Boundary-bulk patterning in three-dimensional confluent cellular collectives

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Organoids are \textit{in vitro} cellular collectives from which brain-like, or gut-like, or kidney-like structures emerge. To make quantitative predictions regarding the morphology and rheology of a cellular collective in its initial stages of development, we construct and study a three-dimensional vertex model. In such a model, the cells are represented as deformable polyhedrons with cells sharing faces such that there are no gaps between them, otherwise known as confluent. In a bulk model with periodic boundary conditions, we find a rigidity transition as a function of the target cell shape index $s_0$ with a critical value $s_0^* = 5.39 \pm 0.01$. For a confluent cellular collective with a finite boundary, and in the presence of lateral extensile and in-plane, radial extensile deformations, we find significant boundary-bulk patterning that is one-cell layer thick. More specifically, for lateral extensile deformations, the cells in the bulk are much less aligned with the direction of the lateral deformation than the cells at the boundary. For in-plane, radial deformations, the cells in the bulk exhibit much less reorientation perpendicular to the radial direction than the cells at the boundary. In other words, for both deformations, the bulk cells are insulated from the deformations, at least over time scales much slower than the timescale for cellular rearrangements. Our results provide an underlying mechanism for some observed cell shape patterning in organoids. Finally, we discuss the use of a cellular-based approach to designing organoids with new types of morphologies to study the intricate relationship between structure and function at the multi-cellular scale.

Introduction

Organoids provide us with an \textit{in vitro} window into organogenesis \cite{1,2}. An organoid starts off as a cellular collective (a clump of cells) consisting typically of cells, such as pluripotent stem cells. The cells are given some induction medium to help them differentiate into either neurons or kidney cells or heart muscle cells. The cellular collective is then transferred to a petri dish containing Matrigel, which contains collagen and, at times, growth factors and morphogens, followed by additional protocol, such as agitation \cite{3,4}. Even at the initial stages, there is some patterning of the collective in terms of cell shape \cite{5}. Over a longer time scale, as the cells divide, the organoid continues to develop additional structure and a brain organoid \cite{6}, for example, ultimately emerges. Intriguingly, brain organoids, exhibit the \textit{in vivo} phenomenon of neuronal diversity \cite{7}. Additionally, at even later stages of development, neurons in the brain organoid begin to fire and fire synchronously, just as in the developing brain \cite{8}. Intestinal organoids, on the other hand, self-assemble into crypt-villus units \cite{9}. In addition to providing a window into organogenesis, organoids also provide an intermediary platform between cells and organs to study disease \cite{11,12}. For instance, scientists are currently exploring how SARS-CoV-2 infects the brain via brain organoid studies to provide guidance for drug treatment of the COVID-19 \cite{10,13}.

We now hone in on the early stages of organoid development. In particular, for quasi-two-dimensional geometries, which are used in the formation of brain organoids \cite{5} and intestinal organoids \cite{12}, for instance, there appears to be differences in cellular structure between the cells in contact with the Matrigel at the edge of the organoid and the cells in the bulk (or interior). In
particular, the cells on the boundary appear to be more elongated with the direction perpendicular to the edge of the Matrigel. The cells in the bulk, on the other hand, appear to be more globular with no apparent, particular orientation [5]. For three-dimensional geometries, a central hole, or lumen, in place of a bulk of globular cells can emerge [6].

Given the apparent ubiquity of the phenomenon, we ask: How does such a bulk-boundary effect in these cellular collectives arise? Theoretical work has been done to demonstrate how an individual versus multiple cortex-core structures materialize from a hydrodynamic description of active forces in cellular collectives to help regulate the overall brain organoid architecture [13]. Here, we implement a cellular-based computational approach to probe the above question, namely, a three-dimensional vertex model [14–18]. Such models are ones with cells represented as deformable polyhedrons and there are no gaps between them. While two-dimensional vertex models have been studied widely [19–28], three-dimensional vertex models have perhaps not been studied as extensively. Presumably, this is, in part, because there is no publicly available code, while two-dimensional codes, such as CHASTE [29, 30], CellGPU [31] and the different, but related, Active Vertex Model [32], are available.

