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# 1 **Staying away from the breaking point: probing the limits of** 2 **epithelial cell elimination**

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7 **Keywords:** epithelium, mechanics, apoptosis, extrusion, rupture, tear, cohesion,  
8 feedback, cell-cell adhesion, ECM

## 9 **Abstract**

10 Epithelial tissues are dramatically remodelled during embryogenesis and tissue  
11 homeostasis and yet need to maintain their sealing properties to sustain their barrier  
12 functions at any time. Part of these remodellings involve the elimination of a large  
13 proportion of cells through apoptosis. Cell extrusion, the remodelling steps leading to  
14 seamless dying cell expulsion, helps to maintain tissue cohesion. However, there is an  
15 intrinsic limit in the system that can only accommodate a certain proportion/rate of cell  
16 elimination as well as certain spatio-temporal distributions. What are then the critical  
17 conditions leading to epithelial rupture/tear/sealing defects upon cell elimination and  
18 which mechanisms ensure that such limits are never reached? In this short review, I  
19 document the conditions in which epithelial rupture has been observed, including in  
20 contexts of epithelial cell death, and the mechanical parameters influencing tissue  
21 rupture, and review feedback mechanisms which help to keep the epithelia away from  
22 the breaking point.

## 23 **Introduction**

24 Epithelia are the central building block of Metazoans which ensure the separation of  
25 organs and their functions through the formation of tight impermeable mechanical and  
26 chemical barriers. While the different junctions along the apico-basal axis and their  
27 coupling to the cytoskeleton help to build a stable scaffold attaching cells together and  
28 generating a mechanical continuum (**Figure 1A**), this barrier also has to accommodate  
29 drastic remodellings such as the one occurring during morphogenesis or during the  
30 spontaneous turnover of some adult epithelia. Accordingly, apoptosis and epithelial

31 cell death is highly prevalent during tissue morphogenesis [1] or in adult tissues with  
32 high cell turnover [2]. The exclusion of dying cells from the epithelia is compatible with  
33 its barrier function thanks to cell extrusion [3-5], a serie of sub-cellular remodelling  
34 steps leading to the contraction of the dying cells and the junctions shared with  
35 neighbours, cell sliding toward the apical or basal side concomitant with junction  
36 remodelling [6]. While extrusion is an efficient process to expel the cells while  
37 maintaining tissue sealing, some extreme conditions in pathological or physiological  
38 contexts are associated with very high rate of cell death that can endanger the  
39 cohesion of the tissue. For instance, infection and inflammation can trigger gap  
40 formation in the mice and human gut through the high frequency of cell shedding [7].  
41 Alternatively, cytotoxic stress can trigger epithelial tissue rupture *in vitro* when reaching  
42 a threshold rate of cell elimination [8]. Repetitive loss of sealing will not only endanger  
43 the mechanical continuum of the tissue and perturb morphogenesis, but can also  
44 triggers chronic inflammation [9].

45 Yet, epithelial tissue can sustain very high cell elimination rate could it be during gut  
46 homeostasis where billions of cells die daily [10], or during  
47 embryogenesis/metamorphosis where some epithelial tissues can be completely  
48 cleared within 24h while maintaining cohesion at any time [11]. What are then the  
49 intrinsic limits of the systems and the conditions that lead to tissue rupture? Which  
50 tissue mechanical parameters can tune the susceptibility to gap formation? Are there  
51 any feedback mechanisms that ensure that such critical points are never reached in  
52 physiological contexts? I address these points in this short review by first documenting  
53 conditions in which epithelial rupture has been observed and discuss the relevant  
54 mechanical parameters that can tune the susceptibility to gap formation. I then discuss  
55 some examples where epithelial cell death has been associated with gap formation in  
56 light of the relevant parameters described in the first part. Eventually, I describe  
57 feedback mechanisms acting on different spatial and temporal scales, could it be  
58 through the modulation cell death, cell extrusion, cell division or the local and global  
59 mechanical properties that help to sustain high death rates.

