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Computational modeling of organoid development

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Abstract

Organoid developmental processes encompass the coordination of multicellular communication to achieve physiological functionality. Questions regarding the hierarchy of communication mechanisms and unknown contributions to variability in successful growth and maturation makes the organoid systems ideal for computational analysis and optimization. Until recently, progress has been hampered by the limitations of computational tools to handle highly complex networks. Advances in experimental tools for characterization, live-cell monitoring, and environmental manipulation are timely for enhancing spatiotemporal predictions of organoid properties. We discuss recent approaches to address these challenges with novel computational methods and biophysical principles that have yielded insights into the emergence of structure and spatial organization. Computational organoid modeling across multiple levels of emergence offers promising potential for understanding, improving, and designing ab initio multicellular engineered systems.

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Multicellular systems, Computational methods, Organoids.

Introduction

Recent developments in tissue engineering through control and manipulation of the extracellular environment and/or cell–cell communication have yielded success in producing organoids. These functional microtissues provide attractive platforms for iPSC-derived organ-on-chip screening, interrogation of developmental principles of organization and lineage specification, and may provide a springboard for future engineering of at-scale organs from modular components. To date, the strategies for yielding self-assembled organoids from pluripotent precursors have not been guided by computational prediction or design. Simulation of complex behavior arising in multicellular constructs could provide critical insight in improving reproducibility [1] or guidance toward desired form and function [2] within an experimentally insurmountable search space. Engineering of inducible transcription factors, precise morphogen presentation, and novel biomaterial surfaces provide examples of the numerous potential experimental factors available for deriving organoid systems (see Figure 1).

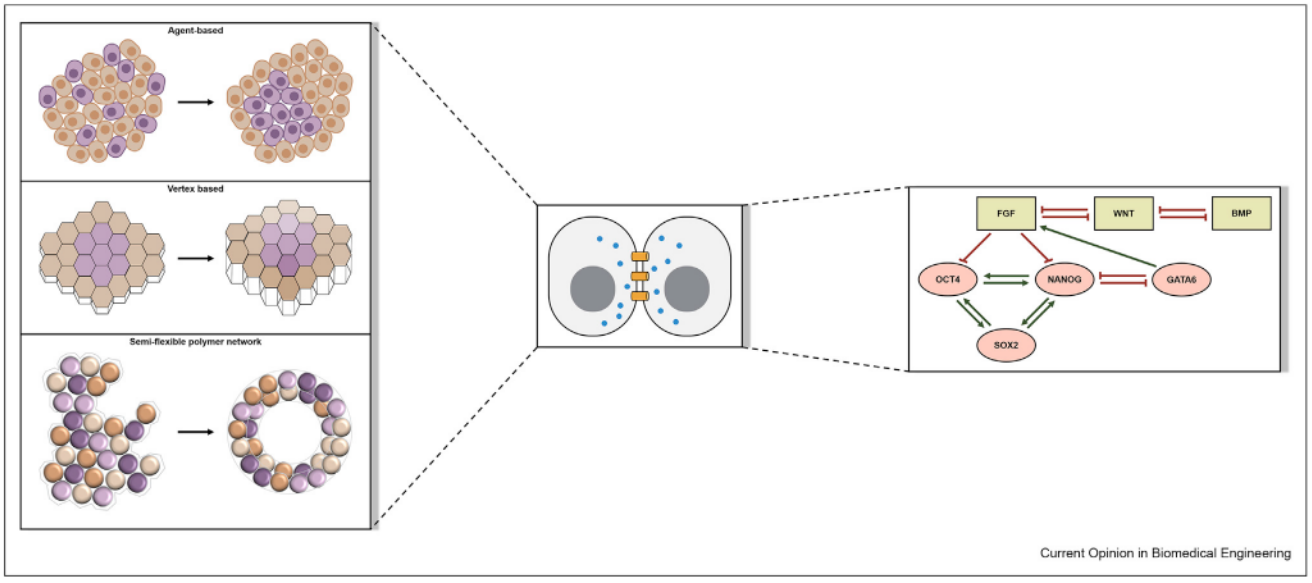
Modeling two-dimensional cultures vs. three-dimensional organoids

