Regionalized tissue fluidization is required for epithelial gap closure during insect gastrulation

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Many animal embryos pull and close an epithelial sheet around the ellipsoidal egg surface during a gastrulation process known as epiboly. The ovoidal geometry dictates that the epithelial sheet first expands and subsequently compacts. Moreover, the spreading epithelium is mechanically stressed and this stress needs to be released. Here we show that during extraembryonic tissue (serosa) epiboly in the insect Tribolium castaneum, the non-proliferative serosa becomes regionalized into a solid-like dorsal region with larger non-rearranging cells, and a more fluid-like ventral region surrounding the leading edge with smaller cells undergoing intercalations. Our results suggest that a heterogeneous actomyosin cable contributes to the fluidization of the leading edge by driving sequential eviction and intercalation of individual cells away from the serosa margin. Since this developmental solution utilized during epiboly resembles the mechanism of wound healing, we propose actomyosin cable-driven local tissue fluidization as a conserved morphogenetic module for closure of epithelial gaps.
Epiboly is one of the hallmark morphogenetic movements during animal gastrulation. It involves spreading of an epithelial sheet over the spherical or ellipsoidal egg. The sheet eventually forms a continuous layer that entirely surrounds the embryo and the yolk sac. During this morphogenetic event, fundamental geometrical and mechanical problems arise. First, in order to cover the entire egg, the epithelium has to expand in surface area. However, once the egg equator is reached, the expanding tissue must also undergo a regional compaction at its leading edge in order to seal seamlessly at the bottom of the sphere.
**Results**

*Tribolium* serosa undergoes inhomogeneous expansion during epiboly. To visualize serosa epiboly, we imaged transgenic embryos expressing a nuclei-marking enhanced green fluorescent protein (eGFP) with multi-view light-sheet microscopy (Fig. 1c and Supplementary Movie 1). Taking advantage of the serosa’s topology as a superficial egg layer, we unwrapped the three-dimensional (3D) data into two-dimensional (2D) cartographic time-lapse projections and segmented the serosal part of the blastoderm tissue (Fig. 1d, Supplementary Fig. 1A–D, and Supplementary Movies 2 and 11). The serosa covered initially about 35% of the egg surface and spread to cover 100% of the surface (Fig. 1e). In order to examine the expansion at the cellular level, we imaged embryos expressing LifeAct-eGFP that labels cortical F-actin and segmented the apical surface of all serosal cells at the five reference stages (Fig. 1b) during serosa expansion (Fig. 1f, g). The results showed that the ~3-fold expansion in serosal tissue surface area was mirrored by a ~3-fold expansion of the apical area of serosal cells from Stage 1 to Stage 4 (Fig. 1f). Strikingly, serosal cells did not expand uniformly: at Stage 3, the apical area of ventral cells in the vicinity of the serosa window was on average 29% smaller compared to dorsal cells (Fig. 1f, g and Supplementary Movie 11). We conclude that serosa epiboly exhibits inhomogeneous apical cell area expansion in order to accommodate the ventral area compaction required by the elliptical geometry of the egg.

Ventral leading edge of the serosa exhibits local tissue fluidization. An alternative but not mutually exclusive mechanism to achieve ventral area compaction is by reducing the number of marginal cells at the serosa window (Fig. 2a). While it is in principle possible that leading cells are not excluded and converge to a multicellular rosette, such a rosette has not been observed during *Tribolium* serosa window closure. Our cell tracking experiments showed that the initial number of approximately 75 leading cells progressively decreased to only 5–6 cells during final serosa closure (Fig. 2b, c) and that these cells originated from all around the periphery of the window (Fig. 2d and Supplementary Movie 3). Careful examination of individual cells at the leading edge in time-lapse recordings of embryos of the LifeAct-eGFP transgenic line revealed frequent rearrangement of cells resulting in cells leaving the serosal edge (Fig. 2e, f and Supplementary Movie 4). The leaving cells shrank their leading edge facing the serosa window and elongated radially in the direction approximately orthogonal to the window (Supplementary Fig. 2A–C). Upon leaving the edge, the cells gradually relaxed to a hexagonal shape as they reintegrated into the bulk of the tissue (Supplementary Fig. 2D). Mapping of those behaviors onto the time-lapse cartographic projections revealed that the serosa was regionalized into two distinct territories. Dorsal cells, several cell diameters away from the edge, were hexagonally packed, isotropically stretched, and showed no significant neighbor exchanges. By contrast, ventral cells surrounding the serosa window were irregularly packed, showed anisotropically stretched
shapes (Supplementary Fig. 3A and Supplementary Movie 11), and frequently exchanged neighbors (Fig. 2h and Supplementary Movie 5).

