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<td>Rights</td>
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Orientational Coupling Locally Orchestrates Cell Migration Pattern for Re-epithelialization

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Keywords: Migration pattern, spatial organization, repair efficiency, orientational coupling, epithelial bridge

Collective cell migration promotes the multicellular organization that is essential for a plethora of physiological processes, such as wound healing, tissue regeneration and embryonic development.[1] The prompt orientated movement of cells upon wound creation is a critical process in the restoration of tissue integrity and its corresponding physiological function.[2] Epithelial wound healing ensures that the skin cells form a barrier for protecting organisms against infection.[3] Misregulated wounds closure would result in hypertrophic scars, inflammation and even tumorigenesis.[4] When damage is inflicted on the tissue, the following healing process is triggered. To prevent blood loss and infection, hemostasis is first activated through a platelet plug, followed by fibrin matrix formation, which builds the scaffold for infiltrating cells. This is followed by the stage of new tissue formation, during which the
migration of keratinocytes over the injured dermis constitutes the primary event.\[^5\] In the later stages of repair, granulation tissue replaces fibrin matrix as the substrate to further guide keratinocyte migration (Scheme 1a, upper).

When the cell environment comprises a homogeneous distribution of extracellular matrix (ECM), the collective cell migration during epithelial restoration is commonly driven through a combination of protrusion-based cell crawling and purse-string contraction of supracellular actomyosin cables.\[^6\] These two closure mechanisms have been investigated through the closing of wounds in arbitrary shapes.\[^7\] However, for many instances \textit{in vivo}, ECM is discontinuous and separated with micro-scale gaps.\[^8\] Cells capable to probe and respond to their surrounding structural heterogeneity are important for tissue homeostasis.\[^8b\] Particularly, for skin dermis, how cells migrate through the gap-involved ECM and develop bridges above the network are of physiological importance in wound closure.\[^9\] A recent progressive study showed that human keratinocytes are able to migrate over such non-adherent regions by building multicellular bridges via intercellular adhesion, thus enabling re-epithelialization over spatially discontinuous ECM.\[^2a\] The formation of such multicellular bridge is promoted by the large-scale tension from actomyosin contractility.

Having understood the driving force behind collective cell migration across spatially discontinuous ECM,\[^10\] the next challenge is to enhance the efficiency of the re-epithelialization, realizing mechanically stable recovered epithelium and more rapid healing. By investigating how cell-cell junctions develop into multicellular bridge,\[^2a\] and promoting collective movements in cell sheet on sparsely and randomly distributed ECM, one may be able to obtain a significant clue for this challenge (Scheme 1a, lower). It has been established
that for individual cell migration, the direction of migration is directed by an initial polarization of the cell to form a protrusive front edge and retracting rear section.\textsuperscript{[11]} However, it is not clear whether the general direction of collective cell migration in a heterogeneous environment correlates to such polarization of cells.\textsuperscript{[12]} This is because the areas where ECM is absent may constitute ‘barriers’ disrupting the polarization of the cells.

Herein, we hypothesize that the collective migration of keratinocytes, including the migration pattern and the efficiency, is dependent on the collective polarization of cells,\textsuperscript{[13]} which is directed by the ECM morphology (Scheme 1b). Although the general distribution of discontinuous ECM is geometrically random,\textsuperscript{[3]} within a local area of heterogeneous ECM, we can simplify the adherent paths of the cell migration into three types: (1) parallel adherent stripes that rarely occur in nature, (2) adherent stripes that converge to a junctional region (defined as converging paths), and (3) those that become more separated in the direction of cell migration (defined as diverging paths). The spatial organization of these adjacent paths would determine the polarization of cells migrating over these paths, which in turn determines the migration pattern of cells (Scheme 1b, upper). Through systematically studying the way keratinocytes migrate over converging and diverging paths of various angles, we deciphered the relationship between ECM-induced cell polarization and epithelial bridge formation involved in collective cell migration. This study about how the spatial organization of heterogeneous ECM affects the orientational coupling between cell polarization and collective cell migration could provide more insight on enhancing the efficiency of restoring skin continuity and integrity,\textsuperscript{[14]} thereby proving helpful in tissue repair and regeneration. Specifically, by investigating the effect of this orientational coupling on the formation of
epithelial bridges, one may be able to gain a deeper understanding on the complex integrated events underlying collective cell migration, as well as optimize the migratory strategies for wound healing.\textsuperscript{[15]}

