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Balance of Mechanical Forces Drives Endothelial Gap Formation and May Facilitate Cancer and Immune-Cell Extravasation

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July 18, 2018

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Abstract

The formation of gaps in the endothelium is a crucial process underlying both cancer and immune cell extravasation, contributing to the functioning of the immune system during infection, the unfavorable development of chronic inflammation and tumor metastasis. Here, we present a stochastic-mechanical multiscale model of an endothelial cell monolayer and show that the dynamic nature of the endothelium leads to spontaneous gap formation, even without intervention from the transmigrating cells. These gaps preferentially appear at the vertices between three endothelial cells, as opposed to the border between two cells. We quantify the frequency and lifetime of these gaps, and validate our predictions experimentally. Interestingly, we find experimentally that cancer cells also preferentially extravasate at vertices, even when they first arrest on borders. This suggests that extravasating cells, rather than initially signaling to the endothelium, might exploit the autonomously forming gaps in the endothelium to initiate transmigration.

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Introduction

Immune and cancer cells alike are characterized by their ability to migrate within the vasculature and then to leave the vasculature into different tissues. These processes are crucial for a functioning immune system to fight acute infections [1] or participate in wound healing [2]. However, chronic inflammation or tumor metastases are ultimately also initiated by extravasating immune or cancer cells, respectively [3, 4, 5]. Hence, while extravasation is critical to cure communicable diseases, it is also a critical contributor to virtually all non-communicable disease, ranging from cancer to asthma, atherosclerosis, rheumatoid arthritis and heart diseases [6, 4, 7].

Much of the research on extravasation (often termed diapedesis in the context of immune cells) has focused on the role of the extravasating cell during this process, and how it interacts with the endothelial cells of the vasculature through which it is transmigrating. First, the extravasating cell needs to arrest in the vasculature. This may occur through single cells or clusters getting physically stuck in small capillaries, through the formation of adhesions, or both [8, 9, 10, 11, 12]. Such adhesion is mediated by molecules including P-and E-selectin, ICAM, VCAM or integrins [13]. The actual process of transmigration can occur through a single endothelial cell (transcellular extravasation) or, more commonly, in between two or more endothelial cells (paracellular extravasation) [14, 1].

During paracellular extravasation, it was investigated how the extravasating cell signals to the endothelial cells, leading to weakening of VE-cadherin-mediated cell-cell junctions and subsequently gap formation, through which the cells can transmigrate [8, 1]. Gap formation may, for instance, be stimulated by thrombin [15]. As such, molecular signaling events are firmly established as important contributors to extravasation of immune cells.

However, on a fundamental level, all the processes involved in extravasation are mechanical processes. Transmigration, like other forms of cell migration, involves the generation of mechanical forces through the actomyosin cytoskeleton [16]. Moreover, the mechanical properties of the endothelium provide passive mechanical resistance [16]. For instance, increased endothelial cell and junctional stiffness will reduce paracellular extravasation rates [17, 14]. Interestingly, recent research established that active mechanical properties of the endothelial cells are also critical during endothelial gap formation [18, 19, 20], and the rearrangements of cytoskeletal structures are associated with changes in barrier function. For instance, a rich actin cortex parallel to cell-cell borders is associated with stabilized VE-cadherin junctions and thus tight barriers [21, 22], whereas actomyosin stress fibers pulling radially on junctions can lead to junctional remodeling [18, 23]. Additionally, actin-rich pores can actively contract to prevent leakage during extravasation [24]. However, there is still a lack in mechanistic and systems level understanding of the different roles

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of active and passive mechanical properties of the endothelium.

Mathematical multiscale models are powerful tools to investigate the interplay of different physical drivers of biological processes. Many different approaches have been employed to model and understand the dynamics of epithelial monolayers. Agent based models, where individual cells are explicitly taken into account, include center based models (CBM)[25], vertex models [26, 27] and deformable models (DFM)[28, 29]. However, these models do not explicitly model cell-cell adhesion dynamics in a way that leads to the experimentally observed gap formation in monolayers of endothelial cells, and can thus not easily be employed to study this problem so crucial for cancer and immune transmigration.

In this paper, we introduce a mathematical multiscale model of the mechanics of an endothelial monolayer where each endothelial cell contains contractile actin structures that may contract radially or in parallel to the plasma membrane. Then, cells are tethered to neighboring cells by cell-cell junctions that can dynamically form and break in a force-dependent manner. We employ this model to investigate the mechanisms of gap formation in an endothelial monolayer. Interestingly, we find that gaps open dynamically in the absence of any extravasating cells. These gaps form preferentially at the vertices where three or more endothelial cells meet, as opposed to the borders in between two cells. This is in line with our experimental data obtained in-vitro from quantifying gap formation of monolayers of HUVECs seeded on glass. We quantify the frequency of gap openings as well as the duration of gap openings, obtaining good agreement between numerical predictions and experiments. Moreover, through multi-dimensional parameter studies, the mathematical model is able to give us insights into the physical and molecular drivers of the gap formation and gap dynamics. The model predicts that active and passive mechanical forces play an important role in the initial gap formation and in controlling size and lifetime of gaps once they initially formed. The catch bond nature of the cell-cell adhesion complexes as well as the force-dependent reinforcement of adhesion clusters may both stabilize junctions in response to forces acting on them. However, while the catch bonds ultimately weaken when forces are increased beyond the maximal lifetime of a single molecular bond, the force-dependent reinforcement will increase adhesion strength with increasing force [30, 18]. While the catch bond nature and the force dependence of the adhesion clustering processes both crucially influence gap opening frequencies, we find that gap lifetime and gap size are even more sensitive to the passive mechanical properties of the cell. Increased stiffness of the membrane/cortex and, even more notably, of the actin stress fibers will reduce lifetime and size, since the cells will then increasingly resist opening gaps through counteracting forces. On the other hand, we find that bending stiffness of the membrane/cortex may have gap promoting or inhibiting effects.

Our model predictions of gap opening frequency and lifetime at both cell vertices and borders are validated by experiments observing such gaps in endothelial monolayers in the absence of any extravasating cell.

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The results thus challenge the paradigm that all extravasating cells primarily cause gap opening through interactions with the endothelium [1, 8, 31]. We then show experimentally that extravasating cancer cells indeed primarily extravasate at vertices, in line with similar observations for neutrophils [32]. Moreover, we show that cancer cells prefer to extravasate at vertices even when they initially attached to the endothelium at two-cell borders. This suggests that rather than actively opening a gap at the cell-cell border, they instead wait for the occurrence of a gap at a tricellular vertex, as predicted and verified to occur more frequently than gaps at borders by our model and experiments. In summary, our work highlights the importance of taking the dynamic and autonomous mechanical properties of the endothelium into account when trying to understand gap formation and extravasation.

Results

Computational Model of Endothelial Monolayers

We present a novel model of an endothelial cell (EC) monolayer that incorporates different intracellular mechanical structures and dynamical cell-cell adhesions. The intracellular mechanical state is determined by radial contractile actin stress fibers and the cell membrane together with the actin cortex. For simplicity, we combined membrane and cortex into single viscoelastic elements, composed of an elastic spring and a viscous damper, that we refer to, from now on, as membrane elements. The radial stress fibers are also modeled by viscoelastic elements with different mechanical properties from the membrane, similar to a model of epithelial cells [28] (see Fig. 1A). Neighboring cells may form cell-cell adhesions at adjacent nodes, and the resulting adhesion bond is modeled through a spring. The passive mechanical properties of the monolayer are thus modeled through a network of connected elastic and viscoelastic elements, similar to models of epithelial sheets [29, 28]. Since we are interested in studying the opening dynamics of gaps in the endothelial barrier, we explicitly simulate the dynamical binding of adhesion complexes. Contractions represent myosin motor activity that is known to exhibit randomness [33], so we employ Monte-Carlo simulations to estimate the occurrence of such forces as well as that of protrusive forces due to actin polymerization. The forces are then redistributed across the network of connected viscoelastic elements. Cell-cell adhesion complexes that mechanically link neighboring cells can dynamically bind and unbind in a force-dependent manner. The adhesion complexes in the model provide an effective description of both bonds of cell-cell adhesion molecules (such as VE-cadherin) and bonds of these adhesion molecules to the cytoskeleton. Cadherins and adhesion-cytoskeleton bonds are known to increase their binding strength in response to smaller forces, before they ultimately rupture [34]. This catch-bond type behavior is included in our model, and unbinding is thus

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simulated through a force-dependent Monte-Carlo simulation. Moreover, the number of VE-cadherins in an adhesion complex is modeled through a force-dependent adhesion clustering mechanism, as described in [18, 23, 35, 36, 37]. A more detailed description of the mathematical model and its numerical implementation is given in the supplementary information (SI).