Movement of cells past each other during neighbor exchange has been linked to increased tissue fluidity\textsuperscript{18–21}. A useful theoretical framework to assess the behavior of the serosal tissue is the shape index analysis that infers solid-like or fluid-like tissue states from cell shapes in epithelia\textsuperscript{22–24}. Based on the vertex model, the leading theoretical framework for studying the mechanical behavior of epithelial tissues\textsuperscript{25}, the theory predicts a
critical value of shape index $p = 3.81$ marking the transition from a solid-like ($p < 3.81$) to a fluid-like behavior ($p > 3.81$ but see also ref. 26 and below). Our results showed that at Stage 3 ventral cells had on average a high shape index $p$ of 4.25 characteristic of fluid-like tissues, unlike dorsal cells that had a significantly lower $p$ value of 3.93. The changing values of the shape index suggest a gradient of tissue properties along the dorsal–ventral axis of the embryo, where the ventral region is much more fluid-like compared to the dorsal region (Fig. 2g, i and Supplementary Movie 11). These results raise the hypothesis that during serosal epiboly the tissue in the vicinity of the window undergoes a solid-to-fluid structural transition (fluidization) that unjams the tissue and enables seamless closure.

**Serosa shows distinct mechanical properties along the dorso-ventral axis.** We next asked what the mechanical function of the ventral serosal fluidization could be. If the dorsal serosa behaves as a solid-like material, we expect that while being pulled over the egg it would increasingly build up tension. This rising tension would make it increasingly more difficult to further close the serosa window. The function of the ventral cell rearrangement in the proximity of the serosa window could then be in releasing this tension to facilitate closure. Consequently, we would predict a difference in tissue tension between dorsal and ventral serosa. To test this, we performed laser ablations inflicting large incisions across 3–4 cells at different reference stages and positions and determined the recoil velocities of the bordering tissue immediately after the cut (Fig. 3a, b). The cuts were oriented perpendicular to the axis along which the serosal cells were stretched.

Our results showed that the tissue recoil velocity in the dorsal side increased progressively as the serosa expanded posteriorly and ventrally around the posterior pole and plateaued after the serosa window formed (Fig. 3c). Intriguingly, when we performed incisions at the ventral side of the serosa at a stage where this tissue exhibits cell rearrangements (Stage 3), the recoil velocities were significantly lower compared to the dorsal side (Fig. 3d). Because the tissue recoil velocity depends on both the tension in the tissue and the material properties of the tissue, we further analyzed the time-dependent decay of the tissue recoil (28). Such an analysis can discern between fluid-like or solid-like recoil patterns of the severed tissue. Our data suggested that the ventral tissue exhibits more fluid-like behavior than the dorsal tissue (Supplementary Fig. 4 and “Methods”). Therefore, the laser cutting experiments support the view that the dorsal tissue behaves like an elastic solid. Assuming that its properties do not change dramatically over the course of serosa expansion, the tension in this part of the serosa increases as the tissue gets stretched. This notion is further corroborated by our observations that the intact cells neighboring an ablation site responded to the release in tissue tension post-ablation by immediately decreasing their apical areas by one-third (Supplementary Fig. 5). In contrast to the dorsal region, the ventral portion of the serosa is transitioning from a solid-like to a fluid-like state, which is connected to the local reduction of tissue tension.

