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ORGANOIDS

Mini organs in a dish

Organoid models include three-dimensional (3D) cell culture systems that closely resemble the *in vivo* organ or tissue from which they are derived. These 3D systems replicate the complex spatial morphology of a differentiated tissue, and allow biologically relevant cell-cell and cell-matrix interactions; ideally, sharing similar physiological responses found within *in vivo* differentiated tissues. This is in contrast to traditional two-dimensional (2D) cell culture models that often bear little physical, molecular, or physiological similarity to their tissue of origin.

Although the earliest 3D organoid models were first described over 40 years ago, their utility has remained limited until recently. Early organoid models required large numbers of starting cells, were not amenable to high-throughput screening, and often exhibited limited *in vitro* viability [1]. These drawbacks have now been largely eliminated as advances in multipotent stem and progenitor cell isolation have allowed researchers to develop highly reproducible, long-lived organoids.

The rapid developments in organoid technology, and the wide usage of the term organoid for a variety of both *in vitro* and *in vivo* structures, led Lancaster and Knoblich to suggest a basic definition for organoids. They defined organoid as:

> "A collection of organ-specific cell types that develops from stem cells or organ progenitors and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*".

According to Lancaster and Knoblich, an organoid should possess several important features characteristic to the respective organs: "[1] it must contain more than one cell type of the organ it models; [2] it should exhibit some function specific to that organ; [3] the cells should be organized similarly to the organ itself" [2].

In 2009, Hans Clevers and Toshiro Sato used adult stem cells from mouse intestine to create the first mini-gut organoids from murine cells [3] and later extended their method to human epithelial organoids [4]. These organoids were expected to allow researchers to gain new insights into the biology of gut health and disease, including colorectal cancer.

This method inspired many other scientists to create a variety of organoids from mouse and human tissues. These clumps of cells are small enough to survive without blood supply, yet large and complex enough to teach us something about tissue and whole-organ development and physiology.

A typical organoid protocol starts with isolated embryonic or pluripotent stem cells, which are then cultured in a supporting scaffold (such as Matrigel) that enables three-dimensional growth. Organoids are comprised of multiple differentiated cell types that are found in the relevant organ *in vivo*. For example, all cell types of the intestinal epithelium are represented in the Matrigel-based model described by Sato et al. [3]. The signaling pathways governing organoid formation were found to be identical to those used during *in vivo* organ development and homeostasis; thus, cytokines, growth factors and small molecules were also included in the culture medium in order to activate or inhibit specific signaling pathways. Even tissues that are closely related, such as the small intestine and colon, require different combinations of signaling molecules in the process of organoid formation [4].

There are different ways to obtain an organoid culture, and some of these are just beginning to be explored. On the following pages you will find some examples of various organoid models that have been developed, with an emphasis on the cytokines, growth factors and small molecules that were used.

GASTROINTESTINAL (GI) ORGANOIDS

Historically, preclinical GI medical research has relied entirely on animal models and cancer cell cultures that are of limited relevance to human physiology; thus, the ability to obtain GI organoids from human cells is of great importance [4, 5].

The intestinal epithelial layer is made up of tiny, slender projections, called villi. The niches formed at the bases of the villi, known as crypts, are home to the intestinal stem cells responsible for constant renewal of the intestinal lining. In the original study that generated murine small intestine organoids, epidermal growth factor (EGF), R-Spondin-1 and Noggin were included in the medium [3]; whereas a later study that demonstrated the formation of murine colon organoids added Wnt-3a to the above three growth factors. The generation of small intestine and colon organoids from human cells also required two small molecules, TGF-β inhibitor (A 83-01) and p38 MAP kinase inhibitor (SB 202190), in addition to the above mentioned cytokines [4].

In a recent study, human intestinal organoids (HIO) that were produced *in vitro* from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), were engrafted *in vivo* and became functioning mature intestinal tissue. For induction of definitive endoderm (DE), human ESCs or iPSCs were treated with medium containing Activin A, followed by a medium containing Activin A, FGF-4 and the GSK3 inhibitor, CHIR 99021, to form spheroids. The spheroids were then plated in Matrigel, and maintained in intestinal growth medium supplemented with EGF and Noggin to generate the HIO that were later engrafted into immunodeficient mice [6].

BRAIN ORGANOIDS

The complexity of the human brain, which made it difficult to study many brain disorders in model organisms, called for the establishment of an *in vitro* model of human brain development.