## 60 **Documenting the mechanical conditions associated with epithelial rupture.**

61 Epithelial rupture requires the local disassembly of adherens and septate junctions that  
62 will lead to permanent or transient hole formation between cells. These holes can form  
63 at different spatial and temporal scales, from small subcellular cracks, to massive

64 tissue tear, and will either expand or be resorbed by wound healing. Intuitively, rupture  
65 can be triggered when tension or shear stress imposed by cell contractility or external  
66 forces exceed the loading capacity of cell-cell junctions, which will disassemble and  
67 detach (**Figure 1A**). Hence, epithelial rupture will be determined by the ratio between  
68 pulling forces generated by tension and cell-cell adhesion strength. Along this line,  
69 depletion of core components of adherens junctions (here, E-cadherin) rapidly leads  
70 to epithelial rupture in *Drosophila* gastrulating embryos, especially in the mesoderm  
71 region where contractility is developmentally upregulated [12]. The tears appear  
72 preferentially along antero-posterior axis where tension is higher, and can be  
73 abrogated by the depletion of core-regulators of Rho activation in the mesoderm. Tears  
74 were also recently observed in *Drosophila* follicular epithelia upon rapid aPKC  
75 depletion by optogenetics, a core regulator of apico-basal polarity [13]. aPKC depletion  
76 leads to rapid increase of junction tension, which leads to gap formation near cells  
77 undergoing cytokinesis. The extensive adherens junction remodeling for *de novo*  
78 junction formation during cytokinesis [14-16] may generate transient weak adhesive  
79 spots permissive for tissue rupture. These few examples illustrate the strong spatial  
80 heterogeneity for the susceptibility to gap formation associated with local and transient  
81 modulation of adherens junctions and/or local modulation of contractility.

82 The mechanical limit of cell-cell adhesion can be better assessed using simplified  
83 systems *in vitro*. Dual pipette aspiration can be used to detach cell doublets and  
84 measure the absolute forces required for cell-cell detachment [17] which vary by  
85 several orders of magnitudes depending on the cell type and tissue (from 5 to 15 nN  
86 between zebrafish gastrula cells [17] to 200 nN in S180 cells transfected with E-cad  
87 [18]). However, this does not reflect the collective effect that may reinforce tissue  
88 stability. Suspended MDCK layers (without Extra Cellular Matrix, ECM) embedded  
89 between two test rods controlled with a micromanipulator has been used to apply  
90 controllable forces to a single epithelial layer [19]. The monolayer could withstand up  
91 to a 110% strain before tissue rupture, leading to an estimation a 1.7 $\mu$ N forces required  
92 to detach cells, close to 9 times the forces required to separate cell doublets [18].  
93 Downregulation of cell-cell adhesion through Calcium chelation by EDTA reduces the  
94 strain threshold to 25%, confirming the essential role E-cadherin and adherens junction  
95 for stabilising the tissue [19]. While these results underscore the strong reinforcement  
96 of epithelial stability provided by collective effects, they do not address the role of the

97 interactions with the ECM which should influence forces distribution and tissue  
98 robustness.

99 Accordingly, epithelial cracks were also observed in the context of cyclic stretching of  
100 MDCK cells placed on circular patterns on PDMS substrate [20]. Surprisingly, cracks  
101 are not formed during the stretching phase and do not correlate with tissue tension,  
102 but rather occur during substrate relaxation. Contact rupture is driven by the poro-  
103 elasticity of the substrate which upon relaxation de-swells and release water, hence  
104 building up hydraulic pressure on the epithelial basal side. However, contrary to the  
105 stretching of suspended monolayers, these cracks do not lead to large scale tissue  
106 opening. Yet, this suggests that tissue lateral compression could also lead to local  
107 sealing defects.