Multiple cell types undergo proximal (direct neighbor) and distal interactions from the diffusion of morphogens across tissue scales that affect cell fate decisions. While morphogen reaction-diffusion has long attracted mathematical analysis, other drivers of differentiation, such as mechanosensing, membrane voltage, or gap junction communication, can be interrogated through simulations that uncouple these mechanisms from other environmental cues. Bioelectric gradients can predispose the development of multicellular oscillations or ensemble level behavior within non-excitable tissues [3]. Dynamic gap junction-based transport networks as a function of asynchronous cell cycling have been attributed to multicellular patterning by creating an intercellular flow of small molecules [4]. Each of these modeling examples explored fundamental mechanisms of cell–cell communication by limiting the scope of the simulations to a single mode of signaling.

While the above examples are useful for computational modeling and testing properties of spatial organization or symmetry breaking within a population, a challenge to computationally addressing organoid development lies in its intrinsic complexity, which 2-dimensional systems lack. Furthermore, organoid development naturally is a 3-dimensional process. Organoid cultures have been enabled by the use of a variety of methods to leverage surface tension and adhesive forces (or lack thereof) to encourage cell–cell interactions. Consequently, the appropriate computational modeling description must reflect these changes in biophysical forces.

Agent-based modeling (ABM) uses autonomous agents to represent cells capable of making independent, context-dependent decisions with respect to their position and state [5]. In agent-based modeling (ABM) of cells attached to a planar surface, a descriptor for colony growth dictates the choice of dividing cells to seek out

Figure 1



From top left to bottom right, the figure is depicting a series of magnifications of a multicellular system, intercellular and intracellular levels. Left: Organoid representation can take many forms with computational multicellular modeling. Agent-based, vertex based, or a semi-flexible polymer network description has utility depending on biological questions addressed. Middle: The large-scale emergent patterns of the multicellular system (hundreds of microns) are critically dependent on the successful completion of cell–level processes and cell–cell interactions (tens of microns). As an example, the cell–cell interaction is depicted to represent the gap–junction communication. Right: Embedding of molecular networks allow feedback into higher macro-level behavior. The scales of consideration and system inter-dependence create unique challenges for computational modeling efforts.

open surface versus piling up on top of each other. These features are observed to different degrees in various phenotypes and are generated by physical confinement (such as micropatterned areas). In contrast, computational models of 3D organoids require that the spheroid volume expands upon cell divisions [6,7], as the addition of new agents dictates a "jostling" effect outward to maintain the neighbor-to-neighbor distances that are a consequence of Hookean forces between cells. Changes in a spring constant parameter can result in the degree of cellular packing that occurs in simulating organoid growth.

An additional feature of 3D computational modeling of organoids—both experimentally and *in silico*—is the introduction of limited of nutrient availability. Glucose, oxygen and other metabolic precursors necessary for anabolism are subjected to reaction-diffusion biotransport across a multicellular construct. Organoid researchers refer to necrotic cores as evidence of these limitations. Simulations with partial differential equations are often appropriate for describing these properties [8]. Future enhancement of computational models will require features that result from the engineering of vascularized organoids. Prior computational methods developed in the cancer biology field for recapitulating tumor angiogenesis [9,10] provide guidance in how these features may be implemented.

Likewise, the accumulation and diffusivity of secreted molecules such as growth factors or chemokines differ in 2D culture versus 3D. For example, reaction-diffusion modeling of BMP4 in 2D culture suggests that hPSC colony organization under micro-patterned geometric constraints perform intrinsic scaling (i.e. holds true across dimensions) and is not specific to the morphogen concentration [11]. This computational model assumes that a fixed concentration of BMP4 is maintained at the periphery of the colony and equivalent to the concentration in the bulk medium. Under 3D conditions, however, internal pockets of high concentrations in intercellular regions/extracellular matrix deposited within an organoid could provide a stable source of localized influence on cell fate decisions. The omission in the model description of ECM and/or the resultant hindered diffusivity in the extracellular spaces could lead to deviation in behavior from experimentally observed properties of organoids. Immersed boundary methods, such as described for interstitial flow and transport, eliminate the description of cells and fluid as a continuum, allowing for locally discontinuous and inhomogenous regimes of concentration [12]. While not yet applied to organoid computational models, this may be a future strategy for evaluating localized accumulation of morphogens, especially in regimes of high convection such as culture under agitation/rotation.