A protocol for generating 3D brain tissue, so-called cerebral organoids, which closely mimics the endogenous developmental program used patient-specific iPSCs to form a 3D organoid culture model of microcephaly, a disorder that had been difficult to reproduce in mice. iPSCs, resulting from reprogrammed patient skin fibroblasts, were incubated in a medium supplemented with FGF-basic, CHIR 99021 and MEK inhibitor (PD 0325901) for 21 days. The outgrowing colonies were picked and passaged on inactivated CF-1 mouse embryonic fibroblasts (MEFs). Later, single cells were plated in media containing low levels of FGF-basic and high levels of ROCK inhibitor (Y 27632). The neuroepithelial tissues that were formed were transferred to droplets of Matrigel, and after a period of stationary growth these tissue droplets were transferred to a spinning bioreactor containing differentiation medium supplemented with retinoic acid. Analysis of these patient organoids demonstrated premature neuronal differentiation, which could explain the disease phenotype [7].

Recently, a miniaturized spinning bioreactor (SpinΩ) was developed to generate forebrain-specific organoids from human iPSCs. Detached human iPSC colonies were transferred on day 1 to a 6-well plate with stem cell medium containing A 83-01. On days 5-6, half of the medium was replaced with induction medium containing Wnt-3a, CHIR 99021 and a selective TGF-β inhibitor (SB 431542). On day 7, organoids were embedded in Matrigel and grown in induction medium for 6 more days. On day 14, organoids were dissociated from Matrigel and 10 – 20 organoids were transferred to wells of a 12-well spinning bioreactor (SpinΩ) containing differentiation medium. At day 71, differentiation medium was changed to maturation medium, containing BDNF, GDNF and TGF-β1. The organoids could grow beyond 110 days in maturation medium with medium change occuring every other day. These organoids were used to study the effects of Zika virus exposure on the brain, and could be employed in the future for drug testing.

In addition, this platform was also used to generate midbrain and hypothalamus organoids from human iPSCs [8].

B CELL FOLLICLE ORGANOIDS

When naïve B cells encounter antigens, they form clusters of cells called germinal centers in a lymph node or in the spleen; here they proliferate, mutate to produce high-affinity antibodies, and undergo clonal expansion. Until now, recreating this process using 2D cultures *in vitro* was difficult.

Instead of using the conventional Matrigel for 3-D culture, a gelatin and silicate-nanoparticle mix that mimics the environment of the body's lymphoid organs was developed. Naïve B cells obtained from splenocytes were co-cultured with engineered stromal cells expressing both CD40L and B cell activating factor (BAFF) in a medium containing murine IL-4. Much faster than in 2D cultures, the B cells in these organoids matured and displayed class switching within days [9].

LIVER ORGANOIDS

Liver development involves an intricate interaction of tissues derived from both the endoderm and mesoderm. The liver is initially derived from endoderm hepatic bud structures, which develop from foregut epithelium. The hepatoblasts derived from hepatic buds contribute hepatocytes and biliary epithelium, whereas liver fibroblasts and stellate cells originate from nearby mesoderm-derived mesenchyme [2, 10]. A recent approach that was established to generate human liver bud-like tissues employed a mixture of three cell populations, mimicking the early cell lineages of the developing liver: human PSC–derived hepatic cells, human mesenchymal stem cells, and human endothelial cells. For endodermal differentiation, human iPSCs were seeded on a Matrigel-coated dish in medium containing Activin A. Human iPSC-derived endodermal cells were then treated with a medium containing human FGF-basic and BMP-4 for differentiation of hepatic endoderm cells (iPSC-HEs). Human iPSC-HEs were then cultivated with two stromal cell populations: human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (MSCs). The cells spontaneously formed 3D liver buds when mixed at a high density on a layer of Matrigel. When these liver buds were transplanted into mice they displayed vascularization and showed liver-specific functions, and transplanted mice survived the drug-induced lethal liver failure model [2, 11].

RETINA ORGANOIDS

The eye is a highly complexed organ and consists of a variety of cells that are combined in an organized three-dimensional fashion. The light-receptive neural region of the eye, the retina, is derived from the neural ectoderm. Two adjacent epithelial layers are formed early in retinal development: the outer retinal pigmented epithelium and the inner neural retina, which eventually become a tissue containing layers of photoreceptors and supportive cell types [2, 12].

Optic cup organoids were generated from human ESCs, and were compared to similar organoids generated from mouse ESCs. For retinal differentiation, hESCs were reaggregated in retinal differentiation medium containing Y 27632. Matrigel was added from day 2 to day 18. For optic cup formation, CHIR 99021 (or recombinant Wnt-3a) and recombinant human Sonic Hedgehog (Shh) were added to the differentiation medium from day 15 to day 18. These human retinal organoids shared many characteristics displayed by mouse retinal organoids; however, they showed several human- specific differences. In particular, the human retinal organoids were larger than mouse organoids, they developed more slowly, and they grew into tissue comprising multilayers that contained both rods and cones (cone differentiation is rare in mouse ESC culture) [2, 13].

