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SPOTLIGHT

Ethical issues in human organoid and gastruloid research

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ABSTRACT

Research involving human organoids and gastruloids involves ethical issues associated with their derivation as well as their current and future uses. These include unique issues related to the extent of maturation that can be achieved *in vitro* or through chimeric research, as well as fundamental ethical considerations such as those concerning the provenance of human biomaterials and the use of gene-editing technologies. Many of these issues are not specifically addressed by existing ethics oversight mechanisms, but these mechanisms might be easily extended to help ensure that human organoid and related research moves forward in an ethically appropriate manner.

KEY WORDS: Stem cells, Organoids, Gastruloids, Ethics, Regulation, Chimera

Introduction

Considerable research activity is currently focused on a variety of human organoids and similar self-organizing structures such as gastruloids (hereafter collectively referred to as 'organoids', unless otherwise specified). Such research has already led to an enhanced understanding of human development and disease (Lancaster et al., 2013; Matano et al., 2015; Takasato et al., 2015; Garcez et al., 2016). Nevertheless, like other biomedical research in general and human development research in particular, research involving human organoids raises important ethical issues. In this Spotlight article, after briefly describing the current scientific context of human organoids, we describe the ethical issues associated with their derivation and potential research uses. These include the extent of organoid and gastruloid maturation, chimera research, the use of gene-editing technologies in organoid research, and the provenance of human materials. Finally, we consider what type of oversight might be helpful in advancing scientific understanding in an ethically appropriate manner.

The scientific context

The term 'organoid' has conventionally been used to describe three-dimensional (3D) cultures used to study organogenesis *ex vivo*. Recent research employs stem cells to drive *in vitro* development of refined cellular microstructures that architecturally 'resemble their *in vivo* counterparts and recapitulate at least some functions of the organ' (Huch and Koo, 2015). Organoid development can be initiated from resident epithelial stem cells or from pluripotent stem cells (PSCs), including embryonic stem cells and induced PSCs. The source of stem cells dictates the derivation and culture methods, the extent to which organ architecture and function are replicated, and the potential clinical applications of these structures.

Although organoids from freshly isolated explanted tissue provide a means to identify and explore stem cell niches as well as to model disease and organogenesis, clonally generated organoids from selected epithelial stem cells open up new possibilities. Compared with organoids from explanted tissues, those generated from tissue stem cells more closely recapitulate the epithelia of the organ from which they are derived and can be kept in culture indefinitely (Huch et al., 2015). Because these structures can be readily established from biopsy tissue, are easy to manipulate and can be banked, they have been proposed as an *ex vivo* platform for personalized cancer treatment (Gao et al., 2014; Boj et al., 2015; van de Wetering, 2015; Soragni et al., 2016) and as a source of cells for the correction of genetic defects in monoallelic conditions (Schwank et al., 2013) and for transplantation (Yui et al., 2012).

By contrast, organoids derived from PSCs are complex structures displaying discrete pockets of morphologically and functionally distinct cell clusters. Although human PSC-derived organoids represent an unprecedented level of complexity for directed differentiation, they do not fully recapitulate organogenesis. Current PSC-based techniques result in organoids that are a 'haphazard approximation of their *in vivo* counterparts' (Bershteyn and Kriegstein, 2013). Further, their cellular architecture resembles that observed in first-trimester development rather than adult phenotypes and there is usually an absence of vascularization, innervation (in non-neural organoids) and other important supporting cell types. A recent study involving PSC-derived kidney organoids showed some evidence of early vascularization; however, the essential capillary loops in the nephrons were lacking, as well as other structural components (Takasato et al., 2015). Despite these limitations, media reports enthusiastically proclaimed scientists had grown a 'miniature human kidney' in a dish (Gallagher, 2015). Such an achievement would necessitate surmounting the formidable challenges of replicating *in vitro* the sophisticated patterning events that govern developmental biology and sustaining these structures in culture for a sufficient time to allow them to functionally mature. These technological challenges have yet to be overcome.

