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Learning the mechanobiology of development from gastruloids

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Gastruloids acquire their organization and shape through cell biochemical and mechanical activities. Such activities determine the physical forces and changes in material properties that transform simple spherical aggregates into organized tissues. In this Perspective, we discuss why the concepts and approaches of mechanobiology, a discipline that focuses on cell and tissue mechanics and its contribution to the organization and functions of living systems, are essential to the gastruloid field and, in turn, what gastruloids may teach us about mechanobiology.

- Gastruloid organization relies on cell biochemical and mechanical activities that drive physical forces and material property changes, shaping simple aggregates into organized tissues.
- Mechanobiology concepts and approaches are essential for understanding self-organized systems such as gastruloids.
- Gastruloids offer insights into how mechanobiology influences tissue organization and differentiation during development.

Gastruloids are self-organized multicellular systems that recapitulate early events of mammal embryogenesis. They are obtained from small groups of mouse, human, or other mammalian embryonic or induced pluripotent stem cells (a few 100s) that aggregate, divide, rearrange, and differentiate to give rise to the different germ layers and the outline of body axes [1]. As they are self-organized, they are ideal systems for studying the emergence of tissue patterns and morphogenetic movements from basic rules of cell-cell interactions. Over the last two decades,
the field of mechanobiology has enlightened how forces applied to different cell components  
(cytoskeleton, membrane, nuclei, junctions) feedback on biochemical signaling thanks to state-of-the-art stress generation and measurement techniques. Over the last decade, powerful  
genomic approaches have identified multiple signaling pathways and differentiation trajectories  
involved in the patterning and morphogenesis of gastruloids. We are thus at an exciting and  
challenging time where the combination of imaging and mechanical measurements could unravel  
how mechanics contributes to patterning and morphogenesis in complex 3D multicellular  
systems. In this perspective, we highlight recent findings on gastruloids mechanobiology and  
what we can learn about the mechanobiology of development from such systems.  
The transformation of 3D cellular spherical aggregates of pluripotent cells into elongated and  
patterned gastruloids can be separated into two stages: (1) symmetry breaking, defined as the  
loss of radial symmetry and the emergence of a primary axis characterized by the polarized  
expression of the mesoderm marker T/Brachyury (T/Bra) and (2) axis elongation and  
morphogenesis (See Figure (a)). Mechanics is involved at various levels during these two stages.  
Symmetry breaking results from signaling mechanisms relayed and amplified by changes in cell  
behaviors, including cell sorting and collective flows. Cell surface mechanics is essential in this  
response. We will describe recent evidence of the contribution of these mesoscopic mechanisms  
to symmetry breaking and early elongation. Zooming to the subcellular scale, we will examine  
how nuclear mechanics might couple stress and differentiation in gastruloids when they undergo  
patterning and morphogenesis. Finally, at the tissue scale, we will discuss how global external  
mechanical cues could impact axis formation, elongation, and morphogenesis.  

**Sorting between cell populations.** We highlight here several physical mechanisms, including local  
cell-cell mechanical interactions and global mechanical cues (e.g. tensile, shear stress or tissue  
stiffness), that guide gastruloid organization. Recent works have shown that cell-cell adhesion  
and cell contractility differences control local cell sorting in embryonic organoids [2]. Differences  
in adhesive and contractile properties result in differences in surface and interfacial tensions  
between cell populations that determine the extent of contact between tissues and their relative  
positions [3]. In gastruloids cultured in neural differentiation (NDiff) medium complemented with
fibroblast growth factor (FGF) and activin, E-cadherin based-adhesion maintains highly compact and cohesive cell clusters that promote the formation of an endoderm-like region (Figure (a)) [4]. Complementary to endoderm, mesoderm-like regions in these gastruloids lack E-cadherin and express both Snail1, N-Cadherin and OB-Cadherin [5]. Similarly, in the standard gastruloid protocol (NDiff medium), E-cadherin is observed in specific regions, while complementary regions express Snail1 and other cadherins [6–8]. Such observations and other recent reports in vivo [9] suggest the existence of a cell type-specific cadherin code that would ensure sorting [10] (Figure (b)).

In vitro observations show that T-Bra cells are highly motile compared to pluripotent cells [11]. During chick presomitic mesoderm formation, high levels of T-Bra confer high motility to cells, enabling them to separate from less motile cells (Figure (b)) [12]. These in vitro and in vivo observations suggest that motility-based sorting could be an alternative mechanism to differential adhesion- or contractility-based sorting to explain the segregation of T/Bra expressing cells at the posterior pole of gastruloids. Identifying the cell mechanisms (adhesion, contractility, motility) that underlie gastruloid organization will require biophysical and live imaging approaches. A critical step will be determining differences in surface and interfacial tensions associated with distinct cell populations. Gastruloids undergo rapid cellular growth and division. Thus, they are interesting systems to investigate the interplay between cell sorting mechanisms and growth —whether they exhibit competitive or convergent dynamics.