We employ our endothelial monolayer model to explore the dynamics of endothelial cell junctions. We predict the frequency, size and duration of gaps, as well as the preferred geometrical locations of the gap formation, and compare the predictions with our experimental measurements. The parameters used in the simulations are detailed in Table S1. After comparing our predictions with the experimental results, we perform sensitivity analyses to investigate how cell mechanical properties, cell-cell adhesion characteristics and myosin generated forces regulate the formation, lifetime and size of gaps in the endothelium.

Gaps open preferentially at vertices

Fig. 1B,C and Movie S1 show typical simulations of the monolayer dynamics of the computational model. We observe that gaps open preferentially at vertices, i.e. the intersections of three or more cells, as opposed to the border between two cells. We have quantified this by counting the total number of gaps formed as well as their lifetime at borders and vertices of the cell in the center of the monolayer, and showed that our model predictions are in line with the experimental observations (Fig. 1G,H). These experiments were performed by seeding HUVEC cells on glass that formed a continuous monolayer, and then extracting the gap statistics through image analysis (see Methods for further details of the experimental setup and quantification). Vertices are points where more than two cells exert forces and where tangential force components naturally propagate to. Therefore, it is expected that stress concentrates at the three cell vertex rather than at the two cells borders, and the simulations confirm this hypothesis (Supplementary Fig. S8 and Movie S3). The forces on adhesion clusters at the vertices are thus more likely to exceed the corresponding force of maximal lifetime of the bonds, as will be discussed in more detail below.

Mechanical properties of cell-cell adhesion complexes limit endothelial gap opening frequency

We study how variations in the mechanical properties of the cells, the cell-cell adhesion complexes or force variations affect the rate of gap formation. Fig. 2A,B show how passive mechanical properties of the cell affect both the frequency (Fig. 2A) and the location of the gap openings (Fig. 2B). Increasing stiffness of either the membrane or the stress fibers provokes a decrement of the gap generation frequency (Fig. 2A and Movies S4,S5). This is intuitive, since increasing stiffness stabilizes the movements of cells and

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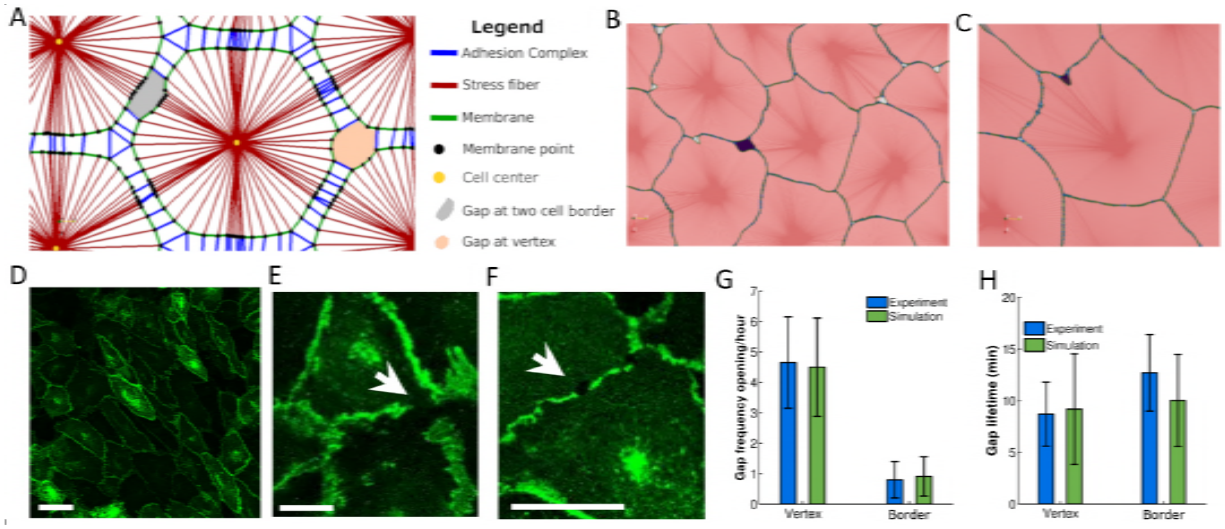

Figure 1: **Endothelial Gaps open preferentially at vertices.** A: Main components of the model. Cells are formed by stress fibers (red), membrane segments (green) and membrane points (black). Membrane points are binding sites at which cell-cell adhesion complexes (blue) may connect to membrane points on adjacent cells and thus mechanically link these cells. When these adhesions break as a consequence of the force-dependent binding law, gaps in the endothelium are generated. Gaps can be generated at a two cell border (grey) or at a vertex between three or more cells (orange). B, C: Simulation of endothelial monolayer dynamics. Green denotes cell membrane and red cell body (darker red are the stress fibers that compose the cell). Dark purple denotes a detected gap. B: Gap at a vertex. C: Gap at border. D-F: Endothelial monolayer of HUVEC cells expressing VE-cadherin-GFP on glass. Gap opened at vertex (E) and border (F). Scale bars are $50\mu m$ (D) and $20\mu m$ (E,F), respectively. G, H: Quantification of gap opening frequency and gap lifetime at vertices or borders, respectively

makes the monolayer less dynamic. On the other hand, the location of the gap openings (i.e. whether they occur at a vertex or border) is critically affected by membrane stiffness at low range of values, until it stabilizes for intermediate and high membrane stiffness. In contrast, stress fiber stiffness affects gap location for very high stiffness, where gaps are almost fully prevented from opening at the borders (Fig. 2B). Interestingly, increasing bending stiffness first increases gap generation up to a maximum point, before it leads to a decrease in gap opening frequency (Fig. 2A). For small to intermediate bending stiffness, the frequency of gap openings increases, since bending stiffness is critical for effective force propagation between neighboring adhesion sites at vertices. When a single adhesion complex ruptures, bending stiffness leads to increased forces on neighboring adhesion complexes. After a peak in gap opening frequency at intermediate bending stiffness, a drop in the gap formation is observed for higher bending stiffness. This is caused by the resulting stabilization of the existing gaps at vertices. This high bending stiffness opposes sharp corners of the membrane at vertices and thus favors stable gaps that are permanently open, implying no new gaps are formed (Movie S7). On the other hand, at cell borders, a high bending stiffness implies that if a single adhesion cluster is ruptured, the forces on it are redistributed across many neighboring adhesion sites and this stabilizes the borders (Fig. 2B).

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Turning to the role of cell-cell adhesion complex properties, our model shows that as the junctions become more stable, gaps open less frequently (Fig. 2D). To increase cell-cell junction stability, we increase the mechanical stiffness of individual adhesion bonds, or the density of adhesion molecules. These results are in line with previous experimental work [14], which reported that more stable cell-cell junctions result in fewer transmigrating cells. While the total number of gaps at either vertex or border decreases with increasing cell-cell adhesion complex stiffness or cell-cell adhesion density available for binding, we see that there are no significant differences between gaps generated at the vertex and gaps generated at the borders (Fig. 2E).

