Describe how organoids are being used to understand fundamental biology questions

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A fascination for the understanding of life is undisputedly unique to our own species. Natural philosophy is a deep-rooted origin of biology as it asks the same fundamental questions in life: human origins, how we are formed and how we can live better. This dates all the way back to the earliest Greek philosophers but without the means to answer such questions, they could merely speculate based on their limited observations. Technological advances are the cornerstones in biology that lay the path for scientists to enhance observation and exploration of theories. The development of the microscope in the 17th century expanded the world of biology [1]. In modern biology, novel technologies such as X-ray diffraction, genomic sequencing and gene editing CRISPR/Cas9 continue to revolutionise biological studies. Organoids are believed to be the next emerging technology: miniaturised 3D cultures artificially grown from stem cells that model organs.

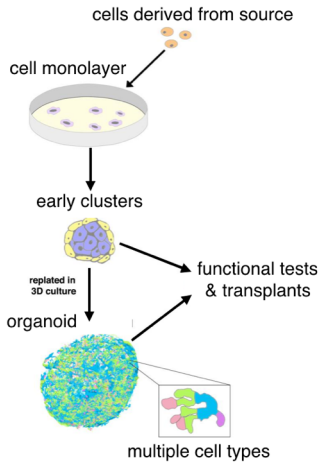

Fig. 1 Organoid formation (modified from ref. 25)

The conventional approach to ex vivo studies involves the isolation of specific cells in a 2D culture. This has been successfully used to address a broad range of questions about cellular activities such as cell cycle regulation and signal transductions [2]. However, this approach encounters its limitations when we ask questions at the level of an organ as it lacks the hallmarks of organs– having multiple cell types and tissues as well as three-dimensionality. Organoids, by definition, are induced from progenitor cells, embryonic stem cells, or induced pluripotent stem cells (iPSC) to self-organise into 3D culture possessing all the necessary cell and tissue types in order to mimic functionality of a specific organ [3] (Fig. 1). Though still imperfect, organoids, through their increased dimensionality, better imitate natural cell environment. It increases cell-cell and cell-matrix interactions which are responsible for cellular functions - cell proliferation, differentiation, gene expression, cell signaling, and drug metabolism [2, 4]. Analysis of functioning human specific tissue, which was once only made possible for a short time with biopsies, can now be done using organoids over a longer period in a more environmentally natural setting.

Although still in its infancy, the surge of organoids has opened exciting avenues in many fields of biology and holds major potential in developmental biology, regenerative medicine, drug testing and disease modelling. Organoids also offer a depth of analysis on par with non-human model organisms and with benefits of more accurate studies on more complex human-specific diseases which are not mimicked accurately in animal models [5]. This technology provides possible solutions in every context of human biology from basic research, translational research and therapies [3].

Organogenesis is an inherently fascinating developmental process and critical for human function. It demands the formation of intricate three-dimensional structures from a collection of distinct cell types, all of which assemble without a template to form a fully functioning organ [6]. However, organogenesis in mammals, especially human, is very difficult to observe as it happens within a uterus. Ethical

implications have repressed the advancement of developmental biology of human, limiting researchers to observational studies on preimplantation embryos, progenitor cells and tissue from aborted fetuses [7]. Organoids provide dynamic observations at every step of early development mimicked in human embryo.

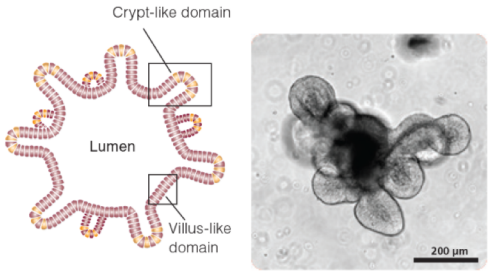

Fig. 2 Intestinal organoid (from ref. 26)

Whilst some organs, such as the brain, largely conserve their original cells, other organs keep replacing their cells throughout their lifetime. From a macroscopic view, the organ maintains complex structure and functionality but on a microscopic level, cells are constantly regenerating autonomously. One such example is the intestine, which facilitates a fast 4 to 5-day turnover of all cells (thus, the first form of organoids was developed for this organ) [8] (Fig. 2). Within the crypt of the epithelium are intestinal stem cells interspersed between Paneth cell which controls differentiation of the stem cells via Wnt signaling. These singular stem cell niches are able to self-organise to mimic morphological structure of crypt-villus, independently from surrounding structures such as mesenchyme [9, 10]. This disproved a commonly accepted theory that mesenchyme cells are imperative in the epithelial morphogenesis through Wnt signalling. This system will, in future, give further access to live observations to help us understand fundamental mechanisms of organogenesis such as intricate relationships between cell division and morphology.

The development of iPSC technology has opened a gateway in tissue engineering and regenerative medicine with the power to promote differentiation into desired cell lineage. Although organoid implantation as cell therapeutics is still on the cusp of reality, there is hope that it could be used genetic correction strategies to enable autologous replacement of tissue affected by genetic disorders using patient-specific tissue [3]. In 2014, the first patient derived retinal pigment epithelial cells were transplanted to treat age related macular degeneration. Organoid technology in the coming years hopes to follow these feats [11].

Hope is displayed in the introduction of colonic organoids into damaged mice colon. The transplanted organoids successfully integrated to cover the damaged areas in the epithelium and overtime, formed self-renewing crypts resembled the same functionality and histology as the surrounding tissue [12]. Over an extended period, at least 6 months, epithelium was continually monitored and showed no signs of cancerous tendencies. This study implies that, although further testing on non-animal models is needed, there is great promise in the regenerative powers of organoids that can be exploited for stem cell therapies. Additional tissues/organs that have been shown to have regenerative potential are the lung, skin, and hair. An ultimate goal along this line would be whole livers or kidneys that can be maintained effortlessly throughout a lifetime but whether that is possible still remains uncertain.

