoso-

mal-associated protein of 25K $M_r$ (SNAP-25) (41), as did also botulinum toxin C1, which specifically cleaves syntaxin (1). In all cases the block of resealing could be delayed by peptides mimicking the respective proteolytic peptide site for each specific neurotoxin protease (1, 41). These results suggest that proteins homologous to vesicle-associated membrane protein/synaptobrevin, syntaxin, and SNAP-25 mediate targeted vesicle exocytotic events that are induced by wounding (1, 41).

Imaging of individual exocytotic events confirmed that a high rate of $Ca^{2+}$-regulated exocytosis was required for resealing and that vesicle fusion had to be targeted to the cell wound site (1). Upon wounding by a laser beam, both eggs and embryos showed a rapid local burst of $Ca^{2+}$-regulated exocytosis dependent on toxin-sensitive soluble *N*-ethylmaleimide–sensitive factor attachment receptor docking proteins. The rate of localized exocytosis was correlated quantitatively with successful resealing (1). In wounded 3T3 cells, vesicles of a variety of sizes rapidly accumulated in large numbers within the cytoplasm surrounding the disruption site (33). At these sites, disruption induced a local $Ca^{2+}$-dependent exocytosis (33). It appears that $Ca^{2+}$-dependent mechanisms for cell membrane resealing may involve vesicle delivery, docking, and fusion, very similar to the exocytosis of neurotransmitters. The extra membrane added by exocytosis persists for 15–60 s or more, but its function is unknown (1).

At higher levels of membrane disruption, such as cutting a large chunk or severing an axon, cells appear to survive at first by forming a "plug" of vesicular material and cytoskeleton (16, 40). Transection of axons creates a locally very high rise in $[Ca^{2+}]_i$ and the local accumulation of vesicles (21). The formation of this tightly packed vesicular plug presumably allows restoration of $Ca^{2+}$ homeostasis, preventing cell death. However, some large gaps are rapidly (seconds) resealed by the construction locally of a sheet of new surface membrane derived from a massive fusion event involving internal membranes (44 and Terasaki and P.L. McNeil, unpublished observations).

The similarities of membrane resealing with neurotransmission imply that features formerly thought to be specializations of the nervous system may be elaborations of a more fundamental cell survival mechanism. Secretory systems and developmental responses that remodel the architecture of the cell surface might be evolutionary descendants of this ubiquitous exocytotic repair mechanism. This viewpoint would generalize our understanding of regulated cell surface events. Now to understand a particular class of cell surface event, we might be looking for relatively minor additions and/or subtractions to the proteins of this basic membrane addition machinery.

Organism-level adaptations for surviving plasma membrane disruptions may also exist. Normally, actin is present in blood at micromolar concentrations, but it can rise to even higher levels after traumatic injury and even exercise (39). Normal blood also contains filamentous actin severing proteins, such as gelsolin. It seems likely that cell wounding, in addition to cell death, is responsible for the presence of actin in normal blood, and that the role of gelsolin is to prevent the occurrence of microthrombi that otherwise might be induced by this exogenous actin.

## Preventing Plasma Membrane Disruptions

Intermediate filaments, spectrin, and dystrophin may protect cell membranes from damage by mechanical stress. For example, a human keratin 14 "knockout" showed a high degree of cell fragility resulting from the lack of an extensive basal keratin network (5). Erythrocytes lacking spectrin are mechanically highly fragile and lyse during exposure to normal hemodynamic stresses. Because of its homology to spectrin and association with both the actin-based cytoskeleton and extracellular matrix, dystrophin has been thought to play a primarily structural role in protecting the integrity of the sarcolemmal membrane from the considerable stresses induced during contraction. Dystrophic muscle cells, which lack dystrophin, are more susceptible to mechanically induced membrane ruptures (9, 31, 36). It has been suggested that increased membrane tearing leads to necrosis by eventually overwhelming repair processes (36), but there is little evidence that membrane wounding leads directly to cell death. Rather, while muscle from *mdx* mice show sixfold greater incidence of wounding after eccentric exercise than control mice, both normal and dystrophic myofibers quickly reseal the membrane wounds and survive (5, 9, 31, 36). Increased membrane wounding is not the direct source of the higher cytoplasmic $Ca^{2+}$ found in dystrophic muscle, since tears that would raise global cytoplasmic $[Ca^{2+}]_i$ should also allow an influx of sodium ions, but free sodium levels and sodium influx rates are normal in *mdx* myotubes (20, 42). Sarcolemmal tears are thus not the proximal cause of cell death; instead, their higher incidence must somehow become translated over time into a longer-term necrotic process. Transient localized $Ca^{2+}$ influxes through membrane wounds may lead to a long-lasting activation of $Ca^{2+}$ leak channels via $Ca^{2+}$-dependent proteolysis (43), which, in turn, could lead to eventual necrosis by persistent high levels of $[Ca^{2+}]_i$ just under the sarcolemma (43).