Fig. 2G,H show the impact of the cortical and radial forces, where the total force is kept constant (when the radial force decreases, the cortical force is increased by the same magnitude). This is biologically relevant since cells are known to shift their cytoskeletal compartments in a context dependent manner [38]. In fact, cell monolayers subjected to shear flow have been reported to increase cortical actin while decreasing stress fibers [14]. Endothelial cells in particular, are known to exhibit both radial and tangential stress fibers with a different effect on gap opening dynamics [39]. As the force shifts from radial to cortical forces, total gap formation fluctuates with a slight increase as cortical forces increase (Fig. 2G). For high cortical forces, the gaps also clearly tend to localize more at the vertices (Fig. 2H). This is because contractions parallel to the membrane result in force concentrations at the vertices. For very high cortex forces, the typical stresses on adhesion clusters at the vertices may thus be higher than the force where the lifetime of catch bonds peaks (Supplementary Fig. S11), explaining the small increase in the number of gaps formed (Fig. 2G). On the other hand, we will later show that these gaps formed at high cortical forces are typically small and have a short lifetime, limiting their potential for extravasation (see Fig. 3I,J).

To take into account that molecular or physical perturbations may simultaneously affect multiple parameters, we now study how variations of two of these parameters at the same time may influence the monolayer integrity and the localization of the gap formation. Although, we have previously seen in Fig. 2A that membrane and stress fiber stiffness have a similar effect on the gap opening frequency, in Fig. 2C we can observe how the effect of varying stress fiber stiffness is clearly predominant over the effect of varying membrane stiffness. Fig. 2F shows the impact of varying cell-cell adhesion stiffness and cell-cell adhesion complex density available for binding. Interestingly, there is a synergy between both parameters on regulating gap opening frequency, as evident through the convex shape of the levels of equal gap opening frequency (Fig. 2F). In Fig. 2I we show the combined role of cortex and radial forces, thus not keeping total force fixed as in Fig. 2G,H. This confirms that total force is the main driver of gap opening frequency, as opposed to a redistribution of forces between cortex and stress fibers (Fig. 2I).

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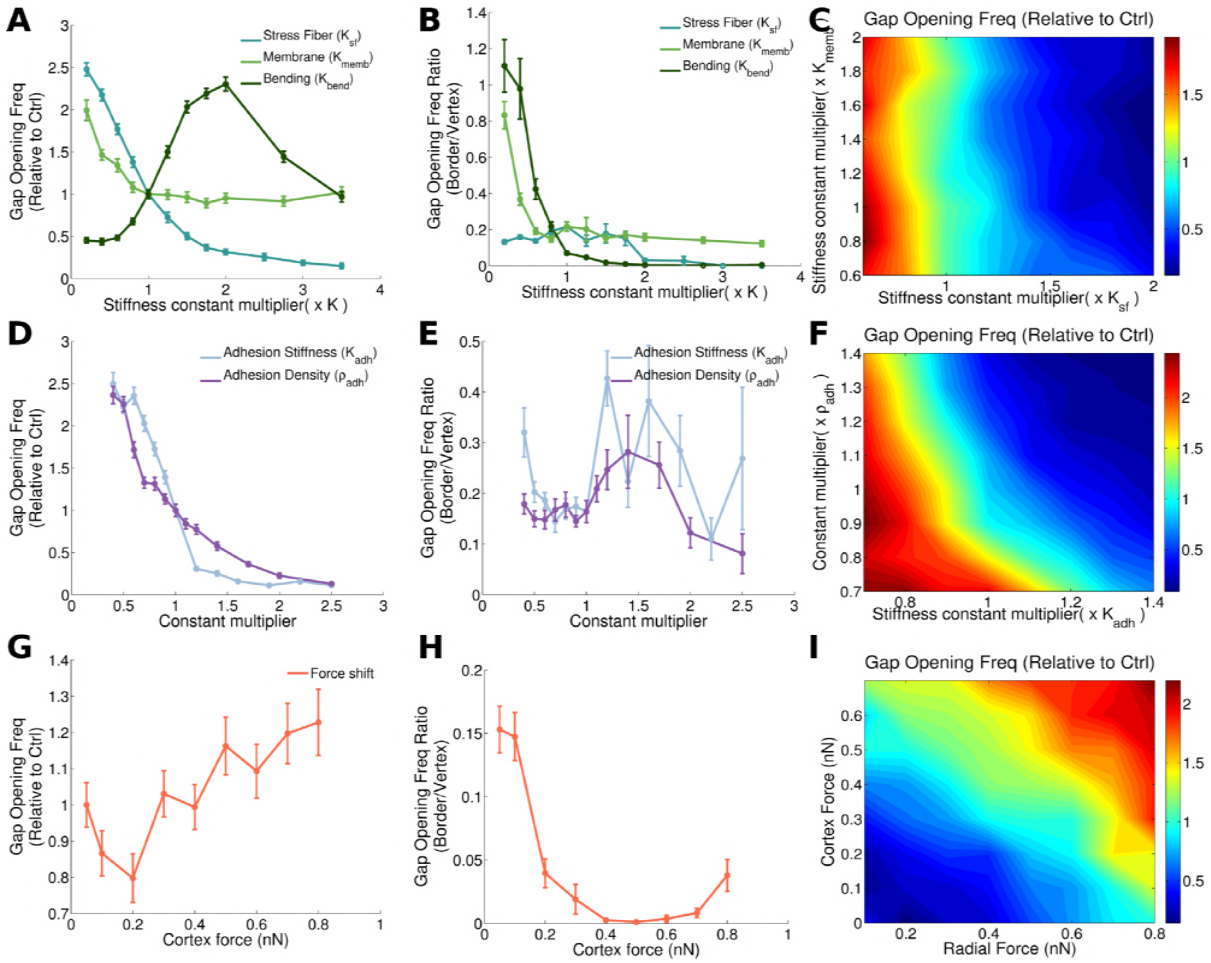

Figure 2: **Intracellular and cell-cell junctional mechanical properties dictate gap opening dynamics.** The first column (A,D,G) correspond to the total number of gaps (vertex plus border) generated per time, compared to the reference case. The second column (B,E,H) shows the ratio of gaps that occur at a two cell border to the gaps that originate at a three cell vertex. The third column (C,F,I) shows the impact of a two parameter variation in the gap opening frequency. The first row shows results from varying cell mechanical properties (stress fiber, membrane and bending stiffness (A,B)), and simultaneous variations of stress fiber and membrane stiffness (C). In the second row (C-F) properties of cell-cell junction are changed: adhesion stiffness and adhesion density. The third row shows results for increasing cortical force, keeping the total force constant (G and H) or varying both forces simultaneously (I).

Passive cell-mechanical properties dictate endothelial gap lifetime and size

The lifetime and size of a gap are physical parameters that may also limit a cancer or immune cell's potential to extravasate through the monolayer. Here, we show how the lifetime and size of a gap are influenced by cell mechanical and junction properties, without the presence of extravasating cells (Fig. 3). We observe that membrane stiffness has a marginal influence on the life of the gap, whereas increasing stress fiber stiffness clearly reduces the time that a gap is open and the gap size (Fig. 3A,B). Indeed, higher stress fiber stiffness will result in mechanical resistance to an opening gap and thus inhibit the propagation of the defect in

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the cell-cell junctions, leading to a stabilization of the monolayer (see Movies S4, S5). The dominance of stress fiber stiffness over membrane stiffness in regulating lifetime and size remains valid in a broad range of parameter space (Fig. 3C,D).

Interestingly, increasing bending stiffness to high values may increase gap lifetime (Fig. 3A). This is because higher bending stiffness will resist deviations from straight membranes. Thus, at straight borders, higher bending stiffness will resist gap openings whereas at vertices with high curvature, cells are more likely to adapt their shape resisting high curvature, thus favoring opened gaps. The dynamics of the monolayer for low bending stiffness is shown in Movie S6.

