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REVIEW ESSAY

Pluripotent stem cell-derived organoids: A brief history of curiosity-led discoveries

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Abstract

Organoids are quickly becoming an accepted model for understanding human biology and disease. Pluripotent stem cells (PSC) provide a starting point for many organs and enable modeling of the embryonic development and maturation of such organs. The foundation of PSC-derived organoids can be found in elegant developmental studies demonstrating the remarkable ability of immature cells to undergo histogenesis even when taken out of the embryo context. PSC-organoids are an evolution of earlier methods such as embryoid bodies, taken to a new level with finer control and in some cases going beyond tissue histogenesis to organ-like morphogenesis. But many of the discoveries that led to organoids were not necessarily planned, but rather the result of inquisitive minds with freedom to explore. Protecting such curiosity-led research through flexible funding will be important going forward if we are to see further ground-breaking discoveries.

KEYWORDS
development, morphogenesis, organoids, stem cells, tissue engineering

INTRODUCTION

There are many fundamental biological differences between humans and other organisms. Nothing exemplifies this better than clinical trial failure rates. At least 90% of drug candidates fail in clinical trials despite successes in animal disease models.[1] For example, rodent models of spinal cord injury have demonstrated functional recovery in at least 69 studies of various therapeutics,[2] yet human trials have so far been rather disappointing, with one such strategy going as far as phase III but failing due to lack of therapeutic effect.[3] Reasons for this failure are myriad, but can essentially be boiled down to human-specific differences, be they differences in drug kinetics, toxicity, efficacy, or even more fundamental differences in human biology.

This highlights the need for human models. Naturally, these must be in vitro models, made up of human cells or tissues of varying complexities. Organoids represent the more complex of these, being made up of multiple cell types and exhibiting a tissue architecture and function seen in the actual organ.[4] The early organoids of Sato et al.,[5] and Ootani et al.[6] in 2009, were derived from already mature tissue of the gut by isolating and culturing adult stem cells in a three-dimensional basement membrane protein-rich gel. These intestinal organoids demonstrated the ability of even committed adult progenitors to form functional tissue in a dish. However, unlike the rapid turnover of the intestine, most other organs do not contain such potent progenitors. Hence, to model such tissues, embryonic or induced PSCs can be differentiated to the relevant cell types in vitro. This makes PSC-derived organoids fundamentally different from adult-derived organoids. The use of PSCs introduces variability in the types of cells that are made, but it also enables impressive self-organization and can capture the complex processes of patterning and morphogenesis.

PSC-derived organoids are possible because of the remarkable robustness of tissue development, even in the absence of the embryonic environment. But such self-organization was not necessarily predicted even by those who first established these models. Instead, it was through curiosity-led studies simply aimed at observing developing cells and tissues that such organoids were made possible. And it was rather difficult to predict that these in vitro oddities would turn out to be such a fundamental tool for human biology and drug discovery. Like so many ground-breaking discoveries, PSC-derived organoids were borne out of curiosity and a search for fundamental insight, without constraints or even a clear plan for translational impact. In this es, I provide a historical perspective highlighting key advances from early tissue reaggregation studies to embryoid bodies (EBs) and in vitro histogenesis, and finally to more advanced organ-like morphogenesis. I argue that freedom to explore was an integral part of enabling this technology, and that going forward, scientific curiosity should be protected rather than stifled by a push for immediate translation.

EARLY FOUNDATIONS OF THE FIELD

Although the term "organoid" as it is used today is a relatively recent one, self-organizing in vitro tissues were described as early as the beginning of the last century. Wilson demonstrated that individual cells and small masses of cells could be physically isolated from a particular species of sea sponge, and upon reaggregation would form a new sponge.[7] Then, in the 1940s, Barth and Holtfreter discovered that the amphibian animal cap could differentiate into neural tissue even when isolated from its embryonic environment.[8–10] For many years, Holtfreter and others searched fervently for the inducer, suggesting even rather bizarre possibilities such as sand.[10] A hint that there may be an intrinsic ability of cells to form such tissues came from the work of Moscona in 1952 while at the Strangeways Research Laboratory in Cambridge who performed dissociation-reaggregation of embryonic chick tissues,[11,12] revealing a remarkable ability of completely isolated cells to re-form new tissues. Although these experiments were performed in the middle of the last century, the results led to debates similar to those currently at the forefront of developmental biology. As Weiss and Taylor wrote in 1960[13]:

