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Plasma membrane disruption (PMD) formation and repair in mechanosensitive tissues

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Abstract

Mammalian cells employ an array of biological mechanisms to detect and respond to mechanical loading in their environment. One such mechanism is the formation of plasma membrane disruptions (PMD), which foster a molecular flux across cell membranes that promotes tissue adaptation. Repair of PMD through an orchestrated activity of molecular machinery is critical for cell survival, and the rate of PMD repair can affect downstream cellular signaling. PMD have been observed to influence the mechanical behavior of skin, alveolar, and gut epithelial cells, aortic endothelial cells, corneal keratocytes and epithelial cells, cardiac and skeletal muscle myocytes, neurons, and most recently, bone cells including osteoblasts, periodontal ligament cells, and osteocytes. PMD are therefore positioned to affect the physiological behavior of a wide range of vertebrate organ systems including skeletal and cardiac muscle, skin, eyes, the gastrointestinal tract, the vasculature, the respiratory system, and the skeleton. The purpose of this review is to describe the processes of PMD formation and repair across these mechanosensitive tissues, with a particular emphasis on comparing and contrasting repair mechanisms and downstream signaling to better understand the role of PMD in skeletal mechanobiology. The implications of PMD-related mechanisms for disease and potential therapeutic applications are also explored.

Keywords

mechanotransduction; mechanosensation; cell membrane; mechanical loading; myocyte; muscle; osteocyte; bone; skeleton

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1. Introduction

Environmental adaptation is paramount to survival from the whole organism down to the cellular level, and a key component of this process is recognizing and responding to physical stimuli. Mechanotransduction is an evolutionarily conserved process that describes the conversion of mechanical stimuli into cellular signaling (1,2). This process informs how we perceive and respond to everyday life, from sensory information like touch, balance and proprioception, to cellular adaptation responses such as muscular hypertrophy, where mechanical loads initiate downstream signaling cascades that alter cellular behavior (3–6).

Mechanosensation mechanisms, or the processes by which cells detect and recognize mechanical stimuli, remain an area of rigorous study in many fields. Cells concurrently utilize myriad mechanisms to recognize mechanical stimuli, such as stretch-gated ion channels, chemical or force-mediated conformational changes of the cytoskeleton, molecular transport through channel proteins like gap junctions, hemichannels, and pannexins, and activation of voltage-gated calcium channels (7–20). Another such mechanosensation mechanism is the loading-induced development of small, transient discontinuities in the cell membrane called plasma membrane disruptions (PMD) (21). These PMD can be induced through several different mechanisms, including but not limited to exposure of the cell to physical deformation, debris, or fluid shear stress (12,22–25). Once formed, PMD initiate mechanotransduction signaling cascades similar to other known mechanosensation mechanisms (4,12,25–35). PMD facilitate the release of secondary messengers including ATP, nitric oxide (NO), and various growth factors (e.g., fibroblast growth factors) (34,36,37). Mechanotransduction can also be induced by transmission of signals from PMD-affected cells to nearby, non-wounded neighboring populations, providing a mechanism by which signaling from a small population of wounded cells may be amplified. For example, in MDCK kidney epithelial cells, PMD induced a paracrine purinergic signaling event that increased cyclic AMP synthesis in neighboring cells (34,35). Following PMD, neighboring cells displayed an initial decrease in cAMP, before experiencing a transient ATP-dependent increase in cAMP. In wounded cells, cAMP activity increases membrane repair rate, in anticipation of repetitive disruptions (38). The increased cAMP signaling in neighboring cells has been hypothesized to play a protective role in response to PMD events in nearby cells by preparing for their own PMD events to subsequently occur.

Resealing of PMD is necessary for cell survival, as without resealing, the cell is vulnerable to an unregulated influx of cytosolic toxins (such as calcium and oxidants) while simultaneously allowing ATP and proteins to escape (4,25,27,28,32,34–36,39–50). PMD repair (which will be discussed in the next section) therefore represents a fundamental, biological response to cell injury (4,6,12,29,30,33,51,52). Mechanosensitive cell populations utilize PMD-initiated signaling cascades to coordinate inflammatory mediators, release of secondary messengers, and recruitment of repair machinery to the site of the disruption and to initiate subsequent downstream signaling events. Calpains, annexins, growth factors, and NO have all been reported to play roles in signaling downstream of PMD in multiple types of mechanosensitive tissues (12,25,34–36,48,53–55).

PMD have been detected in skin, alveolar, kidney, and gut epithelial cells, aortic endothelial cells, corneal keratocyte fibroblasts and epithelial cells, cardiac and skeletal myocytes, neurons, and most recently in the periodontal ligament cells, osteoblasts, and osteocytes of the skeletal system (4,12,25,30,31,33,35,52,56) (Figure 1). A common feature of these cell types is that they are regularly exposed to varying magnitudes of shear stress (9,57–62). For example, epithelial cells in the gastrointestinal tract are affected by gut motility, which subjects the tract to compressive and shear stresses (29). Likewise, aortic endothelial cells are exposed to shear stress as blood circulates through the body (33,63), and osteocytes are exposed to fluid shear within the lacunocanalicular system (64,65). This review will address the similarities and differences between PMD in mechanosensitive tissues, with a focus on bone, comparing repair mechanisms, downstream signaling pathways, and the implications of these properties for disease states.