Nevertheless, a number of studies have begun to explore a higher level of organoid complexity and functionality (Assawachananont et al., 2014; Takebe et al., 2013; Workman et al., 2017). Retinal organoids generated from mouse PSCs can functionally engraft, exhibiting synaptic connections with the retina in host mice (Assawachananont et al., 2014). In addition, cells from liver progenitors (so-called liver buds) derived from human PSCs co-cultured in 3D with endothelial cells have been shown to engraft and vascularize in host mice, where they undergo further maturation to form more complex structures (Takebe et al., 2013). Subsequently, complex vascularized organ buds for kidney, pancreas, intestine, heart, lung and brain have been developed through co-culture of mouse PSC-derived progenitors, endothelial cells and mesenchymal stem cells following engraftment in host mice

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(Takebe et al., 2015). Innervation of PSC-derived organoids has also recently been achieved (Workman et al., 2017). Specifically, developing PSC-derived intestinal organoids were co-cultured with PSC-derived neural crest cells in order to recreate a functional enteric nervous system. Research involving human cerebral organoids has also been used to rapidly identify mechanisms by which Zika virus can cause microcephaly in fetuses and for screening drugs that may attenuate infections (Garcez et al., 2016; Xu et al., 2016; discussed in this issue by Qian et al., 2017).

Another phenomenon observed under certain *in vitro* conditions is the self-organization of mouse and human PSCs into structures that recapitulate key developmental features of gastrulation (van den Brink et al., 2014; Warmflash et al., 2014, reviewed in this issue by Simunovic and Brivanlou, 2017). Referred to as 'gastruloids', these complex structures are distinct from organoids in that they do not recapitulate an organ per se, but rather a developmental process. Nonetheless, their ability to self-organize *in vitro* into complex 3D structures that resemble developing tissue *in vivo* has earned them a place within the organoid field. Gastruloids contain markers of primitive streak formation and representative cells from each of the three germ layers, including the ectoderm that will give rise to the central nervous system. Although gastruloids lack the intricate 3D patterning associated with establishing a body plan during postimplantation embryonic development, it is possible that more advanced *in vitro* development could be achieved by mimicking interaction with extra-embryonic tissues, of either human or non-human origin. Such research could in the future provide a unique insight into early human embryo development and disorders associated with first-trimester pregnancy loss (Pera et al., 2015).

Ethical issues

The ethical issues related to human organoids are linked fundamentally to those already identified in developmental biology and related fields of research and clinical practice. Prior debates, particularly in regard to *in vitro* fertilization, embryo research, and the derivation and use of PSCs, have highlighted the importance of attending to the provenance of materials used in experiments (including that explicit informed consent is obtained for particular uses), privacy, limitations on certain uses, and proper oversight for selected research and clinical applications. While lessons learned in these fields inform the ethics of research involving human organoids and other similar complex structures, specific consideration should be given to the extent of maturation (including those obtained through chimeric research), the provenance of human biomaterials, and gene-editing technologies. Such issues are relevant no matter the source of the organoid or *in vitro* structure.

Extent of maturation

Scientific work to date hints at possible moral concerns about the 'creation of life' and/or the acquisition of human qualities in research involving human organoids. Accordingly, it is important to consider potential ethical issues that may become relevant depending on scientific progress and the analogies used to understand the moral status of these structures. Here, we consider those entities that are especially salient in this regard: gastruloids, cerebral organoids, and the engineering of multi-organoid complexes.

