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Mechanics of epithelial tissues during gap closure

Simon Begnaud[1], **Tianchi Chen**[2], **Delphine Delacour**[1], **René-Marc Mège**[1], and **Benoît Ladoux**[1,2]

Kenneth M Yamada [Editor] and **Roberto Mayor [Editor]**

[1]Institut Jacques Monod (IJM), CNRS UMR 7592 & University Paris Diderot, Paris, France

[2]Mechanobiology Institute (MBI), National University of Singapore, Singapore

Abstract

The closure of gaps is crucial to maintaining epithelium integrity during developmental and repair processes such as dorsal closure and wound healing. Depending on biochemical as well as physical properties of the microenvironment, gap closure occurs through assembly of multicellular actin-based contractile cables and/or protrusive activity of cells lining the gap. This review discusses the relative contributions of 'purse-string' and cell crawling mechanisms regulated by cell–substrate and cell–cell interactions, cellular mechanics and physical constraints from the environment.

Introduction

Epithelia have important roles in shaping tissues and organs during embryogenesis, as well as in protecting tissues from homeostasis loss during wound healing [1]. Many physiological and pathological processes involve the (re-)sealing of epithelial gaps. From single cell apoptosis to macroscopic wound, discontinuities of the epithelial barrier occur continuously throughout the lifetime of organisms and in various scales and geometries.

Our review hence focuses on how epithelium maintains its own integrity by examining diverse gap closure scenarios. Such discontinuities can arise either intrinsically (e.g. ventral closure and dorsal closure during development, cell extrusion during homeostasis maintenance) or extrinsically (e.g. physical and chemical injury, infection). Due to its physiological importance, a wide range of studies has strived to elucidate the mechanism of epithelial gap closure with both *in vivo* and *in vitro* techniques.

Various morphogenetic events require the collective migration of neighboring epithelium into an opening to form a continuous monolayer, including D. *melanogaster* dorsal closure, *C. elegans* ventral enclosure, eyelid closure, neural tube closure and trachea invagination [2,4••,5•,6]. In all these processes, an actin cable assembles apically to form a contractile 'purse-string', and actin-based structures drive basal protrusion [7–10]. Lessons learnt from other gap closure processes studied *in vitro*, thanks to their striking similarities, helped understand the analysis of tissue morphogenesis *in vivo* [3].

Corresponding authors: Mège, René-Marc (rene-marc.mege@ijm.fr) and Ladoux, Benoît (benoit.ladoux@ijm.fr).

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Wound healing takes place during embryogenesis but also during adult life after a stress, for instance a skin cut, asthma or acute lung injury in the airway system. Independent of the tissue, healing processes share similarities [11]. However, due to its prevalence and tissue accessibility, epidermal wound healing has been the most studied: a multi-step process including tissue growth and remodeling leading to the reconstruction of the wounded area [12]. In adult skin injuries, re-epithelization can last days, during which activated keratinocytes migrate collectively over the wound area, dragging their own basal lamina as they move forward [13]. Keratinocytes in the front remodel the underlying ECM by secreting proteolitic enzymes such as metalloproteinases and depositing new ECM proteins [14]. Cell crawling seems to be more prominent here, with leader cells extending broad lamellipodia [15–17]. Interestingly, wound healing mechanisms vary with the age of the tissue. Much attention has been devoted to the study of embryonic wound healing due to its lack of scarring, reminiscent of gap closure events during morphogenesis, typically by a purse-string mechanism including rapid recruitment and assembly of actin and myosin into a thick cable in neighboring cells around the wound [18–20].

Finally, a particular case of epithelial gap closure is apoptotic cell extrusion, in which a dying cell is excluded from an epithelial monolayer. Cell extrusion also occurs recurrently in adulthood during tissue turnover and homeostatic processes [21–23]. When one or more cells undergo apoptosis, a purse-string mechanism triggers contraction that squeezes the apoptotic cell out of the epithelium.

From the examples discussed above, it appears that two main mechanisms contribute to the restoration of the epithelial integrity: (1) acto-myosin cable contraction in a purse-string manner and (2) cell crawling driven by lamellipodial and/or filopodial protrusions. Sometimes one mechanism dominates but often the two are both present and not mutually exclusive, making it challenging to distinguish their individual contributions [24,25•] (Tables 1 and 2). Fortunately, recent development of *in vitro* approaches allowed great progress in the understanding of the relative and synergistic effects of the two mechanisms as well as their regulation, by means of applying mechanical and geometrical constraints [25•,26•,27••, 28•,29,30•,31].