> "The results re-emphasize internal 'self-organization' as one of the most basic problems in the study of development, in contra-distinction to contemporary preoccupation with external 'inductions.'"

Such a statement is as true now as it was then. Much of current developmental biology has focused on extrinsic signaling cues as the key determinants of cellular behaviors, yet these self-organizing cellular aggregates demonstrate a robust, intrinsic developmental programme whose mechanism is still largely unclear.

Dissociation-reaggregation studies provided important insight into how adhesion proteins like cadherins allow cells to sort out into different domains.[14] But the intrinsic developmental programmes that govern such self-organization remained unknown, and for the past several decades, dissociation-reaggregation studies were largely ignored. The connection to stem cell biology and its translational potential was certainly not evident at the time.

EMBRYOID BODY BEGINNINGS

As cultures of embryonic stem cells (ESCs) became possible,[15–17] attention turned to ways of differentiating stem cells to particular cell types with therapeutic relevance, again with a predominant focus on external signaling and inductive cues.

As more insights into morphogenetic cues became clear from embryonic studies of organizers and the role of patterning factors, it became possible to direct the differentiation of PSCs to various organ identities, such as cardiomyocytes,[18] pancreatic cells,[19] and neurons.[20–22] The fact that PSCs could be maintained in culture and in theory differentiated to any cell type of the body held great promise for regenerative medicine. There was a lot of hope but unfortunately also hype, and many have argued that the stem cell field has failed to live up to its promises.[23] This may reflect the fact that extrinsic inductive signals are simply not sufficient to generate bona fide functional cells. In fact, we now know that cells have a more faithful identity when they develop within a tissue context.[24]

Early work on 3D differentiation involved structures called EBs[25,26] (Figure 1), often by aggregating single cell suspensions of PSCs in a hanging drop of media. EBs exhibit spontaneous differentiation to give rise to rudimentary structures of endo-, meso-, and ectoderm, representing the three germ layers of the embryo.[27] While early studies recognized the histogenic potential of EBs to form structures reminiscent of tissues,[25] their heterogeneity and complexity made it difficult to control cell differentiation. For example, while contractile cardiomyocytes could be observed to form spontaneously in EBs[28] their appearance was rather unpredictable.[29]

DIRECTING 3D HISTOGENESIS

A focus on more control over the cell types generated in EBs led many researchers to perform such 3D differentiations only transiently and to plate EBs on 2D substrates where the cells could be more evenly exposed to inductive cues to force differentiation down particular lineages.[30] Such was the approach of several groups, including the lab of Yoshiki Sasai, whose work laid important foundations for the organoid field. In the early 2000s, Sasai and others were largely focused on determining inductive cues for directed differentiation. For example, EBs grown in a serum-free medium with Wnt and Nodal antagonists and then exposed to adherent culture generated forebrain neurons.[31] Likewise, application of other inductive cues after initial directed neural differentiation generated cells of the retina[32] and cerebellum.[33] However, such strong extrinsic patterning and the 3D-to-2D approach naturally disrupts self-organization processes and the resultant cell clumps did not display signs of histogenesis.

