Tissues do not exist in isolation—they interact with other tissues within and across organs. While cell-cell interactions have been intensely investigated, less is known about tissue-tissue interactions. Here, we studied collisions between monolayer tissues with different geometries, cell densities, and cell types. First, we determine rules for tissue shape changes during binary collisions and describe complex cell migration at tri-tissue boundaries. Next, we propose that genetically identical tissues displace each other based on pressure gradients, which are directly linked to gradients in cell density. We present a physical model of tissue interactions that allows us to estimate the bulk modulus of the tissues from collision dynamics. Finally, we introduce TissEllate, a design tool for self-assembling complex tessellations from arrays of many tissues, and we use cell sheet engineering techniques to transfer these composite tissues like cellular films. Overall, our work provides insight into the mechanics of tissue collisions, harnessing them to engineer tissue composites as designable living materials.
biological tissue is a cellular community or, as Virchow wrote in the 19th century, “a cell state in which every cell is a citizen”. This concept is increasingly apropos as interdisciplinary research pushes deep into the coordinated cell behaviors underlying even “simple” tissues. Indeed, cell–cell interactions give rise to behaviors such as contact inhibition\(^2\)\(^{-}\)\(^4\), collective cell migration\(^5\),\(^6\), and cell-cycle regulation\(^7\)\(^{-}\)\(^9\), which underlie physiological functions such as tissue development and healing\(^10\)\(^{-}\)\(^12\), organ size control\(^13\)\(^{-}\)\(^14\), morphogenetic patterning\(^15\), and even pathological processes such as tumor invasion\(^16\)\(^{-}\)\(^17\).

In places where tissues meet, the resulting tissue is a living composite material whose properties depend on its constituent tissues. In particular, tissue–tissue interfaces underlie both biological processes, such as organ separation and compartmentalization\(^18\)\(^{-}\)\(^19\), as well as biomedical applications such as tissue-mimetic materials\(^20\)\(^{-}\)\(^22\) and engineered tissue constructs\(^23\)\(^{-}\)\(^25\). Thus, recent research has focused on the formation and dynamics of tissue–tissue boundaries. For instance, the interplay between repulsive Eph/ephrin and adhesive cadherin–cadherin cell interactions regulate tissue boundary roughness, stability, and cell fate\(^26\)\(^{-}\)\(^30\). Furthermore, colliding monolayers with differences in Ras gene expression were able to displace one another\(^31\)\(^{-}\)\(^32\), while epithelial tissue boundaries were found to induce waves of cell deformation and traction long after the tissues had collided\(^33\).

Here, we sought to broadly investigate questions such as: how do tissues of different shapes interact with each other, and what happens when many tissues simultaneously interact? Our goal was to then develop these fundamental concepts into broad “design principles” for assembling composite tissues in a controlled way. Specifically, we sought to harness mechanical tissue interactions in the context of both tissue biophysics and cell-sheet engineering, where the latter aims to produce and harvest intact monolayers to create scaffold-free, high-density tissues\(^34\)\(^{-}\)\(^35\). Such cell sheets are typically produced by allowing cells to come to confluence within a stencil or patterned substrate to form a monolayer with a desired geometry\(^34\)\(^{-}\)\(^35\). Alternatively, tissue composites can be produced by harvesting arrays of small, autologous epidermal patches to fuse with time over burn wounds\(^36\).

To facilitate this work, we developed a rapid tissue patternning and arraying approach based on low-cost razor writing of silicone micro-stencils\(^10\). Using this technique, we created arrays of individual epithelial monolayers and then allowed them to grow out and collide, fuse at the interfaces, and ultimately self-assemble into tessellated patterns. During this process, we performed live imaging as these tissue arrays self-assembled into patterns over 2–3 days, which we were able to predict by extending our earlier model of tissue expansion\(^10\) to account for multi-tissue interactions. We then characterized the dynamics of the boundary in collisions of tissues with different sizes and cell densities. Moreover, we proposed a physical model for understanding the resulting boundary motion and extracting tissue mechanical properties from it. We next introduced a design framework for the systematic assembly of many-tissue composites (3 cell types and 30+ tissues), and investigated more complex cases such as the singular dynamics of tri-tissue junctions. Finally, we introduced heterotypic tissue collisions as an area for future development, which enabled additional features such as tissue engulfment.

**Results**

**Collisions between archetypal tissue pairs.** Our first experiment aimed at uncovering the basic rules governing tissue collisions, with the goal to understand and then fully predict the shape and motion of the tissue–tissue boundary. As classical “healing” assays exclusively focus on collisions between rectangular tissue domains, we first investigated the importance of tissue shape and size in collisions between homotypic tissues (i.e., of the same cell type). Specifically, we characterized interactions between growing pairs of millimeter-scale epithelial tissues, including equal-size rectangles, circles, small vs. large circles, and circles vs. rectangles (Fig. 1 and Supplementary Video 1). We used MDCK epithelial cells, a standard model\(^5\)\(^{-}\)\(^7\), and labeled each tissue in a pair with a different color (Methods) to clearly distinguish the boundary. Imaging over 2–3 days, we observed no mixing (Supplementary Video 2).

