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Modeling human disease using organotypic cultures

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Reliable disease models are needed in order to improve quality of healthcare. This includes gaining better understanding of disease mechanisms, developing new therapeutic interventions and personalizing treatment. Up-to-date, the majority of our knowledge about disease states comes from *in vivo* animal models and *in vitro* cell culture systems. However, it has been exceedingly difficult to model disease at the tissue level. Since recently, the gap between cell line studies and *in vivo* modeling has been narrowing thanks to progress in biomaterials and stem cell research. Development of reliable 3D culture systems has enabled a rapid expansion of sophisticated *in vitro* models. Here we focus on some of the latest advances and future perspectives in 3D organoids for human disease modeling.

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In vitro organoid models

When cultured in supportive conditions, many types of somatic stem cells (SSCs) can grow into multicellular structures that self-organize into mini-tissues with differentiation patterns and cellular architecture that resembles the corresponding normal tissue. These spheroid structures are commonly referred to as organoids and were first established from tissues such as neuronal cells, lung and intestinal epithelia [1–3]. However, the term 'organoid' itself had been used prior to that to describe structures formed by complex mixtures of normal or cancerous cells when grown in a 3D matrix or put into suspension. Over the last decade, self-organizing organoids have been derived from many organs based on the successful expansion of SSCs using 3D extracellular matrix (ECM) scaffolds or air–liquid interface conditions (Table 1). The fast rates of expansion and self-renewal of these structures under growth-permissive conditions most likely reflect states characteristic for development, hyperplasia or regeneration rather than homeostasis, and should be considered when interpreting results obtained from *in vitro* organoid systems.

In parallel to SSC-based cultures, 3D tissue organoids have been obtained by directed differentiation of pluripotent stem cells (PSCs), including patient-derived induced pluripotent stem cells (iPSCs) (Table 1). PSC differentiation protocols primarily utilize liquid culture conditions and in some case result in heterotypic organoids, where more than one cell type is present. Other protocols resort to either an intermediate purification step or defined differentiation conditions to derive pure cultures of the appropriate cell type for subsequent analysis. Irrespective of differentiation strategy, there appears to be a general shortcoming in the efficient differentiation of cell types from PSCs with functional properties that match those observed in the adult tissues, and the derived cells often have resemblance to fetal tissues. Therefore, some PSC-derived organoids may currently be better suited for modeling developmental disorders rather than diseases with a later onset. This might change in the future as more efficient differentiation methods are developed, focusing not only on generating the right tissue type, but also on tissue maturation.

Several aspects of 3D organoid modes have been recently extensively reviewed [4–7]. In this article we focus on different aspects of using 3D organoids to model human disease (Figure 1).

Organoid models of disease

Organoid systems offer unique possibilities for disease modeling *in vitro* as they enable primary cells isolated directly from tissues or PSC to self-organize in a 3D structure akin to their normal tissue morphology. Under these conditions, a cellular micro-niche directs appropriate cellular differentiation and stem cell maintenance. One example is the establishment of mucin-rich barriers inside 3D organoids from the intestine and stomach, which have been exploited in studies of infectious diseases (Table 2A). A recent series of reports described co-cultures of gastric organoids with *Helicobacter pylori*, and provided evidence that *H. pylori* on its own elicits inflammatory responses and stimulates pro-proliferative signaling pathways within the co-cultured epithelial cells [8•,9,10•,11,12•]. Similarly, some virulent, but not non-virulent, strains of *Salmonella* and *Clostridium* have been shown to interfere with intestinal organoid growth [13,14,15•,16,17,18]. Taken together, these results clearly demonstrate the potential for wider studies of the human