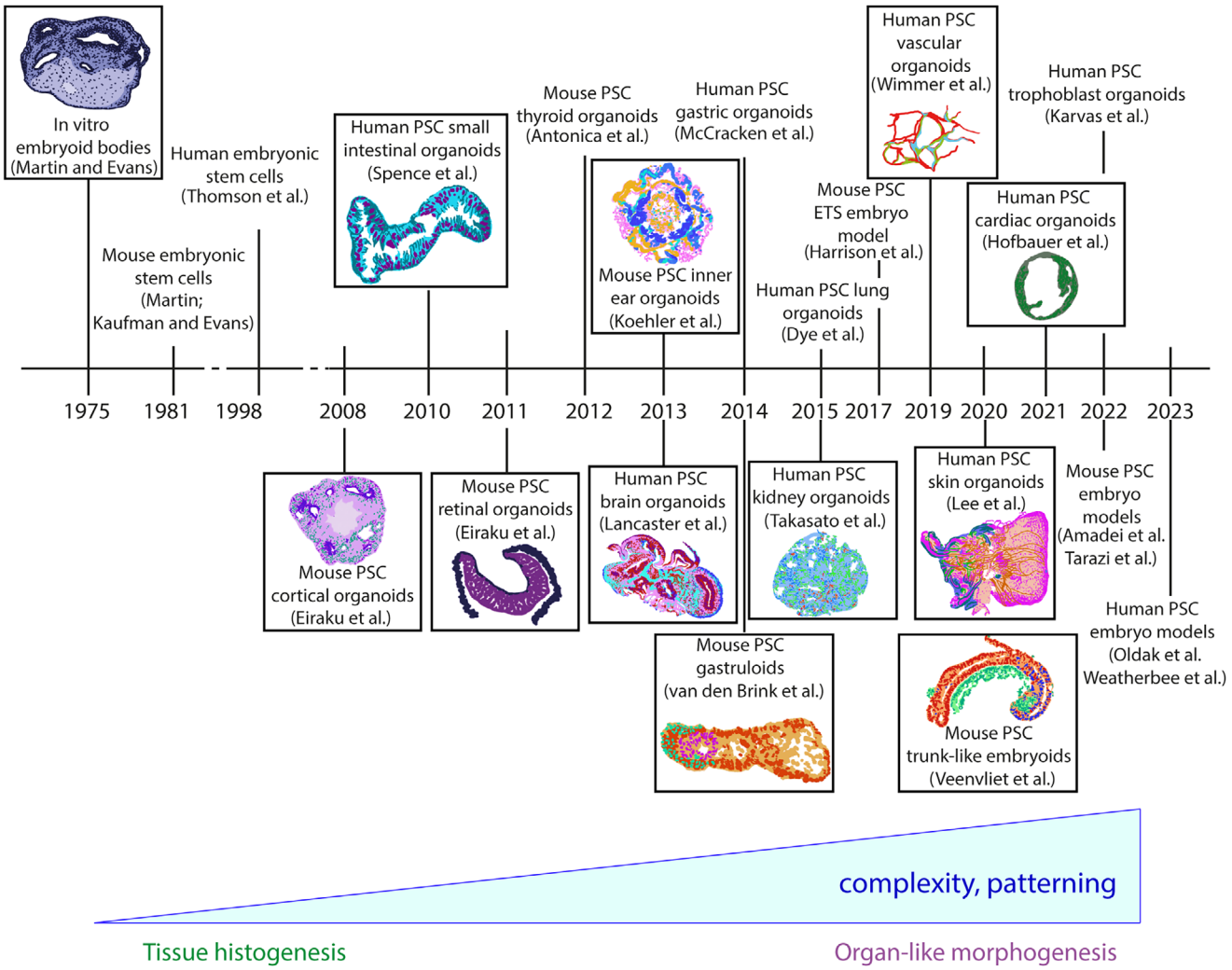

FIGURE 1 A brief history of pluripotent stem cell (PSC)-derived organoids. A timeline of PSC-derived tissues and their evolution from embryoid bodies. A selection of methods is illustrated with drawings based on actual data shown in the referenced publications. The progression from histogenesis to organ-like morphogenesis in more complex organoids with regional patterning (i.e., brain/cerebral organoids, gastruloids, kidney organoids, skin organoids, and trunk-like embryoids) is highlighted below.