Owing to the extent to which they resemble embryos, human gastruloids raise potential conceptual and ethical concerns related to the creation of early human life *in vitro* (Pera et al., 2015). As previously suggested, if human gastruloids are considered to be functionally akin to human embryos, an array of ethical and regulatory concerns arises about the appropriateness of creating these PSC-derived constructs in jurisdictions that prohibit the generation of research embryos and their destruction, as well as the limitations over the extent to which human gastruloids may be permitted to mature. Even in locales where the creation of research embryos is permitted, work with human embryos *in vitro* is generally limited to the first 14 days of development or the appearance of a primitive streak, whichever comes first. Since human gastruloids are meant to recapitulate precisely this postimplantation stage of human development, the relevance of the 14-day rule might well apply to research actually directed at deriving gastruloids or 'embryo in a dish' model systems (Hyun et al., 2016). From recent reports it appears that gastruloids can mimic a similar stage of gastrulation in a much shorter window – days rather than weeks – than human embryos (Warmflash et al., 2014). However, it is the developmental stage rather than the exact number of days that have passed that matters for the 14-day rule. Therefore, greater clarity around the limitations posed by the 14-day rule is necessary if gastruloid research is to proceed in the future. At least currently, gastruloids derived from PSCs seem unlikely to be fully functionally equivalent to a postimplantation stage embryo, but this might change if researchers seek to employ 3D culture systems involving extra-embryonic tissue (of human or animal origin) as well as other refinements.

Similarly, although considerable complexity of human cerebral organoids has been achieved (Lancaster et al., 2013), at least for the time being these *in vitro* constructs are far removed from the structural and functional sophistication of the human brain (Yin et al., 2016). Current cerebral organoids lack mature neural networks, have no sensory input and output and are therefore unable to interact with and react to the environment, making concerns about cognitive function or 'thinking' of cerebral organoids unfounded at present (Bersenev, 2015). However, if current limitations were overcome through customized bioengineering strategies to refine spatial development and enhance maturation through increased vascularization and/or perfusion, resulting in afferent sensation and complex neural networks, research involving human cerebral organoids would begin to raise moral concerns (Cheshire, 2014). Such concerns could be further intensified if these structures were to be transferred into chimeric animal models, as discussed below.

Although it has not yet been reported, it might soon become technologically feasible to link numerous human organoids into working complexes, taking the concept of 'organ-on-a-chip' microfluidic devices (Bhatia and Ingber, 2014) to a whole new level. If this occurs, then the degree of integrated biological functioning might become morally relevant. Multi-organoid complexes that approximate large interconnected swaths of the human body might trigger moral sentiments about the appropriateness of creating and experimenting with such familiar, biologically humanized entities. As a related matter, it has been suggested that organoids could be 'programmed' via artificial genetic circuits (Yin et al., 2016). By applying logic gates, the cells within the *in vitro* structure could be 'trained' to respond to certain cues in culture or upon transplantation, thereby fine-tuning organoid development. Here, the extent of autonomous functioning might at some point raise relevant concerns about the ambiguous moral status of these self-developing constructs.

Chimera research

Although various forms of chimera research have been taking place without much debate for decades, such as transplantation of human cancer cells into mice or functional engraftment of cells derived

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from human PSCs, ethical concerns may arise when cells and complex *in vitro* structures of human origin are introduced into the brains or reproductive systems of non-human animals. The chief worry seems to be that in the process of biologically humanizing a research animal, scientists might inadvertently morally humanize the resulting chimera (Hyun, 2016). For instance, substantial ethical concerns have long been raised by 'humanized mice', especially with regard to the central nervous system (Greely et al., 2007). Also, the introduction of human gonad-like organoids into animal models might raise concerns about the possibility of inadvertent cross-species fertilization involving human and non-human gametes. Accordingly, organoid and related research that proposes chimeric integration in an animal's central nervous or reproductive system should be designed in such a way as to mitigate these ethical concerns. For example, the transfer of human or cerebral organoids into non-human animals should be conducted through incremental research and be closely monitored to determine the physical and behavioral changes that might occur in the host animal (Hyun, 2016). Likewise, if there is a possibility of integration into reproductive systems, it will be crucial to take measures to ensure that such chimeric animals are unable to breed.

Provenance of human biomaterials

Longstanding debates regarding *in vitro* fertilization and PSC research underscore the need for human biomaterials to be obtained in an ethically appropriate manner (Daley et al., 2016). In these settings, at a minimum this has included the need to ensure that human biomaterials are obtained with explicit and voluntary informed consent that is consistent with the proposed use of the biomaterials and in a manner consistent with local norms and policies. While high ethical standards should apply for organoid and related research, it is possible in some countries to obtain biomaterials for research without specific consent. For example, U.S. federal research regulations currently permit research involving 'pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects' (United States Government, 2009). Thus, in the absence of local policies or regulations that may pose more stringent requirements, tissue discarded during clinical procedures can be used for research without explicit consent of the patient, provided that the tissues are anonymized and the patient's admission form or consent for diagnostic or surgical procedures stipulates that biomaterials collected during the course of treatment may be used for 'education and research'. This highlights the importance and challenge for the researcher of ensuring the ethical provenance of human biomaterials used for organoid and related research, especially in relation to biobanking, commercialization and global distribution (Boers et al., 2016).

