



## Review

## Engineering a second brain in a dish

Maxime M. Mahe<sup>a,b</sup><sup>a</sup> Department of Pediatric General and Thoracic Surgery, Cincinnati Childrens Hospital Medical Center, S6.609, Building, 3333 Burnet Avenue, Cincinnati, OH, USA<sup>b</sup> Inserm UMR 1235 TENS, INSERM, University of Nantes, Rm427, Faculty of Medicine, 1 Rue Gaston Veil, Nantes, France

## ARTICLE INFO

## Article history:

Received 1 November 2017

Received in revised form 13 April 2018

Accepted 16 April 2018

## Keywords:

Human pluripotent stem cells  
 Intestinal organoid  
 Enteric nervous system  
 Tissue engineering  
 Translational embryology  
 Neurogastroenterology

## ABSTRACT

The utilization of human pluripotent stem cells holds great promise in elucidating principles of developmental biology and applications in personalized and regenerative medicine. Breakthroughs from the last decade have allowed the scientific community to better understand and successfully manipulate human pluripotent stem cells using distinct differentiation strategies into a variety of target tissues. This manipulation relies solely on our understanding of developmental processes occurring in model organisms. The *in vitro* translation of our developmental knowledge upon stem cells provides a new means to generate specific tissue to understand developmental and disease mechanisms, as well as physiological processes. The generation of an integrated human intestinal tissue is one such example. In this review, we highlight the biological motivation behind the generation of human intestinal organoids. We further describe the integration of an enteric nervous system within the organoid to generate a functional intestine. Forthcoming strategies to add additional complexities to the intestinal tissue so as to better understand how our second brain functions within the gut are also discussed. The organoid system offers a promising avenue to understand how the enteric nervous system works and patterns the human intestine during both physiology and disease.

© 2018 Elsevier B.V. All rights reserved.

## Contents

1. The use of pluripotent stem cells to make human tissue . . . . .	165
2. Making intestinal organoids from human pluripotent stem cells . . . . .	166
3. Building an enteric nervous system in intestinal organoids . . . . .	166
4. Engineering environmental complexity in innervated intestinal organoids . . . . .	167
5. Future challenges and perspectives . . . . .	167
Acknowledgements . . . . .	168
Funding . . . . .	168
References . . . . .	168

## 1. The use of pluripotent stem cells to make human tissue

The ability to isolate and culture human stem cells in three dimensions equips us with an exciting avenue to study organ physiology and diseases. As such, the growing field of organoid research

has been pivotal in addressing important questions related to human biology (Dutta et al., 2017). Organoids are tridimensional cellular structures that resemble tissue-specific phenotypes, both in form and function. These organoids can be grown from isolated fetal and adult stem cells or generated through the directed

**Abbreviations:** BMP, bone morphogenic protein; EGF, epidermal growth factor; ENS, enteric nervous system; ESC, embryonic stem cell; iPSC, induced Pluripotent Stem Cell; FGF, fibroblast growth factor; HIO, human intestinal organoid; HIO+ENS, human intestinal organoid with an enteric nervous system; hPSC, human Pluripotent Stem Cell; NCC, neural crest cell; PHOX2B, paired-like homeobox 2b; TGF-β, transforming growth factor-β; WNT, wingless-related INtegration site; WNT3A, Wnt family member 3A.

E-mail addresses: [maxime.mahe@cchmc.org](mailto:maxime.mahe@cchmc.org), [maxime.mahe@inserm.fr](mailto:maxime.mahe@inserm.fr)

<https://doi.org/10.1016/j.brainres.2018.04.015>

0006-8993/© 2018 Elsevier B.V. All rights reserved.

differentiation of human pluripotent stem cells (hPSCs, for review (Bartfeld and Clevers, 2017; Huch and Koo, 2015; Koo and Huch, 2016; McCauley and Wells, 2017)). Intestinal organoids derived from adult intestinal stem cells are a promising tool to study gut-brain interactions (Bellono et al., 2017). Although of importance, we have chosen to limit our focus to intestinal organoids derived from human pluripotent stem cells.

For a few decades, developmental biologists have made significant contributions in elucidating the intricacies of vertebrate development. This has been possible through the study of multiple model organisms including fish, xenopus, chicken and murine models. These discoveries have allowed the scientific community to build a detailed encyclopedia of vertebrate development from the formation of an embryo to the organisms completion (Tseng et al., 2017). Studies performed using these different models have identified essential pathways that govern the formation of the initial germ layers, organs and tissues. These pathways include, but are not limited to, Transforming growth factor-beta (TGF-beta)/bone morphogenic protein (BMP), Fibroblast growth factor (FGF), Wnt-related integration site (WNT) and Retinoic acid pathways.

Major breakthroughs included the isolation and culture of human embryonic stem cells (ESC, (Thomson et al., 1998)) and the discovery of pluripotency factors which can reprogram cells, like fibroblasts, into induced human pluripotent stem cells (iPSC, (Takahashi et al., 2007)). The use of hPSCs has facilitated the study of developmental processes and disease-mechanism in humans that were impossible to study ethically until recently. Importantly, the use of iPSCs constitutes an ideal material for personalized medicine as they can be generated from virtually every patient. The manipulation of the aforementioned developmental pathways and their application to pluripotent stem cells *in vitro* has resulted in the highly specific derivation of tissues of interest, including the human intestine and the enteric nervous system lineages.

