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SPOTLIGHT

The physics of organoids: a biophysical approach to understanding organogenesis

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ABSTRACT

Organoids representing a diversity of tissues have recently been created, bridging the gap between cell culture and experiments performed *in vivo*. Being small and amenable to continuous monitoring, they offer the opportunity to scrutinize the dynamics of organ development, including the exciting prospect of observing aspects of human embryo development live. From a physicist's perspective, their ability to self-organize – to differentiate and organize cells in space – calls for the identification of the simple rules that underlie this capacity. Organoids provide tractable conditions to investigate the effects of the growth environment, including its molecular composition and mechanical properties, along with the initial conditions such as cell number and type(s). From a theoretical standpoint, different types of *in silico* modeling can complement the measurements performed in organoids to understand the role of chemical diffusion, contact signaling, differential cell adhesion and mechanical controls. Here, we discuss what it means to take a biophysical approach to understanding organogenesis *in vitro* and how we might expect such approaches to develop in the future.

KEY WORDS: Organoids, Organogenesis, Biophysics, Stem cells, Modeling

Introduction

In the era of big data, we are identifying more and more components and interactions in highly complex biological systems. Making sense of this complexity is challenging. Physicists like to use simple models and derive universal laws, which is attractive for a conceptual understanding of biological systems. Making sense of complexity can involve the development of theories that offer explanations for patterns in nature and can be supported or disproven through observation and experimentation. The analysis of reaction-diffusion by Turing is a telling example of how a mathematical theory can move biology forward (Turing, 1952). To support theories and help visualize phenomena, physicists often use models that are representations of a biological system that is too difficult or indeed impossible to display directly.

In recent years, organoids – miniaturized, simplified versions of organs and tissues that are grown *in vitro* – have emerged as a powerful model of bona fide organogenesis. They can be derived from one or a few adult or embryonic tissue cells, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), which are cultured in three dimensions and can self-organize *in vitro* owing to their self-renewal and differentiation capacities (Eiraku et al., 2011; Fatehullah et al., 2016; Greggio et al., 2013; Lancaster et al., 2013; Sato et al., 2009; Spence et al., 2011; Takasato et al., 2015). As with their *in vivo* counterparts, organoids contain multiple cell types organized in structures that resemble the organ of interest and exhibit some of the organ function. Many different types of organoids have been generated, including cerebral, intestinal, kidney and pancreatic organoids (for a review see Huch and Koo, 2015).

From the physicist's perspective, the use of organoids for understanding organogenesis offers several advantages over traditional two-dimensional (2D) culture systems. The fact that some organoids can be initiated from very low numbers of cells makes them most suitable for a physicist to quantitatively define the initial state of the system and the subsequent interactions. Unlike organs in model organisms, organoids are free from the influence of other cells or signals in the body. The culture medium can be designed and modified to either maintain the progenitor – or stem cells – in their initial state or enable scenarios in which a subset of progenitors expands while others differentiate, thereby mimicking the system of a developing embryo (Greggio et al., 2013). The culture medium for growing organoids is relatively simple and well defined compared with the corresponding *in vivo* milieu. That said, the frequent use of serum or Matrigel in growing organoids introduces complexity and batch-to-batch variation. Being *in vitro* systems, organoids often facilitate continuous observation, including imaging, yielding high temporal resolution in the observation of self-organization steps. As compared with traditional 2D *in vitro* culture, organoid conditions enable the maintenance and expansion of many more primary cell types, including stem and progenitor cells, without feeders. Moreover, the 3D growth environment provides the cells with more degrees of freedom. In a successful organoid setup, the group of cells recapitulates many aspects of *in vivo* differentiation – some proliferating, polarizing, adhering to chosen neighbors and forming tubes, pits, bulges or folds.

Despite their advantages, there are some downsides to organoid systems. As with any *in vitro* system, there is a risk that our observations have limited relevance to organ formation or homeostasis. Therefore, it must be noted that organoids cannot replace the study of *in vivo* organogenesis in model organisms. Another drawback is that controlling self-organizing systems is inherently difficult because they develop their own endogenous interactions between elements. Small changes in the initial conditions, such as the initial number of cells, will lead to variability in outcomes between different organoids under the same culture conditions, so efforts should be made to standardize the initial conditions (Todhunter et al., 2015; Ungrin et al., 2012).