Keratinocytes, like HaCaT cells, are capable of forming epithelial bridges while collectively migrating over non-adhesive regions within suitable sizes. To study this, we rationally designed an experimental platform that can provide parallel, converging and diverging paths for collective migration of keratinocytes. Figure S1 provides a brief illustration on the fabrication of such a platform. A polydimethylsiloxane (PDMS) substrate was first created with elevated features (5 $\mu$m in height, lower height may introduce over-printed fibronectin in unexpected regions and larger height could lead to deformation of the elastomeric substrate) in the microscale, which were coated with fibronectin such that they served as adhesive regions for cell migration;\textsuperscript{[16]} whereas the basal triangle regions were left non-adhesive (Figure 1a, upper). To form a linking layer connecting the substrate and fibronectin, a layer of ethylenediaminetetraacetic acid (EDTA) was coated onto the substrate through immersing it in EDTA solution (Figure S1a). Subsequently, the fibronectin transferred from a flat stamp could stably bind to the elevated regions and create a heterogeneous environment for keratinocytes (Figure S1b, c).\textsuperscript{[17]} For the channel in the middle, the two elevated sides of every triangle serve as converging paths for keratinocytes migration. The channel on the right is geometrically the same as that in the middle but in a reversed orientation, thus changing it to diverging paths for keratinocytes migration (Figure 1a). As shown in the sectional view (Figure 1a, lower), the width of each adhesive path is 20 $\mu$m, with a basal non-adhesive gap in between (gap width is 120 $\mu$m, which is suitable for
multicellular bridge formed over the gap). The apex angle between the two paths determines the extent of convergence or divergence for cell migration. For our experimental study, five different angles, namely 20°, 30°, 60°, 90° and 120°, were chosen (Figure S1d).

To carry out the cell migration studies on our experimental platform, HaCaT cells were first seeded in the confined bottom square regions in each channel. After the cells have reached confluency, the confinement was lifted off in order to trigger cell migration (Figure 1a). When the cells at the leading front had migrated into the adhesive stripes of triangle region, the cell sheet would be pulled up over the non-adhesive basal regions, thus forming epithelial bridges (Figure S2a, b). The formation of such suspended structures was proven by scanning electron microscopy (SEM) measurement, through which the shadow of epithelial bridges was observed (Figure S2c). Furthermore, the nanoindentation test (see Experimental Section in supporting information for more detail) showed that the extracted rigidity and modulus are almost the same for both the epithelial bridge and the epithelial cells attached on the substrate, which suggests that the epithelial bridge is considerably stiff (Figure S3). The formed epithelial bridge is sustainable to mechanical perturbations, as repeated indentation only resulted in slight reduction of the rigidity of epithelial bridge, without any observable breakage. Strikingly, the bridges are formed more easily and rapidly over basal triangles between converging paths (Figure 1b, middle), as compared to that formed on channels with parallel (Figure 1b, left) or diverging paths (Figure 1b, right). In order to analyze the migration dynamics for cells migrating on different channels, we investigated the displacements of the leading cells at the midpoint over basal non-adhesive regions (Figure 1c). This is because cells at the midpoint advanced at slowest pace, as seen from the curvature of
the front edge with the cells protruding at both ends. The displacement for cells migrating on channels with parallel (black) paths showed a linear progression with time. It is noteworthy that reducing the gap width of the parallel channel by half only resulted in slight increase of the average migration velocity (~10%), as the cells would bridge over not only inside the gaps but also at both side wings (Figure S4a, b). However, progression of displacement for cells migrating on channel with converging paths increases with time (red), which suggests that the cells had ‘accelerated’. Conversely, the progression was found to decrease with time for cells migrating on the channel with diverging paths (blue), thus implying that the diverging paths created a ‘barrier’ that caused the cells to slow down. The velocity chart of the advancing cell sheet further proved that converging paths could promote keratinocyte migration as the velocity of cells kept increasing as they progressed on converging channels (Figure 1d). These results confirmed that the spatial organization of adjacent adhesive paths exerts a strong influence on the collective migration patterns of keratinocytes.