Fig. 3E,G show that adhesion complex stiffness and density at low values do not have a big impact on lifetime, however as they increase, lifetime starts to decrease. Both stiffness and density have a similar effect, since the total stiffness of an adhesion complex depends on both density and single bond stiffness (Eq. (S10)). Higher stiffness of the adhesion complex leads to more passive mechanical resistance to gap openings, and this effect dominates for high stiffness. The level of noise due to repeats of our MC simulations is higher for these adhesion parameters than for the parameters determining cell mechanics. Likewise, for the gap size, the stabilizing effect of both adhesion complex stiffness and density dominates and leads to a reduction in gap size (Fig. 3F,H). However, the effect of increasing the density is slightly stronger than that of increasing single bond stiffness. This is because the density affects not only adhesion complex stiffness (Eq. (S10)), but also the rate of forming new adhesion complexes (Eq. (S9)) and the rate of reinforcing existing bonds (Eq. (S11)). These effects together thus synergize to stabilize gaps and prevent them from growing too large.

Earlier, we have shown that a shift in the force (from radial to cortical) produces an increment in gap formation (Fig. 2G). Fig. 3I,J show that this shift in the force reduces gap lifetime and size. This indicates that, although the frequency of opening is increased, these gaps are smaller and last shorter in time which may reduce paracellular extravasation, as suggested in previous experimental work [14]. Combined changes of cortical and radial force show that although both kinds of forces are needed to increases gap size and lifetime, the impact of radial forces is clearly predominant over the impact of cortex forces (Fig. 3K,L). This is intuitive, since radial forces clearly separate cell borders generating bigger gaps and make them harder to close, whereas cortical forces distribute forces to vertex regions. This does not provoke large cell deformations, which is reflected in the low impact on the gap size and lifetime observed.

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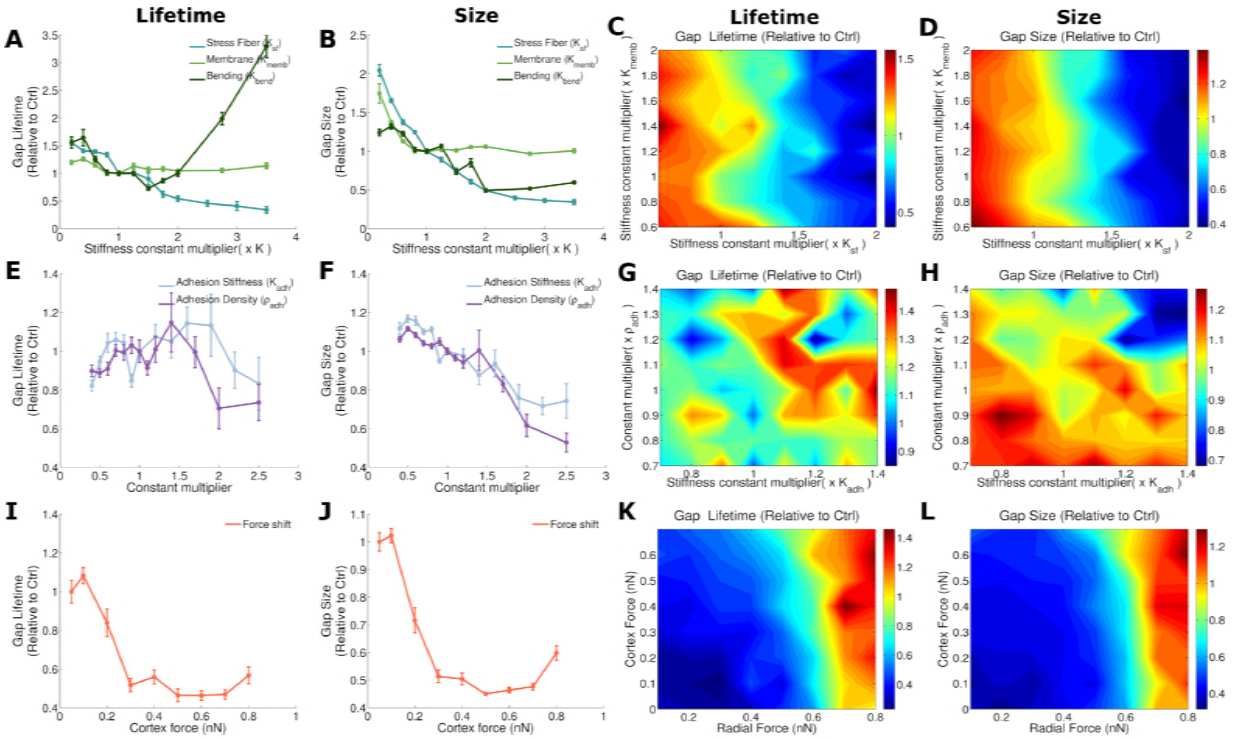

Figure 3: **Impact of Cell mechanics and cell-cell junctions properties on lifetime and size of gaps.** Average lifetime of gaps ratio (divided by control case) in first column and average size in second column. A,B: Impact of membrane, stress fiber and bending stiffness. E,F: Impact of cadherin properties: stiffness and cadherin density (which affects to the binding probability). I,J: Increasing cortical force, keeping the total force constant. Effect of two parameter variation on gap lifetime (third column) and size (fourth column). C,D: variation of membrane and stress fiber stiffness. G,H: Adhesion stiffness versus catch bond unbinding constant variation. K,L; Cortical and radial force variation.

Catch bonds facilitate regimes of maximal endothelial stability

In Fig. 4A-C we show the impact of varying the catch-bond unbinding parameter k_c^0 that shifts the location of the peak of maximal lifetime of a single catch bond, while we maintain the actual maximum value through simultaneously shifting the slip-bond unbinding parameter k_s^0 (Eq. (S12) and Fig. S11). We observe that for a pure slip bond (corresponding to $k_c^0 = 0$), gaps occur at a higher rates than for small nonzero values of k_c^0. Increasing k_c^0 further leads to a minimum in gap opening frequency, from which the frequency increases again. This minimum corresponds to a maximum of stability, where forces on the adhesion complexes are similar in magnitude to the peak of stability of the catch bond. Consequently, shifting the location of that peak even further towards higher forces (by increasing k_c^0 even further) means we destabilize the catch bonds again. Note that the gap lifetime and size of gaps are much less influenced by the location of the catch bond maximum than the gap opening frequency.

In Supplementary Fig. S10, we show histograms of the forces on adhesions comparing the number of

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bound clutches, the number of unbinding events, and the ratio of unbound to total bonds for slip bonds ($k_c^0 = 0$) to the catch bond with reference values ($k_c^0 = 0.27s^{-1}$). We see that adhesions in the catch bond case bear and disengage at higher forces than for the slip bond case, confirming that the typical forces on bonds are of such magnitude that the catch bond nature stabilizes the junctions. Obviously, shifting to even higher values of k_c^0 would result in the unbinding of most bonds (not shown).

In Fig. 4D-F we modify the reinforcement binding rate k_{reinf}^0 to check the influence of the reinforcement. This is different from the previous analysis where the adhesion complex density available for binding was changed, since now the binding probability based on distance is not affected (Eq. (S9)). However, we see the same trend of increasing stability with increasing k_{reinf}^0 (Fig. 4D), in line with the result obtained from varying cadherin density (Fig. 2D), suggesting that binding is mainly regulated by this reinforcement process. Similar to the catch bond, we see that adhesion reinforcement is less important in determining gap size or lifetime (Fig. 4E,F) than in regulating gap opening frequency.

Force fluctuations and distribution regulate gap opening dynamics

We have shown that both the magnitude of forces and the cytoskeletal compartment that generates the forces (stress fibers or cortex) affect gap opening frequency, size and and lifetime. Besides these broad compartments, many other biological and physical parameters affect how forces ultimately act on cell-cell junctions: Forces may act in a directed manner due to larger parallel actin bundles and synchronous myosin activation, e.g. initiated through waves of activators [15], or may act more randomly [33]. We test variations in force applications through parameters that affect the transition time when forces change ($t_{Transition}^{Force}$), through spatial force distributions and through the velocity at which forces are modified. In Fig. 4G we observe how increasing the force transition time $t_{Transition}^{Force}$ slowly reduces the gap opening. This is due to the fact that a slower, persistent application of forces leads to a redistribution of the forces through rearrangement and remodeling of the cell. It is consistent with experimental works that showed that force fluctuations influence gap opening dynamics [15].