As for prior three-dimensional vertex model work that has been done, researchers have found that introducing polarized interfacial tension allows for cells to migrate individually, or even as a cluster, through a tissue [33]. In addition, studies of branching in tissue to form a lung have also been explored in such models [34]. And yet, prior studies of the three-dimensional vertex model have focused on a particular energy functional that is different from a three-dimensional extension of the two-dimensional energy functional in which a rigidity transition was uncovered [23] and with support from experimental observations of asthmatic bronchial epithelial tissue [35]. More recently, the same two-dimensional vertex model extended to include two cell types predicted a new micro de-mixing phenomenon which was supported by experiments [26]. We will use the same version of the energy functional as the one exhibiting a density-independent rigidity transition in two dimensions to ask first whether or not there exists a rigidity transition in a bulk model. Prior work has demonstrated that there exists a rigidity transition in a three-dimensional Voronoi model as a function of the three-dimensional shape index [36]. We will then explore how deformations of a confluent cellular collective affect the morphology and the rheology of the collective. Our efforts represent a substantive leap from earlier, elegant work studying the shapes of shells composed of the polyhedrons as we now can probe the interior [37].

Results

Model. Cells are biomechanical and biochemical constructs that are not in equilibrium, i.e., they are driven by internal, or active forces. The biomechanics of the cellular collective is given by the energy functional:

$$E = K_V \sum_j (V_j - V_0)^2 + K_A \sum_j (A_j - A_0)^2 + \gamma \sum_\alpha \delta_{\alpha,B} A_\alpha,$$

where $A_j$ denotes the $j$th cell total area, the $j$th cell volume is denoted by $V_j$, and $\alpha$ labels the faces of the cells, with $\delta_{\alpha,B} = 0$ if a face is not at the boundary $B$ of the collective and 1 otherwise. Given the quadratic penalty from deviating from a cell’s preferred volume and area, $K_V$ and $K_A$ are volume and area stiffnesses, respectively. Physically, the volume term represents the bulk elasticity of the cell with $V_0$ denoting a target volume, while the area term represents the isotropic contractility of the acto-myosin cortex with $A_0$ denoting a target area. The larger the $A_0$, the less isotropically contractile the cell is, and vice versa. The contractility is isotropic in the sense that all faces are equivalent. Note that this translation from the mathematics to the biology differs slightly from prior translations for two-dimensional models in which the effective target perimeter $P_0$ (assuming $A_0 = 1$) translates to a competition between cell-cell adhesion at larger
FIG. 1. **3D vertex model** (a) Snapshot of the bulk model with $s_0 = 5.60$. (b) Snapshot of the cellular collective (light green) with $s_0 = 5.60$ and empty cells (dark green). (c) Snapshot series of a reconnection event between cells in which the edge composed of the vertices labeled 7 and 8 becomes the triangle composed of vertices labeled 9, 10, and 11, and vice versa to result in a change of neighbors while remaining confluent. The numbers indicate serial vertices, and solid lines indicate edges. The point labeled 0 indicates the center of the reconnection event.

$P_0$ and contractility at smaller $P_0$. Indeed, cell-cell adhesion and contractility are coupled \[38\]. For instance, knocking out E-cadherin in keratinocytes, effectively changes the contractility \[26\]. However, since cells share edges in the vertex model, we cannot tune for cell-cell adhesion directly, and so we stick with isotropic contractility instead. Presumably, the less isotropically contractile a cell is, the more likely it can develop contractility in a particular direction, or anisotropic contractility as modulated by stress fibers, for example \[39\]. As for the linear area term, the cells at the boundary of the cellular collective, there is an additional surface tension term for faces interacting with the “vacuum”. One can nondimensionalize any length $l$ in the simulation with $l = V_{0,\beta}^{1/3}$. An important parameter in these models is the dimensionless shape index $s_{0,\beta} = A_{0,\beta}/(V_{0,\beta})^{2/3}$. A regular tetrahedron has a dimensionless shape index of $s_0 \approx 7.2$, for example.

Now that we have addressed the biomechanical aspect of cells. We must also account for their dynamics. Cells can move past each other even while the tissue remains confluent. In two dimensions such movements are known as T1 events. Understanding such events are key to understanding the rigidity transition in two dimensions \[23\]. In three dimensions, such movements are known as reconnection events. Prior work has developed an algorithm for such reconnection events focusing on edges becoming triangles and vice versa that may occur for edges below a threshold length $l_{th}$ \[40\]. Very briefly, there are three conditions that must be met to ensure that
the reconnection event is physically plausible, namely, that the event is geometrically, energetically, and topologically irreversible. See the Methods Section for a few more details and see Ref. [40] for full story.