Despite the similar geometry shared between channels with converging and diverging paths, the migrating keratinocyte sheet on converging channels could form suspended bridges to completely occupy the upright triangles up to the base. However, the epithelial bridges demonstrated only partial occupancy over the inverted triangles on the channel with diverging paths, leaving a ‘hole’ above the inverted triangle region. This implies that the orientation dependent cell migratory behavior involves a ‘zipper-like’ mechanism: the full occupancy over non-adhesive upright triangle regions constitutes the ‘zip on’ function for cells migrating along converging paths; whereas the unoccupied ‘hole’ above inverted triangle region is a manifestation of the ‘zip off’ function. The activation of ‘zip on’ function could enhance the
efficiency for re-epithelialization and vice versa.[7, 20]

To address the essential components maintaining epithelial bridges, we investigated the impact of myosin-II-regulated contractility and E-cadherins-mediated intercellular adhesion on the bridges, respectively. We first perturbed the stress fibers by adding blebbistatin (50 µM) to inhibit myosin II. As shown in Figure S5 (upper row), the well-formed epithelial bridge in the converging channel collapsed. Meanwhile, the epithelial bridges in the parallel and diverging channels also relaxed as the unoccupied regions had been significantly enlarged (Figure S5, middle and lower rows). To test whether the integrity of epithelial bridge is mediated by the intercellular adhesion, we reduced the intercellular adhesion by replacing the normal culture medium to calcium-free culture medium. As shown in Figure S6, relaxation of the bridges also occurred. Together, these results demonstrated that both contractility of stress fibers and cell-cell adhesion are essential for the maintenance of epithelial bridges, which contribute on collective cell migration over non-adhesive regions.

Our quantitative study on the occupancy of epithelial bridges revealed the difference between converging and diverging paths is also dependent on the apex angle, as the largest difference was located in the range between 30° and 60° (Figure 2a). The occupancy over the basal triangle region in the converging channel was almost 100% for all five different angles. However, in the diverging channel, the occupancy was down to ~70% for apex angles of 30° and 60°. For angles larger than 60° (90° and 120°), the occupancy remained nearly 100% as the distance between two neighboring adhesive squares has been significantly reduced. It is worth noting that the gap width is a critical parameter with a fixed value, which in turn make the distance between neighboring squares be a function of the apex angle. On the other hand,
when the apex angle is down to 20°, the occupancy in diverging channel was also nearly 100% as both the converging and diverging paths are geometrically close to parallel stripes, and thus display little difference in guiding cell migration. It is worthy to note that during the keratinocyte migration over the non-adhesive regions in the diverging channel, the circular gap (hole) left behind the leading front may further be gradually resealed by the contraction of actin cables (Figure S7a),\textsuperscript{[9a]} resulting in local backwards cell migration and reduction of efficiency (Figure S7b). Moreover, cytokinesis induced holes could occasionally be observed, which caused the initially occupied regions to open. Both of these circumstances must be excluded from consideration as they are not driven by the forward collective cell migration.

This biphasic relationship between occupancy difference and apex angle revealed that the aspect ratio (non-adhesive gap length/adhesive square width) of the non-adhesive region plays a role in cellular bridges based re-epithelialization. When the apex angle is in the range between 30° and 60°, the aspect ratio is mathematically close to 1 (1.7 for 30° and 0.8 for 60°). An aspect ratio of value far from 1 would strongly expand the non-adhesive region in either lateral or longitudinal axis, reducing the difference between the included converging and diverging paths (Figure 2a).

Having established the relationship between the ECM geometry and efficiency of re-epithelization, we next studied the mechanism of how the velocity field of collective keratinocyte migration is determined by the orientation of the guiding paths. This is important as it may help in developing a strategy to optimize the collective migration of keratinocytes in a heterogeneous environment. By recording the displacement of HaCaT cells on channels with various apex angles, it was revealed that the velocity of migrating keratinocytes is
consistently higher on the converging channel than that on the diverging one (Figure 2b, Figure S8). This orientation dependent velocity difference does not exist for simple epithelial cells such as Madin-Darby canine kidney (MDCK) cells, as such cells would simply move along the adhesive paths without forming epithelial bridges over non-adhesive regions (Figure S9). Statistical study further demonstrated that for apex angles of 30° and 60°, the average velocity of collective cell migration on the converging channel is more than 20% higher than that on the diverging channel, whereas the velocity differences between the converging and diverging channels were reduced for the other three apex angles (Figure 2b). The chart of average velocity showed a similar trend as that of occupancy (Figure 2a), which suggests the contribution of epithelial bridges towards the velocity of collective cell migration in a heterogeneous environment.