Then, distributing the same radial forces over several adjacent stress fibers reduces gap opening frequency (Fig. 4H). More spatially distributed forces are less capable of damaging cell-cell junctions than localized peak forces, since such high peak forces are required to overcome the catch bond maximal lifetime. Likewise, high peak forces lead to longer lifetime and larger size of the resulting gaps (Supplementary Fig. S12C,D).

Next we observe the effect of force persistence in time. We vary the force recalculation time parameter (equally for all forces) in Fig. 4I. Results show that the time that forces are applied does not have a big influence on gap formation. This suggests that cells are able to adapt to forces in longer time scales and

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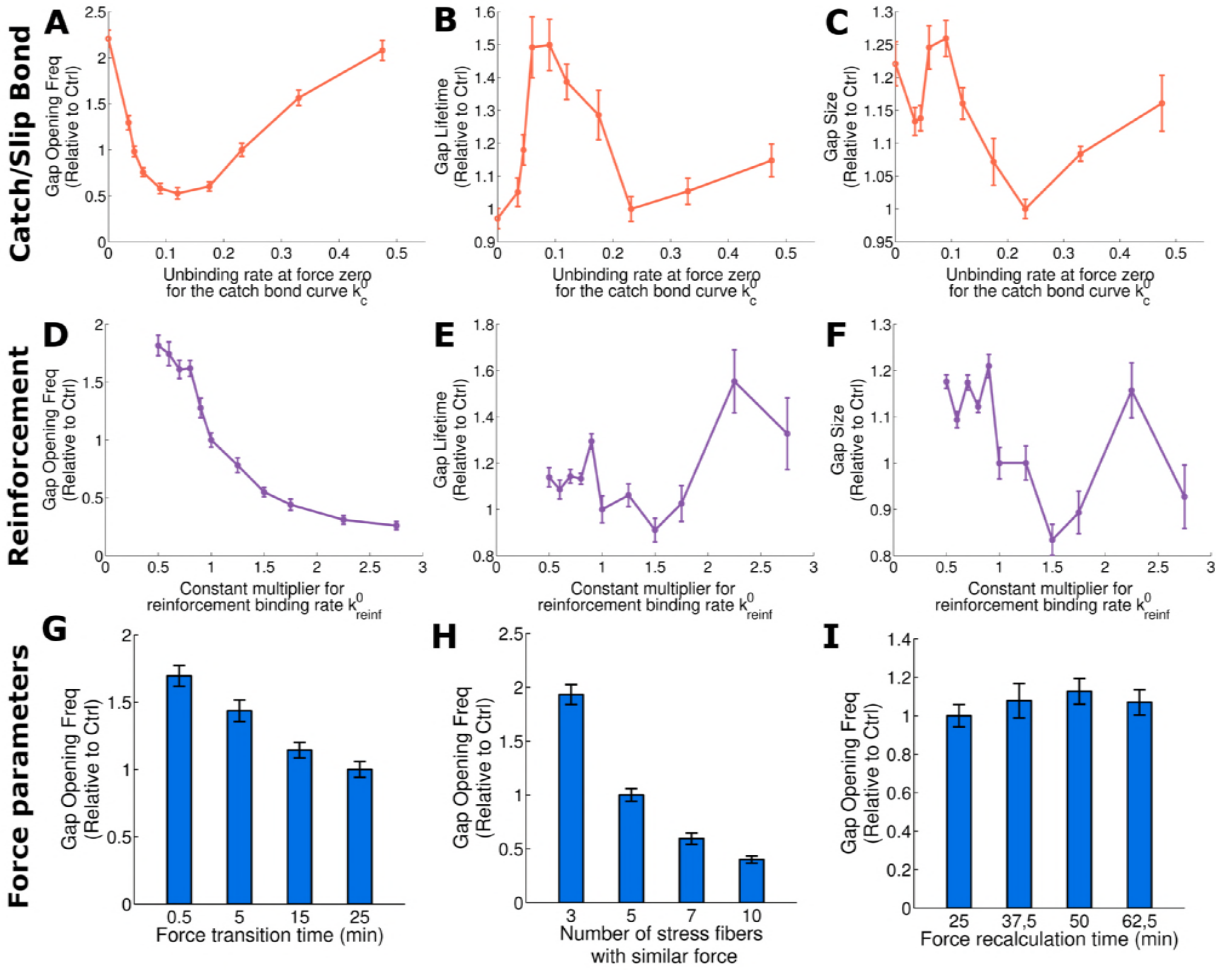

Figure 4: **Effect of the maximal lifetime of a catch bond, the cadherin reinforcement and the force application on the gap opening dynamics.** First row (A-C) shows the impact of shifting from a pure slip bond ($k_c^0 = 0$) to a catch bond. As k_c^0 increases, the peak of stability moves to higher force while we fix the magnitude of a single bond lifetime. Second row (D-F) shows reinforcement analysis varying k_{reinf}^0. Results are normalized with reference case values and are shown for gap opening frequency (A, D), gap lifetime (B, E) and gap size (C, F). Third row shows the effect of force application on total gap opening frequency. G: Total gap opening frequency depending on the time that takes to make the force transition. Longer time means smoother force changes. H: Total gap opening frequency depending on the number of stress fibers at which the same force is distributed. I: Variation in force recalculation time for all types of forces considered in the model.

therefore it is not the time that forces are applied what regulates gap formation, but the transitions of force fluctuations and their spatial distribution.

Cancer extravasation mimics autonomously occurring endothelial gap dynamics

To demonstrate that the geometry of the gap opening dynamics is physiologically relevant, we quantified the characteristics of extravasating cancer cells through monolayers of HUVECs, as shown in Movie S9. Here, a tumor cell is seen transmigrating through an endothelial monolayer at a tricellular junction as delineated

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by VE-cadherin GFP, followed by gap-closure after the tumor cell has completely cleared the barrier. Fig. 5A shows the ratio of tumor cells that extravasated at vertices, relative to borders. We see that tumor cells preferentially extravasate at the vertices, in line with the previously observed increased frequency of gaps opening there (Fig. 1G) and similar observations of extravasating neutrophils [32]. Moreover, even if cancer cells initially arrest at the border between two endothelial cells, they are much more likely to extravasate at a vertex at later points in time, rather than at the border where they initially attached to, perhaps first through migration on the surface of the endothelium and subsequent preferential attachment to points of exposed basement membrane as a result of inherent EC junctional dynamics (Fig. 5B). This could suggest that in addition to the possibility of cancer cells actively signaling to open a gaps in the endothelium, endothelial barrier dynamics itself can also present the cancer cells with opportunities to begin the transmigration process.