In addition to reconnection events, there is an underlying Brownian dynamics for each vertex. Specifically, the equation of motion for the position $r_I$ of a single vertex $I$ is

$$\dot{r}_I = \mu F_I + \mu F^B_I,$$

with $F_I$ and $F^B_I$ denoting the conservative force and the random thermal force on the $I$th vertex respectively. The force $F_I$ is determined from both the area and volume energetic constraints and, hence, includes cell-cell interactions. In addition, each $I$th vertex performs a random walk with an effective diffusion coefficient of $\mu k_B T$, where $T$ is an effective temperature. Unless otherwise specified, the mobility $\mu = 1$. Finally, the Euler-Murayama integration method is used to update the position of each vertex. See Table I for the listing of the parameters used in the simulations and their corresponding values.

To explore the properties of a cellular collective, one must consider boundaries. For instance, as cellular collectives become embedded in Matrigel to ultimately become organoids, they interact with the deformable Matrigel as they develop [6]. Here, we do not explicitly consider Matrigel, though prior work in two dimensions of a cellular collective embedded in a spring network has been done [41]. Instead, we construct a confluent cellular collective, or a clump of confluent cells, by making a cut-out of the bulk periodic system that contains cells with empty cells beyond the boundary between cells and empty space (see Figure 1(a) and (b)) as has been done in two dimensions [41]. The cut-out is initially cylindrical. For larger $s_0$, the cut-out readily balls up into a sphere, for smaller $s_0$, the cut-out approximates a sphere. For those cells at the boundary of the clump, the interfacial vertices contain an additional interfacial surface tension $\gamma$. Moreover, we impose the constraint that only one empty cell can perform a reconnection event involving other cells. For particular geometries, such as monolayer, allowing reconnection events with two empty, or phantom, cells, creates a hole in the confluent cellular collective. While it is an interesting route of study, we will not pursue effects on the emergence of holes here.

Finally, we explore the rheological properties of the three-dimensional vertex model in bulk as well as the confluent cellular collective. For the latter, we consider deformations on the cellular collective on the boundary by having the boundary vertices undergo constant speed $v$ outward. In other words, a constant extensile strain/deformation rate is imposed. In terms of direction, we explore two kinds of extensile strain, one that is uni-axial and one that is in-plane, radial. One can readily interpret the deformation as an external one, or one that is applied to the clump. Alternatively, one can interpret the deformation as an internal one due to, for example, leader cells whose polymerization of the cytoskeleton is in an outward direction much like a flexocytes model with internal structure [42]. Finally, these boundary vertices do not exhibit thermal-like fluctuations in their motion.

A rigidity transition in bulk. Prior studies have found a density-independent phase transition in two-dimensional vertex models as well as a three-dimensional Voronoi model with the same form of the energy functional [23, 36]. Is there then a similar transition in the three-dimensional vertex model presented here? To determine whether or not there is a transition, one can look at energy barriers to neighbor exchanges. In this approach, one can determine a necessary condition for cellular rearrangement in two dimensions. For cells with area set to unity, for example, having a target shape index that is regular pentagon involves no energy cost for 4-point vertex to emerge—the geometry at which the cells perform a neighbor swap. Alternatively, one can measure the shear modulus of the system [23, 36]. Since we are implementing a dynamical approach, with each vertex undergoing Brownian motion in a many-body, cell-cell interaction potential, one can also determine whether or not the system is a fluid or solid by looking at the mean-squared displacement of cells.
However, it is sometimes difficult to pinpoint the transition point given that the crossover time to a caging can be rather long \cite{24}.

Instead, we use the concept of an overlap function, combined with trajectories, to determine whether or not the cells remain localized or not \cite{43}. In particular, $Q_n$ is defined as

$$Q_n(t) = \frac{1}{N} \sum_{j=1}^{N} w,$$

where $w = 0$ if a cell has lost two or more neighbors and $w = 1$ otherwise. Should the system be solid-like and so the cells do not change neighbors, then $Q_n(t) = 1$. Should the system become more fluid-like and so cells do change neighbors, then $Q_n(t) < 1$. The smaller $Q_n(t)$ becomes, the more cells change neighbors. We must also couple this measurement with observations of the trajectories of cells since the changing of neighbors could result in some localized movement, i.e., caging, as opposed to system-spanning trajectories, which are indicative of something more akin to a fluid, as opposed to a glassy state. Note that we have not yet incorporated a mechanism to prevent back-and-forth reconnection events. Recent work implements such a mechanism in two dimensions \cite{44, 45}.