Based on our experimental study, a mechanical model was built to illustrate our orientational coupling effect on the collective migration of keratinocytes. The whole process of cells migrating over the triangle region could be divided into the protruding stage and the remodeling stage.[21] The former refers to the state in which cells protrude towards basal non-adhesive triangle region, pulling up the cells in the middle to bridge over the basal region (Figure 2c). The leading cells in the converging channel would be migrating from both ends of the upright basal triangle until they have joined in the middle, and that is when the initial cellular bridges are developed and remodeled to occupy the whole basal triangle region, forming the latter remodeling stage (Figure 2d). In the protruding stage, we assume that cells in both the converging and diverging channels take the same time to reach the midpoint as they basically migrate along the adhesive paths. The difference in migration arises in the
remodeling stage due to the unequal area of the basal region to be bridged over by the cells. As illustrated in Figure 2d, the remaining basal area for the cells to bridge over in the converging channel is:

\[ \int_{0}^{r \tan \beta} \Delta x_c \, dy_c = r^2 (\tan \beta - \sin \beta) \]  

(1)

Whereas for cells in the diverging channel, the remaining basal area is

\[ \int_{r \cos \beta}^{r \tan \beta} \Delta x_D \, dy_D = r^2 (\tan \beta - \cos \beta \sin \beta) \]  

(2)

Thus, in consideration of the whole basal triangle area, the differentiation factor should be calculated as:

\[ \frac{\int_{r \cos \beta}^{r \tan \beta} \Delta x_D \, dy_D - \int_{0}^{r \tan \beta} \Delta x_c \, dy_c}{\Delta \beta} = \cos \beta (1 - \cos \beta) \]  

(3)

Where \( \beta \) is the base angle of the basal triangle, and \( r \) is the half length of the base of the triangle. It is worth noting that the cells would also bridge over to form side wings (curved red arrow in Figure 2d) in diverging channel, as demonstrated in keratinocytes migration assay (Figure 1b, right channel, from 6h to 14h). The side wings would generate a bypass flow of cells from the reservoir and cause a further slowdown of the migration velocity. By using this model that analyzes the progress of keratinocytes migrating over heterogeneous ECM in terms of two critical stages, we decoded the biphasic relation between \( V_C/V_D \) (the average velocity ratio of cells migrating over converging paths to cells moving over diverging paths) and the apex angle. Our calculations demonstrated good agreement with the experimental results, as the value of \( V_C/V_D \) reaches maximum when apex angle is 60°.

To understand how the velocity of cell migration is affected by the formation of epithelial bridges, we utilized particle image velocimetry (PIV) to map the velocity field and vorticity for the HaCaT cells migrating over the heterogeneous triangle regions (Figure 3, Figure S10).
Herein, an apex angle of 60° was chosen as the example to be demonstrated as it results in the largest velocity difference between migration in the converging and diverging channels. In the protruding stage, significant vorticity could be found at the bottom adhesive squares for both the converging and diverging channels, suggesting the non-adhesive regions created barriers for advancing cells (Figure 3a, b). The scatter diagram of velocity vectors and the histograms of velocity magnitude showed that both the velocities along the lateral axis ($V_\perp$) and the longitudinal axis ($V_\parallel$) were significant, further demonstrating that the non-adhesive barrier induced random directionality of advancing keratinocytes (Figure S10a). However, during the remodeling stage, the velocity of cells migrating on the converging channel would be polarized along the longitudinal axis (Figure 3c), with the distribution of $V_\parallel$ in a larger range of 1 to -5 nm/s (a negative value indicates advancing movement, whereas a positive value refers to retrograde movement). In contrast, the velocity of cells migrating on the diverging channel exhibited polarization along the lateral axis at both the leading front and the region behind the triangle (Figure 3d), thus generating lower $V_\parallel$ (mainly within the range of 2 to -2 nm/s) and significant vorticity (Figure 3e, Figure S10b). These results reveal that, with respect to the formation of epithelial bridges over non-adhesive regions, the collective migration of keratinocytes is faster when the leading cells move on converging paths.