While the recoil pattern after laser ablation supports the hypothesis of ventral tissue fluidization suggested by the shape index analysis, it has been recently shown that the relationship between shape index and tissue fluidity is non-linear when the tissue is under tension26. Since we obtained from laser ablations evidence that the *Tribolium* serosa exhibits a spatially inhomogeneous tension profile, we applied this extended theoretical framework. Moreover, we observed that the cells close to the window are strongly elongated in direction radial to the window (Fig. 2e, f and Supplementary Fig. 2) which could indicate local anisotropy in the tension profile. Therefore, we calculated a local cell alignment factor $Q$ across the serosal tissue as a proxy measure of local tissue tension anisotropy (see “Methods”)29. The theory predicts that for a given value of $Q$ the shape index $p$ needs to exceed an adjusted threshold value in order for the tissue to be fluid-like. For each local value of $Q$ across the cartographic maps, we plotted the difference between the actual shape index value ($p$) of the cell and the local threshold signifying solid-to-fluid transition (Fig. 3e, f and Supplementary Movie 11). This analysis revealed that, also when taking tissue tension anisotropy into consideration, the ventral cells lining the rim of the serosa window exhibited a distinct fluid-like state during closure, in stark contrast with the rest of the epithelium exhibiting a solid-like state. Therefore, both experimental and theoretical evidence support the local fluidization of the ventral-most serosal tissue.

**Ventral serosa fluidization is mediated by an actomyosin cable.** We next asked what induces the local tissue fluidization. Recent live imaging studies of *Tribolium* gastrulation suggested that an accumulation of actin, resembling a cable, emerges at the leading edge of the serosa13,30. To test whether this accumulation indeed represents a contractile actomyosin cable31,32, we imaged the distribution of non-muscle myosin II (hereafter referred to as myosin) in gastrulating embryos injected with the *Tribolium* myosin regulatory light chain (*Tc-sqh*) mRNA fused to eGFP (*Tc-sqh-eGFP*). During epiboly, myosin accumulated at the boundary between the serosa and the embryonic primordium (Fig. 4a, b, Supplementary Fig. 6A–C, and Supplementary Movie 6). Actomyosin enrichment at the serosa–embryonic boundary initiated shortly after epiboly started and became more pronounced as the
boundary stretched around the posterior pole. It peaked during serosa window closure and at this stage appeared as a contiguous supra-cellular cable (Fig. 4b and Supplementary Fig. 6A–C). The actomyosin cable lined the rim of the serosa window and underwent shape transformations from triangular to circular during closure (Fig. 4c and Supplementary Figs. 6B and 7A). The cable formed at the boundary of the serosa–embryonic (prospective amnion) region and the serosal cells forming the cable often appeared bent inwards over the window rim (Supplementary Fig. 7). By segmenting and measuring the length of the cable based on LifeAct-eGFP or Tc-sqh-eGFP enrichment, we found that the cable first increased its length until the serosa–embryonic boundary reached the posterior pole and then decreased in length to zero during window closure (Supplementary Fig. 6D). As the cable shrunk, the total myosin intensity normalized by cable length stayed the same or increased over time (Supplementary Fig. 6E). Laser cutting experiments of individual cell edges contributing to the actomyosin cable revealed that the cable was under tension and that this tension increased over time (Fig. 4d-f and Supplementary Movie 7). If the cable acted as a contiguous
Abolishing the cable halts ventral serosa fluidization and closure. Such a model predicts that, in the absence of the actomyosin cable, the serosa window would fail to close ventrally. A previous study suggested that the juxtaposition of normally proportioned extraembryonic (serosa) and embryonic (amnion and germband) rudiments in the differentiated Tribolium blastoderm is required for proper emergence and constriction of the actomyosin cable at the extraembryonic/embryonic boundary. Furthermore, it has been demonstrated that the transcription factor encoding zerknüllt-1 gene (Tc-zen1) has an early function in specifying serosal cell fate and that RNAi knock-down of Tc-zen1 results in serosa-less embryos that are not covered by extraembryonic membranes ventrally. Based on this evidence, we hypothesized that Tc-zen1 RNAi embryos would be lacking the actomyosin cable. Although Tc-zen1 knock-down is expected to impact multiple cellular properties in the anterior blastoderm, where cells are transformed from serosal into embryonic (most likely amniotic) fate, Tc-zen1 RNAi embryos exhibit a very specific early morphogenetic defect without significantly compromising the morphology and viability of late embryos. Live imaging of transgenic embryos expressing LifeAct-eGFP obtained after knock-down of Tc-zen1 revealed indeed the absence of the actomyosin cable (Fig. 5a, b and Supplementary Movie 9). While such Tc-zen1 RNAi embryos started the contraction and folding of the embryonic primordium as wild-type embryos, the epibolic movement halted and a ventral serosa window failed to form and close (Fig. 5a, b, e and Supplementary Movie 10). Compared to wild type, the dorsal spreading cells in Tc-zen1 RNAi embryos became larger, presumably due to their lower number (Fig. 5c). The cells on the ventral leading edge, however, were much smaller (Fig. 5d, f), did not elongate anisotropically (Fig. 5b and Supplementary Fig. 3B), did not exchange neighbors, and were not evicted from the leading edge. Finally, although the shape index of the dorsal cells in Tc-zen1 RNAi embryos was comparable to wild type (Fig. 5g, h), the ventral region showed a significantly lower shape index compared to wild type (Fig. 5g, i) with less pronounced regionalization around the serosal window (Fig. 5g, j). We propose that one reason why the serosa window fails to close in the absence of the actomyosin cable is because tissue fluidization fails to occur and the epithelial tissue cannot remodel to close its gap.