Collisions between identical rectangular tissues are well characterized from the traditional wound healing scratch and barrier removal assays\(^5\)\(^{-}\)\(^33\), and our data here confirmed the expected symmetric collision and fusion along the midline (Fig. 1a), allowing us to move on to unstudied configurations. We next compared identical circles, where we observed that a straight boundary formed at the midline as before (Fig. 1b), but it was nearly twice as smooth as the boundary between colliding rectangles (Supplementary Fig. 1, \(p\) value 0.014, see Methods). We suspect that this is due to the difference in collision dynamics: while parallel strips of tissue collide all along the collision line at once, circles collide at a single point and gradually extend the boundary line outward (Supplementary Videos 2 and 3).

Finally, we introduced significant asymmetry by replacing one of these circles with either a much larger circle, or a long, thin rectangle (Fig. 1c, d). In each case, we observed a curving of the boundary away from the larger tissue, which was especially...
Fig. 1a as a control (Fig. 2a), we prepared tissue pairs with an density. Using the same con of homotypic tissues with relative mismatches in either size or cell.

Homotypic tissue boundary dynamics and collision memory. Having established a platform for reproducibly studying the formation of complex boundaries between homotypic tissues, we next aimed to connect key biophysical factors to the behavior of these tissue–tissue boundaries. To this end, we studied collisions of homotypic tissues with relative mismatches in either size or cell density. Using the same configuration of two rectangles as in Fig. 1a as a control (Fig. 2a), we prepared tissue pairs with an initial mismatch in tissue width of 1000 vs. 500 μm (Fig. 2b), and other pairs with an initial mismatch in cell density of ~2600 vs. ~1800 cells/mm² (Fig. 2c).

First, we determined how asymmetry in either tissue width or density affected boundary motion upon collision. We tracked the mean tissue boundary and found that wider and denser tissues displaced narrower and less dense tissues, respectively. Boundary motion was pronounced, directed, and sustained for 15–20 h before stopping (red and blue in Fig. 2d and Supplementary Video 5). In contrast, control experiments with symmetric tissue collisions showed larger boundary fluctuations with very little average drift (black in Fig. 2d and Supplementary Video 5). Prior studies have noted similarly biased boundary dynamics, but only in heterotypic tissue collisions, for example between wild-type and Ras-transformed endothelial cells. Here, we show that collisions between homotypic tissues—genetically identical—also produce boundary motion due to asymmetry in tissue size or cell density. In contrast to heterotypic collisions; however, homotypic tissue boundary motion eventually stops.

We related boundary motion to tissue flow using particle image velocimetry (PIV) to measure the velocity field. We represented these data in kymographs of the velocity component along the collision direction, v, averaged over the tissue length, across multiple tissue collisions (Fig. 2e, see Methods). With identical (control) tissues, cells around the tissue boundary symmetrically reversed their velocity shortly after collision; convergent motion became divergent. We defined the “center of expansion” as the position from which tissue flow diverges (Methods). For control tissues, the center of expansion lies very near the tissue centroid shortly after collision (Fig. 2e, left).

A key biological question for tissue healing and fusion is at what point, to any, do two fused tissues act as one in terms of their overall dynamics? We investigate this question in collisions between tissues with size or density mismatch, which exhibited tissue flow towards the less dense or narrower tissues. In these cases, the centers of expansion began at the centroid of wider or denser tissues rather than at the overall centroid or collision boundary (Fig. 2e, center and right). The center of expansion then gradually shifted towards the centroid of the fused tissue. After the center of expansion reached the overall centroid, the fused tissue expanded symmetrically without memory of the collision. Thus, by comparing expansion centers to geometric centroids, we demonstrated that a transition exists beyond which two colliding tissues cease functioning independently and shift to behaving as one larger tissue.

Cell density gradients drive boundary motion. We hypothesized that collision boundary motion was driven by differences in tissue pressure, which are directly linked to cell density gradients. To test this, we quantified local cell density (Methods) and represented it in kymographs for each collision assay (Fig. 2f). In all cases, we found collision boundaries moved down local density gradients, consistent with our hypothesis.

While tissues in the size-mismatch assay had the same initial density, the larger tissue had a higher density at the time of collision (Fig. 2f, center and Supplementary Fig. 3). This observation is consistent with our prior work showing that, even when prepared with the same density, larger tissues develop higher cell densities than smaller tissues as they expand due to increased relative tissue spreading in smaller tissues. Accordingly, initially larger tissues displaced initially smaller tissues. Respectively, in the density mismatch assay, the tissues that are initially denser remain denser at the time of collision (Fig. 2f, right). Accordingly, the density gradient at the onset of collision correlates with the initial density mismatch (Supplementary Fig. 4), culminating in the denser tissues displacing the less dense ones.