Organoids can be used as disease models to provide a unique platform for the analysis of mechanisms of human-specific diseases. Scientists often rely on animal models to study diseases. This not only has ethical implications but is also limiting our understanding since their biology is different from human's, especially in organs like the brain. Since organoids can be grown from human stem cells, they can model

specific diseases and facilitate a range of research from basic mechanistic observations to drug development and therapies [7].

Cystic fibrosis is a congenital condition caused by a mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes a chloride channel. Although this mutation is associated most with the defected lungs, CFTR is also expressed in the epithelial cells of the gut. When intestinal organoids were grown from the patients, they could recapitulate the dysfunction of the disease in vitro. A swelling inducing drug called forskolin is known to increase the amount of intracellular cyclic AMP (cAMP) and consequently activates CFTR channel in healthy, control organ. The healthy organoids respond to forskolin by transporting fluid to the lumen and subsequently swells; this is a typical response in human gut control. In contrast, this response does not take place in the patient-derived organoids due to the mutated CTFR gene. This work demonstrated the excellent predictive value of intestinal organoids in screening CTFR targeting drugs and has become the first personalized treatment test for CF patients with rarer CTFR mutations [13, 14]. CF patient-specific organoids can then be stored for primary cell banks which in future can be used to for further cellular research and development for personalised drugs. Disease modeling with organoids provides a tool with which fundamental mechanisms of origin and growth of disease can be studied. This not only accelerates drug discovery but also opens an avenue towards genome editing treatments involving CRISPR/Cas9.

Brain organoids were able to unravel the mystery of the ZIKA virus' (ZIKV) effect on microcephaly which devastatingly affected over 1600 babies during the ZIKA epidemic in Brazil throughout 2015 and 2016 [15]. Upon exposing developing forebrain organoids to the ZIKA virus, it revealed that the pathogen selectively targets neural progenitor proliferation and therefore delays cell cycle. This increases cell death to make smaller, underdeveloped organoids which resembles the effects of microcephaly in the ZIKV diseased brains of babies [16]. These models are of great importance for identifying and verifying the potential compounds for preventing microcephaly such as inhibitors for the potential ZIKA receptor, AXL [17]. In addition, infected organoids provide a platform for drug testing to treat ZIKA virus. However, it is important to highlight discrepancies in infectivity of brain organoids compared to the human brain. ZIKV was found to affect both sets of neural progenitors equally but where infection to the astrocyte cells were sparse, were abundant in human tissue. Although brain organoids hold massive potential in neuroscience, of which we know so little, they are models and thus caution should be taken when drawing conclusions.

Out of all the diseases in the human body, neurological diseases have had the slowest progression in our understanding since they often affect cognitive and behavioural traits which are distinctive to humans. One such example would be with autism spectrum disorders (ASDs): a developmental brain disorder where there is no concrete genetic bases or etiology and instead is stereotyped by language deficits, repetitive behaviours and difficulties in social interactions present in early childhood. By reprogramming skin fibroblasts of people with severe idiopathic autism into iPSC, researchers can add specific growth factors to encourage the development of cerebral organoids and study them at a level of scrutiny that was previously unachievable. When compared to control organoids from unaffected relatives of the patients there is an upregulation of genes responsible for cell proliferation and neural differentiation and thus exhibiting an accelerated cell cycle. There is an upregulation of the transcriptional factor FOXG1 which overall causes an overproduction of GABAergic neurons. GABAergic

neurons produce an inhibitory synapse called GABA so this study supports the hypothesis that an imbalance in excitatory/inhibitory neurons is a pathophysiological mechanism contributing to ASD and thus suggests FOXG1 is a potential biomarker of ASD [18, 19].

The ever-evolving research in neuroscience leads us back to the most basic question: what makes us fundamentally human? A better understanding of species-dependent differences will improve the understanding of mechanisms that make humans unique, and this may help the translation of findings made in animal models into therapeutic treatments. Studies have already established key differences among our primate relatives- chimpanzee and macaque cerebral organoids have a faster neural development than humans which explains the difference in brain size and neuron numbers [20]. It has also been recently established that there are at least 261 differences in gene expression between the organoids and notably in the outer radial glial cells [21]. Studies of these genes will help reveal the molecular basis of the uniqueness of human.

Since the publication of the Neanderthal genome in 2010, it is confirmed that Neanderthals were interbreeding with modern human until they became extinct which leads us bigger questions to uncover how modern humans evolved [22, 23]. By merging several biological techniques from extracting ancient DNA from fossils to sequence genomes, and with CRIPSR/Cas9 gene editing tools, scientists are hopeful in growing mini Neanderthal brains – coined 'neanderoids' [24]. What sounds like science fiction may become a reality within the coming years and could answer a plethora of evolutionary questions.

Organoid technology is rapidly expanding and breaking boundaries in biological research which previously seemed impossible. With the capacity to mimic specific features of 3D dimensionality, cell types composition and functionality of an organ, organoids hold great promise in biological and translational application. It provides an ability to study human development and disease which was restricted due to the lack of functioning in vitro models. Perhaps more excitingly, organoids also give a unique access to the workings of brains in the early stages of development which we hope will provide answers to cognitive traits that sets humans apart from other species. As revolutionising as organoid research may be, there are still limitations in reproducibilty, lack of vascularisation and nervation which we hope to overcome in the near future.

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