Discussion

The epibolic expansion of the Tribolium serosa to envelop the entire egg surface is a dynamic morphogenetic process constrained by the ellipsoidal geometry of the egg and the mechanical properties of the tissue. Our data suggest that the regionalized tissue fluidization at its leading edge solves the geometrical and mechanical problems associated with serosa epiboly. First, it addresses the geometric constraints necessitating both the expansion and regional compaction of the tissue to close the gap. While the bulk of the tissue expands in a manner similar to an elastic solid material, the fluid-like ventral region remodels, halts the increase in cell area, and therefore can remain compact. Second, in the absence of cell divisions, which have been implicated as a stress-release mechanism in fish, local cell rearrangements induced by actomyosin contractility at the leading edge release the mechanical stress in the non-proliferative serosal sheet and maintain epithelial integrity during closure.

While here we focused on the gradient of serosa properties along the dorsal ventral axis, the pulling of the epithelial sheet over an ovoidal shape is an inherently 3D process and thus the tissue likely experiences stresses in many directions. More systematic probing of the mechanical properties of gastrulating Tribolium embryos will be required to understand the source of the forces acting on the serosa. We expect that the condensation of the adjoining embryonic primordium, together with...
additional forces generated by the attachment of the blastoderm to the vitelline envelope, the yolk, active crawling of the serosa on the vitelline envelope, and regulated changes in the shape and stiffness of the serosal cells, may also contribute to the serosal epiboly.

Independent of the forces involved in serosa expansion, our results suggest that the serosa closure is mediated by a heterogeneous actomyosin cable operating at the single-cell level to exclude marginal cells individually from the serosa window. The order in which cells are evicted correlates with local myosin...
accretion at each cable-forming edge. This is consistent with previous findings that myosin intensity correlates with tension in wound-healing cables.\textsuperscript{36,37} Furthermore, it has been suggested that a non-uniform stepwise contractility of individual edges is necessary for efficient epithelial closure during wound healing in Drosophila embryos and neural tube closure in chordates.\textsuperscript{38,39} This kind of sequential contraction is likely occurring during window closure to dissipate serosal resistance. Last but not the least, a recent study proposed that tissue fluidization is required for seamless wound healing in damaged Drosophila imaginal discs.\textsuperscript{40} Similar to the actomyosin cable of the Tribolium serosa window, the cable that assembles at the leading edge of the wound evicts cells from the wound periphery and promotes cell intercalation resulting in tissue fluidization and acceleration of epithelial gap closure. All these striking similarities point toward a general morphogenetic function of actomyosin cables in shaping and repairing epithelia by local tissue fluidization.