In 2008, Sasai's group demonstrated that rather than plating 2D substrates, neural induced mouse EBs maintained in 3D culture could exhibit histogenic properties reminiscent of the embryonic cortex[34] (Figure 1). The resultant structures exhibited a progenitor zone and a neural plate, similar to but larger than 2D neural rosettes, and reminiscent of previously described structures seen in neuroectodermal regions of teratomas and EBs[26], highlighting the fact that the foundations of PSC-derived organoids were already evident in these earlier structures. While more advanced patterning and organ suprastructure was still lacking, it highlighted the robust nature of neural ectoderm to form a polarized neuroepithelium in 3D.

Turning to the endoderm, in 2010 Jason Spence and James Wells produced PSC-derived gut organoids by inducing human PSCs in 2D culture to definitive endoderm and then directing them to a hindgut fate.[35] Cells self-organized into 3D growths that budded from the dish. Rather than aspirate these spheroids with media changes as would have typically been done previously, the authors maintained them in 3D culture conditions similar to those used for adult-derived gut organoids. The result was the formation of a convoluted epithelium reminiscent of embryonic intestinal epithelium (Figure 1), but unlike adult-derived organoids the PSC-derived hindgut tissues also developed associated mesenchyme.

Sensory organs were also an early tissue type of focus. In 2011, the Sasai lab developed impressive retinal structures from mouse EBs.[36] As with cortical directed EBs, the authors observed that maintenance of retinal induced mouse EBs in 3D culture resulted in more advanced histogenesis, but in this case the tissue architecture went beyond that seen in the more rudimentary EBs or teratomas. The retinal tissues displayed impressive stratified cytoarchitecture with an outer nuclear layer, inner nuclear layer, and ganglion cell layer composed of the major retinal cell types exhibiting advanced morphologies. Turning to another type of sensory epithelium, Koehler et al. in 2013 directed mouse EBs to inner ear fate which developed impressive tissue cytoarchitecture when similarly maintained in 3D self-organizing culture rather

than simple 2D culture.[37] This work highlighted the power of self-organization, and in 2013 Sasai introduced the concept of "cytosystems dynamics" indicating a shift in focus more in favor of the viewpoint of Weiss and Taylor, suggesting a more holistic approach may be needed to uncover the core modules governing self-organization[38]:

> "Multicellular systems involve huge numbers of regulatory components, and the complexity of these in multidimensional networks is beyond comprehension. Such highly robust and regulative phenotypes could never be explained by strict control of each regulatory component in the system."

Self-organization is an emergent property that cannot be understood or recapitulated by simply combining the parts. This makes it in many ways a black box, but nonetheless a powerful tool to grow, rather than build, potentially any tissue of the body.

As with many areas of regenerative medicine, experiments in mouse or other model organisms provided the foundation on which to develop human systems. Human EBs similarly subjected to directed differentiation to retinal identity also exhibited striking histogenesis with stratified retinal cell types.[39] In the same study that described cortical induced mouse EBs kept in 3D, human ESCs were similarly subjected to the same inductive cues and also formed intriguing cortical zones, though the authors reverted to 2D plating of the human EBs rather than maintaining them in 3D culture.[34] These studies demonstrate a gradual move away from the rather heavy-handed extrinsic control over differentiation that was the norm at the time. Although Yoshiki Sasai's work still focused on inductive cues to direct differentiation, the tissues his lab produced demonstrated the power of the 3D environment, a realization that began to permeate the field.

MORPHOGENESIS IN VITRO

In vivo studies have demonstrated that neural differentiation is the default for PSCs,[40] and in vitro cultures have revealed that even serum can influence differentiation and direct nonneural identities.[22,41] Thus, while the early PSC-organoids utilized small molecules and growth factors to direct differentiation, evidence was building that directed differentiation may not be necessary in the case of neural identity. Because such extrinsic manipulation could override intrinsic developmental programs, an undirected 3D differentiation aimed at supporting, rather than directing, differentiation could provide more extensive self-organization.