2. Generalized PMD repair mechanisms

PMD resealing is the outcome of an active, dynamic mechanism that is heavily reliant on a series of cytoplasmic components, including membranes, the cytoskeleton, and repair proteins (34–36,41,54,66–68). While some aspects of PMD development and repair are cell-type specific, the majority of known PMD repair processes follow a core set of basic principles (69,70). The first and immediate response of any cell to a PMD event is an influx of extracellular fluid, which under physiological conditions contains an abundance of calcium (Ca^{2+}).

The resealing mechanism initiated to repair a PMD is dependent upon its size. With very small PMD (< 1 nm), the exposure of the hydrophobic domains within the lipid bilayer results in rapid resealing of the membrane via diffusion of the lipids surrounding the PMD site (21,71–73); this resealing process is calcium-independent. However, in eukaryotic cells the plasma membrane is tethered to an underlying cytoskeleton, which produces an opposing membrane tension force (proportional to the disruption diameter cubed) that impedes spontaneous repair once the disruption exceeds a certain size (21). In mammalian cells, Ca^{2+}-initiated de-polymerization of cortical actin in the cytoskeleton is a prerequisite for PMD resealing (54,66,68,74,75). Disassembly and reassembly of microtubules also occurs in a Ca^{2+}-dependent fashion following a PMD (48). Uniform across mechanisms linked to mechanically induced PMD is the requirement of extracellular Ca^{2+} to initiate plasma membrane repair (32,43). Ca^{2+}-dependent exocytosis induces a decrease in membrane tension leading to resealing via lipid diffusion of small disruptions (10 to 100 nm in size) (49,76). Larger PMD (up to microns in size) rely on calcium-dependent exocytosis vesicle-vesicle and vesicle-plasma membrane fusion events to repair the damaged membrane (46,47,53). It has been proposed that during repair of large PMD, intracellular membranes form a “patch”, which then fuses to the plasma membrane surrounding the defect site (21,32) (Figure 2). Other proposed repair mechanisms, which vary across cell types and with PMD size, include caveolae-mediated constriction of the membrane around the disruption site, budding and blebbing of the wound site, and endocytosis of the PMD via invagination of caveolar vesicles (21,77–79). These repair mechanisms integrate with utilization of molecular motors and annexin proteins such as Annexin A1, A5, and A6 (41,53,80). A recent publication, using an array of visualization techniques (high-speed atomic force

microscopy, fluorescence recovery after photobleaching, confocal laser scanning microscopy, and molecular dynamics simulation) demonstrated that Annexin A5's particular role in PMD repair is to stabilize membrane defects so that subsequent vesicle-fusion resealing processes can occur (81). In contrast, Annexin A6's role in PMD repair has been hypothesized to involve promoting the folding and curvature of the extensions of cell, helping to refill the membrane disruption (82).

Protein machinery is recruited to the site of PMD repair. In the immediate aftermath of a physiological PMD event, increased cytosolic Ca^{2+} levels activates proteins including calpains and nitric oxide synthase. Various growth factors (e.g., fibroblast growth factors, FGFs), may also be released and promote tissue adaptation responses (43). Depending on the repair mechanism employed, additional protein machinery may accumulate about the PMD site such as ALG-2 interacting protein X (ALIX), charged multivesicular body protein (CHMP), vacuolar protein sorting-associated protein 4 (Vsp4), and endosomal sorting complex required for transport (ESCRT, particularly ESCRT III) proteins (32,34,36,40,46,79,83). SNAP Receptor (SNARE) proteins, comprised of syntaxins, soluble N-ethylmaleamide attachment proteins (SNAPs), and vesicle associated membrane proteins (VAMPs) are thought to drive membrane fusion (83–85) (Figure 2). Calpains are a critical component of PMD repair at this stage, as calpain small subunit (*Capns*) knockout cells demonstrated repair failure; calpains facilitate localized remodeling of the cytoskeleton at the site of the PMD, removing exposed cytoskeleton from the site of PMD and clearing the way for exocytotic vesicular fusion to reseal the cell membrane (54,67,68) (Figure 2). Within one to two minutes after formation of the PMD, the cytoskeletal network is re-established and the cell returns to normal function (49,79,86,87). Interestingly, a cell experiencing a second PMD soon after the first will reseal the latter PMD more quickly than the initial disruption (76,88) due to an increased amount of exocytosis triggered by the second insult to the plasma membrane; this process is dependent on signaling molecules like protein kinase C and protein kinase A (23,76,88). For more information on generalized PMD repair mechanisms, the reader is referred to several previously published reviews (21,71–73).

3. Cell specific PMD repair and signaling pathways

In this next section, we will describe the process of PMD formation, repair, and associated signaling cascades initiated by PMD that have been documented across several types of mechanosensitive tissues. Relevance to disease states and potential therapeutics, when known, will also be described.