Using computational models to study organoid formation

Unlike the modeling of an early-stage blastocyst, in which each cell division results in daughter cells with prior memory of the parent cell and their position, the seeding of organoids is initialized as a seemingly disorganized group of cells. The delay in differentiation events compared to embryogenesis stages reflects the time required to dynamically coordinate downstream morphogenic fates amongst themselves and the environment. Several studies highlighted here have focused on specific points in the embryonic and organoid development process to computationally model, illuminating the numerous emergent processes that faithfully produce specific cell and organ fates.

After cells form into an aggregate, shape deformations are critical for organoid morphogenesis to yield structures that resemble the organ system of interest. Vertex models have successfully described how spatial patterns of apical cell contractility induce deformations of epithelial shells mathematically and computationally at three dimensions and two dimensions. Hexagonal prism-like cells formed together to simulate epithelial shells, with each cell having a fluid-filled cavity and a solid membrane. Both 3D and 2D vertex models have demonstrated principles of evagination or invagination - either the apical side comprising the inner shell surface or the apical side comprising the outer shell surface [13]. In contrast, agent-based models considering the interactions between different cell types, and their behaviors reflect a variety of phenotypes together in a tissue. Germann et al. developed ya||a (yet another parallel agent-based model) and used concepts of tissue polarity for mesenchymal cells and apical-basal polarity for epithelial cells to describe interactions between epithelial cells and mesenchymal cells and their behaviors [14]. Moreover, to reduce computational costs, the model was developed for operating on graphics processing units (GPUs), which enabled faster large-scaled simulations of morphogenesis. To combine the mechanical properties of each cell and chemical interactions between the cells during morphogenesis, Okuda et al. proposed a 3D vertex model with expressions of intercellular signaling molecules for growth. Upon simulating signal-dependent epithelial morphogenesis, multicellular deformations, and biochemical patterns resulted in four types of 3D morphogenesis—arrest, expansion, invagination, and evagination [15]. In later work, the approach was changed to incorporate a 3D vertex model with a mathematical model of Turing reaction-diffusion dynamics [16]. With a 3D monolayer cellular sheet, the model yielded various morphogenesis, including tubulation, branching, and undulation, and showed diverse morphologies within the same type of tissue depended on different time scales. An unconventional multicellular computational modeling approach is to consider the organoid structure as a semi-flexible polymer network [17]. Simple rules, such as the differentiation of intestinal stem cells into Paneth cells as a function of the local mean curvature, yielded realistic outcomes in intestinal organoid structure and highlighted the role of Wnt/Notch activation in regulating elasticity of cell—cell interactions. The formation of branched versus cyst forms during intestinal organoid development was discovered to be sensitive to a Wnt-dependent elastic modulus parameter. Importantly, identification of differentiation drivers that map to biomechanical outcomes alone was insufficient for capturing complexity in form. Knowledge of an intermediary cell type and spatially constrained clonal expansion of the subpopulation was instrumental in the success of the model predictions.