To further elucidate the reasons why the orientation of extracellular adhesive paths results in different migratory patterns, we stained the actin network of HaCaT cells with phalloidin during both the protruding and remodeling stages (Figure 4). We found that, in the converging channels, the F-actin bundles were mainly polarized along the lateral axis during the cells’ protruding stage (Figure 4a), thus suggesting that the cells suspended between the
adhesive paths were subjected to tension.\[23]\) Strikingly, the actin network of cells in suspended region at remodeling stage had been polarized along the longitudinal axis (Figure 4b). Quantifying the angular orientation of actin bundles further indicated that the principal orientation turned from lateral to longitudinal during the transition from the protruding to the remodeling stage (Figure 4c). Meanwhile, in the diverging channels, the principal orientation of actin bundles within suspended cells remained lateral during both stages (Figure 4d-f). Furthermore, quantification of the orientation of myosin II (Figure S11) revealed that the epithelial bridge was supported by the tension from actomyosin stress fibers as the orientation of myosin II also turned from lateral to longitudinal during the conversion from the protruding to the remodeling stage (Figure S11a, c); whereas the orientation remained lateral during both stages for epithelial bridge in the diverging channels (Figure S11b, d). The mapping of the local Young’s modulus of epithelial sheets in both the converging and diverging channels (Figure 4g, h) have been conducted through indentation studies by atomic force microscope using a spherical bead. As shown in Figure 4g and Figure S12, the distribution of Young’s modulus of epithelial sheet reflects the forces transmitted from pulling cells on the adherent paths of the converging channels; whereas the distribution of higher modulus is concentrated along the lateral direction for the epithelial sheet in the diverging channel (Figure 4h). Statistical study further confirmed the orientational distributions of higher modulus for epithelial bridges during remodeling stages are consistent with the organization of stress fibers, in both the converging and diverging channels (Figure S13). These indentation studies not only confirm that the suspended epithelial sheets are subjected to tension, but also correspond the local modulus difference with the organization of actin bundles. It is worth noting that
the same tendency was also found for the collective polarization of one primary keratinocytes (normal human epidermal keratinocytes). As shown in Figure S14, the polarization of the advancing primary cell sheet is remodeled to align along the direction of cell migration in the converging channel, facilitating the formation of epithelial bridge over non-adhesive regions and thus promoting the integrity of the whole cell sheets. In contrast, the principle orientation of epithelial bridge in diverging channel remains in lateral direction, forming a barrier, which in turn reduces the integrity of the advancing cell sheets. Overall, as the directional collective cell migration requires the establishment and maintenance of collective polarization,\textsuperscript{[24]} by inducing the leading cells to be elongated towards the front (in longitudinal polarization), the suspended cells in the converging channel could act as ‘joint-leading’ cells in collective migration,\textsuperscript{[12b]} thus enhancing the migration velocity and efficiency (Figure 4i).

The guidance of collective cell migration relies on establishment of leader-follower polarization and integration of ECM cues.\textsuperscript{[24, 25]} Progressive studies on collective keratinocyte migration suggested that the leading cells on adhesive stripes exert pulling forces on the following suspended cell sheet,\textsuperscript{[2a]} thus promoting migration of the whole cell sheet.\textsuperscript{[1c, 6b]} Herein, to further understand the angular distribution of the traction forces, we used traction force microscopy (TFM) to measure the average longitudinal ($T_\parallel$) and lateral ($T_\perp$) traction-force components of the cells at the leading front (defined as the cells moving on the adhesive paths at the side), and the following row (defined as the cells located at the marginal region between the adhesive squares and basal triangles), respectively.\textsuperscript{[26]} Consistent with previous results,\textsuperscript{[1a, 2a, 26b]} the cells at leading front on both the converging and diverging channels exerted significant traction forces directed backwards toward the following row (Figure S15). We
also observed cells immunostained for vinculin and detected maturated focal adhesions at the leading front,\(^{[27]}\) which confirmed the contribution of leader cells in guiding and instructing the follower cells to facilitate a coordinated movement (Figure S16). The traction forces exerted by cells at the following row exhibited remarkably different distributions between the converging and diverging patterns (Figure 5a-d). The cells at the following row on the converging channel exerted polarized forces mainly pointed towards the front, which suggests that these cells were cooperatively pushing forward and driving wound closure above the basal upright triangles (Figure 5b).\(^{[12a]}\) In contrast, the cells at the following row on the diverging channel exerted polarized forces lateral to the forward direction, contributing minimally to wound closure above the basal inverted triangles (Figure 5d). The laterally polarized traction forces exerted by the following row also provided evidence suggesting that the epithelial bridges on the diverging channel were under tension. The concentrated distribution of vinculin at intercellular junction sites in the suspended region of the diverging channel further confirmed that tension\(^{[28]}\) was being exerted by epithelial bridges (Figure S16b). To sum up the aforementioned results, it can be interpreted that the remodeling of actin network for suspended cells on the converging channel is driven by the longitudinal polarization of forces, whereas the organization of actin network of suspended cells on diverging channel is maintained by the lateral polarization of forces (Figure 5e).