**Methods**

**Tribolium rearing and stocks.** _T. castaneum_ stocks were kept at 32°C and 70% relative humidity on whole-grain or white flour supplemented with yeast powder according to standard procedures.\textsuperscript{41,42,43} All mRNA injections were performed into embryos of the _vermilion\textsuperscript{white}_ strain. The following transgenic lines were used for live imaging: (i) _Efa-nGFP_, ubiquitously expressing a nuclear-localized GFP reporter;\textsuperscript{44} (ii) _αTub-H2A::eGFP_, ubiquitously expressing a nuclear eGFP reporter (kindly provided by Peter Kitzmann from Gregor Bucher’s laboratory); (iii) _EFA-Gap43-YFP-2A-Histone-RFP_, ubiquitously expressing both a membrane YFP and a nuclear GFP reporter (kindly provided by Johannes Schinko and Anna Gilles from Michalis Averof’s laboratory); (iv) _αTub-LifeAct-eGFP_, ubiquitously labeling filamentous actin with GFP\textsuperscript{45}; (v) _αTub-αflucor-GFP_, ubiquitously labeling the Tribolium non-muscle myosin II through its regulatory light chain. The predicted _Tribolium spatiogloss squama_ gene (Tc-sqh) encoding the non-muscle myosin II regulatory light chain was identified by Blast analysis against the _Tribolium_ genome\textsuperscript{46} and shares 93% similarity with the _Drosophila melanogaster_ sqh gene. The Tc-sqh open reading frame was amplified from cDNA and cloned in-frame with GFP downstream of the _Tribolium_ α-Tubulin1 promoter\textsuperscript{47} in a pIggyBac transgenesis vector, kindly provided by Peter Kitzmann and Gregor Bucher. The pIggyBac-aTub-Tc-sqh-eGFP vector was injected into the _Tribolium_ _vermilion\textsuperscript{white}_ strain together with a helper plasmid expressing the pIggyBac transposase and eight transgene lines were established by the TriGenes gUbuntu service (https://trigenes.com). Experiments were conducted using two selected transgenic lines expressing ubiquitous and uniform expression of Tc-sqh-eGFP.

**RNA injections.** Actin and myosin dynamics were visualized in _vermilion\textsuperscript{white}_ embryos injected with in vitro transcribed capped mRNAs encoding LifeAct-eGFP or Tc-sqh-eGFP that were synthesized from linearized plasmid templates pTrich-LifeAct-eGFP and pCs2+-Tc-sqh-eGFP, respectively.\textsuperscript{30,32} For the RNAi knockdown experiments of _Tc-zelen_, the double-stranded RNA (dsRNA) against the _Tribolium zerknelli_ 1 gene (TCC000921) was synthesized with primers optimized for gene specificity (a 203-bp amplicon outside of the conserved homeobox region).\textsuperscript{48} The mRNAs and the dsRNA were each injected at a concentration of 1 mg/ml. Eggs from the _vermilion\textsuperscript{white}_ strain were collected for 2 h at 30°C aged for another hour at 30°C and dechorionated in 16% commercial Klorix bleach for 1–2 min. Dechorionated pre-blastoderm embryos were mounted on a 1% agar bed and were microinjected in air through their anterior pole under a brightfield upright microscope, as previously described (Supplementary Fig. 1A). Injected eggs were incubated in humid chambers at 30°C for 2–3 h and the most homogeneously labeled and bright embryos were selected for imaging. For parental knock-down of _Tc-zelen_ by RNAi, dsRNA was injected into the abdomen of female pupae collected from the _αTub-LifeAct-eGFP_ transgenic line.\textsuperscript{49} Injected adult females were crossed to males from the same line and their eggs were collected for imaging. For the embryonic RNAi knock-down experiments, 3–5-day-old embryos were co-injected with mRNA encoding fluorescent reporters and dsRNA against _Tc-zelen_.