108 More recently, spontaneous holes formation was described in confluent MDCK and  
109 Caco2 cells plated on circular patterns on soft gels [21]. A large proportion (~90%) of  
110 these ruptures are initiated near cells undergoing cytokinesis and their frequency is  
111 drastically reduced upon mitosis inhibition, in agreement with the observations  
112 performed in *Drosophila* follicles upon aPKC depletion [13] (see above). Importantly,  
113 increasing substrate stiffness completely abolishes holes formation [21]. This is  
114 explained by the enhanced substrate adhesion, which through stabilised focal  
115 adhesion and high friction dampen the stress applied at transcellular junctions.  
116 Accordingly, the monolayer switches from a dominant tensile state on soft substrate to  
117 a compressive state on stiff substrate. Importantly, ruptures are associated with local  
118 peak of tensile stress driven by cell outward movements which are frequently  
119 associated with local  $-1/2$  topological defects (a so-called tri-radius alignment  
120 associated with high tension also described in liquid crystal). Altogether, this study  
121 outlines the importance of local tension fluctuations which can trigger cell detachment  
122 and gap formation. Epithelial stability is enhanced on stiff substrate and at high  
123 substrate adhesion which dampen junction tensile stress through energy dissipation  
124 by friction.

125 To conclude, these studies outline a restricted number of parameters that can  
126 modulate the susceptibility to gap formation: while high cell-cell adhesion as well as  
127 high substrate adhesion stabilise the epithelium, high junctional tensile stress as well  
128 as local fluctuations increase the probability of tissue rupture. Local and transient  
129 inhomogeneity of cell-cell adhesion and/or tensile stress, which can be generated by

130 cellular events such as cell division or spontaneous collective movements, produce  
131 numerous local weak spots that further enhance the probability of hole formation. This  
132 relatively simplistic picture is obviously complexified by the strong interconnections and  
133 cross-regulation of these parameters, where for instance high tension can directly  
134 enhance cell-cell adhesion [22] or substrate adhesion [23] through  
135 mechanotransduction.

### 136 **Creating gaps through epithelial cell death**

137 After discussing the conditions that can lead to tissue rupture *in vivo* and *in vitro*, I will  
138 now focus on sealing defects that were associated with epithelial cell death while trying  
139 to interpret them in the mechanical framework of tissue rupture described above. Of  
140 note, I will not focus here on conditions affecting core regulators of cell extrusions,  
141 which is also associated with transient sealing defects [24]. In absence of cell division  
142 and with fixed boundaries, cell elimination should lead to a progressive extension of  
143 cell apical area and increased tensile stress until reaching a critical strain leading to  
144 tissue rupture (**Figure 1B**), specially without protective feedback (see below). This  
145 situation can occur *in vitro* with cytotoxic treatment in MCF10A cells when death rate  
146 reaches a critical value [8], or upon induction of high rates of apoptosis through global  
147 induction of pro-apoptotic genes *in vivo* in the *Drosophila* pupal notum (a single layer  
148 epithelium) [25]. More recently, epithelial rupture and detachment was also associated  
149 with the high density of cell extrusion triggered by the alveolar constriction during  
150 metacholine treatment on *ex vivo* cultured slices of mice lung as well as *in vivo*, an  
151 experimental condition simulating asthmatic bronchioconstrictive attacks [26]. Cell  
152 extrusions are here driven by the activation of the stretch sensitive channel Piezo upon  
153 tissue crowding [26, 27]. Accordingly, cell extrusion and tissue detachment can be  
154 significantly reduced with Gadolinium<sup>3+</sup> (an inhibitor of TRP channels) [26]. In this  
155 context, the constriction of the smooth muscle layer is reminiscent of the PDMS  
156 substrate relaxation that was triggering water outflux and crack formation in MDCK  
157 islands [20]. Thus the poro-elasticity of the basal layer and the dewetting triggered by  
158 hydraulic pressure may be more relevant to explain epithelial detachment in the lung  
159 rather than an increase of tensile stress driven by massive extrusion.