To obtain a better understanding on the role of mechanical forces from leading cells on the regulation of collective migration, we performed a theoretical simulation based on treating the migrating cell sheet as an elastic material.\(^{[29]}\) According to previous studies,\(^{[2a]}\) the Young’s modulus of the cell sheet was considered to be 2 kPa. Our measurement by TFM estimated
that the force exerted by the leading cells at each side path was about 300 nN. Since the largest difference between the converging and diverging channel was found to be in the range of apex angles between 30° and 60°, our model tested the strain distributions for these two angles (Figure S17a). The calculated results demonstrated that, for the converging channel, the cells within the suspended bridges were under compression, which suggests that the cell density was enhanced in the region. In contrast, for the diverging channel, the suspended cells were almost in expansion, which indicates lower cell density (Figure S17b). This model is in good agreement with our experimental results (Figure S17c). It also enables us to explain our proposed ‘zipper-like’ mechanism based on a ‘mechanical coupling’ perspective: at high cell density, the cells repel each other and facilitate coordinated migration, which enabled more effective migration in the converging channel. At low cell density, the cells demonstrated attraction rather than repulsion, which creates a barrier for forward coordinated migration.

By using our fabricated micro-environments, we demonstrated that during re-epithelialization, the collective cell migration over complex extracellular matrix relies significantly on the spatial organization of the adhesive paths that lie ahead of the advancing cell sheet. Based on our experimental results, we proposed a ‘zipper-like’ mechanism: cells migrating in the converging channel demonstrate the ‘zip on’ process and exhibit higher efficiency for re-epithelialization, whereas cells migrating in the diverging channel undergo the ‘zip off’ process and result in unsealed ‘holes’ and migration at lower velocity. We believe that revealing and decoding this mechanism would pave the way for the development of biological or synthetic skin substitutes in wound care and post-injury reconstructions.

Furthermore, integrating the cutting edge 3D printing technologies with our designing principle
on precisely tailoring ECM with fine details at a micron level, new light could be shed on strategies for wound convers and implantable scaffolds to optimize collective cell migration.\textsuperscript{[30]} Leveraging the finding that collective cell migration is a function of adhesive path orientation will optimize the spatially local properties of the scaffolds and better replicate the heterogeneous and complicated nature of the 3D cellular environment, thus helping to recapitulate the integrity and function of native tissues. This integrability in our microfabrication approach will contribute to bridge the gap between the current state of tissue engineering and the ultimate goal of realizing rapid regeneration and even synthetically rebuilding impaired tissue or organs.
Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
This project was supported by NTU-Northwestern Institute for Nanomedicine and National Research Foundation, Prime Minister’s Office, Singapore, under NRF Investigatorship (NRF-NRFI2017-07).

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))
Reference


Scheme 1. Schematic of the principle affecting polarization of migrating cell sheets on sparsely distributed ECM. **a,** During dermal wound healing, epithelial cells coordinately move to close the wounds for re-establishing tissue integrity (upper). Zoom-in view of the epithelial cells migrating on the randomly arranged and sparsely distributed ECM, where multicellular bridges are formed to aid in the wound healing (lower). **b,** The spatial organization of adjacent adherent paths ahead of advancing cells could be classified to three type: converging, parallel and diverging (upper). The ECM organization acts as a compass on the cytoskeletons, guiding the polarization of epithelial bridges over the non-adhesive regions, determining the orientational coupling between cell polarization and collective cell migration (lower).
Figure 1. The dynamics of migrating keratinocytes on channels with various organization. 