To understand the mechanics of the collision boundary motion, we modeled the expanding tissue as an active compressible medium. Tissue expansion is driven by polarized active cell-substrate forces, which are known to be maximal at the tissue edge and decay over a distance Lc ~ 50 μm as we move into the cell monolayer. Here, we ignore active traction forces at the tissue boundary after collision, which is ~1 mm away from the outer tissue edges. At the collision boundary, we establish a force balance whereby pressure gradients drive tissue flow as

\[ -\nabla P = \xi \nu. \]  

Here, ξ is the cell-substrate friction coefficient, which for simplicity we assume to be density-independent. Including a density-dependent friction would affect the speed but not the direction of boundary motion, which is determined by the tissue pressure profile. We assume that the tissue pressure P increases with cell density ρ as specified by an unknown equation of state P(ρ), with P′(ρ) > 0. Hence, we obtain

\[ \nu = -\frac{P′(\rho)}{\xi} \nabla \rho, \]  

which predicts that the collision boundary moves from high to low cell densities with a speed proportional to the density gradient (Fig. 2g).
To test this prediction, we measure both the velocity and the density gradient at the boundary for each experiment in our three different assays (Methods). Consistent with our prediction, the results show a negative correlation between the boundary velocity and the cell density gradient (Fig. 2h). To more strongly connect density gradients to boundary displacement, we explored the absolute cell densities of the colliding tissues, ranging from 1200 to 3300 cells/mm², while preserving the mismatch ratio. We found that the absolute densities did not significantly affect collision boundary displacement (Supplementary Fig. 5c). Altogether, we conclude that cell density gradients drive boundary motion, which is consistent with pressure-driven tissue flow.

These pressure-driven tissue flows could be driven either by passive volume-exclusion forces between cells, which result from cell crowding, or by active cell-generated forces, which can also be regulated by cell density. To probe the cellular origin of the forces that drive boundary motion, we conducted density mismatch collision assays between tissues treated with blebbistatin (Methods). This treatment inhibits myosin-II activity, hence decreasing active forces by active cell-generated forces provide a major contribution to boundary motion. Myosin, then, is part of the molecular pathway that translates the density mismatch into a pressure gradient that moves the tissue boundary (Supplementary Video 7), which suggests that myosin-generated active forces provide a major contribution to boundary motion.
Fig. 3 TissEllate approach to design complex tissue composites. a, b Target tricolor tessellation (a) and chosen initial condition (solid black lines) with accompanying final pattern simulated using TissEllate (b). c, d In vitro realization of the tissue composite, which self-assembles from the initial pattern (c) to the final tessellation by collision (d), representative of three independent experiments with similar results. e, f Initial pattern and final tessellation for a two-dimensional hexagonal lattice (e, one experiment) and another complex pattern (f, representative of six independent experiments with similar results). The white outlines indicate the simulated tissue shapes. See Supplementary Videos 8-10.

boundary. Cell density can also regulate cell–cell adhesion strength. Future work can investigate whether the density mismatch induces differences in cell–cell adhesion between colliding tissues, and whether they contribute to the mechanical forces that drive boundary motion. Overall, these results bridge the tissue-scale mechanical picture of our model with some of the molecular mechanisms underlying tissue collision dynamics.

Estimating tissue mechanical properties from collisions. Based on our model, we use our measurements of cell density and velocity to extract information about the tissue’s equation of state $P(\rho)$. To this end, we obtain the average boundary velocity and density gradient for each assay, and we fit a line to them (Fig. 2h). From this fit, and using $\xi \sim 100 \text{ Pa} \cdot \text{s}/\mu \text{m}^2$ $^{44,46}$, we obtain $P(\rho) \sim 0.5 \text{ Pa} \cdot \text{mm}^2$. This result indicates that, in the conditions of our experiments, for every cell that we add per square millimeter, the tissue pressure goes up about 0.5 Pascal.

Next, we use these results to estimate the mechanical properties of the cell monolayer. To this end, we assume a specific equation of state $P(\rho)$. Whereas the functional form of $P(\rho)$ for tissues remains to be established in experiments, here we use the form proposed in theoretical work by Recho et al $^{47}$:

$$P(\rho) = K \ln \left( \frac{\rho}{\rho_e} \right),$$

where $K$ is the bulk modulus of the monolayer around the reference cell density $\rho_e$. Accordingly, $K$ is a density-independent parameter that characterizes the linear mechanical response of the cell monolayer around its reference state. Deriving generalized equations of state with a density-dependent bulk modulus $K(\rho)$ remains a challenge for future work. Equation (3) was justified theoretically for growing tissues around their homeostatic state, around which the cell proliferation rate varies linearly with cell density $^{47}$. Although we cannot test the accuracy of this equation of state in our experiments, its assumptions are quite generic and could be met in our system; hence, our choice. From Eq. (3), we have $P(\rho) = K/\rho$. Using the average cell density measured in our experiments during boundary motion, $\rho = (3.4 \pm 0.2) \times 10^3 \text{ mm}^{-2}$ (SD), we estimate $K \sim 2 \text{ kPa}$.

This order-of-magnitude estimate falls in between two previous measurements of bulk moduli of MDCK cell monolayers. First, in-plane stretching of suspended cell monolayers yielded a stiffness $E = 20 \pm 2 \text{ kPa}$ $^{48}$. Because these monolayers have no substrate, cells do not migrate, and hence suspended monolayers might have different mechanical properties than monolayers on a substrate. Second, in spreading cell monolayers, a linear relationship between tension and strain revealed an effective tensile modulus $\Gamma = 2.4 \pm 0.4 \text{ mN/mm}^4$ $^{49}$. Using a monolayer height $h = 5 \mu \text{m}$ $^{44,50}$, this value translates into a stiffness $E = 0.48 \text{ kPa}$.