3.1 Epithelial cell and fibroblast PMD

Epithelial cells are often directly subjected to environmental mechanical forces, and these loading scenarios can lead to formation of PMD. For example, mechanical brushing of the gingivae and tongue induced PMD in the oral cavity of rats, which increased expression of the mechanically responsive transcription factor c-fos in junctional epithelial cells (89). Likewise, keratinocyte epithelial cells in the skin developed PMD when mechanically lifted from the substratum (90). This process activated phospholipase D (particularly the

phospholipase D2 isoform), an enzyme involved in membrane trafficking. Additionally, keratinocyte PMD repair was inhibited in the presence of phospholipase D inhibitors, suggesting that induction of phospholipase D activity is critical for keratinocyte membrane repair mechanisms (90). Carrying these studies *in vivo*, provision of phosphatidylglycerol (a lipid signal generated by phospholipase D) to a mouse full-thickness skin biopsy wound enhanced the rate of wound healing (90).

In addition to skin and oral cavity epithelial cells, PMD have been observed in the mucosal epithelial cells of the gastrointestinal tract, epithelial cells and keratocytes in the cornea, alveolar epithelial cells of the lung, renal proximal tubular epithelium, and in epithelial breast cancer cell lines. Epithelial cells in the gastrointestinal tract are subjected to compressive and shear stresses during gut motility that can lead to PMD formation (29). In rat gastric epithelial cells, an apparent reduction in F-actin was visualized at disruption sites and flow cytofluorometric analysis of the cytoskeleton using phalloidin staining showed that this decrease in F-actin was Ca^{2+}- dependent (74). PMD formation in gastric mucous epithelial cells promoted mucous secretion both in isolated cells and in organ culture models, and both PMD repair and the resulting mucous secretion were Ca^{2+}-dependent (63). Carbohydrate-binding lectin proteins, which can be toxic following ingestion, impaired PMD repair in gastric epithelial cells; this was suggested as a novel form of protein-based toxicity that could explain mechanisms of lectin-associated food poisoning (91). Corneal epithelial cells develop PMD, which reseal via vesicular fusion events, in response to ultrasound exposure (52). Likewise, corneal keratocyte fibroblasts develop PMD from rubbing motions (i.e., rubbing the eye), and these PMD initiate Ca^{2+} signaling both in the wounded cell and in non-wounded adjacent cells (22). In the lung, PMD in alveolar epithelial cells have been hypothesized to play a role in ventilator-induced lung injury (92,93). The protein tripartite motif containing 72 (TRIM72) is critical for repair of lung alveolar epithelial cells following induction of PMD (94), where TRIM72-mediated repair inhibited stretch-induced p53 activation by promoting its proteasomal degradation, consequently reducing cell apoptosis (95). A recent report demonstrated that MDA-MB-231 breast cancer epithelial cells developed PMD during migration associated with metastatic invasion, and that annexin proteins, particular Annexin A5 and A6, were critical to the repair of these PMD (96). Moreover, silencing Annexin A5 and A6 via shRNA impaired PMD repair in these cells and led to cell death, suggesting a potential novel avenue for preventing cancer metastasis (96). Specialized secondary messengers, such as NF-kB, are activated following the induction of a PMD in epithelial cells (70).

3.2 Endothelial cell PMD

Caveolae play an important stabilizing role in endothelial cell membranes subjected to physiological hemodynamic forces, where disassembly of caveolae protects against PMD formation during loading (97). Not surprisingly, endothelial cells from *caveolin 1*-null mice were more susceptible to PMD formation when challenged with increased cardiac output from treatment with dobutamine (97). Using endogenous albumin as a PMD tracer, approximately 6.5% (ranging from 1.4 to 17.9% across a group of n=14 animals) of aortic endothelial cells in young male Sprague-Dawley CD rats presented with PMD under baseline *in vivo* conditions (33). These PMD occurred in longitudinal streaks aligned along

the axis of blood flow or in clusters around vascular bifurcations. Surprisingly, the abundance of endothelial cell PMD did not increase with exercise, although sample sizes were quite small (n=5 exercised animals) to detect this change (33), suggesting further study would be necessary before forming conclusions regarding the role of exercise in vascular endothelial PMD formation. Endothelial cells produce and respond to basic fibroblast growth factor (bFGF), also known as FGF2. Interestingly, bFGF lacks a key signaling peptide required for exocytotic export, and therefore is believed to be released primarily via PMD (36). It has been proposed that PMD-induced release of bFGF from endothelial cells promotes proliferation either in the wounded cell or in adjacent non-wounded neighboring cells (33).