We measure $Q_n(t)$ for the bulk system for different target shape parameters, averaging over 20 realizations. See Figure 2(a). We observe that for $s_0 = 5.1$, $Q_n(t) = 1$ and remains at unity throughout the duration of simulation on average. However, for $s_0 = 5.8$, it is clear that $Q_n(t)$ is decaying to a value less than unity. The overlap function is fit to an exponential decay with $\tau_n$ defined as the decay time. In Figure 2(b), we plot the decay time $\tau_n$ as a function of $s_0$. We find that $\tau_n$ approximates zero around $s_0^* = 5.39 \pm 0.01$, which is an estimate for the rigidity transition point since the typical time scale for cellular rearrangements becomes zero. Additionally, some cell trajectories are plotted in Figures 2(c) and (d). Some trajectories span larger than one cell.
FIG. 3. **Boundary-bulk effect with lateral extensile deformation.** (a) Cross-sectional snapshots of the cellular collective before and after the lateral extensile deformation, where the black colored rods indicate the orientation of cells. (b,c,d,e) The correlation function $C(\Delta r)$ as a function of the distance $\Delta r$ between the centers of two cells before and after the lateral extensile deformation for (b) bulk cells with $s_0 = 5.6$; (c) boundary cells with $s_0 = 5.6$; (d) bulk cells with $s_0 = 5.0$; (e) boundary cells with $s_0 = 5.0$.

length, for the larger $s_0$, to indicate that the cells are not caged, at least on that scale, and so not simply switching back and forth between neighbors. We, therefore, find that both solid-like and fluid-like behaviors as a function of $s_0$, suggesting a density-independent rigidity transition in this three-dimensional vertex model. We also study the cell shape index distribution to find that for $s_0 = 5.40$ and above the average of the distribution tracks the target $s_0$ indicating that cells can achieve their target shape while in the fluid-like state, while in the solid-like state, they cannot. See Fig. S1.

**Boundary-bulk morphology with lateral extensile strain** While understanding additional bulk behavior in three dimensions is important, we now focus on a confluent cellular collective, or a clump of cells. As discussed above, we take the initial Voronoi construction (based on randomized points in three dimensions) and then carve out a cluster of cells such that any polyhedron that is
not part of the cellular collective is simply empty space. The faces of cells in contact with empty
have an extra energetic interfacial surface area contribution.

To explore the structure and rheology of the cellular collective as it undergoes a deformation,
we assign the vertices in contact with “empty space” a deterministic velocity $v$ outward along
a particular, uni-axial direction. As these vertices move outward, the structure of the cellular
collective adjusts so that the collective remains confluent. Moreover, we explore whether or not
a collective that begins as a solid with $s_0 = 5.0$ and then becomes a fluid as a result of the
defformation. In other words, how do deformations affect the rheology of the cellular collective?

As for the structure of the cellular collective following the uni-axial, extensile deformation,
we ask whether or not cells align along the uni-axial direction of extension. Prior studies of
epithelial colonies that are uni-axially pulled are able to identify topological defects within a nematic
background [46]. Such defects are recapitulated by a two-dimensional vertex model with additional
polarization terms added to energy functional to account for polarization, and nematic ordering
is present even before the pulling [46]. While we do not observe nematic ordering before the uni-
axial, extensile deformation, we ask whether or not there exists emergent nematic ordering along
the uni-axial direction. To test for this, we fit each polyhedron to a minimal volume ellipsoid and
then determine the orientation of the long axis, dub it a “rod”, and compute the average of the
absolute value of the cosine of the angle between any two rods for any pair of rods $(i, j)$ some
distance between the two centers $r_{ij} \leq \Delta r$, or

$$C_n(\Delta r) = \frac{1}{M} \sum_{r_{ij} \leq \Delta r} \frac{|\vec{d}(\vec{r}) \cdot \vec{d}(\vec{r} + r_{ij}\vec{n})|}{|\vec{d}(\vec{r})||\vec{d}(\vec{r} + r_{ij}\vec{n})|}, \quad (4)$$

where $M$ is the number of pairs and $\vec{n}$ is the three-dimensional unit vector. The correlation function
$C(\Delta r)$ indicates the correlation in orientation between two rods with the centers a distance $r \leq \Delta r$
from each other.