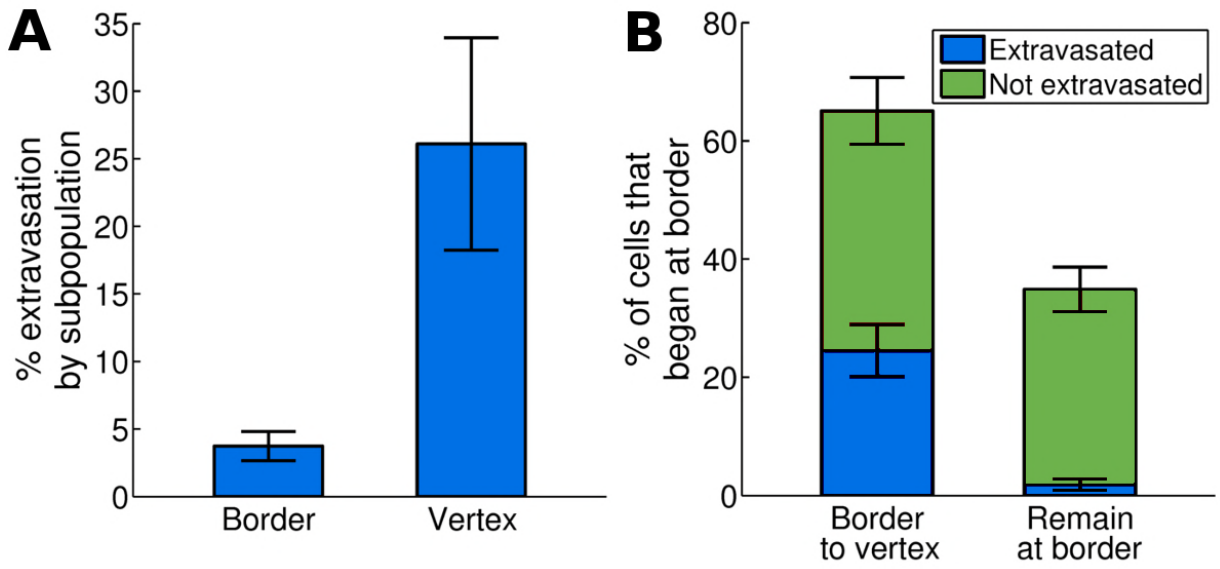

Figure 5: **Cancer cells preferentially extravasate at vertices, even when they first attached to endothelial cell borders.** A: Extravasation of cancer cells in dependence of the location where they transmigrate. B: Extravasation of cancer cells that initially attached to endothelial cell borders, in dependence on whether they extravasate or not and on where they extravasate.

Discussion

The computational model presented in this paper allowed us to study how gaps initially open, grow, stabilize and finally close, and we identified which physical properties dominantly regulate each stage.

Supplementary Fig. S13 summarizes some of our key conclusions: By comparing our reference case with extreme variations of very low stress fiber or membrane stiffness, we see that these passive mechanical

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properties are less important than adhesion properties in increasing gap opening frequency (Supplementary Fig. S13A). On the other hand, the lifetime and especially size of gaps increases significantly with lower stress fiber or membrane stiffness, since the softer cells are more likely to deform and adapt in response to the opened gap (Supplementary Fig. S13B,C). We also verify that stress fiber stiffness influence is stronger than membrane stiffness influence. In contrast, properties of the cell-cell adhesions strongly affect the frequency of the gap openings, but less so their lifetime or size. Indeed, changing the catch bond to a pure slip bond, or decreasing the density of adhesion bonds, strongly increase the frequency of forming gaps (Supplementary Fig. S13A) while only marginally affecting the size or lifetime of the gaps (Supplementary Fig. S13B,C). These data thus summarizes our biological model where adhesion properties control the initial formation of gaps, while cell mechanical properties are critical in limiting the size and duration of opened gaps.

Our results also show that earlier experimental observations, where neutrophils were found to extravasate preferentially at endothelial cell vertices, [32], can be explained through the mechanical dynamics of the endothelial monolayer alone. Consequently, this may be a general mechanism for extravasating cells, and we found a similar behavior with extravasating cancer cells (Fig. 5). This finding is complementary to the intensive literature that suggests that chemical or mechanical signaling of extravasating immune or cancer cells to the endothelium facilitates extravasation [1, 8, 31]. There are many potential hypotheses why both the autonomous dynamics of the endothelial monolayer and the bidirectional signaling with the extravasating cells may play a role during extravasation: It may be that initial autonomously forming gaps are important for extravasating cells to sense a gap and they consequently signal to widen the gap or to keep it open. Alternatively, it may be cell type specific which effect dominates. For instance, it is still a major research question why certain cancer cells preferentially metastasize to certain organs [40]. We may speculate that not only the signaling of the specific primary tumor cells with an organ-specific type of endothelial cells influences the likelihood of extravasation [8]. Also, the mechanical properties of the endothelium of the target organs will likely play a major role. Our flexible modeling framework was tested with a HUVECs monolayer, yet, by changing the physical parameters of the model, it may be quickly adapted to other endothelia.

Besides testing our model with different endothelial cells, some other important steps towards validating our model conclusions in vivo will be to test our model with more realistic three dimensional microvasculature with blood flow, embedded in extracellular matrix and surrounded by supporting cells such as pericytes, fibroblasts or, for brain, astrocytes [40, 1]. Such real, in vivo microvasculature consists of vessels that are curved and exposed to shear stresses due to the flow. That, in turn, may be affected by extravasating cells that may obstruct blood flow. Similarly, matrix stiffness was shown to affect endothelial monolayer integrity [41, 42]. Some complications in validating our results in vivo involve the lack of available in vitro cultures that are required to provide high throughput, microscopy resolution and level of experimental control that

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is lacking in vivo, making direct comparison of computational models to in vivo experiments unfeasible. However, the recent rapid progress in developing more complex and organ specific in vitro assays of 3D microvasculature will make such validations feasible in the near future [43, 44, 45].

Moreover, there are also challenges to the mathematical modeling of complex 3D microvasculature. Modeling of epithelial sheets in 3D has proved challenging, with some recent interesting progress after decades of mainly focusing on epithelial monolayers in 2D [46, 47, 48]. These models are based on frameworks such as vertex models, where the dynamics of each cell is incorporated into the dynamics of vertices between cells. The vertex model framework is thus not suitable to predict the formation of gaps at either vertices or borders. Given these challenges, is was paramount to establish a 2D mathematical model of an endothelial monolayer that was validated with novel experiments and that was able to lead to insights into the mechanisms of endothelial gap formation.

Methods

Generation of HUVEC monolayers and image acquisition

Human umbilical chord vein cells (HUVECs) were transduced with VE-cadherin-GFP using methods described previously [44]. HUVECs at P7-10 were seeded onto 35 mm glass bottom Mattek dishes (at 3×10^5 cells/dish), which had been plasma treated for 30 seconds previously. Cells were allowed to grow to confluence (beyond 100%) in EGM-2MV (Lonza) for 3 days before imaging. Dishes were imaged on an Olympus FV1000 confocal microscope with magnifications of 30X (oil immersion), under an environmental chamber set at 37C and 5% CO2. The chamber was equilibrated for $\sim$ 30 min prior to the start of image acquisition. For time-lapse videos of junctional dynamics, z-stacks of $40\mu m$ ($4\mu m$ steps) were taken at intervals of 3 minutes.

Analysis of junctional disruption dynamics

Time-lapse images were appended and analyzed manually on ImageJ. A single unique junctional disruption is defined as a vertex or border with an observed gap of greater or equal than $3\mu m$, and are preceded and proceeded at some point in time with a closure (no visible gap in fluorescence greater than $0.6\mu m$). The number of junctional disruption events was counted for each border and vertex of an image over a total time period of 2 hours. Vertices and borders belonging to the same cells were still considered to be unique.

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Analysis of tumor cell extravasation

Tumor cells were suspended in EGM-2MV (Lonza) and a concentration of 15,000 cells/mL, and 1mL of the suspension was gently added to each HUVEC monolayer. Cells were allowed to settle first for ~ 10 minutes before acquisition of of $t = 0$ images. For quantification of extravasation, z-stacks were taken at $3\mu m$ steps at an endpoint of 6 hours to image the entirety of the tumor cell and endothelial monolayer. Any tumor cell that has breached the endothelial layer as evidenced by protrusion extension across and beneath the endothelial layer was considered as "extravasated". Delineation of the endothelial barrier is visualized via CD31 staining (Biolegend, Cat # 303103) for 30 min in EGM-2MV at 37C and 5% CO2 prior to imaging.

Author contributions

M.B.C., R.D.K. and F.S. conceived the research problem. J.E., X.C., V.S., J.M.G.-A, R.D.K. and F.S. developed the model. M.B.C., E.M. and R.D.K. designed the experiments. J.E. wrote the computer code and performed all simulations. M.B.C. performed the experiments and analyzed experimental data. J.E., J.M.G.-A, R.D.K. and F.S. analyzed the model predictions. J.E. and F.S. wrote the paper. J.M.G.-A, R.D.K. and F.S. equally supervised the work.