The acto-myosin purse-string in epithelial gap closure

The purse-string mechanism is defined as the accumulation of actin and myosin II forming a contractile cable surrounding the rim of the gap [19]. It is involved in a large variety of situations related to epithelial gap closure.

Single cell wounding is a critical event that must be quickly addressed to avoid leakage of intracellular components and subsequent cell death [32]. Cell repair by purse-string mechanism is conserved from embryonic to adult tissue cells of mammalian and non-mammalian origin [33–37]. As observed in wounded *Xenopus* oocyte, actin and myosin II accumulate at the injury site within the first minute, and then progressively segregate to form two concentric rings surrounding the rim of the gap [33,38]. A repertoire of small GTPases Rho, Rac and Cdc42 localize circumferentially around the gap and actively regulate the reorganization of acto-myosin cytoskeleton in a spatiotemporal manner [39]. During fly

early embryo cell repair, the acto-myosin ring colocalize with E-cadherin at the plasma membrane [20]. In this situation, microtubules play an important role in organizing the acto-myosin ring [20,34,37] and in guiding vesicular transport to the injury site.

For gap closure events involving multiple cells and therefore epithelium healing, a supracellular purse-string has been reported to form in all cells at the wound border (Figure 1Ia). In this case, acto-myosin accumulates at the wound margin, but junctional acto-myosin also participates in the healing process [40]. Acto-myosin fibers are linked between neighboring cells, presumably through adherens and/or tight junctions [41–45], such that the supracellular cables can build-up and maintain tension across several cells. In this way, the contraction of the acto-myosin cable can drive the collective movement of the wound edge cells into the void [45] (Figure 1Ic). A complex spatiotemporal function of Rho GTPases signaling in controlling the closure has been reported [39,45–47].

Purse-string mechanism is mainly found in the closure of small monolayer defects during wound healing or cell extrusion [45,48]. The study of a pure purse-string mechanism *in vitro* has been challenging since it requires preventing cell adhesion and matrix-based migration.

However, recent studies have managed to implement *in vitro* models where epithelial gap closure can occur over non-adherent surfaces [49••,50••] (Figure 1Ib). Here, the contraction of a multicellular actin cable is efficient enough to close large-scale gaps, while the cells at the edge of the pattern are still attached to the ECM. Geometrical cues such as size and curvature of the gap matters, as well as intact intercellular junctions [28•,49••]. Interestingly, it appears that the maximal gap size that can be closed via purse-string differs among different cell types, such as keratinocytes and kidney epithelial cells, possibly due to differences in cytoskeleton and intercellular adhesion associated mechanical properties [28•, 49••,50••]. In the case of skin cells, force measurements revealed that they are first exerting traction forces on the substrate that point away from the gap. Once the cells have extended over the gap, as the contractile ‘purse-string’ cables form across the leading edge cells, the radial component of the force reverts direction with a maximal radial force of proximately 4 μN [48]. These cables contract rapidly, leading to the formation of a suspended cell sheet over the gap and complete closure of the wound. The ‘tug-of-war’ mechanism identified in this study provides a clear demonstration of how cells exert directional forces to facilitate epithelial gap closure.

The role of cell crawling in epithelial gap closure

The crawling mechanism requires the extension of a lamellipodium by leading edge cells, often switching from apico-basal polarity to front-rear polarity [51] (Figure 1IIa and IIb). This process was initially described in monolayer wounding experiments using mechanical removal of a strip of cells, that is, manual scraping with pipette tip or razor blade [16,52,53]. Other studies performed with damage-free stencil removal and surface patterning techniques have shown that gap closure can in fact be triggered by the mere presence of free space [16,54]. The geometry and the size of these gaps can be easily varied with reproducibility [29,30•,55]. First-row cells extend lamellipodia and crawl into the free space in a Rac1-dependent manner [52]. However, cells positioned rows behind the leading edge also extend