Complementary to these measurements, which probe tissue stiffness under extension, our estimate reflects the stiffness of the cell monolayer under the compression that results from a tissue collision. The mechanical response of tissues is strongly nonlinear. Hence, the response to compression and to extension need not be the same. In fact, the cellular mechanisms responsible for extensional and compressional tissue elasticity can be quite different, and hence the corresponding moduli can differ widely $^{51}$. Here, our estimate suggests that the compressional modulus of MDCK cell monolayers on a substrate is of a similar order of magnitude to the extensional one.

Overall, our collision assays provide a way to probe the bulk mechanical properties of migrating cell monolayers, which are otherwise difficult to measure. Remarkably, analyzing collisions between tissues that differ only in their cell density allowed us to infer mechanical properties without measuring any mechanical forces. Rather, we employed our model to relate tissue flows to pressure and density gradients, from which we inferred the relationship between pressure and density. In the future, collision assays might be used to measure the equation of state of cell monolayers, which is a key input for mechanical models of growing and expanding tissues $^{42,52}$.

Large-scale tissue tessellations for cell sheet engineering. The stereotyped dynamics of tissue collisions that we uncovered suggested simple underlying design rules that would allow self-assembled tissue tessellations to be designed first in silico and then realized in vitro with potential applications in tissue engineering.

We tested this idea with a tessellation inspired by the artwork of M.C. Escher—a “dice lattice” (Fig. 3a). To design this tessellation, we used the computational model described above (Fig. 1) to simulate many initial tissue array conditions until
converging on the pattern of ellipses shown in Fig. 3b. We engineered this pattern with tissues (Fig. 3c) and filmed it developing as predicted (Fig. 3d and Supplementary Video 8). This computer-aided design (CAD) process can be generalized to arbitrary tessellations (Fig. 3e and Supplementary Videos 9 and 10), offering a “TissueCAD” approach, which we call “TissEllate,” to designing and building composite tissues.

Composite cell sheets may be particularly useful for tissue engineering where cell sheets are extracted from culture vessels and used as building blocks for larger constructs for therapeutic applications. We demonstrated the compatibility of this process with our tissue composites by culturing a dice lattice on a standard tissue-culture dish (Fig. 3d) and med it with temperature-switchable cell adhesion (UpCell). Created with Biorender.com. b. c Tissellation on UpCell dish (b) is transferred to standard tissue-culture dish (c). Four independent tessellation transfers were performed with qualitatively similar results.

Dynamics at tri-tissue collisions. During the self-assembly of tissue tessellations, we observed a special behavior at tri-tissue collision points: We often found long streams of the inner tissue migrating in between the outer tissues, similar to an extrusion process (Fig. 5a, b). These extruding streams of inner tissue were narrow, progressively necking down to the single-cell scale, visually reminiscent of streams of invasive cancer cells (Fig. 5c–e). However, these events, which we call “escapes,” involve the co-migration of all three tissues rather than the invasion of one tissue into the others. In other words, the inner tissue does not break through the outer ones, rather, it squeezes between them as they expand (Fig. 5c–e and Supplementary Videos 11 and 12). Consequently, the initial relative positions of the three colliding tissues are a strong statistical determinant of escape events (Supplementary Figs. 7 and 8).

We characterized the dynamics of escapes by measuring cell speed fields around tri-tissue collisions, which showed that the escaping inner tissue migrated faster than its neighbors (Fig. 5f–h, see Methods). To determine whether this speed increase was a generic consequence of the local negative curvature of tri-tissue collision points, we compared tri-tissue collisions to a single tissue patterned to match the overall shape of the colliding tissues at the time of escape (Fig. 5i). For the single tissue case, we did not observe any speed increase (Fig. 5j), which rules out local curvature as the sole cause of escape events. Therefore, escapes are not a direct result of the overall geometry of the multi-tissue composite; rather, they emerge from the collision interactions between three tissues with their own expansion histories. Furthermore, our simulations of tissue shape evolution based on binary collision rules fail to capture escapes (Fig. 5b). Therefore, escapes are a result of three-body interactions. Moreover, this extrusive behavior represents an unexpected mechanism for producing fine structures between tissues.

Heterotypic tissue boundary dynamics. Where homotypic tissues reflect fusion events during morphogenesis and healing, heterotypic tissue boundaries (i.e., different tissue types) arise
during compartmentalization, and our fourth experiment highlighted how heterotypic tissue interactions can differ from those between homotypic tissues. Critically, heterotypic tissues can have different cell migration speed, motion patterns, and cell–cell interactions (e.g., adhesion and repulsion). However, these differences were not included in our physical model of tissue collisions, and hence we did not use it to analyze heterotypic collisions. Generalizing the biophysical and simulation models to heterotypic collisions is an interesting and important direction for future work.

Here, we prepared co-cultures of the breast cancer cell lines MCF10A (benign), MCF7 (malignant, non-invasive), and MDA-MB-231 (metastatic) as monolayers of the same size and cell density. We used homotypic MCF10A collisions as a reference, for which we observed non-mixing and boundary dynamics similar to the homotypic MDCK collisions discussed earlier (Supplementary Video 13).