The goal of this Spotlight article is to introduce the theoretical, numerical and experimental approaches inspired by physics that can be used to understand the rules of organoid formation and, by

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extension, organogenesis. We discuss how organoid formation relies on the physical phenomenon of self-organization and explore the concept of organogenesis as a non-linear system. We further outline some of the computational approaches that can be used to understand the unique properties of organoids. *In vivo*, developing organs are subject to a variety of different mechanical forces that influence and even control their development and maturation. We discuss how mechanical perturbations of organoids can be used to better understand how these forces shape bona fide organ development. Finally, we provide a brief outlook to the future as we anticipate how the application of biophysical approaches to organoids might enhance our understanding of *in vivo* organogenesis.

The search for simple rules that underlie organ formation

Organoids exhibit the spectacular property of self-organization into ordered structures. Physicists have had a long-standing interest in self-organization, a process whereby order arises out of the local interactions between smaller components of an initially disordered system (Ashby, 1962). Several examples of self-organization in the physics field include crystal formation, the Belousov–Zhabotinsky reaction-diffusion in thermodynamics, and planetary systems formation, to name just a few. There are many examples of self-organization in the biological world too, such as protein folding, bird flock formations and pattern emergence (Camazine et al., 2003). Since self-organization is based on local interactions, it is best demonstrated in biological systems in which one is able to isolate the components that self-organize. In that sense, organoids are excellent models of cells self-organizing into an organ (Fig. 1). Local interactions have been reported in several organoid systems. For example, the Paneth cells in intestinal organoids fuel the growth of stem cells by secreting Wnt ligands (Sato et al., 2011), and a small group of cooperating cells is needed for embryonic pancreatic organoid growth (Greggio et al., 2013). However, for most organoids, the local interactions remain enigmatic. This is due in part to a lack of investigation and in part to the fact that many organoids are initiated from large numbers of cells and, for those initiated from PSCs, the final organization is likely to be the product of many sequential events of self-organization.

A conceptual interpretation of organoids can be informed by the physics of non-linear systems (Box 1). Most biological systems are non-linear, meaning that their output is not proportional to the input. This non-linearity owes to the fact that many governing mechanisms, such as growth, cell type and cell state, are coupled and therefore one cannot completely isolate their effects on the system. As with many biological systems, organoids are deterministic in the sense that the cells follow a given set of rules that rely on cues from their history, such as expressed genes and proteins and specific chromatin modifications (Turner et al., 2016), as well as from their local environment, such as the presence of other cells and the medium in which they are cultured. Note, however, that to reach this deterministic outcome cells often use cues from random