**Signaling and cell surface mechanics.** In the previous section, we explored how differences in adhesion and contractility can contribute to surface and interfacial tension differences, shaping the spatial arrangement of distinct cell populations. However, to be sorted, the cells must first exhibit differences in biochemical and mechanical states, which change during gastruloid organization. In gastruloids, a pulse of Chiron, a Wnt agonist, usually triggers differentiation and leads to heterogeneities in T/Bra and adhesion levels. The activation of T/Bra expression by Wnt (and Nodal) leads to biophysical changes [13, 14], which can potentially initiate the process of cell sorting effectively. E-Cadherin antagonizes Wnt signaling via the binding and sequestration of β-catenin [15]. There is growing evidence that changes in cell surface mechanics impact early
differentiation. In 2D micropattern colonies, cells at the peripheries have fewer neighboring cells than those inside and, thus, fewer E-cadherin junctions. This pattern in E-Cad mediates a wave of epithelial-to-mesenchymal transition (EMT) from the boundary to the center of colonies [16] (Figure (c)). Disruption of epithelial integrity by downregulation of GLYPICAN-4 locally activates signaling and EMT [17]. Similar mechanisms might be at play in 3D gastruloids (Figure (c)). The stabilization of cell-cell adhesion by the glucocorticoid budesonide was shown to prevent symmetry breaking [18]. E-cadherin mediated junctions might be critical to maintaining clusters of pluripotent cells in growing aggregates by counteracting the exit from pluripotency while ensuring cohesiveness between cells. We envision that mapping cell packing (e.g. volume fraction of the extracellular space) heterogeneities during the Chiron pulse will be instrumental to understanding how cell surface accessibility impacts differentiation wave propagation.

During gastruloid symmetry breaking and elongation, tissue collective flows emerge and generate shear stresses on cells (Figure (a))[4, 5]. How these stresses impact cell shape will depend on the cell's ability to exchange neighbors, modulated by cell-cell adhesion, packing and fluctuations. However, the coupling between cell geometry and differentiation is not trivial. De Belly et al. [19] have shown that cells cultured on 2D substrates exiting pluripotency spread more and have lower membrane tension than pluripotent cells. Modulating membrane tension can alter cell differentiation in both 2D cell cultures and gastruloids. Nevertheless, cell differentiation is triggered by membrane tension decrease, which promotes endocytosis and, consequently, extracellular signal-regulated kinases (ERK) activation, rather than by cell shape changes themselves. How the mechanics of cellular elements (cell membrane, cortex or nuclei) affect gastruloids morphogenesis is still unexplored.

**Nuclear mechanotransduction.** After highlighting the importance of cell surface mechanics, we now delve into nuclear mechanics and its relationship to cell differentiation. Strikingly in gastruloid tissues, (i) cells’ volumes are mainly occupied by their nuclei (volumetric occupation going from 50 to 90% for nuclei), and (ii) nuclei deform strongly and dynamically (Figure (d)). Consequently, a natural question is to which extent nuclei undergo deformations and mechanical
stresses affect cellular differentiation. Over the last few years, the relationship between cell
differentiation status, nuclear envelope structure, and chromatin state has been explored in
various *in vitro* systems, which provides valuable information for the gastruloid field.

Heo and colleagues [20] discovered that during differentiation, human Mesenchymal
stem cell (MSC) nuclei become stiffer due to lamin A/C relocation and increased
heterochromatin-rich nodules. Stiffer nuclei in differentiated cells focus deformation and stress
on nucleo-cytoskeletal and cytoskeletal linkages, possibly enhancing responses to external
mechanical stress. Softer nuclei in undifferentiated cells absorb stress, preventing differentiation
from mechanical cues. Dynamic mechanical loading alone triggers nuclear remodeling and
differentiation. Nava and colleagues [21] found that cyclically stretched human epidermal
stem/progenitor cells experience short-term heterochromatin transition, causing calcium-
dependent nuclear softening and long-term tissue junction and cytoskeletal changes to prevent
genomic damage.

These observations are likely relevant for gastruloids (Figure (d)). More importantly,
gastruloids are complex 3D tissues showing different differentiation states and thus offer a
platform to analyze how the coupling between stress and differentiation through nuclear
mechanics drives patterning and morphogenesis. Recent scATAC-seq data suggest that cell
differentiation heterogeneities in gastruloids are associated with early changes in chromatin
state and NE cell-cell heterogeneities can be observed [22]. Measurements on gastruloids
combining live imaging of nuclear markers (lamins and histones) with controlled deformations
will be a critical step to go beyond the study of 2D epithelium under stretch. Moving from nuclear
mechanotransduction and its role in cell differentiation, we now focus on the impact of external
mechanical constraints on gastruloid development.