We first collided rectangular monolayers of MCF10A and MDA-MB-231 cells, which have the largest phenotypic difference among the cell lines we used. While these tissues have similar expansion speeds, they exhibit radically different collective dynamics, reflective of different cell–cell adhesion strengths57 (Supplementary Video 14). While cells in MCF10A tissues hardly exchange neighbors, the metastatic MDA-MB-231 cells continually undergo neighbor exchanges and even crawl over each other out of the plane. Upon collision, the MCF10A tissue simultaneously displaced and crawled underneath the MDA-MB-231 tissue (Fig. 6a–d).

We next investigated collisions between MCF10A and MCF7 monolayers. The MCF7 monolayer expands about 6 times more slowly than the MCF10 monolayer, which allowed us to explore the effects of different edge speeds on tissue collisions, with implications for multi-tissue design. Surprisingly, we found that the slower MCF7 tissues actually displaced the MCF10A tissues (Fig. 6e and Supplementary Video 15), which may be due to differences in cell–cell and cell-substrate adhesion. Thus, a higher expansion speed does not imply the ability to displace tissues that expand more slowly. In fact, MCF10A cells at the collision boundary reversed their velocity and migrated away from the MCF7 tissue within 8 h after collision, starting at the boundary and progressively moving into the MCF10A monolayer (Fig. 6f).

This behavior seems a tissue-scale analog of the cellular behavior known as contact inhibition of locomotion, whereby a cell stops and reverses its direction of motion upon collision with another cell34–4.

Furthermore, in collisions between tissues with different expansion speed, the faster tissue should be able to engulf the slower tissue, similar to the engulfment between tissues with differential adhesion58. We confirmed this hypothesis in collisions between rectangular strips of MCF10A cells (fast) and circles of MCF7 cells (slow), which we reproduced with our tissue shape model by incorporating different speeds into our simulation (Fig. 6g–j and Supplementary Video 16, see Supplementary Fig. 9 and Supplementary Note 2). Future work is needed to elucidate the biophysical properties of heterotypic tissue interfaces, but here we highlight how differences in expansion speed enable design options in future multi-tissue studies and applications.

**Discussion**

We investigated how tissue–tissue interactions can be harnessed to self-assemble complex composite tissue sheets—tissue tessellations. First, we demonstrated that colliding tissues change shape in stereotyped and predictable ways. Then, we proposed a physical model of tissue–tissue collisions where the critical driver of boundary dynamics is the underlying gradient in cell density across the interface that induces pressure-driven tissue flow down the gradient. Furthermore, we used this model to estimate the material properties of the colliding tissues without any force measurements. In the future, this approach might be used to reveal information about the equation of the state of living tissues.

Regarding the collision dynamics, previous work had shown that heterotypic tissues can displace each other upon collision31,32. Our findings revealed that even homotypic tissues, which are genetically identical, can displace each other based on purely mechanical differences, suggesting a mechanical tug-of-war where cellular pressure gradients across the boundary can compete with tissue-scale contact-inhibition-of-locomotion. Therefore, our collision assays could be used to study mechanical tissue competition37–41,59,60, which might provide biophysical insight into development61,62, homeostasis63, and tumor growth37,39. In our in vitro experiments, the mechanical competition arises from differences in cell density between the colliding tissues, which are imposed in our experimental setup. In vivo, cell density gradients could potentially arise from a number of biological mechanisms. Uncovering such mechanisms and their consequences for tissue collisions in vivo is an interesting avenue for future research.

Furthermore, based on the reproducible and almost algorithmic tissue interactions that we found, we developed computational design tools to create complex tissue tessellations. Whereas previous work had exclusively studied binary head-on collisions, the tessellations allowed us to study collisions between myriad, arbitrary tissues. For example, a finding unique to tri-tissue interactions is that the inner tissue can speed up and...
squeezes between the outer tissues. Such collision dynamics do not follow binary collision rules and are particularly surprising in their ability to produce complex, fine patterns almost at the single-cell scale. Our work thus opens the door to characterizing and understanding the dynamics of increasingly complex multi-tissue collisions.

Finally, the tessellations are obtained by self-assembly, which allows the tissue boundaries to develop naturally. Thus, our work demonstrates how tissue sheets can be treated as “designable” living materials. Specifically, we developed a simulator that, despite lacking both biophysical laws and cellular resolution, predicts tissue patterns accurately at the 100 μm scale. This feature makes the simulator useful to design tissue composites in silico before realizing them in vitro. This approach is compatible with advanced biofabrication strategies such as cell sheet engineering, which we demonstrated by transferring an “Escher” tissue sheet between Petri dishes while preserving tissue integrity and internal boundaries. Tissue tessellation should also be compatible with bioprinting, which could be used to pattern larger arrays of the initial tissue seeds.