**Live imaging with confocal and light-sheet microscopy.** Point scanning confocal live imaging was carried out at 25°C or 30°C on an inverted Zeiss LSM 780 system equipped with a temperature-controlled incubator. Embryos were mounted in 1% agaroze in glass bottom petri dishes and covered in water. Embryos were scanned with a Zeiss x25/0.8 NA Plan-Apochromat multi-immersion objective or a Zeiss C-Apochromat objective with pixel sizes ranging between 0.2 and 0.55 µm, a z-step of 2 µm, and a temporal resolution of 5 min. Multi-view light-sheet imaging, referred to as selective plane illumination microscopy (SPIM) or light-sheet microscopy, was carried out on a Zeiss Lightsheet Z.1 microscope equipped with a x20/0.1 NA Plan-Apochromat water-immersion detection objective and two x10/0.2 NA dry illumination objectives. Embryos were embedded in glass capillaries in 1% low melting agarose dissolved in 1x phosphate-buffered saline together with fluorescent beads, as previously described.\textsuperscript{50,51} For each embryo, z-stacks were acquired from different views with the following voxel sizes: Figs. 1c, g, d, 3c, 4b (3 views every 120° with voxel size 0.381 µm × 0.381 µm × 2.0 µm), Fig. 1d (3 views every 120° with voxel size 0.33 µm × 0.33 µm × 2.0 µm), Fig. 2b, 4a, b, 5e, 6e, 7d (5 views every 72° with voxel size 0.381 µm × 0.381 µm × 2.0 µm), Fig. 4b Tc-sqh-GFP Stage 1 (4 views every 60° with voxel size 0.381 µm × 0.381 µm × 2.0 µm). The starting point in the time stamps used for all experiments was the last (12h) round of synchronous nuclear divisions, which precedes the formation of the uniform blastoderm and all subsequent morphogenetic events.\textsuperscript{52} Parameters for all live imaging experiments are summarized in Supplementary Table 1.
**Laser ablations.** Laser ablations were performed either on an inverted Zeiss LSM 780 NLO with a ×40/1.2 NA water-dipping objective using an 800 nm pulsed infrared laser or on a customized spinning disc confocal unit with a ×63 water-dipping objective using an ultraviolet laser microdissection apparatus similar to the one described in Grill et al.47. On the first system, three planes with 1–2 µm z-spacing were imaged every 1.6 s (Fig. 4f), 2.5 s (Fig. 4g), or 2.6 s (Fig. 3c) and the cut was performed in the middle plane, while on the latter system a single plane was recorded every 0.5 s (Fig. 3d). Tissue cuts were about 12 µm long spanning 3–4 cell diameters, while ablations of single edges were about 5 µm long. The recoil velocity of ablated edges was measured between six post-cut time frames using the manual tracking plugin in Fiji. For each cut, two to three independent tracks of the recoiling tissue edges were averaged. The recoil velocity was estimated using **Shape index**

| Stage 0 | Stage 1 | Stage 2 | Stage 3 dorsal | Stage 3 ventral |
|---------|---------|---------|---------------|----------------|
|         |         |         | ***           | ***            |

**Area (µm²)**

| Stage 0 | Stage 1 | Stage 2 | Stage 3 dorsal | Stage 3 ventral |
|---------|---------|---------|---------------|----------------|
|         |         |         | ***           | ***            |

**Wild type**

**Tc-zen1RNAi**

| Area (µm²) | Wild type | Tc-zen1RNAi |
|------------|-----------|-------------|
| 250 µm²    |           |             |
| 500 µm²    |           |             |
| 750 µm²    |           |             |
| 1000 µm²   |           |             |

**Shape index**

| Stage 0 | Stage 1 | Stage 2 | Stage 3 dorsal | Stage 3 ventral |
|---------|---------|---------|---------------|----------------|
|         |         |         | ***           | ***            |

**Wild type**

**Tc-zen1RNAi**

| Shape index | Wild type | Tc-zen1RNAi |
|-------------|-----------|-------------|
| 3.0         |           |             |
| 3.2         |           |             |
| 3.4         |           |             |
| 3.6         |           |             |
| 3.8         |           |             |
| 4.0         |           |             |
| 4.2         |           |             |

**Wild type**

**Tc-zen1RNAi**

| Tissue fluidity | Wild type | Tc-zen1RNAi |
|-----------------|-----------|-------------|
| 1.0             |           |             |
| 2.0             |           |             |
| 3.0             |           |             |
| 4.0             |           |             |
| 5.0             |           |             |

* denotes significant difference from wild type. ** denotes significant difference from Tc-zen1RNAi.
standard procedure of measuring the total distance traveled by the two recoiling edges between frames 1 and 2 post-cutting and dividing by acquisition frame rate48. The observed differences in absolute recoil velocities between the ultraviolet and two-photon laser ablation set-ups used in this study have been observed before and are thought to reflect difference in the extent of disruption of the actomyosin network36,49. Keeping this in mind, we only compared recoil velocities generated with the same laser ablation set-up, as shown in Fig. 3c, d.

In an attempt to estimate tissue material properties from recoil dynamics after laser ablation, a power-law analysis was performed as previously described30. Briefly, a kymograph perpendicular to the laser cuts was obtained with the Multi-Kymograph plugin in Fiji [https://imagej.net/Multi_Kymograph]. The recoil velocity was quantitated during the first 10 s after the ablation and fitted by a power-law $D(-t^\alpha)$, where $t_0$ is the time of the ablation and $D$ and $\alpha$ are fitting parameters. $D$ correlates with the recoil velocities of the edges, whereas $\alpha$ gives an indication of the mechanical properties of the tissue and can acquire values from 0 to 1. A tissue with a value of $\alpha$ closer to 0 shows properties of an elastic solid, while a value closer to 1 indicates viscous fluid properties.