Originally named after the Latin for brain, so-called cerebral organoids generated with minimal guidance were shown to exhibit not just isolated neural tissues, but a variety of brain regional identities within the same mass, including retinal, forebrain, choroid plexus, and mid/hindbrain tissues.[42] Although current nomenclature consensus[43] is such that "cerebral" should denote the neuroanatomic meaning referring to the telencephalon rather than the etymological origin of the word, the original cerebral organoids exhibited not only isolated histogenesis of cerebral structures but also a degree of organ patterning (Figure 2). For example, forebrain structures exhibited rostral-caudal (i.e., frontal versus occipital lobes) and dorsal-ventral patterning with ventrally produced interneurons migrating into the adjacent dorsal region,[42] just as in vivo. Likewise, mediolateral organization could be observed with the cerebrospinal fluid-producing choroid plexus transitioning into hem neuroepithelium and stratified cortical tissue with fluid-filled ventricles,[44] just as in vivo. This spatial organization demonstrated an intrinsic axial patterning going beyond local cell–cell interactions seen in histogenesis and suggesting even incredibly complex morphogenetic patterning could be accomplished in vitro.

The potential impact of PSC-derived organoids in the biomedical sphere was not necessarily clear at first. Cerebral organoids provided a demonstration of that potential when they were used to model a human condition, microcephaly. By starting with patient-derived induced pluripotent cells (iPSCs), the organoids revealed a pathogenic mechanism in which neural stem cells became depleted resulting in fewer neurons produced.[42] This condition is not well modeled in mice, so it provided a proof-of-concept that brain organoids could uncover human-specific biology. Perhaps more importantly, it established iPSC-derived organoids as a tool for understanding human disease, and opened the flood-gates to a new era of disease modeling and drug discovery.[45,46]

Since the early reports of PSC-derived 3D tissues and organoids, numerous organs including thyroid,[47] stomach,[48] lung,[49] skin,[50] heart,[51] placenta,[52] and vasculature,[53] have been modeled in a similar fashion (Figure 1), often through gently guiding EBs or dense 2D cultures to the organ precursor of interest and allowing self-organization to take over. For example, to generate organoids of the kidney, several labs devised ways to first direct differentiation to the intermediate mesoderm and then to ureteric bud,[54] metanephric mesenchyme,[55] or both.[56] By first establishing the primordial tissue and providing a permissive culture environment, these tissues could self-organize and form impressive regionalized nephric tubules and glomeruli[57] (Figure 2). More recently, these principles have been taken to a new level with the development of embryo-like models, including gastruloids,[58,59] embryonic trunk-like tissues with somites,[60] and models of the peri- and post-implantation embryo.[61–66] Here too, the concept of gentle guidance with a strong reliance on self-organization is leading to impressive new models with axial patterning reflecting true morphogenetic processes in vitro.

THE IMPORTANCE OF CURIOSITY AND FREEDOM

While it is difficult to know what on a personal level led many researchers in this field to begin experimenting with more and more complex in vitro tissues, it is rather safe to say that all had one thing in common: curiosity. Yoshiki Sasai's publication record indicates a highly inquisitive mind and a thirst for fundamental understanding of developmental biology.[67] Similarly, when I began my postdoc in 2010 in the laboratory of Juergen Knoblich, at the Institute for Molecular