While our work focused on the biophysical elements of tissue interactions, our tissue arraying method provides unique advantages relative to other forms of tissue patterning. First, razor-writing allows custom silicone stencils to be cut and seeded within minutes, all at the benchtop. Second, stencils fundamentally allow cells to grow out simply by removing the stencil, which is distinct from many advanced tissue patterning processes. For instance, printing of unique DNA-oligomer pairs or patterned extracellular matrix allows for precise, high throughput patterning of microtissues with unique composition, but does not support outgrowth [64–66]. Similarly, elegant photopatterning methods allow for precise, spatiotemporal control of the microenvironment but require significant infrastructure and are difficult to scale up to many tissues [67, 68]. Ultimately, while razor-writing has limitations below 500 μm, its convenience and low cost make it extremely accessible to the numerous and wide range of research groups studying monolayer biophysics and cell sheet engineering.

Finally, we envision future work generalizing these design tools to tissue interactions with heterotypic tissues and 2D vs. 3D geometries, each of which will necessitate characterizing boundary rules and implementing them in the computational tools. There is also merit in comparing the similarities and differences between interface formation between growing tissues and immiscible, viscous fluids where similar tessellation dynamics can arise [69]. Such extensions will allow the design of increasingly complex, multi-functional tissue patches for biophysical and biomedical applications.

**Methods**

**Cell culture.** MDCK-2 wild-type canine kidney epithelial cells (ATCC) were cultured in customized media consisting of low-glucose (1 g/L) DMEM with phenol red (Gibco, USA), 1 g/L sodium bicarbonate, 1% streptomycin/penicillin (Gibco, USA), and 10% fetal bovine serum (Atlanta Biological, USA). MCF10A human mammary epithelial cells (ATCC) were cultured in 1:1 DMEM/F-12 (Thermo Fisher Scientific, USA) media that consists of 2.50 mM L-Glutamine and 15 mM HEPES buffer. This media was supplemented with 5% horse serum (Gibco, USA) and 1 g/L sodium bicarbonate, 1% streptomycin/penicillin (Gibco, USA). MDA-MB-231 (ATCC) and MCF7 (ATCC) human mammary cancer cells were both cultured in 1:1 DMEM/F-12 (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Atlanta Biological, USA) and 1% penicillin/streptomycin (Gibco, USA). All cells were maintained at 37 °C and 5% CO2 in humidified air.

**Tissue patterning and labeling.** Experiments were performed on tissue-culture plastic dishes (BD Falcon, USA) coated with type-IV collagen (MilliporeSigma, USA). Dishes were coated by incubating 120 μL of 50 μg/mL collagen on the dish under a glass coverslip for 30 min at 37 °C, washing three times with deionized distilled water (DI), and allowing the dish to air-dry. Stencils were cut from 250-μm-thick silicone (Bisco HT-8240, Stockwell Elastomers) using a Silicone Cameo vinyl cutter (Silhouette, USA) and transferred to the collagen-coated surface of the dish. Cells were separately labeled using CellTracker™ (Biotium, USA) red and orange dyes for two-color experiments and CellRite™ (Biotium, USA) red, orange, and green dyes for three-color experiments.

For MDCK experiments, suspended cells were concentrated at ~2.25 x 10⁹ cells/ml and separated into identical aliquots for labeling. We added 8 μL of appropriate membrane dye color per 1 mL of media and briefly vortexed each cell suspension. Then, we immediately pipetted into the stencils at ~1000 cells/mm², taking care not to disturb the collagen coating with the pipette tip. To allow detachment of cells to the collagen matrix and labeling of the cell membranes, we incubated the cells in the stencils for 30 min in a humidified chamber before washing out the dye and filling the dish with media.

For experiments using other cell types, suspended cells were concentrated at ~3 x 10⁹ cells/ml and 10 μL of membrane dye color per 1 mL of media. We briefly vortexed the cell suspension and allowed it to incubate for 20 min at 37 °C. We then centrifuged the suspension and removed the supernatant, replacing it with the fresh media without dye. We next washed out the dye and filled the dish with media. For all experiments, we then incubated the cells for an additional 18 h after cell attachment to form confluent monolayers in the stencils. Stencils were removed with tweezers, with imaging beginning ~30 min thereafter. Media without phenol red was used throughout seeding and imaging for three-color experiments to reduce background signal during fluorescence imaging. For experiments involving blebbistatin (BioGems, USA), 25 μM was mixed into fresh media and added to the dish 2 h after the stencils were removed. To ensure no phototoxicity, no excitation wavelengths below 500 nm were used during live imaging. This was confirmed in our assays by ensuring migration speeds of blebbistatin-treated tissues matched that of control tissues.

**Live-cell time-lapse imaging.** We performed imaging on an automated, inverted Nikon Ti2 with a Nikon Q2i CMOS camera and NIS Elements software (version AR 5.20.01 64-bit). We equipped the microscope with environmental control for imaging at 37 °C and humidified 5% CO2. Final images were composited in NIS Elements from montages of each pair or tessellation.

For experiments from Fig. 5c–j, we used a 10x phase-contrast objective to capture phase-contrast and fluorescence images every 10 min. RFP/Cy5 images were captured at 10% lamp power (Sola SE, Lumencor, USA) and 150 μs exposure time. Phototoxicity was observed under these conditions for up to 24 h.