**a**, Schematic of keratinocytes migrating upwards on a patterned substrate composed of variable types of channels. The included angle between adhesive paths is marked as the ‘apex angle’ (upper). Sectional view of the patterned substrate with elevated regions coated with fibronectin (the elevated height is 5 µm, the width of adhesive paths is 20 µm and the width of basal gap is 120 µm) (lower). **b**, Cells migrating on different channels with parallel stripes (left), converging stripes (middle) and diverging stripes (right). Scale bar: 100 µm. **c, d**, The (c) displacement and (d) velocity versus time of the leading front of keratinocytes migrating on the three different channels (parallel, ‘C-60’ and ‘D-60’).
Figure 2. The effect of orientational coupling on the occupancy by epithelial bridges over the basal non-adhesive triangles and the average velocity of migrating keratinocytes. a, The occupancy over basal triangles remained roughly 100% for cells migrating on channels with converging stripes, whereas the occupancy decreased significantly for cells migrating on the ‘D-30’ and ‘D-60’ channels. b, The velocity ratio of cells migrating on the channel with converging stripes to cells moving on the channel with diverging stripes is dependent on the apex angle. Both experimental and calculated results showed that this velocity ratio reached its maximum value when the apex angle was 60°. c, d, The working model describing the migration trajectory of cells on channels with either converging stripes or diverging stripes at (c) the protruding stage and (d) the remodeling stage.
Figure 3. Comparison of keratinocytes migratory pattern using PIV for both the protruding and remodeling stages. a, b, c, d, Velocity fields and Heat map of velocity within the migrating HaCaT cell sheet on (a) the ‘C-60’ channel and (b) the ‘D-60’ channel at the protruding stage, as well as (c) the ‘C-60’ channel and (d) the ‘D-60’ channel at the remodeling stage. e, Distribution of velocity along the longitudinal axis ($V_1$) for cells on the ‘C-60’ channel (red) and the ‘D-60’ channel (blue) at the remodeling stage. Insert: scatter plot of velocity for cells migrating on ‘C-60’ channel (left) and ‘D-60’ channel (right). All scale bar: 50 µm.
Figure 4. Organization of actin network for cells over the non-adhesive triangle on the ‘C-60’ and ‘D-60’ channels. a, Actin bundles are aligned in lateral axis for HaCaT cells on the ‘C-60’ channel at the protruding stage. b, Actin bundles are remodeled to align in the longitudinal axis for HaCaT cells on the ‘C-60’ channel at the remodeling stage. c, Orientation distribution of actin bundles for HaCaT cells on the ‘C-60’ channel at the protruding stage (left) and remodeling stage (right). d, Actin bundles are aligned in the lateral axis for HaCaT cells on ‘D-60’ channel at the protruding stage. e, Actin bundles remain aligned in the lateral axis for HaCaT cells on the ‘D-60’ channel at the remodeling stage. All scale bars: 50 µm. f,
Orientation distribution of actin bundles for HaCaT cells on the ‘D-60’ channel at the protruding stage (left) and remodeling stage (right). \textbf{g, h}, Indentation study on Young’s modulus of the epithelial bridges in either ‘C-60’ (g) or ‘D-60’ (h) channel (left) and the corresponded zoom-in view (right). Scale bar: 20 µm. \textbf{i}, Model to explain how the ECM organization affects the polarization of epithelial bridge during collective cell migration. The red bars in \textbf{c} and \textbf{f} show the principal orientation.
Figure 5. Tension distribution within the HaCaT cells at the leading front and following row. **a,** Traction forces field within cells at leading front and following row on ‘C-60’ channel. The right amplified view demonstrated CTF were longitudinally orientated. **b,** Orientation distribution shows that the forces within following cells on ‘C-60’ channel were pointed upwards towards the front. **c,** Traction forces field within cells at the leading front and following row on the ‘D-60’ channel. The right amplified view demonstrated that the CTF were longitudinally orientated for the leading cells and laterally orientated for the following cells. **d,** The forces within following cells on ‘D-the 60’ channel are polarized along the lateral axis. **e,** Statistical study on the CTF distribution for both the leading and following cells on the ‘C-60’ and ‘D-60’ channels. The laterally orientated CTF became dominant for the following cells on the ‘D-60’ channels. Scale bar: 20 µm in **a** and **c**.
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Wound healing: The spatial organization of adjacent adherent paths within sparsely distributed extracellular matrix (ECM) has a significant impact on the orientational coupling between cell polarization and collective cell migration. This coupling effect determines the migration pattern and thus the efficiency for human keratinocytes to regain their cohesion. Our findings may help to design implantable ECM to optimize tissue regeneration.

Keywords: Migration pattern, spatial organization, repair efficiency, orientational coupling, epithelial bridge

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Orientational Coupling Locally Orchestrates Cell Migration Pattern for Re-epithelialization

ToC figure (55 mm broad × 50 mm high)