KIDNEY ORGANOIDS

The kidney differentiates from the intermediate mesoderm (IM) through the interaction of IM-derived metanephric mesenchyme (MM) and a formed ureteric bud (UB). Nephron progenitors derived from the MM are the source of nephrons, while the IM itself is derived from the posterior primitive streak [14, 15].

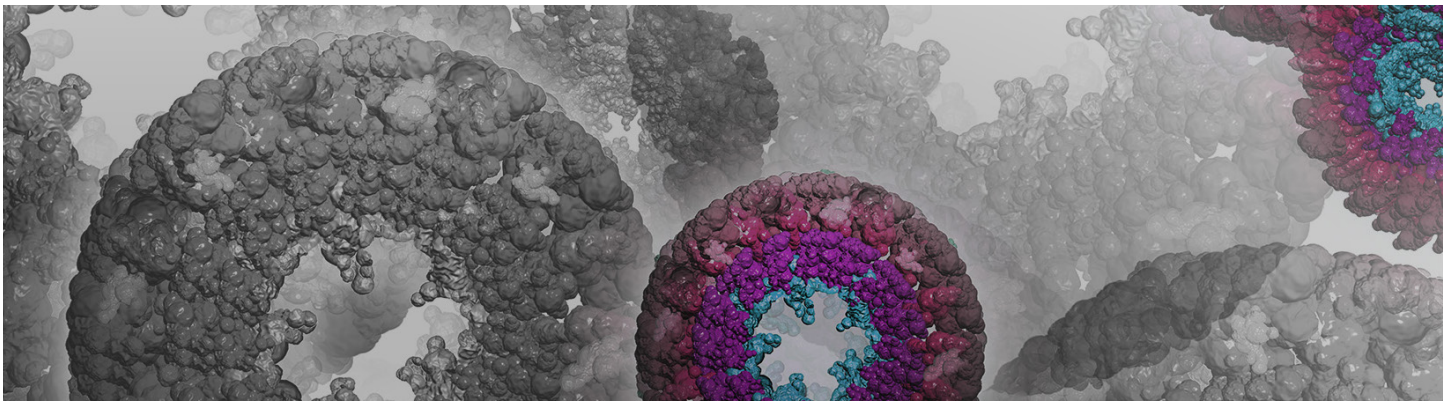
Human ESCs that were cultured on irradiated mouse embryonic fibroblast feeder cells were plated in a Matrigel-coated 96-well plate. After overnight culture, cells were exposed to BMP-4 and Activin A, or alternatively to CHIR 99021, in a serum-free media, then cultured in FGF-9 and heparin- containing media to induce IM cells. These cells were subsequently incubated in a medium containing FGF-9, BMP-7 and retinoic acid (in the case of BMP-4/Activin A-induced cells), or FGF-9 and heparin (in the case of CHIR 99021 induced cells). For the induction of kidney organoids, hESC-derived kidney cells were dissociated into single cells, spun down to form a pellet, placed onto a filter membrane with a collagen IV coat, and, lastly, floated on culture media. This study successfully differentiated hESCs under chemically-defined culture conditions, using growth factors that participate in normal embryogenesis, and resulted in coordinated generation of UB and MM that finally formed, *in vitro*, self-organizing 3D structures, including nephron formation [15].

THE THERAPEUTIC POTENTIAL OF ORGANOIDS

A wide array of organoids from organs of all three embryonic layers have been studied, and include: endoderm-derived organoids of thyroid, lung, pancreas, liver, stomach and intestine; mesoderm-derived organoids of heart, skeletal muscle, bone and kidney; and ectoderm-derived organoids of retina, brain, pituitary, mammary gland, inner ear and skin [2].

The major focus of future organoid studies will continue to investigate and refine the developmental processes and most likely, will subsequently advance into disease modeling. Organoids may be useful to further research in developmental disorders, genetic conditions, cancer and degenerative disease, to name a few [2, 16].

Utilizing patient iPSCs will allow valuable disease modeling, especially when adequate animal models are not available. Organoids can also be used for more efficient testing of drug efficacy and toxicity by removing discrepancies due to the differences between animal and human cells. Organoid drug testing might also dramatically reduce the use of animals for pre-clinical trials. The hope is that organoids are another step in the long journey towards *in vitro* construction of tissues and organs for transplantation into patients; however, many obstacles still need to be addressed along the way, such as proper maturation and the lack of vascularization.



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