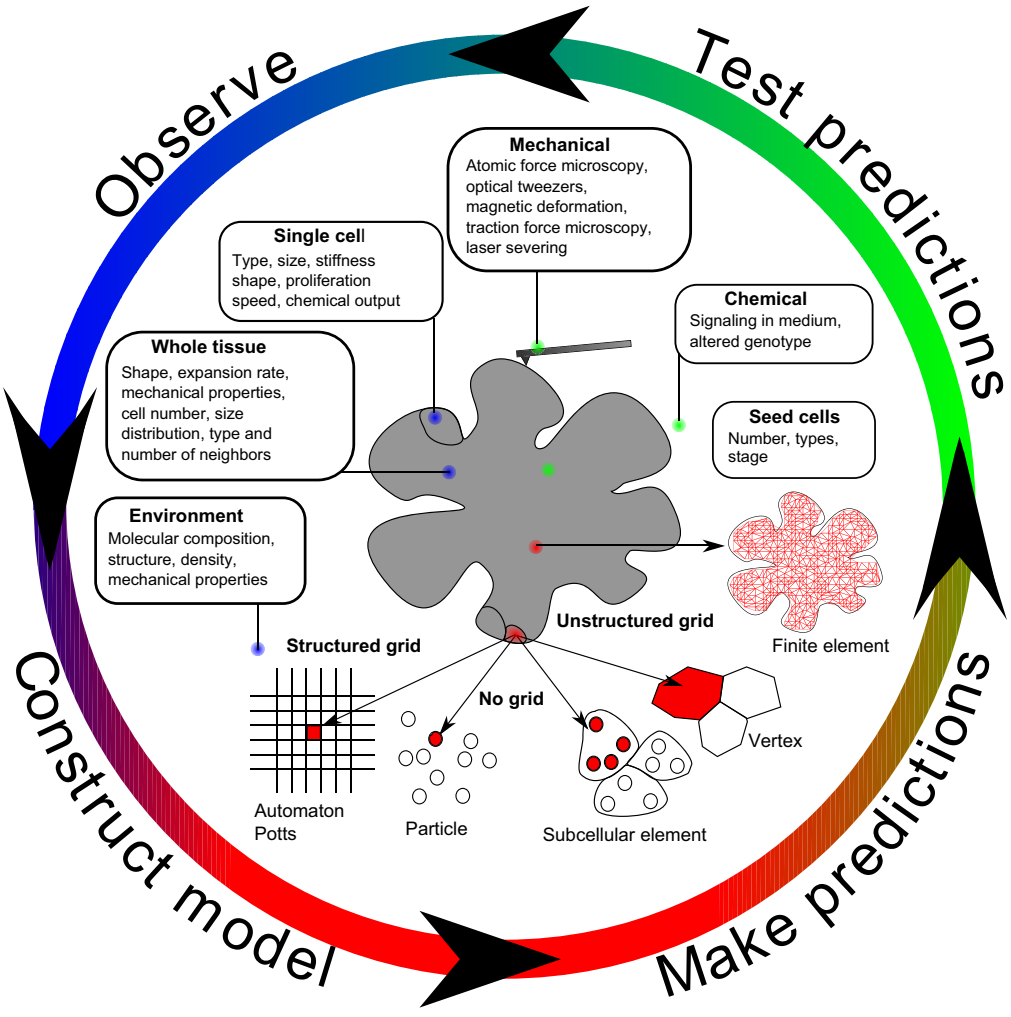

Fig. 1. Using organoids as a platform to deconstruct organogenesis. Owing to the simplicity of the organoid system compared with *in vivo* organogenesis, it is a good choice for *in silico*-supported studies. Step one is to observe the system properties and the parameters that affect them. The next step is to select an appropriate model that incorporates the observations of interest while effectively predicting the system. Finally, the *in silico* predictions can be tested by manipulating the relevant medium or organoid elements. This most often leads to new observations that can be treated in the same manner, leading to either a model refinement or an entirely new model.

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Box 1. *In vitro* organogenesis as a non-linear deterministic system

A non-linear deterministic system can be defined as one in which the input is not directly proportional to the output and in which the system evolves according to a set of rules. The characteristics of a non-linear deterministic system include (Strogatz, 2014):

- Fixed points in phase space: points of equilibrium that the system can settle into, such as an organoid settling into its typical shape or settling into a specific composition of cell types. Note that 'phase space' is applied broadly as the combined state of all quantities in the system, which include biochemical measures such as molecular states of the individual cells or distribution of cell types, and physical properties of the system such as size, shape and weight.
- Bifurcations: points where small changes in the system result in changes to the number of possible equilibria.
- Self-organized criticality: the emergence of fractal geometries, such as on sea shells or romanesco broccoli.
- Limit cycles: oscillations, such as the molecules involved in the circadian clock or the segmentation of somites.
- Attractor: a fixed point, set of fixed points or limit cycle that has a region of attraction in which every set of initial conditions that lies in the region goes to the attractor with time.
- Chaotic behavior: this results from a deterministic system that is extremely sensitive to initial conditions, such as in turbulence, the Barnsley fern, the Lorentz attractor and the double pendulum.

