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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 μ 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 Gadolinium³⁺ (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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366 Using an optogenetic caspase in *Drosophila*, this study first showed that elimination of small
367 clusters of cells is sufficient to generate gaps. Clustered elimination are prevented by local
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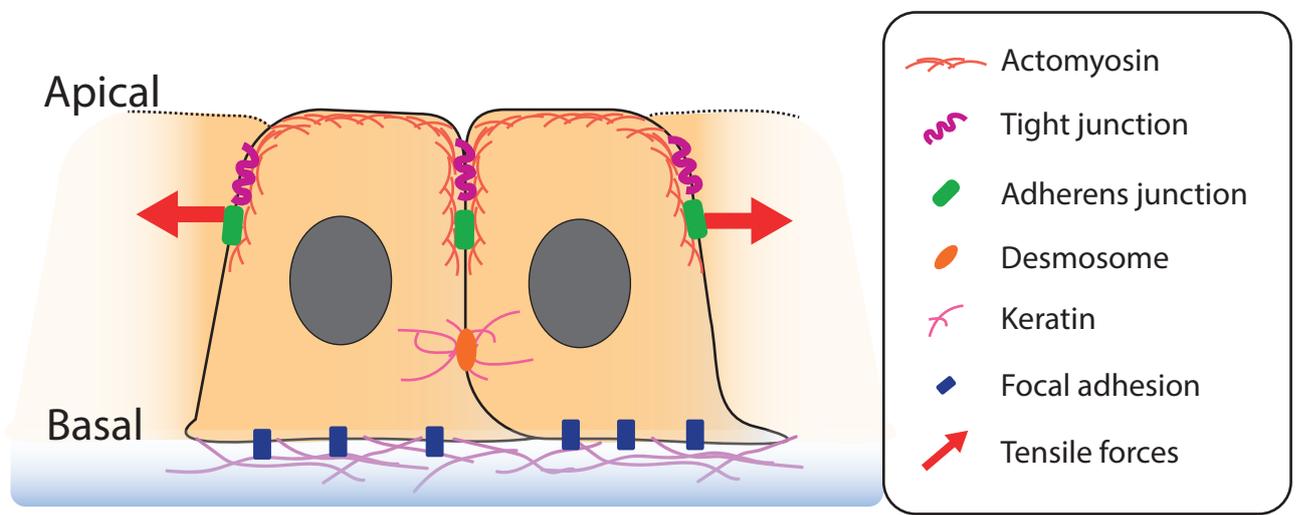
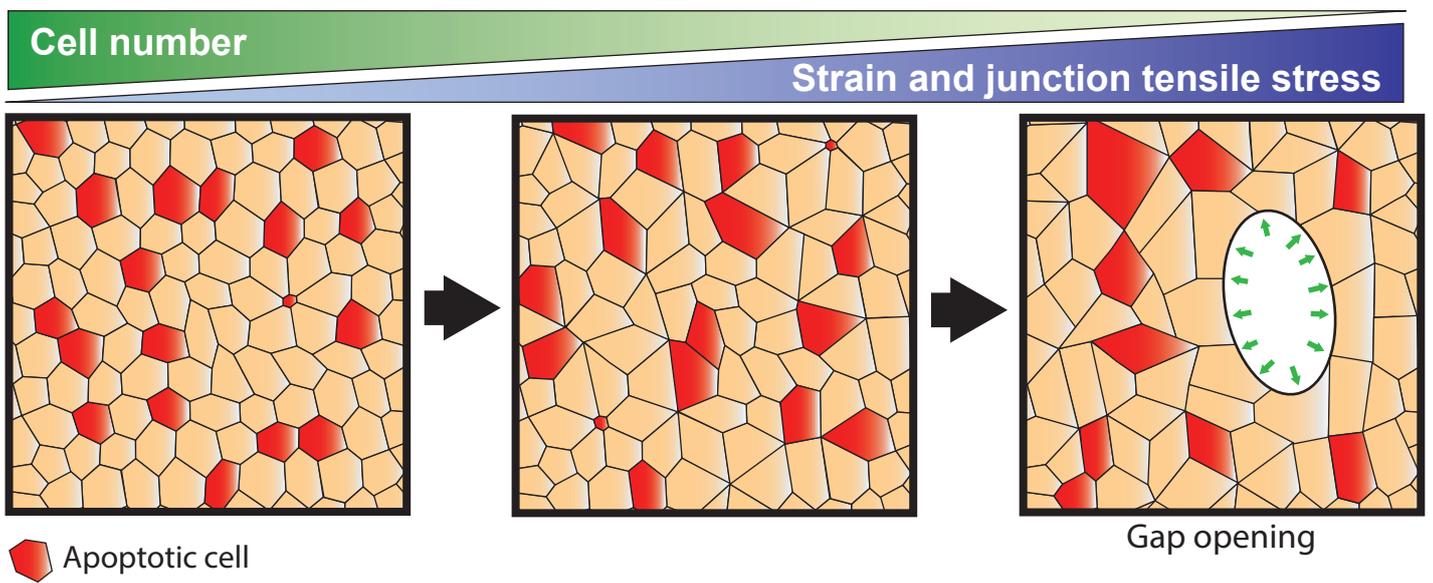
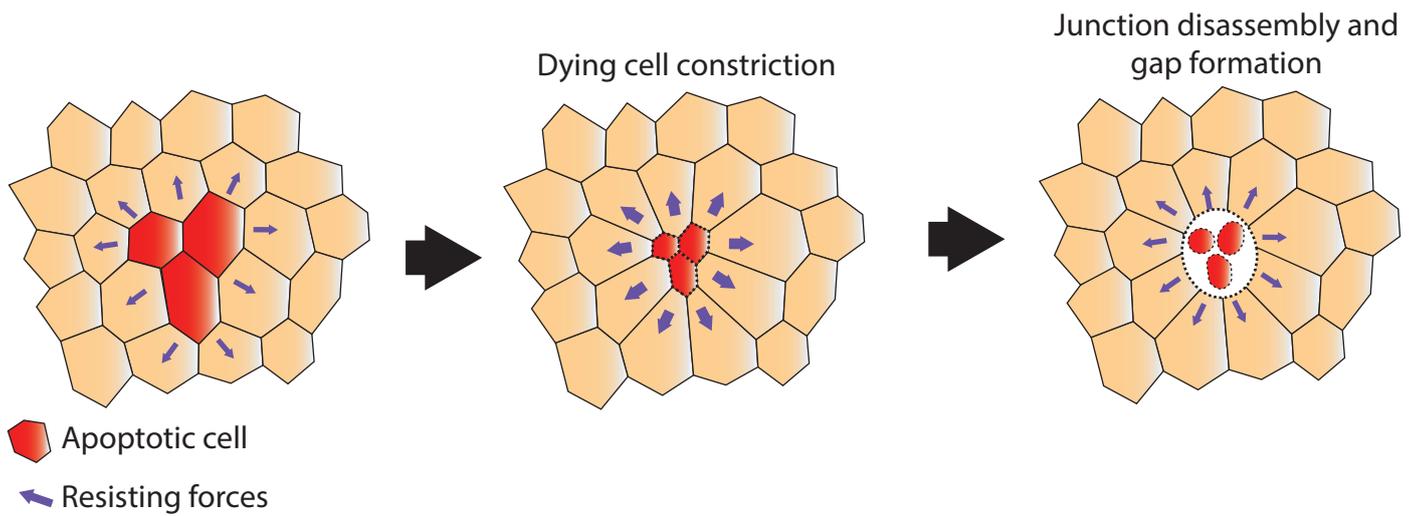
A**B****C**