Use of gene-editing technologies

Gene editing has already been used in organoid research with important results. Consider, for example, the correction of a cystic fibrosis transmembrane conductor receptor and the restoration of function in intestinal organoids generated from the tissue of affected patients (Schwank et al., 2013). Although such efforts provide a proof-of-concept and raise exciting possibilities for future research efforts, CRISPR-Cas9 gene editing has been associated with substantial ethical concerns among scientists and the public, especially with regard to germline-based interventions and enhancement (Reardon, 2015). International discussions about the ethical issues associated with this breakthrough technology are ongoing, and it will be essential for those engaged in organoid research to participate in these discussions and be prepared to respond appropriately to the recommendations that may emerge.

Policy and oversight

Outside of research involving human embryos and human PSCs, much *in vitro* work in developmental biology receives little if any ethics oversight, except to the extent that ethical issues are considered during peer review for funding and publication. Of course, work that raises concerns about biosafety and the use of non-human animals will undergo oversight by appropriate entities. However, given that research involving human organoids can raise ethical concerns that may become more complex as the science evolves, it might be prudent to ensure some type of prospective oversight of this research, perhaps akin to that pertaining to PSC research. Doing so at an institutional level might facilitate a measured review that could identify any ethically troublesome issues and help to navigate them. One way to achieve such a proactive approach would be to utilize a key recommendation offered in the new research guidelines of the International Society for Stem Cell Research (ISSCR). The ISSCR is now urging researchers to submit their proposals to an institutional embryo research oversight (EMRO) process whenever their protocols involve the creation and use of embryos or embryo-like entities with full organismal potential – that is, the potential to create a whole human being (Daley et al., 2016). This latter category could be broadened to include entities such as gastruloids or multi-organoid complexes that might functionally approach early or late-stage human embryos. As with all research involving intact human embryos, an EMRO review of gastruloid and multi-organoid research would include a determination of the scientific rationale and merit of the proposal, the proposed methodology, and whether there are alternative methods for addressing the research question.

Although the clinical translation of human organoids is still on the horizon, at least one group is exploring the use of tissue-derived liver organoids as a source of material for children with inherited metabolic disease (Boers et al., 2016). Thus, it is essential to consider the ethical issues that will arise as bench research moves into clinical trials. As a starting point, consideration could be given to adapting the ISSCR guidelines for stem cell research and clinical translation mentioned above (ISSCR, 2016). In these guidelines, attention is focused on all stages of the research process: the bench, non-human animal studies, first in-human studies, late-stage clinical trials, and clinical use. While the basic features of the guidelines could be maintained, they should be revisited in light of the scientific realities of organoids and gastruloids, rather than PSCs alone.

Finally, and as mentioned earlier, organoids have already piqued public interest with evocative headlines describing 'mini-organs in a dish'. In an effort to avoid public confusion and misplaced expectations of clinical benefit, as well as potential fear about this promising technology, researchers need to avoid the hyperbole that are too often a hallmark of stem cell research and candidly discuss advances and limitations with the public through the popular press and social media (Caulfield et al., 2016). As stated in the ISSCR guidelines, researchers should strive to 'ensure that benefits, risks and uncertainties of stem cell science are not misrepresented' (ISSCR, 2016).

Conclusions

Research on human organoids and gastruloids promises to enhance our understanding of an array of important issues in developmental

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biology. Although the science is fairly nascent and does not currently raise substantially unique ethical concerns, it will be crucial to continue to monitor ongoing efforts and proposed advances that might necessitate new and more detailed ethical analyses. Such work will likely be enhanced with public engagement on these and related issues, so that key perspectives may be incorporated into the process and progress in this promising field is not hampered by unfounded misconceptions and fears.