References

[1] Dietmar Vestweber. How leukocytes cross the vascular endothelium. *Nature Reviews Immunology*, 15(11):692–704, 2015.

[2] Julie E Park and Adrian Barbul. Understanding the role of immune regulation in wound healing. *The American Journal of Surgery*, 187(5, Supplement 1):S11 – S16, 2004.

[3] Sergei I Grivennikov, Florian R Greten, and Michael Karin. Immunity, inflammation, and cancer. *Cell*, 140(6):883–899, 2010.

[4] Peter J Murray and Thomas A Wynn. Protective and pathogenic functions of macrophage subsets. *Nature reviews immunology*, 11(11):723–737, 2011.

[5] William A Muller. How endothelial cells regulate transmigration of leukocytes in the inflammatory response. *The American journal of pathology*, 184(4):886–896, 2014.

[6] Peter Libby, Yoshihisa Okamoto, Viviane Z Rocha, and Eduardo Folco. Inflammation in atherosclerosis. *Circulation journal*, 74(2):213–220, 2010.

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[7] James M Mullin, Nicole Agostino, Erika Rendon-Huerta, and James J Thornton. Keynote review: epithelial and endothelial barriers in human disease. *Drug discovery today*, 10(6):395–408, 2005.

[8] Nicolas Reymond, Bárbara Borda D'Água, and Anne J. Ridley. Crossing the endothelial barrier during metastasis. *Nature Reviews Cancer*, 13(12):858–870, 2013.

[9] Andrew D Luster, Ronen Alon, and Ulrich H von Andrian. Immune cell migration in inflammation: present and future therapeutic targets. *Nature immunology*, 6(12):1182, 2005.

[10] Yvonne Kienast, Louisa Von Baumgarten, Martin Fuhrmann, Wolfgang EF Klinkert, Roland Goldbrunner, Jochen Herms, and Frank Winkler. Real-time imaging reveals the single steps of brain metastasis formation. *Nature medicine*, 16(1):116, 2010.

[11] Denis Wirtz, Konstantinos Konstantopoulos, and Peter C Searson. The physics of cancer: the role of physical interactions and mechanical forces in metastasis. *Nature Reviews Cancer*, 11(7):512, 2011.

[12] Gautier Follain, Nal Osmani, Ana Sofia Azevedo, Guillaume Allio, Luc Mercier, Matthia A. Karreman, Gergely Solecki, Mara Jess Garcia Len, Olivier Lefebvre, Nina Fekonja, Claudia Hille, Vincent Chabannes, Guillaume Doll, Thibaut Metivet, Franois Der Hovsepian, Christophe Prudhomme, Anglique Pichot, Nicodme Paul, Raphal Carapito, Siamak Bahram, Bernhard Ruthensteiner, Andr Kemmling, Susanne Siemonsen, Tanja Schneider, Jens Fiehler, Markus Glatzel, Frank Winkler, Yannick Schwab, Klaus Pantel, Sbastien Harlepp, and Jacky G. Goetz. Hemodynamic forces tune the arrest, adhesion, and extravasation of circulating tumor cells. *Developmental Cell*, 45(1):33 – 52.e12, 2018.

[13] M. J. Allingham, J. D. van Buul, and K. Burridge. ICAM-1-Mediated, Src- and Pyk2-Dependent Vascular Endothelial Cadherin Tyrosine Phosphorylation Is Required for Leukocyte Transendothelial Migration. *The Journal of Immunology*, 179(6):4053–4064, 2007.

[14] Roberta Martinelli, Adam S Zeiger, Matthew Whitfield, Tracey E Sciuto, Ann Dvorak, Krystyn J Van Vliet, John Greenwood, and Christopher V Carman. Probing the biomechanical contribution of the endothelium to lymphocyte migration: diapedesis by the path of least resistance. *J Cell Sci*, 127(17):3720–3734, 2014.

[15] Erik T. Valent, Geerten P. van Nieuw Amerongen, Victor W M van Hinsbergh, and Peter L. Hordijk. Traction force dynamics predict gap formation in activated endothelium. *Experimental Cell Research*, 347(1):161–170, 2016.

[16] Xuan Cao, Emad Moeendarbary, Philipp Isermann, Patricia M. Davidson, Xiao Wang, Michelle B. Chen, Anya K. Burkart, Jan Lammerding, Roger D. Kamm, and Vivek B. Shenoy. A chemomechanical

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model for nuclear morphology and stresses during cell transendothelial migration. *Biophysical Journal*, 111(7):1541 – 1552, 2016.

[17] Antje Schaefer, Joost te Riet, Katja Ritz, Mark Hoogenboezem, Eloise C. Anthony, Frederik P. J. Mul, Carlie J. de Vries, Mat J. Daemen, Carl G. Figdor, Jaap D. van Buul, and Peter L. Hordijk. Actin-binding proteins differentially regulate endothelial cell stiffness, icam-1 function and neutrophil transmigration. *Journal of Cell Science*, 127(20):4470–4482, 2014.

[18] Zhijun Liu, John L Tan, Daniel M Cohen, Michael T Yang, Nathan J Sniadecki, Sami Alom Ruiz, Celeste M Nelson, and Christopher S Chen. Mechanical tugging force regulates the size of cell–cell junctions. *Proceedings of the National Academy of Sciences*, 107(22):9944–9949, 2010.

[19] Antje Schaefer and Peter L Hordijk. Cell-stiffness-induced mechanosignaling–a key driver of leukocyte transendothelial migration. *J Cell Sci*, 128(13):2221–2230, 2015.

[20] Joppe Oldenburg and Johan de Rooij. Mechanical control of the endothelial barrier. *Cell and tissue research*, 355(3):545–555, 2014.

[21] Koji Ando, Shigetomo Fukuhara, Takahiro Moriya, Yutaro Obara, Norimichi Nakahata, and Naoki Mochizuki. Rap1 potentiates endothelial cell junctions by spatially controlling myosin ii activity and actin organization. *J Cell Biol*, pages jcb–201301115, 2013.

[22] Yvonne L Dorland and Stephan Huveneers. Cell–cell junctional mechanotransduction in endothelial remodeling. *Cellular and Molecular Life Sciences*, 74(2):279–292, 2017.

[23] Stephan Huveneers, Joppe Oldenburg, Emma Spanjaard, Gerard van der Krogt, Ilya Grigoriev, Anna Akhmanova, Holger Rehmann, and Johan de Rooij. Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. *Journal of Cell Biology*, 196(5):641–652, 2012.

[24] Niels Heemskerk, Lilian Schimmel, Chantal Oort, Jos Van Rijssel, Taofei Yin, Bin Ma, Jakobus Van Unen, Bettina Pitter, Stephan Huveneers, Joachim Goedhart, et al. F-actin-rich contractile endothelial pores prevent vascular leakage during leukocyte diapedesis through local rhoa signalling. *Nature communications*, 7, 2016.

[25] Jörg Galle, Markus Loeffler, and Dirk Drasdo. Modeling the Effect of Deregulated Proliferation and Apoptosis on the Growth Dynamics of Epithelial Cell Populations In Vitro. *Biophysical Journal*, 88(1):62–75, 2005.

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[26] Alexander G Fletcher, Miriam Osterfield, Ruth E Baker, and Stanislav Y Shvartsman. Vertex models of epithelial morphogenesis. *Biophysical journal*, 106(11):2291–2304, 2014.

[27] Shao-Zhen Lin, Bo Li, Guang-Kui Xu, and Xi-Qiao Feng. Collective dynamics of cancer cells confined in a confluent monolayer of normal cells. *Journal of Biomechanics*, 52:140–147, 2016.