3.3 Neuron PMD

Traumatic brain injury (TBI), caused by high strain rate loading, induces PMD in neurons (98–103). As one example, a 2 atmosphere central fluid percussion injury in rats led to detection of PMD in more than half of the neurons in layers V and VI of the lateral neocortex soon after injury (104). These PMD, also referred to as “mechanoporation” (101,104) occur in both focal and diffuse models of TBI immediately upon induction of the injury (103–105). The severity of the injury correlates with the number of PMD formed, and PMD are formed in the areas of highest principal strain and shear stress in the tissue (98,101). In the event of PMD repair failure, neurons experience uncontrolled calcium influx, ATP dysregulation, and eventual cell death (100,106). However, some wounded neurons are capable of PMD repair, particularly in diffuse TBI models (100,107). For example, in rats subjected to a diffuse TBI (450 g weight dropped from 2 m onto a metallic helmet affixed to the rat’s skull), approximately 40% of the neurons with PMD at 4 hours after wounding demonstrated an ability to reseal the disruption and presented with normal cellular morphology (107). Interestingly, several studies suggest that PMD formation in neurons following a TBI is biphasic, where an additional population of neurons develop PMD as a secondary (non-mechanical) event in the hours to days after injury (104,107).

3.4 Myocyte PMD

Skeletal muscle and cardiac muscle have both been reported to develop PMD with *in vitro* and *in vivo* mechanical loading (25,108–110). Skeletal muscle myocytes are particularly prone to injury during eccentric loading, when actively contracting against a lengthening stretch (4,111). Conversely, at least one human study suggests that PMD formation in skeletal muscle is reduced during simulated microgravity (112). In addition to their role in genetic myopathy conditions such as muscular dystrophy, myocyte PMD explain the physiologically relevant immediate loss of muscle’s capacity for force production after high force exercise (4). PMD are also critical effectors in cardiac muscle. Ischemic-reperfusion injuries, which can occur following stroke or heart attack, induce PMD (113,114). Like bone cells (115), myocytes increase expression of c-fos following exposure to mechanical loading as part of their hypertrophic response (116–118), and formation of PMD is required for this to occur (25). PMD in muscle have also been shown to be critical for release of bFGF / FGF2; this has been hypothesized to be an important mode of bone-muscle crosstalk during musculoskeletal hypertrophy (119).

PMD repair in myocytes requires Annexin A5 (80) and A6 (82). Lipid-directed antioxidant activity, such as the antioxidant Vitamin E or the antioxidant enzyme glutathione peroxidase 4 (GPx4), is also required for both *in vitro* and *in vivo* myocyte membrane repair, as long-term dietary vitamin E deficiency or knockout of the GPx4 enzyme led to PMD repair failure (42,120). Myocyte PMD repair is impaired in microgravity, as acute microgravity exposure (parabolic flight) inhibited membrane-membrane fusion events in human myoblasts (121). Dysferlin, a calcium-sensitive protein important for vesicle fusion, is recruited to the PMD site in skeletal and cardiac muscle (66,122), where it is eventually cleaved by calpain 1 and 2 (54,68). Dysferlin deficiency impairs tethering of lysosomes to the cell membrane, resulting in impaired lysosome exocytosis that delays PMD repair (123). Mitsugumin 53 (MG53), also known as TRIM72, is a member of the tripartite motif family that is also critical for muscle PMD repair (108,124,125), as it accumulates in the membrane at the PMD site and recruits vesicles needed for resealing of the disruption (124). MG53-deficient myocytes are unable to repair PMD, which may explain why MG53 knockout led to progressive myopathy and myocardial damage *in vivo* (108,124,125).

Changes in processes of myocyte PMD formation and repair are associated with muscular disorders. In humans, dietary deficiency in Vitamin E is associated with skeletal myopathy and frailty, particularly in the elderly; this is consistent with Vitamin E's critical role in myocyte plasma membrane repair (120,126). Similarly, mutations in the myocyte PMD repair-associated protein dysferlin result in limb-girdle muscular dystrophy type 2B (109,127) and cardiomyopathy (110). The propensity for myocyte PMD formation and repair is altered in patients with Duchene's Muscular Dystrophy (DMD), a genetic disorder resulting from mutations in the spectrin family member dystrophin (128). Dystrophin is a cytoskeletal structural protein that provides support for the plasma membrane and links the plasma membrane to the extracellular matrix (129). Absence of dystrophin in DMD results in increased susceptibility to PMD development and a reduced chance of surviving the disruption, thereby increasing post-wounding myocyte death that leads to muscle atrophy (129,130). In the *mdx* mouse model of DMD, dystrophin-deficient mice developed significantly greater PMD under conditions of normal cage activity and following mechanical loading from exercise (131,132). These data suggest an intriguing role for dystrophin and the spectrin family of proteins in mechanosensation. Several studies suggest that enhancing plasma membrane stability and repair, via treatment with Poloxamer 188 (also known as Pluronic F-68), can abrogate myopathy phenotypes in dystrophin- and dysferlin-deficient mice (133–137). However, conflicting reports also exist (138,139), suggesting that further research into the potential benefit of Poloxamer 188 therapy in PMD-associated disorders is required.