Table 1

Organoid models of non-transformed tissues and organs

Organ	Reference (source cells used)
Biliary tract	[23•] (hSSC, Gel), [48] (hPSC, Gel), [49] (hPSC, Gel)
Brain	[27] (hPSC, Liq), [50] (hPSC, Liq), [51] (hPSC, Liq)
Colon	[3] (mSSC, Gel), [52] (hSSC, Gel), [41] (mSSC, Gel), [53] (mSSC, Gel), [54•] (mSSC, ALI), [55] (hPSC, Gel)
Eye (Retina)	[56] (mPSC, Liq), [57] (hPSC, Liq), [58] (mPSC, Liq), [59] (hPSC, Liq), [60] (mPSC, hPSC, Liq)
Inner ear	[61] (mPSC, Liq)
Kidney	[29] (hPSC, Liq), [62] (hPSC, Liq), [63] (hPSC, Gel)
Liver	[64] (hPSC, Liq), [44] (mSSc, Gel), [23•] (hSSC, Gel), [65] (hPSC)
Lung	[66] (mSSC, Gel), [67] (hPSC, Gel), [68] (hPSC, Gel, ALI)
Oesophagus	[69] (hSSC, Gel), [70] (mSSC, Gel)
Ovary	[71] (hSSC, Gel)
Pancreas	[45] (mSSC, Gel), [72•] (mSSC, hSSC, Gel)
Pituitary gland	[73] (mPSC, Liq)
Prostate	[74] (mSSC, hSSC, Gel), [75•] (mSSC, Gel)
Salivary gland	[76] (mSSC, Gel), [77] (mSSC, Gel)
Small intestine	[3] (mSSC, Gel), [78] (mSSC, ALI), [79] (hPSC, Gel), [42] (mSSC, hPSC, Gel)
Stomach	[8•] (hPSC, Gel), [10•] (hSSC, Gel), [54•] (mSSC, ALI), [80] (mSSC, Gel), [81] (mSSC, Gel), [82] (mPSC, Gel)
Tongue	[83] (mSSC, Gel), [84] (mSSC, Gel)
Thyroid	[85] (mSSC, Gel), [86] (mPSC, Gel)

The list includes organoid models where evidence exists for the long-term maintenance of cells with normal characteristics.
m, mouse; h, human; SSC, derived form somatic stem cells; PSC, derived from pluripotent stem cells; ALI, air–liquid interface culture; Liq, liquid culture; Gel, embedded 3D hydrogel culture.

microbiome using 3D organoid models of epithelial tissues. Indeed, two reports utilized *in vitro* organoid systems to investigate interactions with non-pathogenic commensal bacterial species [19,20]. In addition to studies of bacterial virulence, 3D organoids have also been used to experimentally model viral infections [21]. Most recently, Garcez *et al.* have exposed iPSCs-derived human neurospheres and cerebral organoids to Zika virus (ZKV) to show that it targets neuronal stem cells, thereby reducing viability and growth. This reinforces the association between ZKV infections and microcephaly [22••].

An important feature of 3D organoids is that they can be established efficiently from genetically normal cells and remain genetically stable even following serial passaging in culture [23•,24]. This is unlike many 2D *in vitro* culture systems, which traditionally utilize naturally or experimentally transformed cell lines. When derived in 2D conditions, even primary cultures undergo extremely strong selective pressure that eliminates more slowly proliferating lineages. In at least some cases, this may lead to spontaneous transformation to overcome replicative senescence following serial passaging. Therefore, genetic stability of organoid cell culture models is an important feature when modeling non-oncogenic disorders such as idiopathic diseases or hereditary disorders caused by specific gene mutations. It is also crucial for the potential exploitation of organoid technologies in regenerative medicine.

3D organoid systems have been successfully used to characterize the pathophysiology of different somatic disorders (Table 2B). In almost all reported cases, the organoid models fatefully recapitulate specific *in vivo* disease phenotypes, which has and most likely will be critical for development of new pharmaceutical interventions. Most notably, Bigorgne *et al.* took advantage of intestinal organoid cultures to delineate the disease mechanism in multiple intestinal atresia/combined immunodeficiency (MIA-CID) associated with loss of function mutations in *TTC7A* and identified potential mode of pharmacological intervention [25•]. Similarly, Dekkers *et al.* used rectum-derived organoids from cystic fibrosis patients to develop a screen that allowed identification of the most efficient drugs for each patient [26]. Disease modeling and personalized therapy based on assays using organoids are relatively cost-effective and quick to establish. As it is simple to maintain normal healthy organoids derived from somatic tissues, these technologies provide promise for future strategies to develop precision medicine based on patient stratification.

In spite of the great progress in organotypic *in vitro* systems, there are clear limitations to using cells derived from somatic tissues in modeling of certain disease phenotypes. First and foremost, besides that tissues have to be available for cell isolation, the derived cells also have to be proliferative and able to build expanding self-organizing organoid structures. Therefore, alternative technologies such as PSC-derived organoid systems should be considered for modeling cellular disease phenotypes in developmental disorders and tissues that do not necessarily self-organize when cultured from somatic cells. This strategy has proven valuable for organs such as the brain, where Knoblich and coworkers established an organoid model, which allowed the team to recapitulate *in vitro* the developmental aspects of CDK5RAP2-dependent pathogenesis of microcephaly [27]. Using