160 While these studies rather focused on the absolute number and rate of cell elimination,  
161 we also recently outlined the important role of the precise spatio-temporal distribution  
162 of cell death. Using an optogenetic Caspase to trigger concomitant eliminations of

163 neighbouring cells, we observed frequent gap formation as soon as more than three  
164 cells organised in clusters were eliminated together [28] (**Figure 1C**). Importantly, gaps  
165 did not form when cells were eliminated in lines, suggesting that the precise topology  
166 of junctions may be causative to gap formation. On the one hand, clustered elimination  
167 may be associated with the high distance between surviving cells, which take too long  
168 to get close enough to build new junctions prior to cell-cell junction disassembly in  
169 dying cells triggered by caspases [28, 29]. On the other hand, the cluster of apoptotic  
170 cells shared a common tricellular junction which may constitute a weak spot  
171 susceptible to detachment. Accordingly, radial intercalation of multiciliated cell  
172 precursors within the epithelium in *Xenopus* embryo occurs preferentially at tricellular  
173 junctions, suggesting that they constitute a weaker point of adhesion more prone to  
174 detachment [30]. Irrespective of the mechanism, this reflects that clustered  
175 eliminations are particularly detrimental for epithelial sealing.

176 Finally, cell extrusion in context of impaired or reduced cell-cell adhesion have also  
177 been associated with holes formation, could it be through the reduction of E-cad  
178 mediated adhesion [31] or by impairing desmosome junctions [6]. Interestingly, tearing  
179 occurs near extrusion in Desmoplakin depleted MDCK cells (a core component of  
180 Desmosomes) despite the global reduction of junctional tension, suggesting that high  
181 tensile stress may not be an absolute requirement for extrusion-driven tissue tear.

## 182 **Staying away from the breaking point through feedback**

183 We have so far outlined two major parameters that can endanger epithelial cohesion  
184 during cell elimination: a critical high rate of cell elimination that will bring tensile stress  
185 up to the limit that can be supported by cell-cell junctions and/or clustered cell  
186 elimination. What are then the mechanisms ensuring that epithelia stay away from  
187 these critical points ? Feedback maintaining cohesion can act on different time scales  
188 that will either ensure on short time scales that the critical death rates are never  
189 reached, or on longer time scales by modulating key mechanical parameters affecting  
190 epithelial stability. This includes: *i*) negative feedback acting on the rate and distribution  
191 of cell death on short and long time scales, *ii*) feedback acting on cell number through  
192 cell division, *iii*) mechanical feedback stabilising the epithelial organisation and  
193 dissipating mechanical stress (**Figure 2**). The first line of defence against epithelial  
194 collapse is to slow-down and/or redistribute cell death (**Figure 2B**). Recently, work in  
195 MDCK, MCF10A and Caco2 cells revealed the existence of ERK and Akt waves

196 triggered near dying cells and propagating over few cell rows [8]. These waves protect  
197 transiently the neighbouring cells from apoptosis hence buffering down the rate of cell  
198 death despite pro-apoptotic treatment. Abrogating the waves through EGFR or  
199 Adam17 inhibition leads to a monotonic increase of cell elimination up to epithelial  
200 collapse. A similar feedback was also described in the *Drosophila* pupal notum, a  
201 single layer epithelium, where cell extrusion triggers EGFR/ERK activation in cell  
202 neighbours and protects from apoptosis the first row of cells for roughly one hour [28].  
203 This transient protection redistributes in time and space cell elimination, hence  
204 preventing the appearance of clusters of cell elimination that would directly endanger  
205 the sealing properties (see above) [28]. These rapid feedback mechanisms (effective  
206 after few minutes) ensure the first line of defence that prevents massive rate of  
207 elimination. On longer time scale, cell elimination will also progressively reduce cell  
208 density and increase tensile stress which can feedback on cell survival and cell  
209 proliferation after few hours, which will both increase cell number and help to reduce  
210 the stress borne by intercellular junctions. This was reported for various  
211 mechanosensitive pathway such as Yki Yap/Taz [32] or through the stretch-activated  
212 channel Piezo [33], all promoting cell proliferation upon tissue stretching. Cell extrusion  
213 and the stretching of direct neighbours also promote heterogenous YAP activation  
214 which increases the probability of cell division near each cell death event [34] (**Figure**  
215 **2C**). Recently, high tissue tension was shown to perturb and block cell extrusion  
216 downstream of apoptosis induction [35], hence revealing another potential negative  
217 feedback that should slow-down extrusion progressively as global tissue tension  
218 increases during cell elimination. While I discussed feedback acting on total cell  
219 number, alternative mechanism may directly modulate mechanical parameters that will  
220 ensure epithelial stability. As discussed above, three main factors could help to  
221 maintain tissue cohesion: an increase of cell-matrix adhesion (which dissipates  
222 junction tensile stress, see above), a direct reduction of junction tensile stress or a  
223 reinforcement of cell-cell adhesion. Several reports outlined the contribution of basal  
224 cell protrusion for cell extrusion [36, 37], which also include long range collective  
225 movements and cell polarisation toward the dying cell triggered by ERK and calcium  
226 waves [38-40] (**Figure 2D**), which may all increase adhesion to the matrix, friction and  
227 dissipate part of the junction tensile stress [21]. Alternatively, apoptosis also triggers a  
228 downregulation of junction tension in the neighbouring cells through Src activation  
229 which should prevent a rapid increase of tensile stress [41] (**Figure 2E**). While this was