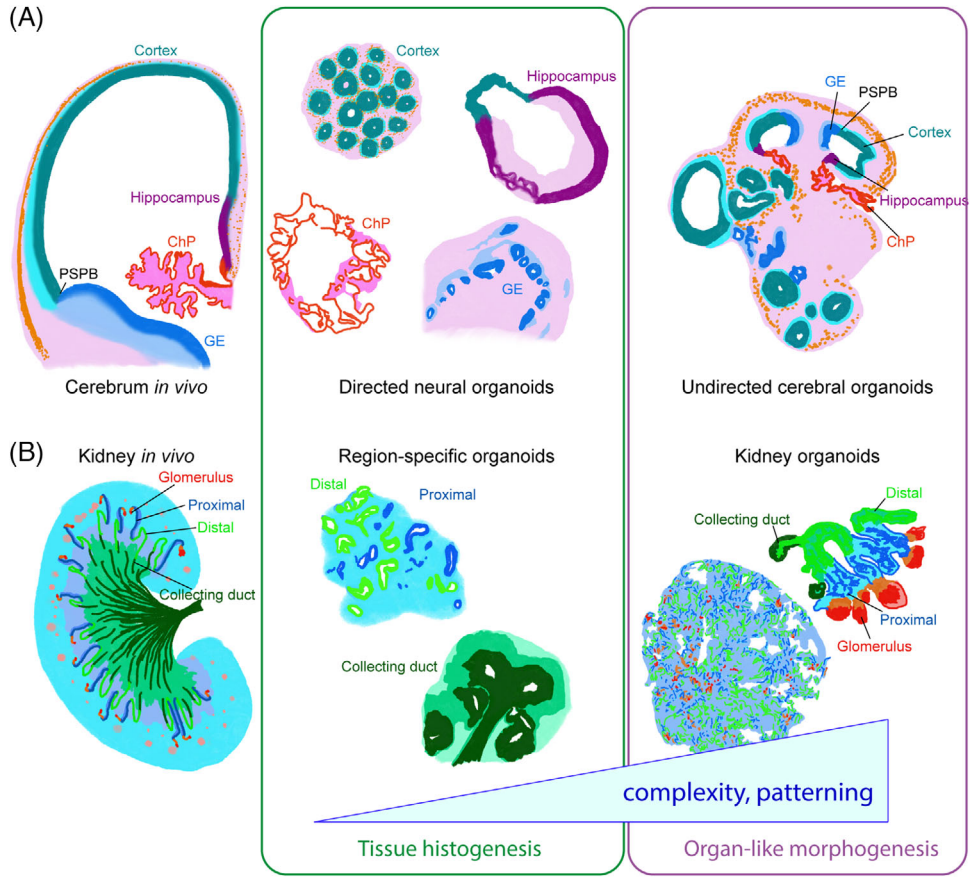

FIGURE 2 Various organoids model different degrees of organ structure and patterning. (A) A diagram of a coronal section of one hemisphere of the developing human cerebrum showing the organization of dorsal and ventral regions progressing from the choroid plexus (ChP) to the hippocampus and cortex, to the pallial-subpallial boarder (PSPB) and the ventral ganglionic eminences (GE). These individual regions can be modeled in guided organoids made through directed differentiation using signaling factors, or can develop intrinsically in close proximity in a similar pattern to in vivo in undirected brain/cerebral organoids. Images are drawings based on actual data shown in: Altman and Bayer[94] (in vivo), Paşca et al.[95] (cortical organoids), Pellegrini et al.[96] (ChP organoids), Sakaguchi et al.[97] (hippocampal organoids), Bagley et al.[98] (ventral forebrain organoids), and Renner et al.[44] (undirected cerebral organoids). (B) A diagram of a kidney showing the hierarchical structure of the nephron and collecting ducts. Specific regions can be modeled independently in single region organoids, but can also be modeled together where they form regionalized tubules like those found in the developing kidney in vivo. Images are drawings based on actual data shown in: Taguchi et al.[55] (metanephric mesenchyme organoids), Xia et al.[54] (ureteric bud organoids), and Takasato et al.[57] (kidney organoids).

Biotechnology in Austria (IMBA), we were driven by a desire for fundamental insight, largely unfettered by concerns about work packages or promises to cure diseases. Knoblich and I sketched out a rather rough outline to develop an in vitro assay to test various orthologs of drosophila genes of interest in mouse neural stem cells.