Understanding developmental processes

Several efforts focusing on cell—cell and cell—environment interaction in the context of organoid development elucidated how molecular regulators of emergence can positively or negatively affect cell fate outcomes, principles that inform fundamentals of developmental biology. We refer the reader to comprehensive reviews [5,18] that cover a variety of developmental systems modeled through agent-based simulations and the utility of this modeling approach for integrating the Turing reaction-diffusion phenomenon with positional information. A multipurpose biomechanical modeling platform to study optic cup morphogenesis relied on biophysical laws of mechanical interactions between cells in geometrically-confined conditions [19]. This computational modeling approach used experimentally derived physical parameter metrics, such as height, volume, curvature, proliferation/apoptosis rate, to parameterize their model, specifically to replicate a self-invagination morphogenic process and subsequent boundary formation between regions of organizing clusters. The authors successfully simulated the dynamic mechanical bending process that dynamically modulates shape outcomes based on individual cell—cell interactions at the regional interface and the robustness of the spatiotemporal outcomes *in vitro*. Other groups have also attempted to create synthetic emergent multicellular structures or organoids, to functionally improve existing methods and predictively control emergent patterning. Structurally, the formation of sheets, tubes, rings, and other macroscopic forms can be guided by modular building with organoid subunits. For example, kinetic Monte Carlo, lattice-based simulations of multiaggregate fusion predicted time-evolved configurations that could arise with cell migration and lumen polarization [20]. The use of this stochastic modeling method could provide distributions of collective spatiotemporal behavior from many simulations; however, this study did not evaluate questions of reproducibility or yield from their initialized structures.

Modeling to inform robust biomanufacturing

The conceptual next step following successful emergent systems control is addressing shortcomings in current biomanufacturing and biotechnological processes, where robust, high yield/high throughput protocols for organoids are needed. Finite element modeling (FEM) is a top-down engineering approach and has been used to model *in vivo* mesenchymal condensation as a multicompartment mesoscale mechanical interaction between components [21]. In this example, the computational model focused on the strain/tensile forces generated by a "single material" mesenchyme and its dynamic effects on ECM biophysical parameters. Despite the usage of minimal parameters to define the behavior of coordinated cell groups, the FEM could still successfully replicate cell interaction and collagen-dependent tensile forces on multiple micro-patterned surfaces. This platform is promising for regenerative medicine manufacturing technologies for predicting which cellular interactions govern invagination upon exposure to different surfaces, as 3D topographies could be used to guide cell growth and mesenchymal self-organization with minimal external guidance. Recently, a Cellular Potts Model (CPM), combined with machine learning and optimization procedures predicted that hiPSCs, engineered with specific genetic perturbations with known biomechanical consequences, follows predictable self-organizational trajectories [22]. Manipulation of CDH1 and ROCK1 led to cell sorting and distinct multicellular organizational features. The data-driven approach mapped specific time-lapsed immunofluorescent images as a training set to determine cell—cell biomechanical interactions and individual cell type-specific parameters in the CPM. Simulations iterated over thousands of solution sets, and an optimization algorithm searched the parameter space for design conditions leading to patterning trajectories. The supervised image classifiers and machine learning rule-based algorithms informed the experimental design process.

Synthetic, novel functionalities

Future organoid synthesis is anticipated to leverage synthetic biology tools that are becoming more widely adopted in human cell biology. Weiss and colleagues reported that synthetic induction of a master differentiation driver resulted in the organization of complex organoid tissues [23]. To achieve the ectopic expression of the transcription factor, GATA-binding protein 6 (GATA6), lentiviral vectors were used to deliver a small gene circuit. Upon doxycycline treatment, variance in transgene expression occurred, resulting in a spectrum of $GATA6^{+}$ progenitors inducing differentiation via complex patterning, ultimately morphing into a liver hindgut organoid. While modeling was not a component of this study, from a computational perspective, this induction strategy provides tight temporal control over state changes, a critical assumption made in many agent-based modeling descriptions.

The Lim lab engineered a novel synthetic gene circuit, capable of eliciting cell—cell communication based on extracellular ligand—ligand interactions and downstream transcription of targeted genes [24]. Programmed synthetic notch signaling and E-cadherin expression within spheroids initiated a multistep reciprocal intercellular communication cascade and resulted in uniform self-organization and cell sorting into geometrically distinct phenotypic subpopulations. Patterning outcomes were completely dependent on the logical design of the genetic components. Again, computational modeling was not a component in the circuit design of this multicellular system, but future simulations of such systems could point to the optimization of the circuit regulation for yielding specific 3D organoids with designed geometries.