230 not demonstrated in the context of cell death, epithelial stability may also be ensured  
231 by stress dissipation through increased tissue fluidification through junction  
232 remodelling, as shown during wound healing [42] (**Figure 2F**), or by absorbing massive  
233 strain through super-elastic behaviour [43]. Along this line, calcium pulses occurring in  
234 WT cells near oncogenic cells were shown to promote cell movement and local tissue  
235 fluidity [44], a phenomenon reminiscent of calcium waves observed near apoptotic  
236 cells which may also modulate fluidity through a similar mechanism [38]. High  
237 heterogeneity and fluctuations of contractility and tension in *Trichoplax*, a rudimentary  
238 marine organism composed on two epithelial layers, have also been proposed to buffer  
239 very large mechanical stress induced by collective migration, a process named “active  
240 cohesion” [45]. Similar fluctuations and acute release of tension could help to buffer  
241 the stress generated by high death rates.

## 242 **Conclusions and perspectives**

243 Epithelia can absorb very high rates of cell death during development and tissue  
244 homeostasis, which would have the potential to bring tissues close to the rupture point.  
245 Recent works *in vitro* and *in vivo* have revealed core parameters that will tune this  
246 rupture point, favoured by high tensile stress, low cell-cell adhesion and low cell  
247 substrate adhesion as well as fluctuations of tensile stress. Yet, these critical points  
248 are avoided thanks to the co-existence of various feedback mechanisms, acting on  
249 different temporal and spatial scales, that will buffer down and disperse cell elimination  
250 and increase mitosis. Whether such feedback also tune local and global mechanical  
251 parameters to dissipate the mechanical stress and favour epithelial stability remain an  
252 open and interesting line of future research. Chronic inflammation has been associated  
253 with repetitive loss of epithelial sealing properties during acute stress. While high rates  
254 of apoptosis were the dominant causal link explored so far, modulation of the precise  
255 spatio-temporal distribution of cell death, disruption of relevant negative feedback and  
256 alteration of core epithelial mechanical properties could all be relevant alternative  
257 causes of tissue susceptibility to rupture and inflammation.