fluctuations in their components or environment as a signal input. The signal input coupled to the cells' regulatory network of feedback loops is then used to move towards their desired state. Positive-feedback loops will lead to the amplification of small differences in or between cells, whereas negative feedbacks will lead to buffering and robustness, which, among other factors, drive organoids to self-organize. The organoid system can thus be expected to exhibit features of non-linear deterministic systems (Box 1). Some of these features have been observed in specific organoid systems, namely fixed points in phase space and bifurcations. If a system has stable fixed points in phase space – defined here as the combined state of all quantities in the system – it can settle into multiple equilibria depending on the initial conditions. For example, pancreatic progenitors either grow into spheres maintaining pancreatic progenitor characteristics with one growth medium, or into organoids forming multiple cell types with another (Greggio et al., 2013). Multiple equilibria can be different outcomes of the culture, such as spheres or organoids composed of different cell types. Bifurcation points are points where small changes in system conditions will change the number of possible equilibria the system has. At such points, multiple equilibria can be observed in the same culture. For example, in embryonic pancreatic and fetal intestinal 3D cultures, some conditions allow the observation of both spheres and organoids at the same time (Fordham et al., 2013; Greggio et al., 2013). This property might explain why organoid cultures are not always reproducible. Indeed, small changes in the initial composition of cells – in their state, their age, their interactions or in the growth medium – can direct organoids to different attractor states (Box 1) between cultures or even in the same culture. Other characteristics of deterministic non-linear systems, such as self-organized criticality, self-sustained oscillations with limit cycles and chaotic behavior (Box 1), are more difficult to assess experimentally. For example, it would be difficult to investigate whether organoids exhibit a chaotic behavior, since one of the definitions of chaos is that two setups with the exact same initial conditions – for example, seeded cells at the exact same state or size – will diverge exponentially with time (Strogatz, 2014). Having identified the organoid system as a deterministic non-linear system that self-organizes, the next step is to identify the core components of this self-organization. A useful tool in this regard is *in silico* modeling, which can help uncover the behavior of these core components and determine the relevance of individual parameters such as the choice of medium and the properties of the initial seeded cells.

Computational approaches for modeling organoid biology

Together with the development of organoids, computational models are emerging to decipher their properties (Fig. 1) with the ultimate aim of underpinning organogenesis. Even though *in silico* models of organoids are, in a sense, 'models of a model', the combination is convenient because organoids can be manipulated to test computational predictions and conditions. We have focused on presenting spatial models (models that deal with volume, shape, and so on) as we deem those the most relevant to the organoid system.

Structured grid models

The first category of models is the structured grid (Fig. 1). These models are often the simplest to implement and are employed when a system exhibits simple geometries and one suspects that the governing factors are local interactions. In a recent study, cellular automata have been used to describe how growth inhibitors acting at a short distance could explain lobule formation in pancreatic organoids (Dahl-Jensen et al., 2016). Cellular automata are lattice-based systems in which a lattice point can be in different states. These states could be, but are not limited to, the presence of a cell or an empty point. Every grid point then behaves according to a given set of logical rules or governing equations given by its state at every time step. These types of models are efficient at describing macroscopic behavior based on local interactions. This type of system is ideal if one is interested in self-organization based on chemical gradients. Indeed, resolving diffusion on a structured grid is well documented (Douglas and Gunn, 1964; Patra and Karttunen, 2006; Strikwerda, 2004). However, it is a poor system to use when implementing mechanical properties, as the structured grid has trouble dealing with deformation and representing complex geometries.

Another grid-based model is the cellular Potts model, which was originally used to describe the Ising model of ferromagnetism. The system is lattice-based and, as for the automata, every grid point has the potential to contain any number of different types with a given set of rules. Unlike the automata setup, however, the system is updated one grid point at a time, usually by a Monte Carlo algorithm (Mosegaard and Sambridge, 2002), minimizing a given energy function. This process assumes that the system is constantly allowed to approach equilibrium. One of the arguments for using the cellular Potts model is that it is unreasonable to assume relaxation to equilibrium at every step, belying the use of a free energy-based model. This system has been popular in simulating self-organization resulting from cell-cell and cell-extracellular matrix (ECM) adhesion, where the function to be minimized is the total surface energy of the cell(s). This model has been employed to understand the growth of epithelial structures and proposes interesting interpretations of how single or multiple lumen form in cysts (Cerruti et al., 2013).