**External mechanical constraints.** There is growing evidence that tissue-tissue mechanical
coupling [23, 24] and tissue-scale gradients in mechanical properties [25, 26] are critically players
in embryo morphogenesis. How ectodermal, endodermal and mesodermal tissues mechanically
interact during gastruloids elongation and morphogenesis remains to be explored (Figure (e)).
Beyond that, how tissue-scale mechanical constraints modulate cell differentiation needs to be taken into account in this system. Indeed, while gastruloids free in solution form an axis, there is evidence that the contact points of gastruloids with their environment influence the expression of T/Bra [27]. Using 2D ellipsoidal micropatterns, Blin et al. [28] have shown that T/Bra+ cells tend to localize in high curvature regions, although the mechanism is not fully understood. Similarly, Muncie et al. [29] have shown that specific tissue geometries (e.g., corners of triangular patterns) induce localization of high tension that promotes nascent mesoderm (marked by T/Bra). These reports suggest that geometry is central in coupling cell signaling and mechanics.

Early mammalian embryogenesis occurs in confined environments in strong contact with extraembryonic tissues. Hiramatsu and colleagues [30] have shown that confining post-implantation mouse embryos in microchannels can control the location of the distal visceral endoderm and therefore reorient the anteroposterior axis. Manipulating parameters within the in vivo environment is especially limited in mammalian embryos, owing to their inaccessibility at early stages of development. This can be envisioned for gastruloids. Several reports have shown that gastruloids embedded in low-concentration Matrigel generate somite-like and neural tube structures [31, 32] (Figure (a,e)). It will be essential to develop synthetic approaches enabling the control of the mechanical boundary conditions more systematically, including size, shape, curvature, and the viscoelasticity of the environment. The realm of organoids has made significant progress in offering tools for manipulating the environment, such as the development of matrices that enable precise spatiotemporal regulation of organoid growth, as highlighted in [33]. In the case of gastruloids, which are soft, similar to the early embryos - their in vivo counterparts, we need to address distinctive challenges: soft viscoelastic matrices might allow gastruloid growth while preventing detrimental stress accumulation, which could lead to apoptosis and necrosis.

In this perspective, we focus on gastruloids, mainly because there is a wealth of literature on them and they showcase advanced morphogenetic processes such as dramatic anisotropic shape changes. However, we expect that investigating the mechanical coupling between gastruloids
and mechanically and chemically controlled environments will give crucial insights and a better intuitive grasp to comprehend more complex systems derived from embryonic and extraembryonic stem cells.

In conclusion, gastruloids, as self-organized multicellular systems mimicking early mammalian embryogenesis, offer a promising platform for studying tissue patterns and morphogenetic movements. While recent advancements in mechanobiology can benefit the understanding of symmetry breaking and morphogenesis of gastruloids, and, thus, of mammalian development, challenges exist as gastruloids have 3D complex organization and are highly dynamic. Firstly, advanced imaging and analysis techniques are crucial to capture the dynamic 3D organization of gastruloids, shedding light on the intricate coupling between cell behaviors and differentiation. Moreover, controlling the environment poses a significant technological challenge, necessitating precise manipulation of size, shape, curvature, and viscoelasticity to study the impact of external mechanical constraints systematically. Furthermore, measuring the mechanics and fate of individual or groups of cells within gastruloids demands cutting-edge methodologies, enabling researchers to probe the mechanical stresses and deformations experienced by nuclei and other cellular components. Overcoming these technological hurdles will enhance our understanding of gastruloid mechanics and pave the way for unraveling the complexity of mammalian development.

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**Figure legend**: The different facets of gastruloids mechanobiology

Typical evolution of a gastruloid other several days from its initial aggregation (0 hours post-formation (hpf)) to its morphogenesis (several days after formation). The gastruloid undergoes over the three first days a self-organization phase called symmetry breaking, where it transforms from a spherical aggregate of pluripotent stem cells to a spatially organized 3D tissue made of different germ layers. T/Bra gene expression (in green) appears first in the aggregate outer layers to propagate deeper in the tissue later. Between 60 hpf and 80 hpf, the emergence of a T/Bra tissue scale gradient is concomitant with the beginning of gastruloid elongation. After 80 hpf, a pole of cells can maintain T/Bra expression while the rest of tissues differentiate further in either epithelial, endodermal, or mesodermal tissues. In the days following symmetry breaking, gastruloids continue to elongate and undergo morphogenesis; their
elongation can be enhanced by embedding them in Matrigel. During symmetry breaking, heterogeneities in cell-cell adhesion, tissue packing and cell differentiation emerge. Tissue flows (collective cell movements) and cell deformation patterns can be quantified during the elongation and morphogenesis phases (b) Symmetry breaking is associated with Epithelial to Mesenchymal transitions. Cell sorting and tissue phase separation can be driven either by differential adhesion/contractility or differential cell motility. (c) In 2D circular micropatterned embryonic stem cell colonies, concentric cell differentiation arises from enhanced cellular receptor availability at the colony edges. However, in the 3D environment of gastruloids, the organization of cellular receptors at cell population interfaces and how their accessibility is influenced by local tissue packing remains unknown (d) During early phases of gastruloids development and symmetry breaking, cell nuclei occupy a large portion of cell volume (between 50 and 85%). Furthermore, nuclear lamins levels are highly modulated by cell differentiation stage within a single gastruloid (personal communication) (e) During gastruloids elongation, both epithelial and mesenchymal tissues are in mechanical contact. Both elongation and somitogenesis are enhanced when gastruloids are grown in Matrigel.

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