unusual lamellipodia, so called ‘cryptic’, under the cell ahead [56]. Moreover, advanced image analysis showed that cells at back of the epithelial cell sheet are also motile [53]. Interestingly, when only the first row of cells are subjected to a dominant negative form of Rac1, closure proceeds normally as cells behind the leading edge, with normal levels of Rac1 activity, can jostle through the first row of cells and become leader cells. Nevertheless, the closure is abrogated when the dominant negative form of Rac1 is expressed in the three first rows of cells at the edge [52]. Therefore, although the role of leader cells remains crucial to locally orient and drive collective epithelial migration [57,58], the closure is not necessarily only led by the leader cells [59,60]. Along this line, particle-based computational simulations relying on the migratory capacity of cells can describe *in silico* coordinated cell movements, as well as the appearance of leader cells at the boundary of cell monolayers [61,62]. In fact, these stimulations have shown that the cell crawling behavior is sufficient to account for gap closure [63].

Controversy remains as to what triggers the activation of the protrusive machinery. In studies where cell death occurs due to the closure process, damage-induced factors can initiate the response through ERK signaling pathway, whereas under conditions without damage, cell crawling may be induced by the presence of free space and self-polarization alone [53,54,64–67]. Along this line, the role of front cells is also important in coordinating the polarization of a migrating tissue through their interactions with their physical environment and neighboring cells as recently reviewed in [68].

Coexistence and interplay between cell crawling and purse-string

Cell crawling and purse-string are both important for closing epithelial gaps, and one can be favored over the other depending on the experimental conditions, including the presence of dead factors, gap size and geometry. Importantly, the two mechanisms are not mutually exclusive (Table 2). For instance, even though wound healing has been shown to mainly depend on purse-string in embryos, the presence of cellular protrusions has also been reported, and both mechanisms are required for efficient closure [3,10,69] (Figure 2a,b). Interestingly, the mode of closure appears to depend on the curvature of the wounded edge [25•].

In vitro systems have provided a novel understanding of the physical and mechanical parameters involved in epithelial gap closure [25•,26•,27••,70•]. The coexistence of cell crawling and actin-based cable contractility has been reported to be crucial for promoting optimal wound closure. Moreover, in model wounds or scratches, the leading edge repolarizes and transforms into crawling cells, with the appearance of leader cells harboring a large forward lamellipodium [15,16]. However, along the side of the protrusive front and in between two leader cells, the assembly of a supracellular actomyosin cable is frequently observed preventing new leader cell formation [70•] (Figure 2c). This cable is reminiscent of the one observed in purse-string process, and its formation also depends on RhoA activity [70•].

Brugues *et al.* studied how actomyosin cables and actin-based protrusions generate mechanical forces during wound repair [27••]. Cells adjacent to the wound generate radial

traction forces pointing either away from the wound or into the wound. The inward pointing forces coincide with the position of protrusions, whereas outward pointing forces coincide with the position of acto-myosin cables. Interestingly, the forces generated by the contraction of the acto-myosin cable around the wound are also transmitted to the substrate. Cells transmit forces to the substrate through specialized structures known as focal adhesions (FAs) [71,72]. During epithelial gap closure, it appears that FA orientation is mostly parallel to the wound edge under the acto-myosin cable but perpendicular in cell protrusions [25•,27••].

The shape of the wound, and in particular the direction of the local curvature of the gap, may be a key determinant of the modes of epithelia gap closure (Figure 2d). Negative curvature, that is, concave border, is related to actin cable assembly and purse-string-based closure, whereas positive curvature, that is, convex border, favors cell crawling [25•,26•,73,74•]. A recent study explored the roles of two gap-closing mechanisms and described how the relative contributions of the two mechanisms are affected by gap geometry [25•]. Cells predominantly crawl at positive curvature, whereas purse-string and crawling mechanisms additively operate to fill the gap in areas of negative curvature, thus leading to faster tissue velocity (Figure 2). To summarize, these two mechanisms can act in concert to close gaps consisted of both concave and convex regions and their relative contribution depends on the local curvature.

Conclusions and perspectives

Purse-string and cell crawling mechanisms have been proposed to drive epithelial gap closure, but a clear picture of their respective functions is masked by the complexity of the closure process and the variety of conditions. However, recent *in vitro* and *in vivo* experiments have shown that physical constraints, such as local tissue curvature are crucial to the regulation of gap closure mechanisms [25•,27••,70•]. Such coupling could be mediated by a differential organization of the actin cortex depending on the shape of the cell membrane, but also by a differential distribution of curvature-sensing proteins, such as BAR domain proteins [75].