Unstructured grid models

Unstructured grid models are employed if one's interest lies in simulating mechanical features such as deformation, stress or strain (Fig. 1). Continuum models describe the dynamics of objects from a

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macroscopic perspective with the use of continuum mechanics equations, treating the object in question as either a visco-elastic solid or a fluid (Zienkiewicz and Taylor, 2005). This premise makes these models well suited to describe the mechanical behavior of tissue and its interaction with its surroundings. However, the model does not keep track of each individual cell. Therefore, the use of continuum models alone to describe cell self-organization is difficult, as individual parameters pertaining to cell polarity, adhesion, proliferation, growth and morphology are all unavailable (Piotrowski et al., 2015).

As an alternative, vertex models are better adapted to biological systems. In the vertex model, every cell consists of multiple vertices spanning a polygon, where the polygon faces are cell surfaces. A requirement of the model is that every vertex must have exactly four connections to other vertices. Like the continuum models, the vertex model can capture some macroscopic mechanical features, but unlike them it retains cellular identity. This enables one to study self-organization as a consequence of adhesion, proliferation, polarity and mechanical input, both globally and locally. The vertex model was recently exploited to investigate how groups of epithelial cells deform as they grow in viscous material (Okuda et al., 2013, 2015). However, the strict vertex requirement makes it difficult to simulate anything more complex than a single lumen structure, restricting its use to that of single epithelial layers (Misra et al., 2016). As many types of organoids do indeed contain only a single lumen during development, this modeling form nonetheless holds great potential.

Models with no grid

Models without a grid offer the possibility of modeling complex geometries while retaining individual cell identity, and encompass the same mechanical features as the unstructured grid models (Fig. 1). Particle models are essentially cellular automata in a lattice-free environment. Here, each cell is represented by one particle. Each particle then interacts through potentials that keep them at a standardized distance from each another. This model can simulate complex geometries with far fewer points than the grid-based models. In subcellular element models, a cell is represented by more than one particle, so each particle represents a sub-element of the cell. This modeling approach captures cell behavior at a microscopic level (particles of the same cell) while retaining a macroscopic perspective (all particles). Self-organization can be studied at any level of detail depending on how many particles are used to represent a single cell. A framework has recently been published that allows one to study self-organization as a function of growth, polarity, migration, juxtacrine and global signals for cells consisting of more than one particle (Milde et al., 2014). Such approaches could be useful in modeling organoid growth. However, the high resolution has a high computational cost, and the model has no direct way of including an ECM that the cells can interact with.

Some of these models become very computationally costly in 3D, but 2D modeling does not take full advantage of the 3D structure of organoids. Therefore, the choice of dimension will depend on the phenomena one wishes to investigate. It should be mentioned that hybrid models can be used as a way of circumventing the weaknesses of individual modeling approaches. This approach was taken by Buske et al. in their work modeling developing intestinal organoids, where they used a continuum approach (unstructured grid) to model the mechanical behavior of the medium and the organoid, while using a particle modeling frame (no grid) to keep track of individual cells and then let them interact with the continuum (Buske et al., 2011, 2012). *In silico* models should be seen as an advanced working hypothesis – guiding experiments in the right direction with their predictive capacity. For more technical details on the individual modeling approaches, along with examples of their application to other biological systems and information about existing software, we recommend the review by Simon Tanaka on the subject (Tanaka, 2015).