Competing interests

The authors declare no competing or financial interests.

References

Assawachananont, J., Mandai, M., Okamoto, S., Yamada, C., Eiraku, M., Yonemura, S., Sasai, Y. and Takahashi, M. (2014). Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Rep.* **2**, 662-674.

Bersenev, A. (2015). All about organoids-interview with Madeline Lancaster. *Stem Cell Assays*, http://stemcellassays.com/2015/09/interview-madeline-lancaster/.

Bershteyn, M. and Kriegstein, A. R. (2013). Cerebral organoids in a dish: progress and prospects. *Cell* **155**, 19-20.

Bhatia, S. N. and Ingber, D. E. (2014). Microfluidic organs-on-chips. *Nat. Biotechnol.* **32**, 760-772.

Boers, S. N., van Delden, J. J. M., Clevers, H. and Bredenoord, A. L. (2016). Organoid biobanking: identifying the ethics: organoids revive old and raise new ethical challenges for basic research and therapeutic use. *EMBO Rep.* **17**, 938-941.

Boj, S. F., Hwang, C.-I., Baker, L. A., Chio, I. I. C., Engle, D. D., Corbo, V., Jager, M., Ponz-Sarvise, M., Tiriac, H., Spector, M. S. et al. (2015). Organoid models of human and mouse ductal pancreatic cancer. *Cell* **160**, 324-338.

Caulfield, T., Sipp, D., Murry, C. E., Daley, G. Q. and Kimmelman, J. (2016). Confronting stem cell hype. *Science* **352**, 776-777.

Cheshire, W. P. (2014). Miniature human brains: an ethics analysis. *Ethics Med.* **30**, 7-12.

Daley, G. Q., Hyun, I., Apperley, J. F., Barker, R. A., Benvenisty, N., Bredenoord, A. L., Breuer, C. K., Caulfield, T., Cedars, M. I., Frey-Vasconcells, J. et al. (2016). Setting global standards for stem cell research and clinical translation: The 2016 ISSCR Guidelines. *Stem Cell Rep.* **6**, 787-797.

Gallagher, J. (2015). Miniature human kidney grown in a dish. *BBC News website*, http://www.bbc.com/news/health-34464477.

Gao, D., Vela, I., Sboner, A., Iaquinta, P. J., Karthaus, W. R., Gopalan, A., Dowling, C., Wanjala, J. N., Undvall, E. A., Arora, V. K. et al. (2014). Organoid cultures derived from patients with advanced prostate cancer. *Cell* **159**, 176-187.

Garcez, P. P., Loiola, E. C., Madeiro da Costa, R., Higa, L. M., Trindade, P., Delvecchio, R., Nascimento, J. M., Brindeiro, R., Tanuri, A. and Rehen, S. K. (2016). Zika virus impairs growth in human neurospheres and brain organoids. *Science* **352**, 816-818.

Greely, H. T., Cho, M. K., Hogle, L. F. and Satz, D. M. (2007). Thinking about the human neuron mouse. *Am. J. Bioeth.* **7**, 27-40.

Huch, M. and Koo, B.-K. (2015). Modeling mouse and human development using organoid cultures. *Development* **142**, 3113-3125.

Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M. M. A., Ellis, E., van Wenum, M., Fuchs, S. A., de Ligt, J. et al. (2015). Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* **160**, 299-312.

Hyun, I. (2016). Illusory fears must not stifle chimaera research. *Nature* **537**, 281.

Hyun, I., Wilkerson, A. and Johnston, J. (2016). Revisit the 14-day rule. *Nature* **533**, 169-171.

ISSCR (2016). *Guidelines for Stem Cell Science and Clinical Translation*. Skokie, IL: International Society for Stem Cell Research.

Lancaster, M. A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L. S., Hurles, M. E., Homfray, T., Penninger, J. M., Jackson, A. P. and Knoblich, J. A. (2013). Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373-379.