[28] Yousef Jamali, Mohammad Azimi, and Mohammad R K Mofrad. A sub-cellular viscoelastic model for cell population mechanics. *PLoS ONE*, 5(8), 2010.

[29] Amit Pathak. Scattering of Cell Clusters in Confinement. *Biophysical Journal*, 111(7):1496–1506, 2016.

[30] Porntula Panorchan, Jerry P. George, and Denis Wirtz. Probing Intercellular Interactions between Vascular Endothelial Cadherin Pairs at Single-molecule Resolution and in Living Cells. *Journal of Molecular Biology*, 358(3):665–674, 2006.

[31] Yi-Ting Yeh, Ricardo Serrano, Joshua Franois, Jeng-Jiann Chiu, Yi-Shuan Julie Li, Juan C. del lamo, Shu Chien, and Juan C. Lasheras. Three-dimensional forces exerted by leukocytes and vascular endothelial cells dynamically facilitate diapedesis. *Proceedings of the National Academy of Sciences*, 2017.

[32] Alan R Burns, David C Walker, Evelyn S Brown, Lisa T Thurmon, Robert A Bowden, Charles R Keese, Scott I Simon, Mark L Entman, and C Wayne Smith. Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. *The Journal of Immunology*, 159(6):2893–2903, 1997.

[33] Ming Guo, Allen J Ehrlicher, Mikkel H Jensen, Malte Renz, Jeffrey R Moore, Robert D Goldman, Jennifer Lippincott-Schwartz, Frederick C Mackintosh, and David A Weitz. Probing the stochastic, motor-driven properties of the cytoplasm using force spectrum microscopy. *Cell*, 158(4):822–832, 2014.

[34] Thomas Lecuit and Alpha S. Yap. E-cadherin junctions as active mechanical integrators in tissue dynamics. *Nature Cell Biology*, 17(5):533–539, 2015.

[35] Jiawen Chen, Jillian Newhall, Zhong Ru Xie, Deborah Leckband, and Yinghao Wu. A Computational Model for Kinetic Studies of Cadherin Binding and Clustering. *Biophysical Journal*, 111(7):1507–1518, 2016.

[36] A. K. Barry, N. Wang, and D. E. Leckband. Local VE-cadherin mechanotransduction triggers long-ranged remodeling of endothelial monolayers. *Journal of Cell Science*, 128(7):1341–1351, 2015.

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[37] Shigenobu Yonemura, Yuko Wada, Toshiyuki Watanabe, Akira Nagafuchi, and Mai Shibata. α-Catenin as a tension transducer that induces adherens junction development. *Nature Cell Biology*, 12(6):533–542, 2010.

[38] Cristian Suarez and David R Kovar. Internetwork competition for monomers governs actin cytoskeleton organization. *Nature Reviews Molecular Cell Biology*, 17(12):799–810, 2016.

[39] Joppe Oldenburg and Johan De Rooij. Mechanical control of the endothelial barrier. *Cell and Tissue Research*, 355(3):545–555, 2014.

[40] Anna C. Obenauf and Joan Massagu. Surviving at a distance: Organ-specific metastasis. *Trends in Cancer*, 1(1):76 – 91, 2015.

[41] R Krishnan, D D Klumpers, C Y Park, K Rajendran, X Trepat, J van Bezu, V W M van Hinsbergh, C V Carman, J D Brain, J J Fredberg, J P Butler, and G P V Amerongen. Substrate stiffening promotes endothelial monolayer disruption through enhanced physical forces. *American Journal of Physiology-Cell Physiology*, 300(1):C146–C154, 2011.

[42] Roberto C. Andresen Eguiluz, Kerim B. Kaylan, Gregory H. Underhill, and Deborah E. Leckband. Substrate stiffness and ve-cadherin mechano-transduction coordinate to regulate endothelial monolayer integrity. *Biomaterials*, 140:45 – 57, 2017.

[43] Jessie S Jeon, Simone Bersini, Mara Gilardi, Gabriele Dubini, Joseph L Charest, Matteo Moretti, and Roger D Kamm. Human 3d vascularized organotypic microfluidic assays to study breast cancer cell extravasation. *Proceedings of the National Academy of Sciences*, 112(1):214–219, 2015.

[44] Michelle B Chen, Jordan A Whisler, Julia Fröse, Cathy Yu, Yoojin Shin, and Roger D Kamm. On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics. *Nature Protocols*, 12(5):865–880, 2017.

[45] Tatsuya Osaki, Vivek Sivathanu, and Roger D Kamm. Engineered 3d vascular and neuronal networks in a microfluidic platform. *Scientific reports*, 8(1):5168, 2018.

[46] XinXin Du, Miriam Osterfield, and Stanislav Y Shvartsman. Computational analysis of three-dimensional epithelial morphogenesis using vertex models. *Physical biology*, 11(6):066007, 2014.

[47] Satoru Okuda, Yasuhiro Inoue, and Taiji Adachi. Three-dimensional vertex model for simulating multicellular morphogenesis. *Biophysics and physicobiology*, 12:13–20, 2015.

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[48] Silvanus Alt, Poulami Ganguly, and Guillaume Salbreux. Vertex models: from cell mechanics to tissue morphogenesis. *Phil. Trans. R. Soc. B*, 372(1720):20150520, 2017.

[49] T. Kim, W. Hwang, and R. D. Kamm. Computational Analysis of a Cross-linked Actin-like Network. *Experimental Mechanics*, 49(1):91–104, oct 2007.

[50] Kristine Manibog, Hui Li, Sabyasachi Rakshit, and Sanjeevi Sivasankar. Resolving the molecular mechanism of cadherin catch bond formation. *Nature Communications*, 5:1–11, 2014.

[51] Craig D Buckley, Jiongyi Tan, Karen L Anderson, Dorit Hanein, Niels Volkmann, William I Weis, W James Nelson, and Alexander R Dunn. The minimal cadherin-catenin complex binds to actin filaments under force. *Science*, 346(6209):1254211, 2014.

[52] Elizaveta A. Novikova and Cornelis Storm. Contractile fibers and catch-bond clusters: a biological force sensor? *Biophysical journal*, 105(6):1336–45, sep 2013.

[53] Christopher J. Staiger, Michael B. Sheahan, Parul Khurana, Xia Wang, David W. McCurdy, and Laurent Blanchoin. Actin filament dynamics are dominated by rapid growth and severing activity in the arabidopsis cortical array. *The Journal of Cell Biology*, 184(2):269–280, 2009.

[54] J. F. Dorn, L. Zhang, T.-T. Phi, B. Lacroix, P. S. Maddox, J. Liu, and A. S. Maddox. A theoretical model of cytokinesis implicates feedback between membrane curvature and cytoskeletal organization in asymmetric cytokinetic furrowing. *Molecular Biology of the Cell*, 27(8):1286–1299, 2016.

[55] Lilia A. Chtcheglova, Linda Wildling, Jens Waschke, Detlev Drenckhahn, and Peter Hinterdorfer. AFM functional imaging on vascular endothelial cells. *Journal of Molecular Recognition*, 23(6):589–596, 2010.

[56] Aleksandr Rabodzey, Pilar Alcaide, Francis W. Luscinskas, and Benoit Ladoux. Mechanical Forces Induced by the Transendothelial Migration of Human Neutrophils. *Biophysical Journal*, 95(3):1428–1438, 2008.

[57] Julian E. Hassinger, George Oster, David G. Drubin, and Padmini Rangamani. Design principles for robust vesiculation in clathrin-mediated endocytosis. *Proceedings of the National Academy of Sciences*, 114(7):E1118–E1127, 2017.

[58] Kai Bodo Grimm, Hans Oberleithner, and Johannes Fels. Fixed endothelial cells exhibit physiologically relevant nanomechanics of the cortical actin web. *Nanotechnology*, 25(21), 2014.

[59] Pierre Sens and Julie Plastino. Membrane tension and cytoskeleton organization in cell motility. *Journal of Physics Condensed Matter*, 27(27), 2015.