## 258 **Declaration of interest**

259 The author declares no competing interest

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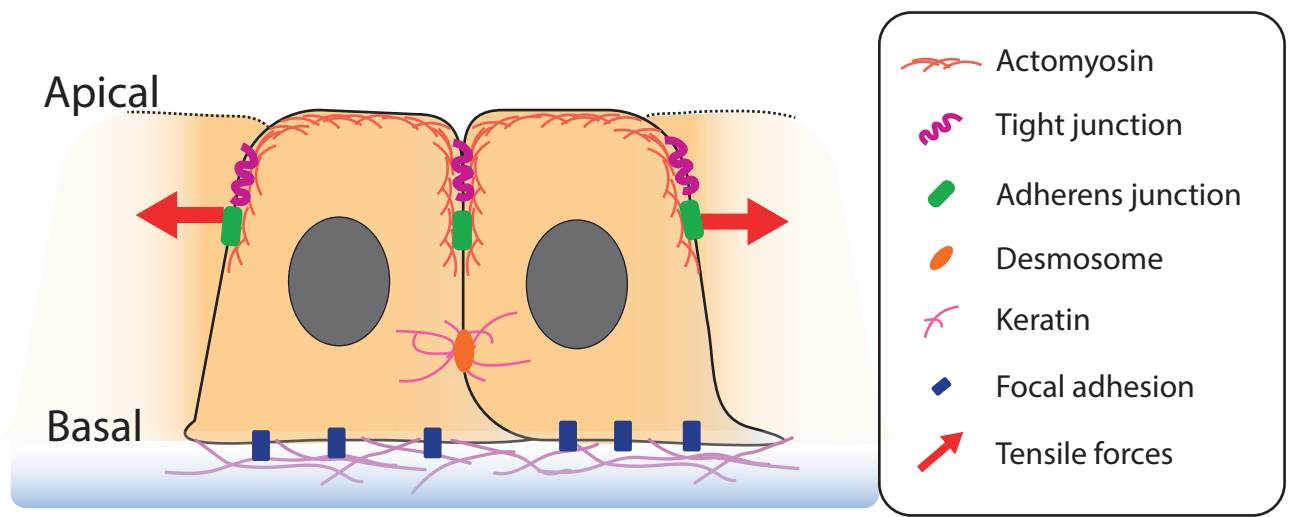
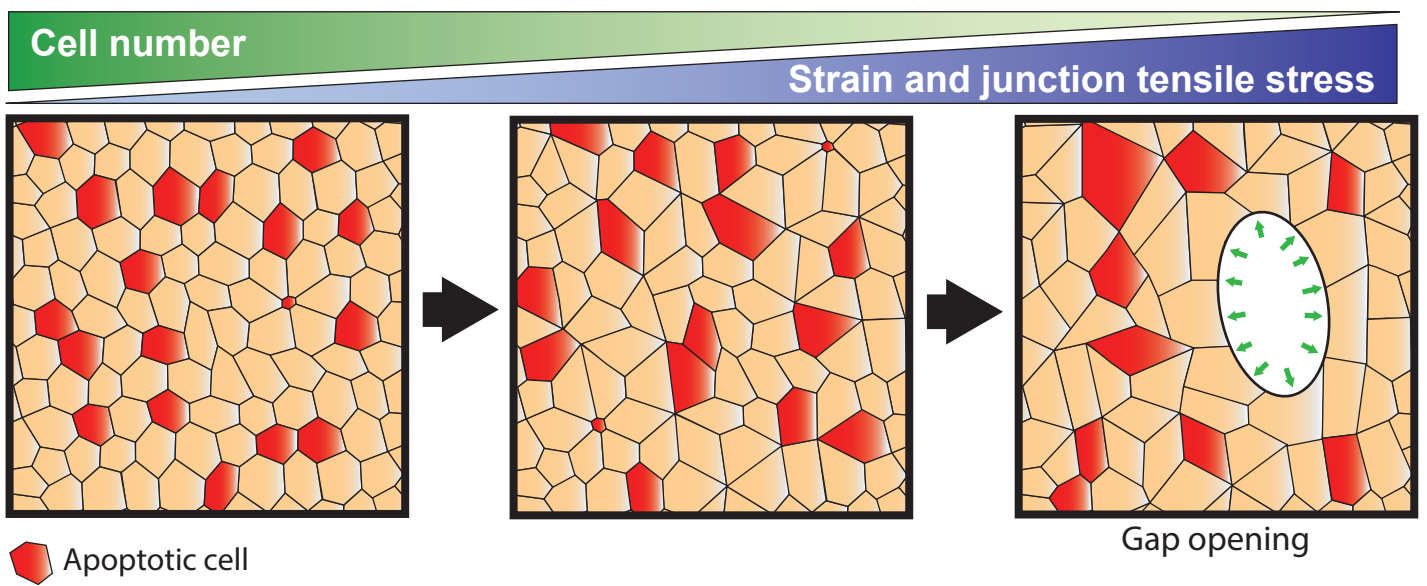
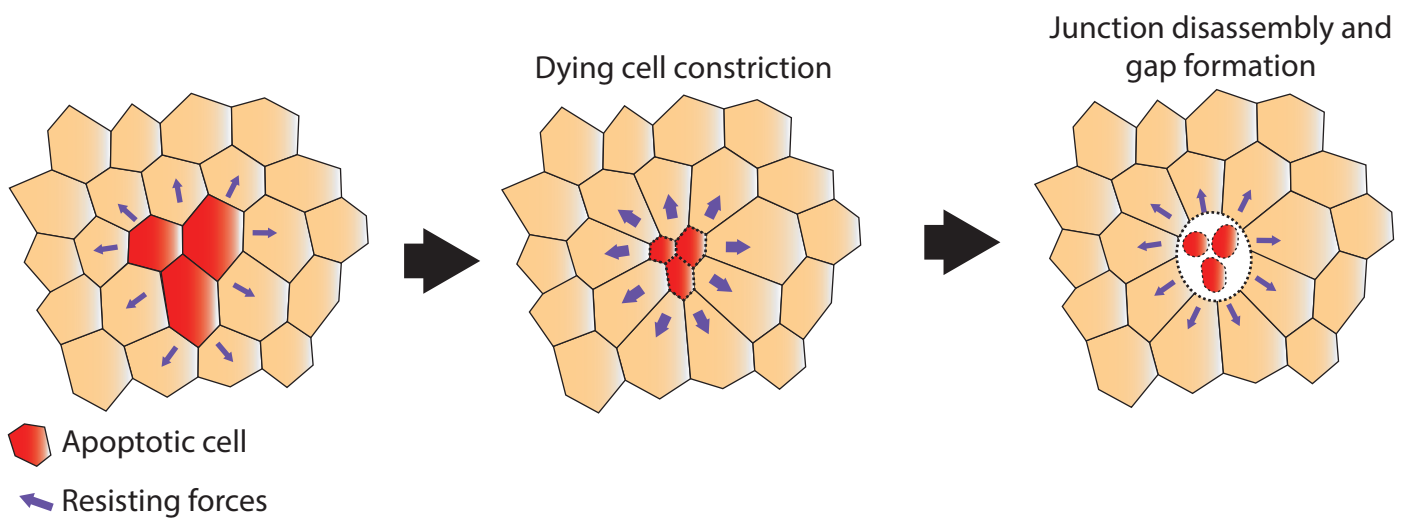
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**A****B****C**