Interestingly, components of cell–cell adhesion, such as E-cadherin, are also dynamically redistributed at the wound edge, which could be mediated by contractile forces exerted by the acto-myosin cable [3,42,76,77••]. Cadherin-based adhesions have been implicated in the transmission of intercellular, as well as in cell–substrate forces [78,79,80•,81], making them indispensable players in the mechanical regulation of multicellular gap closure.

Finally, it would be of great interest to systematically characterize the closure of gaps, depending on the mechanical properties of the surrounding environment, such as how the stiffness of the substrate may affect epithelial wound healing [82,83]. Aside from the passive mechanical properties of the ECM, other cells in the wound microenvironment can also actively provide mechanical cues to the epithelium. Recent works suggest that contraction of underlying cells drives *Drosophila* dorsal closure or *Zebrafish* epiboly [84]. Similarly, myofibroblasts in the dermis beneath an injured epidermis can contract and help the sealing of wounds [85,86].

Venturing into the realm between biology and physics should help us better understand the mechanics guiding epithelial gap closure. With the recent advances in *in vitro* techniques, we have the means to unveil more hidden mysteries in the process.

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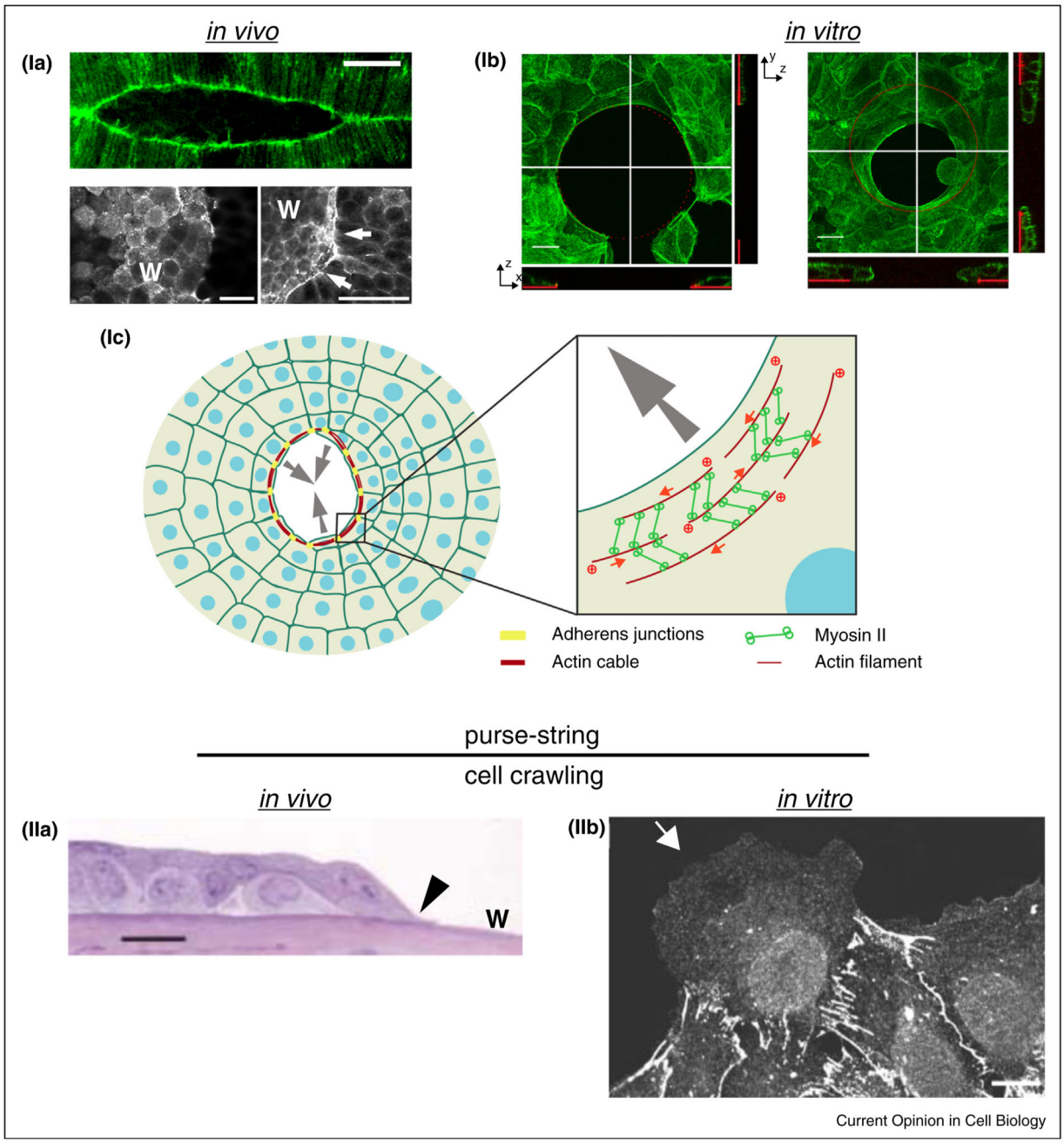