**Image processing.** The multi-view light-sheet datasets were registered and fused using Fiji plugins as previously described50–52. The four-dimensional (3D + time) fused datasets were converted into 3D (2D + time) time-lapse maps by making cylindrical projections using the ImSANE software53. Cells were segmented using a deep learning-based approach called StarDist, which is capable of learning morphological priors54. Different neural networks were trained for different markers (membrane and actin labels). The training data were obtained by generating realistic looking synthetic microscopic images of Tribolium using Generative Adversarial Networks. The generated synthetic data were evaluated visually against the real microscopic data to ensure textural and morphological consistency between the two. After training StarDist networks on such synthetic data, they were applied to the real microscopic images, and the predictions were manually curated with the Labkit plugin in Fiji [http://sites.imagej.net/Labkit/] to fix any segmentation mistakes. After cartoonographic projections, some cells on the edges of the maps were necessarily cut in order to unfurl the 3D embryo to 2D. Those incomplete maps were excluded from analysis. Distortions that are inherent to the mapping of curved surfaces onto a plane were corrected with custom Fiji plugins (available on the “Tomancak lab” Fiji Update site) thereby allowing the measurement of quantities like size, circularity, shape factor, density, velocity, and the local cell alignment (see below). Consequently, the scale bars in map projections are only approximate and reflect accurately the sizes only in the middle portions of the maps. Nuclei in the depth color-coded cartoonographic projections were tracked using MaaMuT36 and Mastodon (both available via Fiji Update site).

**Shape index analysis.** Shape index was calculated for each segmented cell in the 2D cartoonographic projections as $p = P/\sqrt{A}$, where $P$ is the cell perimeter and $A$ is the cross-sectional area22. The measurements were distortion-corrected using the above-mentioned Fiji plugins and plotted onto the segmented cartoonographic projection as a color map. The local cell shape alignment index $Q$ was calculated as described recently30. Briefly, cells in each map projection were converted into a triangular mesh connecting the centers of all adjacent segmented cells, i.e., where three (or more) cells touch, a triangle (or a triangle fan) with vertices coinciding with centers of adjacent cells was formed. For every triangle, a degree $\varphi$ of deviation from equilateral triangle was computed30. For every cell, its shape alignment index $Q$ became a weighted average over $\varphi$ from all triangles whose vertex coincides with this cell’s center. Using this $Q$, an adjusted shape index threshold was determined as $p_{th} = p_0 + 4.0Q^2$ for $p_0 = 3.94$ and $Q = 0.45$30. According to Wang et al.30, simulations of this threshold marks solid-to-fluid transition for a given anisotropy in the tissue (i.e., for a given value of cell shape alignment index $Q$). The tissue fluidity for a given cell was then calculated as a difference between its actual shape index $p$ and $p_{th}$ for a given local value of $Q$. This difference was converted into a color code and displayed on each cartoonographic projection. Green color signifies solid-like local tissue properties ($p < p_{th}$), brown color fluid-like local tissue properties ($p > p_{th}$), and black color marks the vicinity to the theoretically predicted solid-to-fluid transition ($p = p_{th}$).

**Data availability**

The confocal imaging data and cartoonographic maps that support the findings of this study are available on Figshare under the public project “Regionalized tissue fluidization is required for epithelial gap closure during insect gastrulation” ([https://figshare.com/projects/Regionalized_tissue Fluidization_by_an_Actomyosin_Cable_is_required_for_Epithelial_gap_closure_during_insect_gastrulation/86741](https://figshare.com/projects/Regionalized_tissue_Fluidization_by_an_Actomyosin_Cable_is_required_for_Epithelial_gap_closure_during_insect_gastrulation/86741)). Raw light-sheet microscopic data are available on the Image Data Resource ([https://idr.openmicroscopy.org](https://idr.openmicroscopy.org)) under accession number idr0099 or from P.T. upon request. The Figshare and IDR data DOIs are listed in an Image Datatle. All statistics and p values are reported in the Statistics Datatle. Source data are provided with this paper.

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