Like many discoveries in science, an aspect of serendipity played a part, and it is likely that similar serendipity was at play for others developing PSC-derived organoids.[68] But it was only because of academic freedom that we were able to follow where luck would have it. Because I was the first in the lab to culture stem cells, I did not have certain necessary reagents on hand, so I scrounged around for tissue culture plates and coating reagents. These were suboptimal, likely expired and inactive. Lacking experience with stem cell culture, I went ahead anyway, and my first attempts, which involved culturing mouse neuroepithelial cells that I had dissected from E9.5 embryos, failed to form attached neural rosettes. Instead, 3D structures appeared with evident lumens, reminiscent of the neural tube.[69] I was completely enthralled that such complex structures could form in the absence of any extrinsic guidance, setting off the curiosity-driven investigation that led to cerebral organoids.

FUTURE PROSPECTS

With organoids becoming an important part of disease modeling and drug development portfolios, their translational utility is now evident. Patient-derived iPSCs are now routinely used to derive organoids for a range of human conditions, including Hirschsprung's disease,[70] polycystic kidney disease,[71] autism,[72] epilepsy,[73] and tuberous sclerosis,[74] to name a few. Taking this to a new level, Timothy syndrome, a debilitating disorder presenting with autism and epilepsy, has not only been modeled in neural organoids[75] but a new anti-

sense oligonucleotide therapeutic strategy has been developed that has enormous potential for patients.[76] Thus, organoids are providing not only new understanding, but even new therapeutic avenues.

The power of certain organoids to undergo tissue histogenesis and even organ-like morphogenesis all through self-organizing principles is impressive. However, with less extrinsic control comes more intrinsic variability. Thus, while a greater reliance on self-organization can lead to impressive morphogenesis, outcomes can be variable. This fact has influenced the field to begin swinging again in the other direction, with more recent methods generating more simplified structures and exerting greater control through more inductive signals aimed at achieving greater homogeneity.[77–79] History seems to be repeating itself with such heavily directed approaches resulting in a reduction of structural complexity, and a loss of morphogenesis.[80] At the same time, new approaches combining directed differentiation with fusion of organoids of different identities in so-called assembloids[70,81,82] are adding new layers of complexity. It is important to remember that in many cases organs do not develop through fusion of different tissues, but rather through co-development of tissues in proximity to set up the proper organization of different regions within and across organs (Figure 2). Such intrinsic patterning has already been seen in multiregion brain organoids,[44] neuromuscular junction organoids,[83] skin organoids,[50] and kidney organoids,[57] for example. Going forward, it will be important to find ways to accomplish this morphogenesis in a predictable fashion that is reproducible from organoid to organoid.

The combination of organoids with other technologies such as bioengineering and gene editing is further expanding the potential of these tissue models. CRISPR provides an efficient method for precise genetic engineering and can yield functional insight into genes of interest[84,85] and uncover pathogenic mechanisms in disease modeling.[86] Fluid-flow in the form of bioreactors[42] or micro/millifluidics[87] can provide environments conducive to tissue growth and maturation, vascularization, and patterning.[88] Likewise, the combination of organoids with organ-on-chip technologies[89,90] can provide environmental control and cross-talk between different organoids. Furthermore, various materials can impose different conformations on organoids,[91–93] which is enough to change their differentiation and maturation. These types of bioengineering approaches can take organoids in new directions and will no-doubt be vital in going from organoid to in vitro organ, but as with all extrinsic manipulations, the balance between engineering and self-organization will be important to bear in mind.

Thus, the debate between self-organization and induction seems to be alive and well, and the pendulum will likely continue to swing between the two in the future. The important thing will be to maintain a sphere of academic freedom, so that curious minds can continue to tinker without entirely knowing where it will take them. To enable that, it is vital that core funding and grant schemes are provided to talented scientists without necessarily telling them what to do. The recent trend of granting agencies demanding immediate translational impact will do just the opposite. True impact cannot be predicted or directed, but comes from providing a nurturing, permissive environment to enable the very same self-organizing principles at the heart of organoids themselves.

AUTHOR CONTRIBUTIONS

Madeline A. Lancaster conceived and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

Madeline A. Lancaster is an inventor on patents related to brain organoids and is cofounder of a:head bio.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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