Figure 1. Contractile actin cable (Purse-string) or cell crawling mechanisms for epithelial gap closure both *in vivo* and *in vitro* situations.

(Ia) Top panel: Actin labeling during embryonic dorsal closure of D. *melanogaster*. Scale bar: 20 μm (from [87]). Bottom panel: Actin staining during Xenopus *leavis* wound healing. W: wound; scale bar: 50 μm (from [88]). **(Ib)** Actin staining of HaCaT keratinocytes covering a cyto-repulsive area *in vitro* (Top and side views; left: before gap closure; right: during gap closure; fibronectin: red; from [49••]). **(Ic)** Scheme of purse-string gap closure. Cell at the gap margin assemble a supracellular contractile actin cable. Adherens junctions

insure actin cable continuity between adjacent cells. Inset: Myosin II proteins cross-link actin filaments and insure contractility. **(IIa)** Light migrograph of the leading edge of healing mouse corneal epithelium.
Arrowhead: lamellipodium; w: wound; scale bar: 25 μm (from [89]). (IIb) E-cadherin staining of a leader cell at the wound margin of rat liver epithelium cultured *in vitro*. scale bar: 10 μm.
Source: From Ref. [15] ‘Copyright (2003) National Academy of Sciences, U.S.A.

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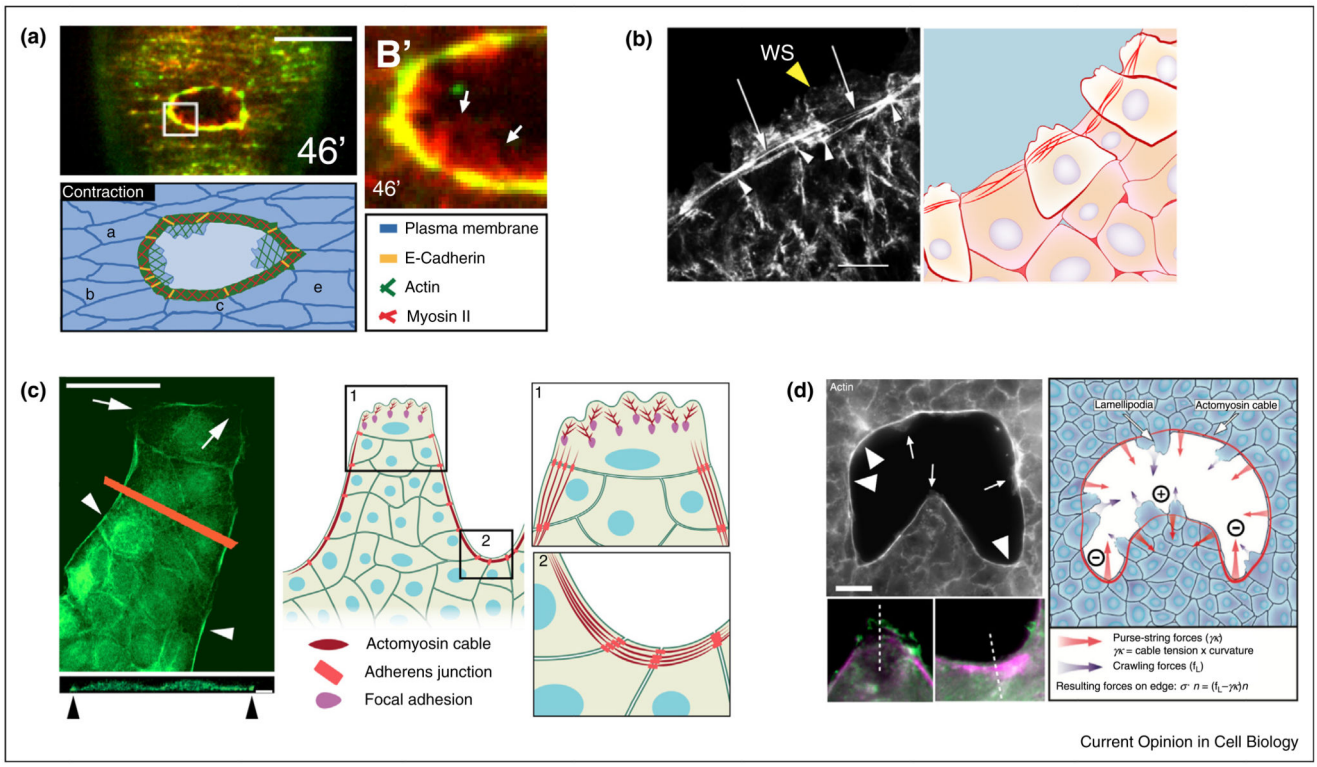