Organoids as models to investigate mechanical aspects of organ development

How cells enact morphogenesis has been a long-standing area of interest in developmental biology. *In vivo* investigations have uncovered many of the molecular components that drive events such as cell deformation, migration, delamination, sheet formation and folding. *In vitro* investigations in 2D have provided settings to dissect molecular engines and monitor mechanical responses of cells to the stiffness of their environment, conditions of stretching, flow and confinement. These mechanical stimuli can change the transcriptional state of cells and their differentiation (Dupont et al., 2011; Mammoto et al., 2012). Organoids provide a framework to study mechanical aspects in 3D, a condition more relevant to what the cells experience *in vivo*, and using more cells that are more similar to their *in vivo* counterparts than is achieved with cell lines. This setting is particularly well suited to the study of how groups of cells intrinsically change shape in the absence of other surrounding organs. The types of questions that can be investigated are illustrated by the pioneering work of Eiraku et al. on retinal morphogenesis (Eiraku et al., 2011, 2012). In an organoid system amenable to perturbation experiments and free of influences from other tissues, these authors recapitulated the process of optic cup formation from ESC-derived neuroepithelial spheres. They observed the local induction of retinal genes, resulting in a local myosin-dependent outward budding vesicle. A secondary relaxation at the tip was followed by a second myosin-dependent contraction, which folds the vesicle inward to generate a double wall. Laser ablation experiments revealed that this inward folding relies on the presence of peripheral hinges, and also on proliferation in the two-layered retinal pigmented epithelium and neural retina to generate compression forces that promote folding. Some differences between mouse and human ESC-derived organoids were observed (Nakano et al., 2012). All of this appeared to occur without external forces and with apparently no input from the surrounding basal lamina (Lowe et al., 2016). Similarly, Pin et al. (2015) investigated crypt fission by modeling intestinal organoids as a viscoelastic monolayer of incompressible cells that deform into a bud above a threshold. The authors showed that Lgr5-positive stem cells, whose stiffness measured by atomic force microscopy is less than that of neighboring Paneth cells, require less linear stress force to deform. As stem cells divide, compression forces are generated and are argued to impose bending on the more deformable stem cells, leading to crypt folding.

These initial attempts by Eiraku et al. and Pin et al. at studying mechanical properties during *in vitro* organogenesis are expected to be the beginning of an emerging field in which the accessibility of organoids can be exploited to measure their mechanical properties or those of the matrix and compare them with those observed *in vivo* or with those of single cells. 3D cultures can also be used to apply strain on cells, using atomic force microscopy, traction force microscopy, laser severing or magnetic-driven deformation (reviewed by Eyckmans et al., 2011; Lee et al., 2011; Lele et al., 2007) (Fig. 1). These techniques function at different scales, some focusing on one cell whereas others target multiple cells in a tissue. Although some techniques are

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challenging to implement in 3D, atomic force microscopy has already been used to probe the mechanical properties of mammary organoids (Alcaraz et al., 2008).

As well as understanding the intrinsic mechanical properties of organoids, it will be interesting to study the influence of the mechanical properties of the environment in which the cells are grown. Such attempts have been initiated and it was discovered, for example, that the differentiation of mammary cells in 3D depends on the stiffness of the environment (Alcaraz et al., 2008; Cassereau et al., 2015). The predilection of most organoids for Matrigel is a hurdle, but moving away from Matrigel to hydrogels, where the mechanical properties can be manipulated independently of biochemical components, will empower such approaches (Gjorevski et al., 2016; Greggio et al., 2013). In this context, intestinal organoids embedded in bovine collagen gels or elastin domain-based engineered hydrogels with varying stiffness grow with various efficiencies (DiMarco et al., 2014, 2015). Collagen gels loaded with polydimethylsiloxane (PDMS) further enable one to control stiffness independently of pore size (Cassereau et al., 2015).

Outlook

The field of directed differentiation has expanded with the ambition of generating replacement cells for regenerative medicine. The organoid field continues to build on these developments and has captured our imagination. Their simplicity allows us to explore the fundamental conditions needed for organogenesis though *in silico* modeling and *in vitro* observation. One important challenge is the reproducibility of organoid formation from different cells, both in a single batch and between batches. However, a quantitative approach that focuses on the initial conditions, such as cell state or type, number or polarity, is expected to increase reproducibility and provide insight into how developmental processes scale with increasing cell numbers. A biophysical approach to organoids can benefit future investigations that examine the role of mechanical forces and their interactions with molecular signaling pathways in organogenesis. We expect that investigations into the role of biomechanics in organ growth will be empowered by the development of more defined hydrogels, which will enable modulation of the mechanical characteristics of the *in vitro* environment without changing its molecular composition.

There is a long way to go before we can use organoids to create organs for transplantation. In closer reach, organoids are becoming available as support for drug development, personalized medicine and disease modeling. The potential applications of organoids in human medicine have somewhat overshadowed the other fantastic opportunities that they are giving us to decode the simple rules that underlie development in model organisms and also in humans.

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Competing interests

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