3.5 Bone cell PMD

We (12,24,140) and others (30,141,142) have shown that various cell populations within the skeletal system likely utilize PMD as a mechanism to sense mechanical stimuli. In teeth, mechanical forces initiated by chewing induce PMD in periodontal ligament cells, which promotes release of factors that may affect bone remodeling like IL-113 and bFGF (142). In the appendicular skeleton, it is commonly understood that fluid shear within the lacunocanalicular system is a primary stimulus for the mechanobiological responses of bone,

and that osteocytes sense this fluid shear via their dendritic processes (62,143,144). Osteocytes likely utilize many different mechanosensation mechanisms to respond to mechanical loads, where the magnitude of loading (i.e., level of strain) may dictate which mechanism dominates (7,10,12,14,145–150). However, consistent with the processes described above, we have shown that osteocytes develop PMD in response to mechanical loading both *in vitro* and *in vivo*, and that these PMD occur on the osteocyte's dendritic processes (Figure 3) (12). This work was verified by an independent laboratory, who showed that PMD formation can also occur in osteoblasts (141).

In osteocytes, PMD likely form in response to the drag forces induced by fluid shear on the osteocyte pericellular matrix (i.e., glycocalyx) (24) (Figure 3). This is evidenced by the fact that osteocyte PMD are most readily formed by *in vitro* fluid shear when cells have been cultured for at least 3 or 4 days prior to loading (*unpublished observation*), consistent with the timeframe needed for pericellular matrix deposition (151). Furthermore, removal of the pericellular matrix with hyaluronidase negated fluid shear-induced osteocyte PMD formation (24). Osteocyte PMD formation is also dependent upon the loading profiles to which the cells are exposed. Most *in vitro* osteocyte mechanobiology studies utilize fluid shear stresses of less than 20 dynes/cm^2, and osteocyte PMD formation is not robust at these levels of loading (12). However, modeling- and tracer-based studies suggest that osteocytes experience much higher shear stresses *in vivo* (between 30 to 70 dynes/cm^2) due to amplification from lacunocanalicular geometry (64,152), and in line with this concept, osteocyte PMD formation was abundant within this range of shear stresses (12). Therefore, PMD may represent a mechanosensation mechanism that is preferentially activated in higher intensity loading scenarios. Consistent with this hypothesis, PMD are only formed in osteocytes when a fluid shear load is applied rapidly; gradual application of shear (such as through a ramped approach to a peak shear stress) prevents the formation of osteocyte PMD (12). Notably, this harmonizes with the previously published finding that osteocytes require a "stress kick" to respond to fluid shear, where a gradual onset of loading blunts downstream mechanotransduction events like release of nitric oxide (153).

PMD induce intracellular Ca^{2+} signaling, release of ATP, and upregulation of c-fos in bone cells (12,141); analysis of other downstream mechanotransduction responses is currently ongoing. Following induction of a PMD in osteoblasts or osteocytes, extracellular Ca^{2+} rushes into the cell and triggers release of intracellular Ca^{2+} stores (12,24,140). Increased cytosolic Ca^{2+} levels activate PLC/PKC signaling, initiating repair of the PMD via Ca^{2+}/PLC/PKC-dependent vesicular exocytosis of ATP (141) (Figure 3). The increased PKC levels "prime" the vesicular pathway, and over time and multiple cycles of mechanical stress, influence the resilience and sensitivity of the cell's response to mechanical stimulation (141,154). Vesicular fusion is promoted by Ca^{2+}/PLC/PKC-dependent exocytosis in bone, similar to its role described in the resealing of PMD in other cell types (35,141). The PKC isoform PKCμ, also known as Prkd1, was particularly identified as critical for mediating PMD repair in bone cells (Figure 3) (141). The rate of PMD repair in osteocytes can be altered, and doing so can affect downstream signaling (12,140). As one example, enhancing osteocyte PMD repair rate via Vitamin E supplementation, while beneficial for cell survival, blunted the downstream mechanotransduction response (12). Carrying this *in vivo,* male mice fed a Vitamin E-deficient diet developed more PMD with

loading, and showed an increased bone formation response to exercise, as compared to mice fed a control diet (140).

PMD formation and repair-related mechanisms may underlie some of the changes in bone mechanobiology with aging, where aged bone becomes less responsive to a given level of loading (155). Bone's mechanosensing osteocytes are lost with aging, and surviving osteocytes demonstrate impaired mechanotransduction (156,157). Consistent with this idea, we recently reported that osteocytes from old mice developed fewer PMD from fluid shear than cells from young mice, and old mice developed fewer osteocyte PMD during exercise *in vivo* (24). This was due at least in part to decreased pericellular matrix production with age, as pericellular matrix was critical for osteocyte PMD formation, and aged osteocytes produced approximately half of the pericellular matrix levels detected in young osteocytes (24). Quite surprisingly, PMD repair rate was faster in old as compared to young osteocytes (24). This observation was associated with a blunted calcium wave propagation to adjacent non-wounded osteocytes and was attributed to increased oxidative stress with age. As oxidative stress impairs osteocyte PMD repair and subsequent cellular survival (158), it was hypothesized that increased oxidative stress during aging promoted a progressive loss in slower- repairing osteocytes consistent with both osteocyte loss and impaired mechanosensitivity in aged bone (24).