Figure 2. Combination of contractile cables and cell crawling for gap closure.

(a) Top: the actomyosin cable and the actin-based lamellipodia (arrows) participate in embryonic gap closure. Myosin and actin are displayed green and red, respectively; scale bar: 20 μm. Bottom: schema of *D. melanogaster* embryo wound healing during contraction phase (from [24]). (b) Left: F-actin staining of the leading edge of adult mouse corneal epithelium during wound healing. At the wound margin cells extend lamellipodial protrusions (yellow arrowheads) and take a part in the assembly of the supracellular actin cable (white arrows). Note the actin reinforcement at the intercellular contacts (white arrrowheads). WS: wound surface, scale bar: 10 μm (from [41]). Right: Scheme of epithelial adult mouse corneal wound healing. (c) Organization of a finger-like protrusion. At the tip of the protrusion, the leader cell extends large lamellipodia (arrows). At wound border and between two leader cells, follower cells assemble a supracellular acto-myosin cable (arrowheads). Pictures shows F-actin staining of the protrusive front of a kidney epithelium *in vitro*; top and side views; scale bar: 50 μm and 5 mm respectively (from [70•]). (d) Local curvature of the epithelium edge induces either lamellipodia extension (arrow) or acto-myosin cable assembly (arrowhead). The amplitude of curvature is correlated with the predominance of the lamellipodia or actin cable (from [25•]); grey: F-actin; purple: phospho-myosin light chain; green: cortactin; scale bar: 20 μm). At the edge of the tissue, the force balance relies on the stress, σ, normal to the edge and the contributions of the crawling forces due to lamellipodium extension, f_L, and purse-string forces, $\gamma\kappa$, where γ is the line tension and κ the local curvature (=1/R).

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Table 1

Purse string and crawling mechanisms are described separately. The articles are ordered first by mechanism of gap closure then by year of publication.