## Adapting to Mechanical Force: Mechanotransduction from Membrane Disruption

The "wound hormone" hypothesis attempts to explain how tissue remodeling, involving cellular hyperplasia and/or hypertrophy, is initiated in response to a stressful mechanical load (30). Chemical mediators of remodeling (growth factors), stored in cell cytosol, are proposed to be released from this location through plasma membrane disruptions. Since disruption frequency is proportional to stress imposed, a force transducing mechanism is built into this "unorthodox" mechanism of mediator release.

Support for the wound hormone hypothesis comes from studies of basic (bFGF)[1] and acidic (aFGF) fibroblast growth factors. These polypeptides lack a signal peptide sequence but, when present in the extracellular environment, are potent growth-promoting factors. Cell fractionation and immunostaining experiments both localize bFGF to the cytosolic and nuclear compartments (34, 37). bFGF is efficiently released into extracellular medium when mechanical force is used to disrupt specifically plasma but not or-

ganelle membranes of endothelial cells (30), and this occurs through disruptions that are survived, as well as those that are lethal (34). Sublethal injuries, such as those that occur for example when the trailing end of a cell is torn off during normal locomotion (7), are difficult to detect using conventional methods such as lactate dehydrogenase release, and this may explain why FGF release has been observed under conditions in which no overt cell death is apparent (32, 38).

Disruption-mediated release occurs also in tissues experiencing high levels of mechanical stress. First, an artery wall loses bFGF upon balloon injury, and the growth response that normally follows can be inhibited, at least in its earlier phases, by the administration of antibodies that neutralize bFGF growth-promoting activity (24). Second, individual skeletal muscle cells (myotubes) are depleted of immunoreactive cytosolic bFGF in proportion to the degree of disruption suffered upon either needle puncture injury or eccentric exercise of a muscle (9). Third, release of bFGF and aFGF into the perfusate of an ex vivo beating heart preparation is increased by isoproterenol simulation of rate and force of beat (11). Fourth, levels of bFGF are markedly elevated in the blood of human patients suffering from Duchenne muscular dystrophy (12), a disease in which myocyte cell wounding is strikingly elevated, as discussed above.

Wounding increases endothelial cell expression of bFGF message and protein (22), and moreover, bFGF protein is upregulated at sites of traumatic injury in vivo (15). Thus, tissues that have previously suffered an acute episode of cell wounding apparently alter their biological state in preparation for a second insult: increased levels of bFGF are stored for release through recurrent plasma membrane disruptions.

bFGF is a potent endothelial, fibroblast, and muscle (smooth, skeletal, and cardiac) growth- and/or multiplication-inducing factor (17). This suggests that its local release at site of mechanical stress could stimulate several essential activities of tissue remodeling/reinforcement: (*a*) provision of new/supplemental vasculature; (*b*) fibroblast hyperplasia and consequent rebuilding of extracellular matrix; and (*c*) increase in muscle cell size and/or numbers.

Potential signals also enter through disruptions. $Ca^{2+}$ entry into the wounded cell is a candidate signal for inducing bFGF expression, and also the expression of other genes that either facilitate cell survival of the disruption injury or promote tissue recovery or protection from the deleterious consequences of mechanical stress. In support of this hypothesis, stretch and shear stresses are known to alter gene expression in a variety of cell types. Though stretch-activated ion channels are generally assumed to transduce these responses, recent work suggests that typical cell stretching/shearing protocols can result in sublethal plasma membrane disruption (8, 10). Fibroblasts contracting a collagen gel in vitro suffer plasma membrane disruptions, and the resulting $Ca^{2+}$ influx leads in turn to cAMP accumulation (23), suggesting another pathway of disruption-initiated mechanotransduction.

## Perspective

Plasma membrane disruption is a common, normal, and

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1. *Abbreviations used in this paper*: aFGF and bFGF, acidic and basic fibroblast growth factors.

therefore biologically important event in many tissues. Specialized adaptations prevent disruption-induced cell death and protect cells from incurring this common injury; an active, exocytotic mechanism rapidly reseals, and specialized cytoskeletal proteins provide mechanical reinforcement. Mechanotransduction occurs when growth factors are released through disruptions. Since other proteins also leave by this route (39) and signals such as $Ca^{2+}$ enter, additional mechanotransduction mechanisms are also potentially initiated by plasma membrane disruption. Since mechanical stress predates cell–cell signal molecules as an important biological stimulus, it may be that $Ca^{2+}$-regulated exocytosis evolved originally as a resealing mechanism, and bFGF may, by similar reasoning, be an evolutionary primitive autocrine signal.

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