Since the vertices on the edge of the cell collective are different from the bulk cells in that there
is an extra energetic contribution, we separate out the boundary cells from the bulk cells and look
for correlation in alignment between cells in the bulk and between cells on the boundary. For
$s_0 = 5.6$, we find that for bulk cells, the spatial correlation in alignment is rather similar to the
bulk system with periodic boundary conditions (see Figure S2). In other words, we do not observe
nematic ordering. The boundary cells, however, do exhibit ordering with a much higher correlation
in alignment as compared to the bulk cells. Intriguingly, the drop in alignment correlation from
the boundary to the bulk is significant given that the boundary is only one-cell layer thick. Figure
S3 plots $C(\Delta r)$ for the outermost layers and the second outermost layer for additional support of
this statement. In other words, there is a one-cell skin depth phenomenon in which the boundary
cells align with the deformation, but the bulk cells do not. While this may not be unexpected for
a fluid-like system, this difference emerges in the solid-like system with $s_0 = 5.0$ as well, however,
the difference in spatial correlation in alignment amongst cells in the bulk and amongst cells in the
boundary, is not as large. Presumably, this trend is because there are fewer reconnection events
in the solid-like phase, so the system overall is less plastic in the presence of the deformation and,
therefore, less responsive to the deformation.

What is the mechanism driving this one-cell layer skin depth phenomenon? Earlier two-
dimensional studies found a sharp, but deformable, boundary between two groups of cells dis-
tinguished solely by an interfacial line tension between them [47]. More specifically, a discontinuity
in the restoring force once a cell has “invaded” the territory of the other group of cells was deter-
mined [47]. More recent work with three-dimensional Voronoi models demonstrates that there is an
energetic barrier to destabilizing the interface between two cells types within a layered geometry
and that within a two cell layer geometry, the centers of the cells align [48]. Here, we observe
FIG. 4. **Boundary-bulk morphology with in-plane, radial extension.** (a) Cross-sectional snapshots of the cellular collective before and after the radial extension, where the black colored rods indicate the orientation of cells. (b,c,d,e) The distribution of $\eta_i(r)$ before and after the in-plane, radial extension for (b) bulk cells with $s_0 = 5.6$; (c) boundary cells with $s_0 = 5.6$; (d) bulk cells with $s_0 = 5.0$; (e) boundary cells with $s_0 = 5.0$.

that the boundary cells are rather responsive to the deformation along with smoothly connected boundary faces given the interfacial surface tension. However, their inner faces can take on a more jagged surface in the absence of an inner interfacial surface tension between the boundary cells and the second inner layer of cells. With this more jagged surface, the interior cells remain insulated from deformations at the surface. Note that this behavior is also distinct from earlier work in which a bulk two-dimensional vertex model undergoes pure shear, which results in alignment of the cells [49]. Here, we allow the faces of the system to deform such the overall cellular collective shape is not constrained and includes curvature such that the cells take on a more varied zoology of shapes beyond the one-sided right prism [48]. Moreover, the difference in patterning between the boundary cells and the bulks cells persists for faster deformations and for smaller interfacial surface tensions, though the difference decreases particularly for the fluid-like case.

As for the rheology in response to the deformation, we find the distribution of the shape index
FIG. 5. Rheology in response to deformation. (a) Snapshots of the cellular collective before and after the lateral and radial extension, where the black colored rods indicate the orientation of cells. (b,c) The overlap function $Q_n$ of the bulk cells as a function of simulation time with $s_0 = 5.0, 5.6$, before and after (b) lateral extension and (c) radial extension. The cell shape index for the boundary cells becomes more broad than for the fluid-like case as the bulk cannot reorganize in response to the deformation, and so the boundary cells remodel even more so in response. In both cases, the enhancement of the cell shape index, particularly in the boundary cells, is due to an increase in surface area and a decrease in volume. Variations in the relative stiffnesses of $K_A$ and $K_V$ will modulate this response with a larger $K_V$ resulting in more changes in surface area than in volume of the boundary cells.

One might initially surmise that with the increase in the individual cell shape index, the cell collective becomes more fluid-like in response to the deformation in both cases. However, with more of the changes occurring in the boundary cells, some of which were initially bulk cells at the start of the deformation, it is not clear. Measurements of the overlap function indicate that for $s_0 = 5.6$, the cellular collective does not become more fluid-like with similar decay of the overlap function $Q_n$ (as a function of time) before and after the deformation. See Figures 5(a) and (b). Moreover, for $s_0 = 5.0$, there also does not appear to be enhanced fluidization of the system. This finding is consistent with the bulk of the cells remaining insulated from the deformations at the boundary. However, it would be interesting to determine if the shear thinning observed in a bulk two-dimensional system [50] occurs here.