## 2. Making intestinal organoids from human pluripotent stem cells

The intestine is a tube divided into anatomical and functional regions associated with specific digestive functions and host-immunity. The intestinal wall is comprised of several layers including an outer layer of longitudinal and circular muscles and an inner epithelial lining which is in contact with the lumens food and bacterial contents. The intestinal epithelium acts as a functional barrier as it promotes the uptake of the nutrients and prevents the passing of the pathogens. Specialized cells within the epithelium participate to the maintenance of the intestinal lining. These cells include absorptive (enterocytes) and secretory lineages (Goblet, Paneth, Enteroendocrine and Tuft cells) (Chin et al., 2017).

Formation of the intestinal tube starts at around the third week of gestation in human embryos and by approximately the twelfth week of gestation is complete. From there, the intestine will continue to expand and lengthen as the fetus grows and thereafter during the first years of life. The intestinal tube originates in the embryo from a germ layer called endoderm (Noah et al., 2011). During the formation of the endoderm, several signaling pathways including Nodal, FGF, and WNT are activated. Nodal is a member of the TGF-beta superfamily and is required for the endoderm formation (Zorn and Wells, 2009). The midgut and hindgut, future small intestine and colon, are then promoted by the activation of Fibroblast Growth Factor 4 (FGF4) and WNT signaling to repress foregut development and push toward an intestinal fate (Wells and Melton, 1999; Zorn and Wells, 2009).

In 2005, a group demonstrated the generation of endoderm from human ESCs. Using the protein activin A, a Nodal mimetic,

they were able to generate a definitive endoderm (D'Amour et al., 2005). In 2011, Spence et al. were able to demonstrate the first known generation of intestinal midgut spheroids from hPSCs. Using a step-wise differentiation process, the formation of definitive endoderm was followed by an activation of the WNT and FGF pathways using FGF4 and Wnt Family Member 3A (WNT3A). This process generated intestinal spheroids that, when included in a 3D matrix and supplied an intestinal media, led to the formation of complex intestinal structures termed human intestinal organoids (HIOs, (Spence et al., 2011)). These organoids are characterized by a hollow lumen delimited by an epithelial monolayer comprised of absorptive cells, enterocytes and secretory cells including Paneth, Goblet and enteroendocrine cells. These 3D structures are functionally competent at processing peptides and sugars. An important distinction from intestinal organoids derived from adult intestinal stem cells, is that the HIO is more than epithelia. HIOs present a layer of mesenchymal cells originating from mesodermal progenies during the differentiation process. Interestingly, when transplanted in immunocompromised mice, with or without a scaffold, the HIO will further grow and mature to form a well-developed intestinal tissue reminiscent of human small intestine (Finkbeiner et al., 2015a; Watson et al., 2014). The tissue, although intestine-like, does not yet bear all the cellular components observed in human intestine. One of the most prominent is the neuronal network that innervates the digestive tract: the enteric nervous system (ENS).

## 3. Building an enteric nervous system in intestinal organoids

Often called the second brain, the ENS is an integrated and autonomous nervous system that controls gut motility. The ENS coordinates complex behaviors of the gut by controlling peristaltic movements and changing blood flow and secretions of water and electrolytes. The developed ENS is distributed along the entire length of the digestive tract in two major plexus (i.e. myenteric and submucosal). Myenteric neurons control gut motility and submucosal neurons, mucosal processes, distortion of the mucosa, and chemical contents in the lumen (Grundt and Schemann, 2007; Neunlist et al., 2013).

Developmentally, the ENS originates from a different germ layer than the intestine: the ectoderm. The ectodermal contributions to the gut are derived from migrating vagal neural crest cells (NCC) that primarily delaminate from the neural tube, with a minor contribution from the sacral neural crest cells (Nagy and Goldstein, 2017). The vagal NCCs colonize the entire gastro-intestinal tract starting at week four and complete their migration by week nine of gestation (Sasselli et al., 2012).

Previous groups have achieved the directed differentiation of hPSCs into NCCs (Bajpai et al., 2010; Curchoe et al., 2010) including enteric vagal NCCs (Fattahi et al., 2016). To that end, FGF and EGF (Epidermal growth factor) pathways are activated to generate migrating NCCs. Retinoic acid signaling is then required to posteriorize the NCCs (Kudoh et al., 2002). To generate innervated HIOs, hPSC-derived vagal NCCs and midgut spheroids were combined to generate a 3D human intestinal organoid with a neuronal component (HIO+ENS) (Fig. 1). These organoids comprised a hollow lumen and were reminiscent of a normal HIO at the exception they were surrounded by neurons and glial cells. When transplanted in immunocompromised mice, these innervated organoids developed into a mature intestinal tissue with crypts, villi, laminated subepithelial, and mesenchymal layers. Strikingly, these HIO+ENS exhibited similar features to the human ENS including neuronal bundles aligned on the stratified muscular layers of the engraftment reassembling the plexus found in the human gastrointestinal wall.

Download English Version:

<https://daneshyari.com/en/article/8839729>

Download Persian Version:

<https://daneshyari.com/article/8839729>

[Daneshyari.com](https://daneshyari.com)