Mechanism of closure	Cell line	Method for gap production	Size of gap	Time/speed of closure	Comments	Reference
Purse-string	Four days chick embryo	Mechanical wound	0.5 mm diameter, ≈ squared	10–15 μm/h		Martin *et al.*, 1992 [19]
Purse-string	Chick embryo stage 23	Mechanical wound	500 μm long, 70 μm wide	6 h	Rho-dependant Rac-independant	Brock *et al.*, 1996 [42]
Purse-string	Xenopus oocyte	Laser ablation	≈10 mm infra-cellular	?	Regulation by concentric exclusive rings of Cdc42/Rhoa	Benink *et al.*, 2005 [90]
Purse-string	Caco2	Mechanical wound	Few cells size, tens of μm in diameter	30–45 min	MLCK and ROCK dependant	Russo *et al.*, 2005 [46]
Purse-string	MDCK (Madine-Darby Canine Kidney cells)	Laser ablation	1–3 cells size	30–60 min	MLCK ROCK dependant, actin cable anchored at tight junctions	Tamada *et al.*, 2007 [45]
Purse-string	Xenopus oocyte	Laser ablation	Infra-cellular	?	Fusion of actomyosin cables, adherens junction role	Clark *et al.*, 2009 [40]
Purse-string	Early drosophila embryo	Laser ablation	Hundreds μm^2 infra-cellular	4 $\mu m^2/s$	E-cadherin anchors actomyosin at membrane	Abreu-blanco *et al.*, 2011 [20]
Purse-string	Drosophila embryos:	Laser ablation	<5 μm width, ≈5 μm long	Fast then slow regime:	Actin cable + medial acto-myosin network	Fernandez-Gonzalez *et al.*, 2013 [91]
	Early			40 then <5 μm^2/min		
	Late			15 then <5 μm^2/min>		
Purse-string	Xenopus embryos	Excision	>50 μm	?	Inositol kinase tune Ca^{2+} wave, RhoA, Cdc42 and Rac1 activity	Soto *et al.*, 2013 [92]
		Laser ablation	<10 μm			
Purse-string + cell contraction	Blastoderm of early chick embryos	Mechanical wound	≈100 μm, circular	10 min (50% in 30 s)	No change in aspect ratio	Wyczalkowski *et al.*, 2013 [93]
		Microscalpel	≈200 μm, elliptical	>10 min		
Purse-string	HaCaT	Non-adherent gap	100 μm diam, circular	4–17 h	Fluctuation during closure	Vedula *et al.*, 2015 [49••]
Purse-string	MDCK	Non-adherent gap	5–75 μm diam, circular	24 to >72 h	Fluctuation actively contribute to closing	Nier *et al.*, 2015 [50••]
Purse-string	Stratified corneal epithelial cell	Mechanical wound	1 mm, circular	48 h 0.20–0.36 μm/min	No proliferation in actively migrating cells	Gonzalez-Andrades *et al.*, 2016 [94]
Cell crawling	MDCK	Mechanical wound	100–200 μm wide, 500–1000 μm long	≈18 h	Rac dependant	Fenteany *et al.*, 2000 [52]
Cell crawling	Corneal epithelium (*in vivo*)	Mechanical wound	2–2.5 mm	≈18 h	notable cell jostling	Danjo and Gipson 2002 [89]
Cell crawling	MDCK	Mechanical wound	250 μm wide	6 h	Src and ERK activation (2 waves)	Matsubayashi *et al.*, 2004 [53]
Cell crawling	Primary culture of corneal cells	Mechanical wound	1 mm wide, 11 mm long	≈15 h	EGFR and JNK activation	Block *et al.*, 2004 [95]
		Agarose removal			EGFR activation, not JNK	
Cell crawling + actin accumulation	Airway epithelial cells 16HBE	Mechanical wound	678 ± 14 μm wide	15–20 h	Rho and Rac dependant at appropriate concentrations	Desai *et al.*, 2004 [47]
Cell crawling	MDCK	PDMS removal (=clean gap)	Infinite	10 μm/h	1 MAPK wave	Nikolic *et al.*, 2006 [54]

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Mechanism of closure	Cell line	Method for gap production	Size of gap	Time/speed of closure	Comments	Reference
		PDMS ripping (=damaged border) Mechanical wound		30 μm/h leader cells, <10 μm/h followers	2 MAPK waves	
Cell crawling	MDCK	PDMS stencil removal	≈400 μm wide, cm long	≈40 h		Poujade *et al.* 2007 [16]
Cell migration	Human oesophaegal epithelial cells het1a	Cell squeezing with PDMS stamps	250 μm, square	15 h		Lee *et al.*, 2010 [96]
Cell crawling	MDCK	PDMS stencil removal	Tens to thousands of μm^2	10–350 min	In small gaps, passive closure w/o cell protrusion nor purse-string	Anon *et al.*, 2012 [29]
Cell crawling	Bovine corneal endothelial cell BCEC-L	Mechanical wound (conservative of basement membrane for BCEC-L)	Tens of μm	6 h	Healing dependant on electrical and ionic modification migration depends on Sodium channel except for BAEC cells	Justet *et al.*, 2013 [97]
	MDCK (+purse-string)			16 h		
	Rabbit corneal epithelial cells RCEp			6h		
	Bovine aortal endothelial cells BAEC			12 h		
Cell crawling	Chick embryo lens epithelium explants	Microsurgery conservative for ECM	2 mm	3 days	Vimentin (not actin) is predominant in lamellipodia	Menko *et al.*, 2014 [98]
Cell crawling	MDCK (HaCaT)	PDMS stencil removal	500 μm width	≈25 μm/h	Merlin (nf2) coordinate collective migration	Das *et al.*, 2015 [99•]
Cell crawling	MDCK	Plastic stencil removal	5 mm diam colonies on coll I	Observation after 2–3 days	Leader cell coordinate finger-like structure migration (PI3K, integrin β1 and Rac1 dependant)	Yamaguchi *et al.*, 2015 [100]