It is worth noting that many key features of bone cell mechanotransduction share considerable overlap with processes related to PMD formation and repair. For example, an intracellular Ca^{2+} spike is one of the earliest responses triggered in an osteocyte exposed to fluid shear, and that response requires the presence of extracellular Ca^{2+} (37,159). This is identical to the influx of extracellular Ca^{2+} as being the earliest detectable event in PMD formation, and the requirement of the presence of extracellular Ca^{2+} for subsequent PMD repair (32,43). Release of ATP is a critical component for transmission of Ca^{2+} signaling between osteocytes during loading (37,150), and is also critical for transmission of Ca^{2+} signaling between wounded and non-wounded bone cells (12,141,160): degradation of extracellular ATP with apyrase prevents propagation of a Ca^{2+} wave from an osteocyte with a PMD to non-wounded neighboring osteocytes (12), similar to mechanotransduction mechanisms previously reported for mechanically-loaded osteocytes (37). As described above, Annexin A5 is critical for proper resealing of a PMD (80,81), which is consistent with the finding that Annexin A5 disruption impairs mechanically-induced calcium signaling in osteoblastic cells (161). The transcription factor c-fos is induced in osteocytes by both fluid shear (115) and PMD (12), and the microtubule network is implicated in both the response of osteocytes to fluid shear (162) and PMD repair (48). Both fluid shear (37,159,163–165), and PMD (141,166,167) induce ATP, NO, and prostaglandin E2 release as secondary messengers downstream of the initial precipitating event. Likewise, other laboratories have shown that pericellular matrix is critical for osteocyte mechanobiology (151,168,169), and that degradation of the osteocyte glycocalyx blunts flow-induced upregulation of prostaglandin E2 release (170), and we showed that the osteocyte glycocalyx is critical for the formation of flow-induced PMD (24). Under physiological conditions, osteocytes repair a PMD in approximately 10 to 30 seconds (12). Cells that experience a second PMD soon after the first repair the latter PMD more quickly (34), and in osteocytes, PMD repair that occurs too quickly blunts the subsequent downstream mechanotransduction

cascade (12,24). Together, these findings could be consistent with the well-accepted principle that insertion of rest periods between bouts of mechanical loading enhances skeletal responses to loading (171–173). Admittedly, these points comparing downstream responses to fluid shear and PMD in bone cells show correlation and not necessarily causation. However, they do highlight the striking similarities in these processes and provide further support for the notion that PMD may be an important mechanosensation mechanism in bone cells, particularly at higher levels of loading. Future studies, designed to alter PMD susceptibility and repair in osteocytes, will be needed to definitively test the role of osteocyte PMD formation in skeletal mechanobiology.

4. Conclusion

In conclusion, this review has highlighted that PMD are a key mechanosensation mechanism in the processes by which mechanosensitive mammalian cells detect and respond to mechanical loading in their environment. While bone may be the most recently discovered tissue to develop and respond to PMD with loading, it is notable that all responses downstream of PMD in bone cells discovered thus far are consistent with PMD responses reported in other mechanosensitive tissues like epithelial cells, endothelial cells, and myocytes. Thus, PMD formation and repair may represent a novel mechanosensation pathway in bone and a target for modifying skeletal adaptation signaling in osteocytes, such as strategies aimed at improving bone mechanosensitivity with aging.

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Highlights

- Plasma membrane disruptions (PMD) develop in mechanosensitive cells and tissues
- Physical deformation, debris, or fluid shear stress can all promote PMD formation
- PMD initiate mechanotransduction and release factors like ATP, PGE2, NO, and FGF
- Repair of PMD is required for cell survival
- PMD repair rate can modulate the cell's downstream mechanotransduction response
- PMD formation and repair processes are relevant to skeletal mechanobiology