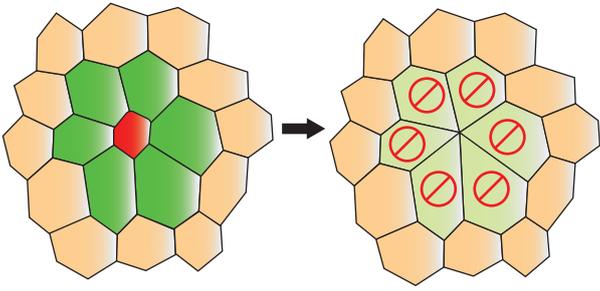
Figure 1: Extreme conditions where cell death leads to epithelial breakage.

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

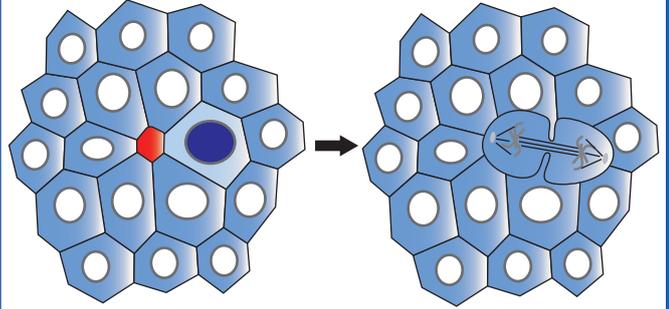


EGFR/ERK

Apoptosis inhibition

C

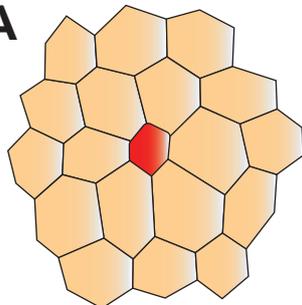
Compensatory proliferation



YAP

Local mitosis

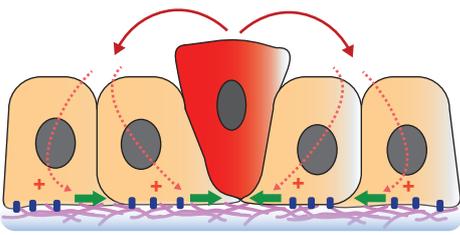
A



Apoptotic cell

D

ECM binding reinforcement



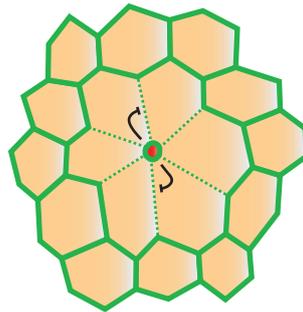
ERK and/or Calcium wave

Polarised protrusion

Focal adhesion

E

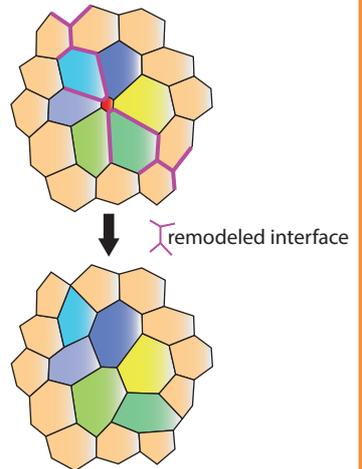
Direct junctional tension relaxation



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.