For all other experiments, we used a 4x phase-contrast objective to capture phase-contrast images every 20 min. For two-color time-lapse images, RFP/Cy5 images were also captured every 20 min at 15% lamp power (Sola SE, Lumencor, USA) and 500 μs exposure time. For three-color time-lapse images, RFP/Cy5 images were captured every 60 min at 15% lamp power (Sola SE, Lumencor, USA) and 300 ms exposure time, while GFP images were captured every 120 min at 15% lamp power (Sola SE, Lumencor, USA) and 300 ms exposure time. No phototoxicity was observed under these conditions for up to 72 h. Final images were composited in NIS Elements from montages of each pair or tessellation.

**Tissue dye segmentation.** The tissue dye becomes diluted as cells divide and spread, so the dye at tissue edges (where cells are more spread and divide more frequently) becomes much more dim than the center of tissues. Because we saw no mixing in our collisions, we segmented the fluorescent channels using a custom MATLAB (Mathworks, version 2018b) script and overlaid them with the phase-contrast images for clear visualization. To segment fluorescence images, we normalized the fluorescence channel histograms to each other and compared relative brightness for each pixel between channels. We then masked with the binary masks obtained from the phase-contrast channel.

**Setting vs. for model.** We set the normal velocity for the model (all shapes and tessellations) according to the outward velocity of the outer edges of the control region collisions. The outward velocity was found to be 2.56 with the FFT window deformation algorithm, employing a standard deviation), so we used the previously reported speed for expanding circles of 29.5 μm/s [19].

**Velocity measurements.** We calculated tissue velocity vector fields from phase-contrast image sequences, rotating each image so that the initial tissue locations in image pairs were horizontal. We used the free MATLAB package PIVLab version 2.5.2.0.01 64-bit. We equipped the microscope with environmental control for imaging at 37 °C and humidified 5% CO2. Final images were composited in NIS Elements from montages of each pair or tessellation.

**Average kymographs.** We first constructed kymographs of each rectangular collision pair, averaging over the vertical direction of each timestep and ignoring the top and bottom 1 mm. We then averaged the individual tissue kymographs, aligning by the initial tissue configuration, and determined the edge extent from the median extent of the individual kymographs.
Center of expansion. We determined the center of expansion by thresholding as $|v_x| < 0.3 \mu m/h$ for individual kymographs of $v_x$. We filtered for the largest contiguous region and took the midline of this region as the center of expansion.

Cell density measurements. We first reproduced nuclear positions from 4x phase-contrast images using our in-house Fluorescence Reconstruction Microscopy tool\(^1\). The output of this neural network was then segmented in ImageJ to determine nuclei footprints and centroids. Local density was calculated for each PIV window by counting the number of nucleus centroids in that window.

Boundary velocity and cell density gradient determination. Boundary velocity was found from the position change of the midline in Fig. 2d. Cell density gradient $\partial \rho / \partial x$ was found as $\frac{\Delta \rho_1 - \Delta \rho_2}{\Delta x}$, where $\rho_1$ and $\rho_2$ are the total density within 300-µm-wide regions immediately to the left and right of the tissue boundary, respectively, and $\Delta x = x_2 - x_1 = 300 \mu m$. We plotted $\partial \rho / \partial x$ for timepoints between 20 and 36 h, which is after collisions and before the boundary stops moving.

Statistical analysis. For all significance testing and corresponding $p$ values reported in this study, we used GraphPad Prism 9.1.0 (GraphPad Software) to perform non-parametric, unpaired two-sided Mann–Whitney $U$ tests. All measurements were taken from distinct samples, where averages and standard deviations were calculated across the series of replicates. In every assay, sample sizes include at least four replicates, across at least three independently performed experiments.

Cell sheet engineering tissue patterning and transfer. We first patterned tissues on a 3.5-cm NUNC\textsuperscript{TM} UpCell\textsuperscript{TM} dish with a supportive membrane (Thermo Fisher, Scientific, USA). We followed the same collagen coating and stencil application process as before, but passivated the underside of our stencils to avoid damaging the UpCell\textsuperscript{TM} surface. To passivate the stencils, we incubated them for 30 min at 37 °C in Pluronic\textsuperscript{TM} F-127 solution (Thermo Fisher Scientific, USA) diluted in PBS to 2%. We washed the stencils three times in DI and gently dried them with compressed air before transferring them to the dish.