Boundary-bulk effects with in-plane, radial extensile strain Now that we have analyzed the confluent cellular collective’s response to lateral, extensile strain, we explore its response to
in-plane, radial extensile strain. Given the sharp boundary patterning in the prior case, we expect the bulk cells to neither align in some arbitrary direction. We find that the nematic alignment correlation function for the bulk cells behaves similarly to the lateral extensile deformation. In other words, there is little alignment in the bulk. Given the radial geometry, however, one may anticipate other types of structural ordering, such as those observed in liquid crystals. For instance, splay, or $S_i(r) = \frac{\|
abla \cdot \vec{d}_i(r)\|^2}{|\vec{d}_i(r)|^2}$, may be relevant. Again, the denominator is present since $\vec{d}_i(r)$ differs in length from cell to cell. While there is very recent elegant work quantifying two-dimensional vertex models as $p$-actic liquid crystals [28], we will take a simpler approach. We compute for each cell,

$$\eta_i(r) = |\cos(\theta_i(r))| = \frac{|\vec{n}_r \cdot \vec{d}_i(r)|}{|\vec{d}_i(r)|}. \quad (5)$$

and plot the distribution of angles $|\cos(\theta_i(r))|$. Should $|\cos(\theta_i(r))| \approx 0$ then the cells exhibit more curl than splay; should $|\cos(\theta_i(r))| \approx 1$, then the cells exhibit more splay than curl. Note that it is easier to compute $|\theta_i(r)|$ than the curl due to numerical inaccuracies. See Figure 4. We find that before the deformation, splay is maximized at the boundary for both $s_0 = 5.6$ and $s_0 = 5.0$. However, after the deformation, the boundary cells are oriented circumferentially to exhibit curl. On the other hand, the orientation of the bulk with respect to the radial direction does not change significantly. So, again, with this alternate type of deformation, we observe a boundary-bulk patterning. Note that the patterning is different than those observed in quasi-two-dimensional brain organoids, where the boundary cells were oriented radially [5]. Bulk cells are indeed moving to the boundary as the deformation occurs, but given the constraint that reconnection events do not occur with more than one empty cell and the deformation rate, bulk cells are not moving to the boundary fast enough. Should more bulk cells move to the boundary during the deformation, we hypothesize that the boundary cells may become oriented radially. In addition, the boundary-bulk patterning persists for faster deformation rates, while smaller interfacial surface tensions, it is not as robust.

We also investigate how the confluent cellular collective rheology is in response to the in-plane, radial extensile deformation. We observe similar trends as in the lateral extension case in which the solid-like system remains solid-like in terms of the overlap function after the extension. However, for the fluid-like case, the system becomes more solid-like. Intriguingly, this result appears to be inline with earlier work demonstrating compression-induced fluidization such that one might expect extension-induced solidification [41]. Presumably, the bulk cells in the cortex-core structure for the quasi-two-dimensional brain organoids are also somewhat solid-like [5]. Changes in the cell shape index distribution before and after the deformation appear to be similar to the lateral extension, despite the change in rheology in the fluid-like case to become more solid-like. See Figure S6. Again, much of the changes in shape index are focused at the boundary of the confluent cellular collection.

**Discussion**

We construct and study a three-dimensional vertex model with a quadratic energy functional in terms of a target surface area and a target volume using over-damped Brownian dynamics simulations. In doing so, we uncover a rigidity transition in the bulk system with periodic boundary conditions. By measuring a discrete neighbor overlap function, we determine the transition location to occur at a target shape index of $s_0^* = 5.39 \pm 0.01$, for the system sizes and temperatures studied. This transition location is slightly lower than the location of the rigidity transition observed in a three-dimensional Voronoi version, where the degrees of freedom are assigned to the cell centers, using energy minimization [36]. Density-independent fluidization transition occurs as isotropic contractility decreases so that anisotropic contractility via stress fibers may ultimately drive cell
motion. Such an effect in single cells has been recently emphasized \cite{39} and is likely to occur at the multi-cellular level \cite{51}.

We have also gone beyond a bulk system to examine confluent cellular collectives and their response to deformations. We indeed observe vestiges of the rigidity transition in the confluent cellular collective case. For both lateral and in-plane radial extension, we observe larger changes in the patterning before and after the deformation for the fluid case. Specifically, the change in the nematic-like, or curl-like, alignment, of the boundary cells is greater in the fluid case. Specifically, for the lateral extension, the cells align with the direction of the extension, while for in-plane radial extension, the boundary cells align perpendicularly to the radial direction. Importantly, there is a significant difference between the bulk cells and the boundary cells in terms of arrangement for both types of deformations. In particular, the bulk cells resemble the cells in the bulk system with periodic boundary conditions, as if there were no boundary deformation at all.