Model validation

The utility of computational models is ultimately defined by the extent to which they satisfy initial questions of critical interacting roles of determining morphogenic and functional emergent fates. A number of methods for generating time-resolved experimental datasets and custom image processing algorithms are defining emergent dynamics and confirming simulation outcomes. Time-lapse microscopy of mixed cell populations with fluorescent reporters of the phenotype is non-invasive and ideal for monitoring the evolution of patterns [22,24]. Other validation methods characterize organoids with assays at multiple spatial levels. For example, single-cell biomechanical metrics, such as cell-type dependent strain and matrix curvature induction, were determined using particle image velocimetry and droplet contraction assays for parameterization of FEMs [21]. In bioelectric studies, e.g., Ref. [25], fluorescent confocal microscopy in parallel with whole-cell patch-clamp quantified membrane potential and spatiotemporal spread across multicellular clusters, validated predicted functional features of bioelectric gradients that preceded neural morphogenesis. Furthermore, canonical variate analysis enabled the authors to incorporate unbiased statistical metrics to study differences between control and pathological conditions. Multiple images were selected for biologically relevant morphologic features; the distances between "landmarks" of organ features in tadpoles were quantified by a statistical metric that relates shape differences, Procrustes distances. The resulting scores yielded information on spatial variation across image sets and showed how much a given perturbation changed emergent patterns.

Machine learning approaches are rapidly being adopted to facilitate more challenging validation efforts in models of multicellular systems. In Villoutreix et al.

[22], the authors created a platform that can handle heterogeneous datasets, meaning that multiple target developmental targets (i.e., gene expression, protein localization, and phosphorylation, and tissue morphogenesis) can be defined as state variables assessed in parallel. Merging state variables in a completion matrix allowed *Drosophila* embryo measurements at distinct timepoints helped to test mechanistic models. Machine learning algorithms were used to fill the missing spaces of the matrix and fully define the multivariate trajectories present within that system. Sparse data matrices are a critical problem in multicellular model validation. Data learning techniques can help to address this problem, improving predictive accuracy. Oyetunde et al. [23] used data learning to improve the predictive power of an underdefined mechanistic genome-scale metabolic model. The large solution space satisfying their physiological constraints did not provide enough insight into optimal bioproduction conditions, so the authors utilized data augmentation and stacked regression techniques to artificially expand their dataset amount and variability. This expanded set was used with ensemble learning techniques to determine the best machine learning approach and critical factors influencing bioproduction. The utility of machine learning techniques is not limited to mechanistic models but also can be incorporated to handle stochasticity. For fitting multicellular patterning outcomes from an ABM of mixed iPSC populations [18], machine learning algorithms were employed in image classification efforts and exploration of the possible parameter solution space. Particle swarm optimization helped pinpoint optimal parameter combinations within a defined experimental design space that produced desired patterns. To match desired *in silico* patterns to experimental data, the authors used Tree Spatial Superposition Logic, a quadtree data structure, to capture detailed local and global spatial relationships in an image. Quantitative measurements of image—image similarity were accomplished by employing a rule-based machine learning algorithm (RIPPER) to give similarity scores to image sets, indicating how strongly simulation patterns correspond to experimentally-produced patterns.

Future directions

Simulating and predicting PSC-derived organoids from initial seeding to maturation and organization is still in its infancy. We are witnessing a parallel growth in the sophistication of experimental tools and perturbations that complement the increasingly complex modeling platforms of multicellular systems. Future integration of multiple modes of communication (e.g., biomechanical, biochemical, bioelectrical) are on the horizon and will inform protocols for an organoid generation that mimic normal physiology or engineered systems with synthetic capabilities. Considerations of biophysical forces that control whole-organoid morphology and integration of nutrient transport limitations will likely be necessary to simulate self-assembly and differentiation from a fully pluripotent aggregate to a mature, functional organoid.

Conflict of interest statement

Nothing declared.

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