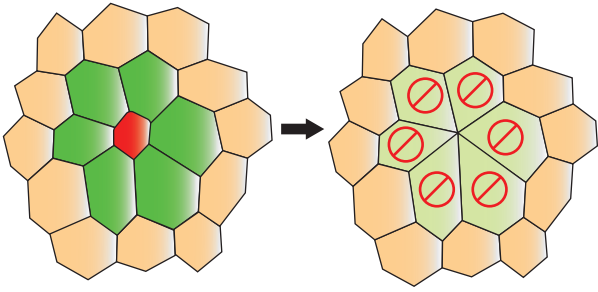
**Figure 1: Extreme conditions where cell death leads to epithelial breakage.**

**A:** Lateral view of two epithelial cells showing the most relevant cellular factors that will affect the stability of the epithelium. Lateral junctions (tight junctions, adherens junctions and desmosomes) stabilise cell-cell contract. ECM binding through focal adhesion helps to reduce the tensile stress at junction through friction, while apical actomyosin builds up tensile stress which pull laterally on junctions (red arrows). **B:** Epithelial tissue experiencing a high rate of cell death (red cells) with fixed boundaries. As cells get progressively eliminated and cell number goes down (green gradient), cell apical area and junction tensile stress progressively increase (blue gradient) until reaching a critical value at which cells detach and a hole is formed (green arrows). **C:** Clustered elimination can trigger transient hole formation. Three cells organised in cluster die simultaneously. Tension increases progressively to constrict the dying cells hence leading to increased resisting forces from the neighbours (purple arrows), however if junctions are disassembled (black dotted lines) before the neighbouring cells are close enough to rebuild new junctions, cells detach and a hole is formed.