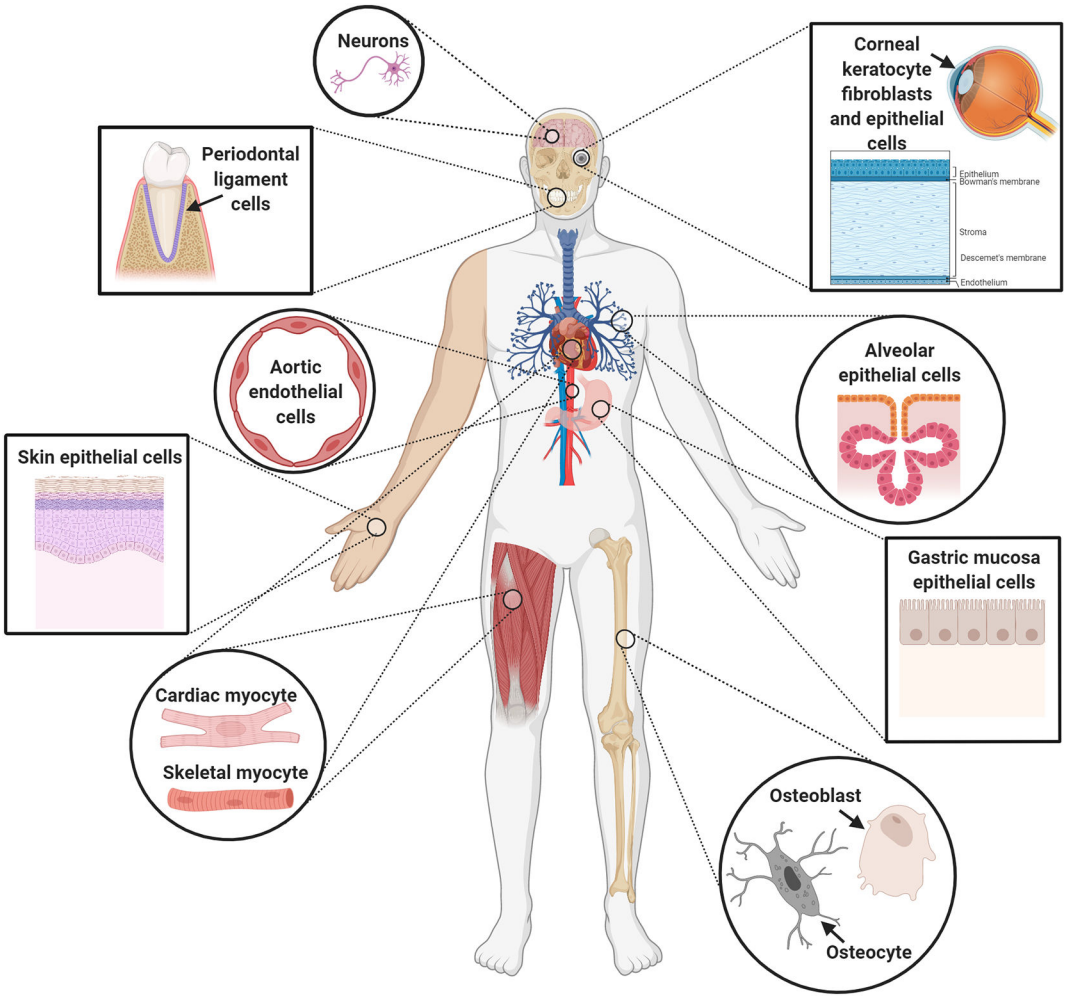

Figure 1.

Tissues and cell types in which plasma membrane disruptions (PMD) have been observed. Figure created with BioRender.com.