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Table 2

Purse string and crawling mechanisms are not exclusive. The articles are ordered by year of publication.

Mechanism of closure	Cell line	Method for gap production	Size of gap	Time/speed of closure	Comments	Reference
Purse-string + cell crawling	Caco2	Mechanical wound	1–8 cell diam (<100 μm)	2–6h	<8 cells diam: purse string	Bement *et al.*, 1993 [10]
Purse-string + lamellipodia	Mouse corneal epithelium	Mechanical wound	4 mm^2	24 h	>8 cells diam: crawling Actin cable anchored at adherens junctions	Danjo *et al.*, 1998 [41]
1st purse-string, 2nd cell crawling	T84 colon carcinoma cells	Pipette tip aspiration	0.018 mm^2, 400 cells size	120–150 min	Dependant on integrin activation	Lotz *et al.*, 2000 [101]
1st purse-string, 2nd cell crawling	Epithelial-like cells in Xenopus embryo	Microsurgery	≈0.2 mm^2, square	60–90 min	Small wounds close at faster rate than larger wounds	Davidson *et al.*, 2002 [88]
Purse-string + lamellipodia + filopodia	Ventral epithelial cells of Drosophila embryo	Laser ablation mechanical wound	10 cells diam, ≈800 $μm^2$, ≈15 μm diam, ≈circular	120 min, 7 $μm^2$/min	KO RhoA x2 slower but closes Rac independent Cdc42 no final zipping adherens junction	Wood *et al.*, 2002 [3]
Syncytium formation + lamelipodia	Drosophila larvae	Mechanical wound	100 μm 6 cells size	24–60 h	JNK dependent	Galko *et al.*, 2004 [102]
Purse-string OR cell crawling	Bovine corneal endothelial cells	Mechanical wound (ECM removal)	150 μm or 2 mm, linear <10 cells, circular	W/o ECM, 6.25 μm/h	W/o ECM, purse string W/ECM cell crawling Irrespective of wound size	Grasso *et al.*, 2007 [103]
Actin polymerization	*C. elegans*	Mechanical wound (ECM maintenance) Laser ablation mechanical wound	20 and 40 μm diam	W/ECM, ≈15 μm/h 2 h	Negatively regulated by myosin	Xu *et al.*, 2011 [104]
Purse-string + lamellipodia	Skin cells of medaka fish (Oryzias latipes)	Explant growth ≈10 $μm^2$	Infinite	3 h	Purse string dependant on RhoA ROCK and MyoII activity	Morita *et al.*, 2011 [105]
Purse-string + lamellipodia + filopodia	Late Drosophila embryo	Laser ablation	≈50 μm width Hundreds $μm^2$, circular	1–4h ≈1 h	4 steps (expansion, coalescence, contration, closure) irrespective of the initial wound size	Abreu-Blanco *et al.*, 2012 [24]
Purse-string + cell crawling	Human corneal limbal epithelial cells	Agarose inserts digestion	Hundreds of μm	11 h	Negative curvature = purse-string positive or no curvature = crawling EGF-dependant cell crawling	Klarlund, 2012 [26•]
Purse-string + cell crawling	HaCaT, primary human keratinocytes and corneal epithelial cells	ECM geometry + stencil removal	Non-adherent 100–120 μm width	≈30 h	Actin cable and crawling leader cells pull on suspended cell monolayers	Vedula *et al.*, 2014 [28•]
Purse-string + cell crawling	MDCK	Stencil removal	50 μm, circular	5vh, ≈300 $μm^2$/h	Rac1 and RhoA dependent	Cochet-Escartin*et al.*, 2014 [30•]
	HEK-HT			3 h, ≈500 $μm^2$/h		