↗ Cell number → ↘ Strain

**B**

Negative feedback on apoptosis/extrusion

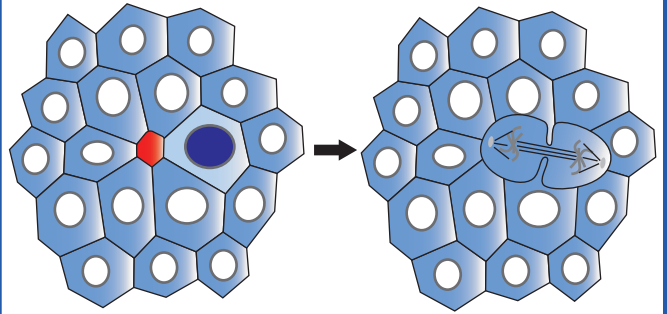


EGFR/ERK

Apoptosis inhibition

**C**

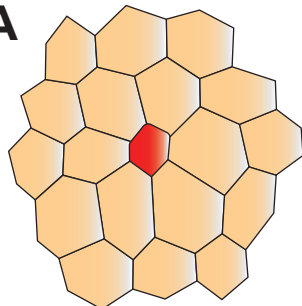
Compensatory proliferation



YAP

Local mitosis

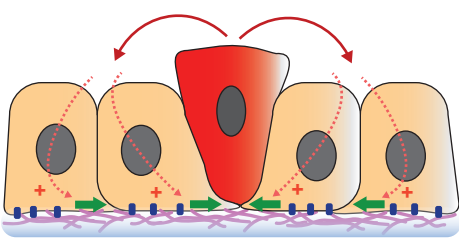
**A**



● Apoptotic cell

**D**

ECM binding reinforcement



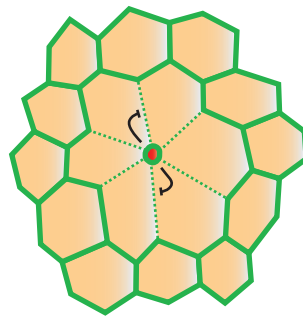
↪ ERK and/or Calcium wave

→ Polarised protrusion

■ Focal adhesion

**E**

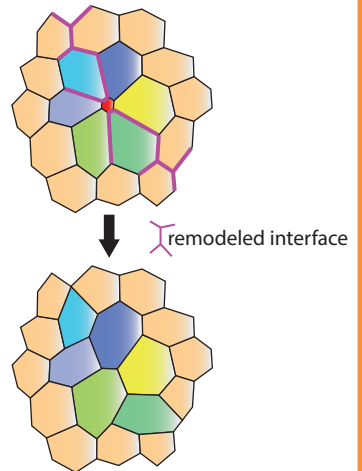
Direct junctional tension relaxation



— actomyosin

**F**

Tissue fluidity



Increased rate of junction remodeling

**Direct mechanical stress dissipation**



## **Figure 2: Feedback triggered by cell death protecting from epithelial collapse**

**A:** Group of epithelial cells with an apoptotic cell in the center initiating cell extrusion. Two categories of feedback can help to maintain the sealing properties, the one affecting total cell number (top, blue) which slow-down the reduction of cell number hence slowing-down the progressive increase of strain/tension, and the one directly promoting mechanical stress dissipation (bottom, orange), which all help to dissipate junction tensile stress. **B:** Negative feedback based on ERK pulses/waves blocking transiently cell death in the neighbouring cells. While not demonstrated, similar feedback could also directly modulate cell extrusion. **C:** Cell extrusion triggers an heterogenous increase of tension and nuclear deformation which promotes YAP (blue) nuclear localisation in a subset of cells and increases mitosis probability. **D:** Feedback promoting ECM binding and basal protrusion which may help to dissipate junctional tensile stress through friction. This may be triggered by ERK or calcium waves, which were both reported to promote basal protrusions. **E:** Feedback triggering a tension release in the neighbouring cell junctions. This can occur through Src activation in orthogonal junctions. **F:** Hypothetical feedback acting on tissue fluidity and promoting junction remodelling, which will help to dissipate mechanical stress. Cell colours are used to better visualise their relative position at each time point.