Matano, M., Date, S., Shimokawa, M., Takano, A., Fujii, M., Ohta, Y., Watanabe, T., Kanai, T. and Sato, T. (2015). Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat. Med.* **21**, 256-262.

Pera, M. F., de Wert, G., Dondorp, W., Lovell-Badge, R., Mummery, C. L., Munsie, M. and Tam, P. P. (2015). What if stem cells turn into embryos in a dish? *Nat. Methods* **12**, 917-919.

Qian, X., Nguyen, H. N., Jacob, F., Song, H. and Ming, G.-l. (2017). Using brain organoids to understand Zika virus-induced microcephaly. *Development* **144**, 952-957.

Reardon, S. (2015). Global summit reveals divergent views on human gene editing. *Nature* **528**, 173.

Schwank, G., Koo, B.-K., Sasselli, V., Dekkers, J. F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C. K. et al. (2013). Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* **13**, 653-658.

Simunovic, M. and Brivanlou, A. H. (2017). Embryoids, organoids and gastruloids: new approaches to understanding embryogenesis. *Development* **144**, 976-985.

Soragni, A., Janzen, D. M., Johnson, L. M., Lindgren, A. G., Thai-Quynh Nguyen, A., Tiourin, E., Soriaga, A. B., Lu, J., Jiang, L., Faull, K. F. et al. (2016). A designed inhibitor of p53 aggregation rescues p53 tumor suppression in ovarian carcinomas. *Cancer Cell* **29**, 90-103.

Takasato, M., Er, P. X., Chiu, H. S., Maier, B., Baillie, G. J., Ferguson, C., Parton, R. G., Wolvetang, E. J., Roost, M. S., Chuva de Sousa Lopes, S. M. et al. (2015). Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* **526**, 564-568.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.-R., Ueno, Y., Zheng, Y.-W., Koike, N. et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* **499**, 481-484.T.

Takebe, T., Enomura, M., Yoshizawa, E., Kimura, M., Koike, H., Ueno, Y., Matsuzaki, T., Yamazaki, T., Toyohara, T., Osafune, K. et al. (2015). Vascularized and complex organ buds from diverse tissues via mesenchymal cell-driven condensation. *Cell Stem Cell* **16**, 556-565.

United States Government (2009). *Code of Federal Regulations* Title 45, Part 46.101.b.2., https://www.hhs.gov/ohrp/sites/default/files/ohrp/policy/ohrpregulations.pdf.

van de Wetering, M., Francies, H. E., Francis, J. M., Bounova, G., Iorio, F., Pronk, A., van Houdt, W., van Gorp, J., Taylor-Weiner, A., Kester, L. et al. (2015). Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* **161**, 933-945.

van den Brink, S. C., Baillie-Johnson, P., Balayo, T., Hadjantonakis, A.-K., Nowotschin, S., Turner, D. A. and Martinez Arias, A. (2014). Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells. *Development* **141**, 4231-4242.

Warmflash, A., Sorre, B., Etoc, F., Siggia, E. D. and Brivanlou, A. H. (2014). A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. *Nat. Methods* **11**, 847-854.

Workman, M. J., Mahe, M. M., Trisno, S., Poling, H. M., Watson, C. L., Sundaram, N., Chang, C. F., Schiesser, J., Aubert, P., Stanley, E. G. et al. (2017). Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat. Med.* **23**, 49-59.

Xu, M., Lee, E. M., Wen, Z., Cheng, Y., Huang, W. K., Qian, X., Tcw, J., Kouznetsova, J., Ogden, S. C., Hammack, C. et al. (2016). Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via drug repurposing screen. *Nat. Med.* **22**, 1101-1107.

Yin, X., Mead, B. E., Safaee, H., Langer, R., Karp, J. M. and Levy, O. (2016). Engineering stem cell organoids. *Cell Stem Cell* **18**, 25-38.

Yui, S., Nakamura, T., Sato, T., Nemoto, Y., Mizutani, T., Zheng, X., Ichinose, S., Nagaishi, T., Okamoto, R., Tsuchiya, K. et al. (2012). Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. *Nat. Med.* **18**, 618-623.