The stark difference between the bulk and boundary cells is a phenomenon that cannot be readily captured in a continuum model. And while there are other ways to depict three-dimensional cellular collectives, such as cellular Potts model \cite{52} or a three-dimensional Voronoi model \cite{36, 53}, the three-dimensional vertex model is our model of choice. The bulk-boundary patterning is a consequence of the absence of any interfacial surface tension at the inner faces of the boundary cells. Moreover, the overall shape of the confluent cellular collective is deformable. With these features, the cells can take on a more varied zoology of shapes beyond right prisms observed a two-layered, two cell-type Voronoi model with interfacial surface tension between the two cell types \cite{48}. In terms of timescales, at least over time scales larger than the time scale for cellular rearrangements, the boundary cells are indeed insulating the bulk cells from the boundary deformation as the confluent cellular collective deforms. Over shorter times, the shape of the cells mimic the boundary deformation, just as an elastic solid. One can therefore observe in a developmental system, for instance, different regimes of deformations at the cell scale in Drosophila epithelial morphogenesis \cite{54}.

The single-cell-layer thick boundary effects provide a mechanism for patterning with a confluent cellular collective. For instance, quasi-two-dimensional brain organoids have a cortex that is approximately one-cell layer thick \cite{5}. And while in our system, the cells orient circumferentially at the boundary after in-plane, radial extension, modulation of strain rate and constraints on the reconnection events may effect the orientation of the cells at the boundary. We leave this for future work. If we can understand the mechanisms behind structure formation in organoids, more generally, we can better control their formation so that it becomes more “deterministic”. This cell-based approach can then ultimately be used to recapitulate more complex organoid structures, such an octopus-like brain with a core brain and its eight mini-brains \cite{55}. While evolution has selected for structures that optimize for specific functions given physical constraints, we can ultimately design brain organoid morphologies, for example, that will allow us to probe the intricate structure-function relationship at the multi-cellular scale.

Even with this simple confluent cellular collective model that does not investigate additional types of interactions between cells such as polarized tension \cite{33, 46}, we observe nontrivial phenomenon. It would be interesting to include cell growth, the opening of holes \cite{27}, polarized interactions between cells \cite{33, 46}, and strain-dependent tension remodeling \cite{56, 57}. Moreover, with cell growth in a confined, but deformable, environment, such as Matrigel, compressive deformations also may arise. But before building too complex a model, which is typically done to recapitulate experiments, we must first understand the most minimal models at hand, as they themselves can exhibit rich behaviors that the biology has discovered long before our own brains have.

Methods
Simulations:

**Bulk case:** An initial state is created using a three-dimensional Voronoi tessellation given randomized cell centers and assuming periodic boundary conditions. The vertices, edges, and faces of each cell are then defined. To compute the force on each vertex due to the energetic contributions in Eq. 1, each polygonal face that has four or more edges is broken up into radially arranged triangles composed of each edge and the center point of the polygonal face $r^p_i$. The pressure of each cell due to the volume term and the tension of each triangular face due to the area term in Eq. 1 are computed. The force on each triangular face is determined by multiplying the pressure with the surface area and multiplying the tension with the edge length. This force is then redistributed to the vertices making up each face. This method was implemented, as opposed to computing the partial derivatives $F_i = \nabla_i E$ of the energy $E$ with respect to the positions of each vertex given the computational efficiency of this latter method and have checked that the two calculations are equivalent. Once the forces are known, the positions of the vertices are updated using Eq. 2 that includes a random thermal force $F^B_i$. The simulation timestep is referred to as $d\tau$. The system is equilibrated for a time period $\Delta t = 10000$ before collecting data for the overlap function plots.

As for how cells exchange neighbors via a reconnection event, following Okuda and company, we focus on edges going to triangles and vice versa. See Figure 1(c). To determine if a reconnection event occurs, we look for edges with lengths less than $l_{th}$ and triangles with all three edge lengths less than $l_{th}$. If there are indeed such edges or triangles, we choose one of the edges randomly and perform an edge-to-triangle reconnection event in which the edge vanishes and is replaced by a triangle whose normal vector is parallel to the initial edge, or choose one of the triangles randomly and perform a triangle-to-edge reconnection event, should the following conditions introduced in the work of Okuda and company be met to ensure whether or not a reconnection event is physically plausible. The *first condition* is that the change in energy before and after the reconnection event should be in the order of $l_{th}$. The *second condition* relates to resolving the topological irreversibility and satisfies the following sub-conditions: (1) two edges do not share two vertices simultaneously, (2) two polygonal faces do not share two or more edges simultaneously, and (3) two polyhedral cells do not share two or more polygonal faces simultaneously. The third sub-condition is implemented given the computational efficiency and was not discussed explicitly in the work of Okuda and company.