After the tissues reached confluence within the stencils, we removed the stencils and allowed the tessellation to collide and heal for ~72 h. To release the tessellation bubbles. We moved the UpCell\textsuperscript{TM} dish with membrane to a 4 °C refrigerator to ensure total release, and prepared a standard 3.5 mm tissue culture dish (BD Falcon, USA) coated with collagen IV as before and filled with warm media. After 7 min at 4 °C, we carefully removed the membrane and tessellation monolayer from the UpCell\textsuperscript{TM} dish and floated it in the tissue culture dish with the tessellation side down. We aspirated the media from below the membrane to initiate bubble-free contact with the dish surface and covered the membrane with 350 μl of warm media. We incubated the membranes at 37 °C overnight before floating the membrane off the surface by filling the dish with medium and removing it with tweezers.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
32. Moirrier, S. et al. Collective stresses drive competition between monolayers of normal and Ras-transformed cells. Soft Matter 15, 537–545 (2019).
33. Rodríguez-Franco, P. et al. Long-lived force patterns and deformation waves at repulsive epithelial boundaries. Nat. Mater. 16, 1029–1036 (2017).
34. Wright, D., Rajalingam, B., Selvarasar, S., Dokmeci, M. R. & Khademhosseini, A. Generation of static and dynamic patterned co-cultures using microfabricated parylene-C stencils. Lab Chip 7, 1272–1279 (2007).
35. Elloumi Hannachi, I. et al. Fabrication of transferable micropatterned co-cultured cell sheets with microcontact printing. Biomaterials 30, 5427–5432 (2009).
36. Joethy, J. et al. Initial impression of the cellutome——epidermal harvesting system in burns management. Burns Open 3, 68–74 (2019).
37. Basan, M., Risler, T., Joanny, J.-F., Sastre-Garasa, X. & Prost, J. Homeostatic competition drives tumor growth and metastasis nucleation. HFSP J. 3, 265–272 (2009).
38. Ranft, J., Aili, M., Prost, J., Jülicher, F. & Joanny, J. F. Mechanically driven interface propagation in biological tissues. N. J. Phys. 16, 035002 (2014).
39. Podewitz, N., Jülicher, F., Gompper, G. & Elgeti, J. Interface dynamics of competing tissues. J. Phys. 18, 083020 (2016).
40. Williamson, J. J. & Sabreux, G. Stability and roughness of interfaces in mechanically regulated tissues. Phys. Rev. Lett. 121, 238102 (2018).
41. Ganai, N., Rüscher, T., Gompper, G. & Elgeti, J. Mechanics of tissue competition: Interfaces stabilize coexistence. N. J. Phys. 21, 063017 (2019).
42. Alert, R. & Trepat, X. Physical models of collective cell migration. Annu. Rev. Condens. Matter Phys. 11, 77–101 (2020).
43. Blanch-Mercader, C. et al. Effective viscosity and dynamics of spreading epithelia: a solvable model. Soft Matter 13, 1235–1243 (2017).
44. Pérez-González, C. et al. Active wetting of epithelial tissues. Nat. Phys. 15, 79–88 (2019).
45. Straight, A. F. et al. Dissecting temporal and spatial control of cytokinesis with high-resolution live cell imaging. Proc. Natl Acad. Sci. USA. 112, 6037–6042 (2015).
46. Guimaraes, C. F., Gasperini, L. Marques, A. P. & Reis, R. L. The stiffness of living tissues and its implications for tissue engineering. Nat. Rev. Mater. 5, 351–370 (2020).
47. Ranft, J. et al. Fluidization of tissues by cell division and apoptosis. Nat. Phys. 12, 2381–2391 (2016).
48. Harris, A. R. et al. Characterizing the mechanics of cultured cell monolayers. Proc. Natl Acad. Sci. USA. 109, 16449–16454 (2012).
49. Vincent, R. et al. Active tensegrity as a mechanical model for epithelial organization. Phys. Rev. Lett. 115, 248103 (2015).
50. Serra-Picamal, X. et al. Mechanical waves during tissue expansion. Nat. Phys. 8, 628–634 (2012).
51. Guimarães, C. F., Pires, M. A., Marques, A. P. & Reis, R. L. The stiffness of living tissues and its implications for tissue engineering. Nat. Rev. Mater. 5, 351–370 (2020).
52. Komai, S. et al. Fluidization of tissues by cell division and apoptosis. Nat. Phys. 12, 2381–2391 (2016).
53. Kobayashi, J., Kikuchi, A., Aoyagi, T. & Okano, T. Cell sheet tissue engineering: cell sheet preparation, harvesting/maintenance, and transplantation. J. Biomed. Mater. Res. Part A 107, 955–967 (2019).
54. Singh, M. et al. Challenging the conventional therapy: emerging skin neoplastic forces in wound healing. Plast. Reconstr. Surg. 136, 5248–5304 (2015).
55. Serena, T., Francius, A., Taylor, G. & MacDonald, J. Use of a novel epidermal harvesting system in resource-poor countries. Adv. Skin. Wound Care 28, 107–112 (2015).
56. Osborne, S. N., Schmidt, M. A., Derrick, K. & Harper, J. R. Epidermal micrografts produced via an automated and minimally invasive tissue form at the dermal/epidermal junction and contain proliferative cells that secrete wound healing growth factors. Adv. Skin. Wound Care 28, 397–405 (2015).
57. Yuki, K., Yoshida, Y., Inagaki, R., Hata, H. & Noda, M. E-cadherin downregulation and RECK upregulation are coupled in the non-malignant epithelial cell line MCF-10A but not in multiple carcinoma-derived cell lines. Sci. Rep. 4, 4588 (2014).
58. Foty, R. A. & Steinberg, M. S. The differential adhesion hypothesis: a direct test. Dev. Biol. 278, 255–263 (2005).
59. Murphy, R. J., Buenzli, P. R., Baker, R. E. & Simpson, M. J. Mechanical cell competition in heterogeneous epithelial tissues. Bull. Math. Biol. 82, 130 (2020).
60. Gradei, D. et al. Cell-scale biophysical determinants of cell competition in epithelia. eLife 10, e61011 (2021).
61. Shraiman, B. I. Mechanical feedback as a possible regulator of tissue growth. Proc. Natl Acad. Sci. USA. 102, 3318–3323 (2005).