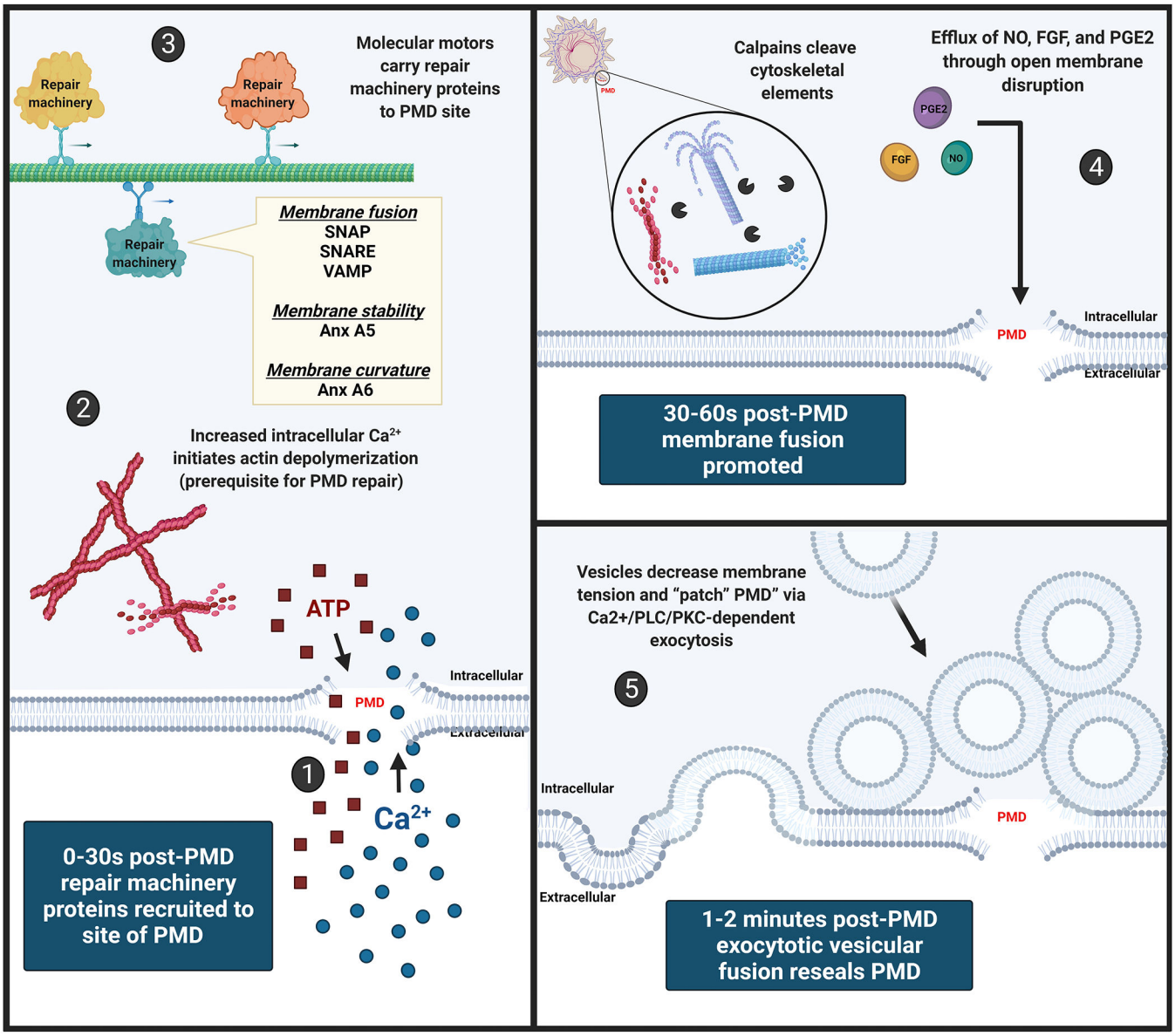

Figure 2:
Generalized molecular mechanisms and relative time frame for PMD repair. Figure created with BioRender.com.

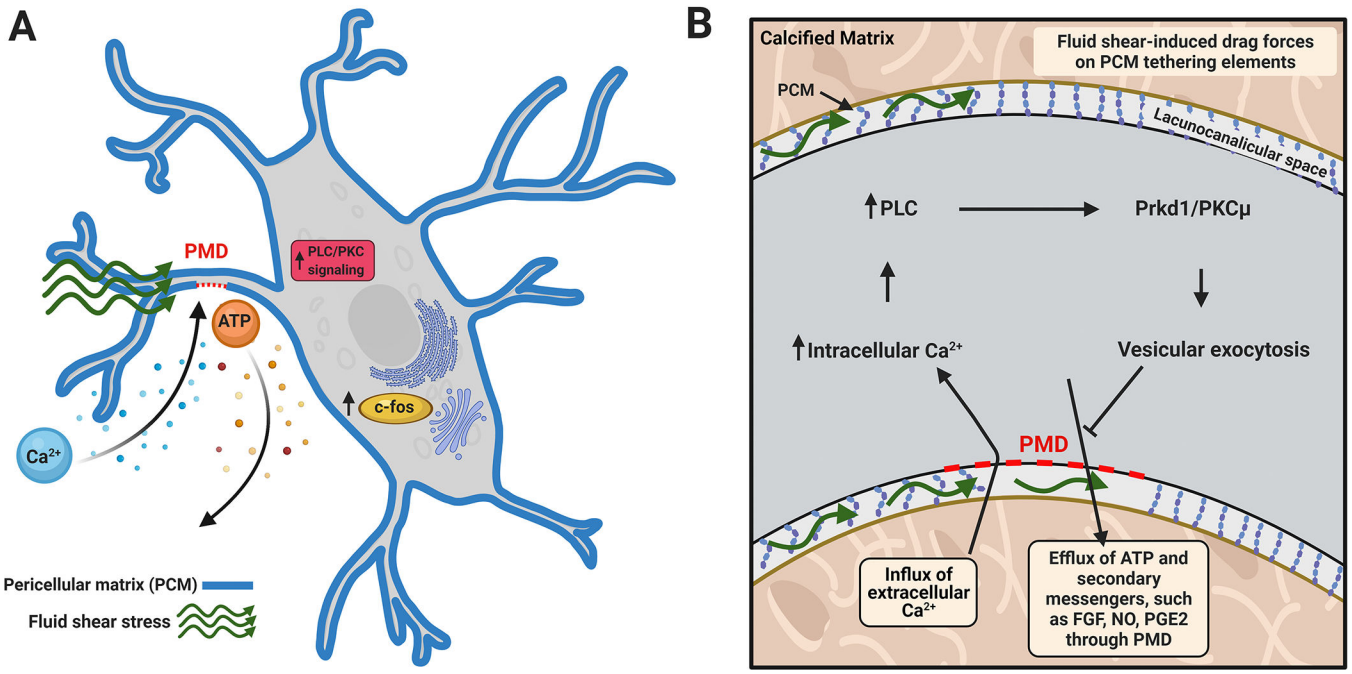

Figure 3: Plasma membrane disruption (PMD) formation and repair in osteocytes.

A) PMD formation in osteocytes is hypothesized to occur via drag forces from fluid flow acting on the pericellular matrix of the osteocyte. B) Magnified view of an osteocyte dendritic process with a PMD, demonstrating mechanisms of PMD formation, downstream signaling, and repair triggered by influx of extracellular calcium through the PMD. Figure created with BioRender.com.