After every ten time steps in the MD simulation are completed, all the edges and triangles that can undergo a reconnection event, given the above conditions, are tagged. First, reconnection events from edge to triangle are performed sequentially. Second, reconnection events from triangle to edge are performed sequentially. We allow for the possibility that some edges and triangles initially tagged can become untagged as the reconnection events occur. Edges and triangles are only subtracted from the tagged list and not added to it.

**Data availability**
The data that support the findings of this study are available upon request.

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**Author contributions**
TZ and JMS conceived the study; TZ wrote the code and conducted the simulations; TZ and JMS interpreted the results and wrote the paper.

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**Competing interests**
Diffusion constant \( D \) 1
Thermal energy \( k_B T \) 10\(^{-4}\)
Simulation timestep \( d\tau \) 0.005
Cell area stiffness \( K_A \) 1
Cell volume stiffness \( K_V \) 10
Cell target volume \( V_0 \) 1
Cell target surface area \( s_0 \) 5.0-5.8
Boundary cell surface tension stiffness \( \gamma \) 1
Boundary cell extension speed \( v \) 10\(^{-4}\)
Reconnection event threshold edge length \( l_{th} \) 0.02
Number of bulk cells \( N_B \) 512
Number of cellular collective cells \( N \) 400
Damping \( \xi \) 1
Number of realizations \( N_R \) 20
Maximum strain of lateral extension \( \epsilon_l \) 122%
Maximum strain of radial extension \( \epsilon_r \) 54%
Long axis sample length after lateral extension \( L_l \) 20
Inplane sample diameter after radial extension \( L_r \) 14

TABLE I. Table of the parameters used in the simulations.

The authors declare no competing financial or non-financial interests.
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Boundary-bulk patterning in three-dimensional confluent cellular tissues
Supplementary Material

FIG. S1. Shape index distribution for the bulk system for different target shape indices.

FIG. S2. Alignment correlation function for bulk cells. The correlation function $C(\Delta r)$ as a function of the distance $\Delta r$ between the centers of two cells for the bulk system with periodic boundary conditions after equilibrium over time period $\Delta t = 110000$ for (a) $s_0 = 5.3$ and (b) $s_0 = 5.6$. 
FIG. S3. Alignment correlation function for outermost layer and second outer layer. The correlation function \( C(\Delta r) \) as a function of the distance \( \Delta r \) between the centers of two cells before and after the lateral extension for (a) second outer layer cells with \( s_0 = 5.6 \); (b) outermost layer cells with \( s_0 = 5.6 \); (c) second outer layer cells with \( s_0 = 5.0 \); (d) outermost layer cells with \( s_0 = 5.0 \).

FIG. S4. Alignment correlation function with high lateral, tensile deformation speed \( v = 10^{-3} \). The correlation function \( C(\Delta r) \) as a function of the distance \( \Delta r \) between the centers of two cells before and after the lateral extension for (a) bulk cells with \( s_0 = 5.6 \); (b) boundary cells with \( s_0 = 5.6 \); (c) bulk cells with \( s_0 = 5.0 \); (d) boundary cells with \( s_0 = 5.0 \).
FIG. S5. Cell shape index and volume distributions before and after the uni-axial, extensile deformation.
\[ s_0 = 5.6 \]

\[ s_0 = 5.0 \]

FIG. S6. Cell shape index and volume distributions before and after the radial, extensile deformation.
FIG. S7. Overlap function $Q$ for different values of $s_0$ and decay time $\tau$ as a function of $s_0$, where (a, b): $w = 0$ if a cell has lost three or more neighbors or (c, d): $w = 0$ if a cell has lost four or more neighbors.
MOVIE S1. The lateral, extensile deformation of the cellular collective with $s_0 = 5.6$, where the black colored rods indicate the orientation of cells.

MOVIE S2. The in-plane, radial, extensile deformation of the cellular collective with $s_0 = 5.6$, where the black colored rods indicate the orientation of cells.