organoids

MDPI

Editorial

Organoids, Assembloids and Embryoids: New Avenues for Developmental Biology, Disease Modeling, Drug Testing and Toxicity Assessment without Animal Experimentation

Süleyman Ergün * and Philipp Wörsdörfer

Institute of Anatomy and Cell Biology, Julius-Maximilians-University, 97070 Wuerzburg, Germany; philipp.woersdoerfer@uni-wuerzburg.de
* Correspondence: sueleyman.erguen@uni-wuerzburg.de

The organs and tissues of our bodies consist of a specific set of cell types. These cells closely interact with each other and the surrounding extracellular matrix. During embryonic and fetal development, such interactions are inductive and are the driving forces of tissue morphogenesis. Mimicking these spatially and temporally complex processes of organogenesis under lab conditions is a young research area, pioneered by the work of Hans Clevers and co-workers. Despite promising progress during the last decade, there is a long way to go before organoids that resemble the entire complexity of their originals, in structure and function, can be generated.

Conventional 2D cell cultures do not display all aspects of the aforementioned complex interplay; therefore, realistic 3D tissue models that recapitulate organ development and morphogenesis and fulfill at least some of the tissue-specific functions are required. Such models are termed organoids. Organoids allow researchers to study the development of tissues and organs, carry out realistic toxicity assessments, drug tests or genome editing strategies, and model human diseases [1]. They enable direct research on human tissues and help to reduce animal testing. Finally, the further development of organoids could also enable their use for replacement therapies.

3D tissue models already have a long history. Early studies from the 1960s and 1970s showed that fetal organs, when removed from the body and dissociated into single cells, have the remarkable potential to reaggregate and resume the shape, tissue architecture and even function of the original organ [2–4].

However, today's boom in organoid models did not begin until 2009 (Figure 1A). During that year, Hans Clevers' lab demonstrated that single primary adult stem cells, isolated from the crypts of the intestinal system, have the potential to regrow a complete intestinal epithelium forming cystic structures [5]. It became clear, that stem cells from most organs and tissues have similar potential, and a variety of publications concerning this topic followed. From this point on, the organoid field developed rapidly.

Primary-tissue-specific stem cells, however, are not the only source for culturing organoids. Another option is so-called pluripotent stem cells (PSCs). In the 1970s, the first PSCs were isolated from teratocarcinomas, and stable PSC cultures were established [6]. Experiments showed that such cells form aggregates in suspension culture, which undergo complex differentiation processes and morphogenetic events [7]. These 3D tissue cultures spontaneously developed different tissues derived from all three embryonic germ layers and were termed embryoid bodies (EBs).

In 1981, the first non-malignant PSC lines were derived from the inner cell mass of mouse blastocysts [8,9], and finally, in 1998 [10], a successful derivation of human PSCs was published. Due to their embryonic origin, these cells were termed embryonic stem cells (ESC). In 2006 and 2007, a series of publications revealed that PSCs can be also artificially induced from terminally differentiated somatic cell types [11–13]. Such induced

Citation: Ergün, S.; Wörsdörfer, P. Organoids, Assembloids and Embryoids: New Avenues for Developmental Biology, Disease Modeling, Drug Testing and Toxicity Assessment without Animal Experimentation. *Organoids* **2022**, *1*, 37–40. https://doi.org/10.3390/organoids1010004

Received: 12 April 2022
Accepted: 14 April 2022
Published: 22 April 2022

pluripotent stem cells (iPSCs) made human PSCs broadly available and boosted the field of stem cell biology.

PSCs have the remarkable potential to give rise to all cell types of the body. For that reason, they can be also differentiated into all multipotent, tissue-specific stem cells; these are the founding populations of the later tissues and organs and, thus, can be used to grow organoids. In 2013, the first PSC-derived organoid models were published. The authors used small molecules and cytokines to specifically direct PSC aggregates towards a neuroepithelial fate, and finally observed the emergence of cerebral structures [14,15]. In the following years, a variety of PSC-based organoid models were published (e.g., of the fetal liver, lung, heart, kidney, and different brain regions). Today, most tissues are created as organoid models in the lab utilizing either tissue-specific or pluripotent stem cells. However, many challenges remain.

Most organoids only represent the organs' parenchyma and are devoid of other important components, such as connective tissue, a vascular and lymphatic network, peripheral nerves, or local immune cells. Moreover, the robust reproducibility of many models is still a problem. Finally, organoid culture is expensive and laborious. The discovery of cheaper reagents and culture media, automatization of the culture process and upscaling of organoid production are interesting research fields and future challenges.

Within recent years, important steps were made in this field, increasing the complexity of organoid culture. Efforts were made to add stromal components and a perfusable vascular system through the incorporation of mesodermal progenitor cells [16]. Moreover, different types of organoids were put into co-culture to generate so-called assembloids [17]. A completely new and fascinating research field is that of mimicking real embryonic development using gastruloids or embryoids, which enables researchers to study the co-development of different organ systems [18–20].

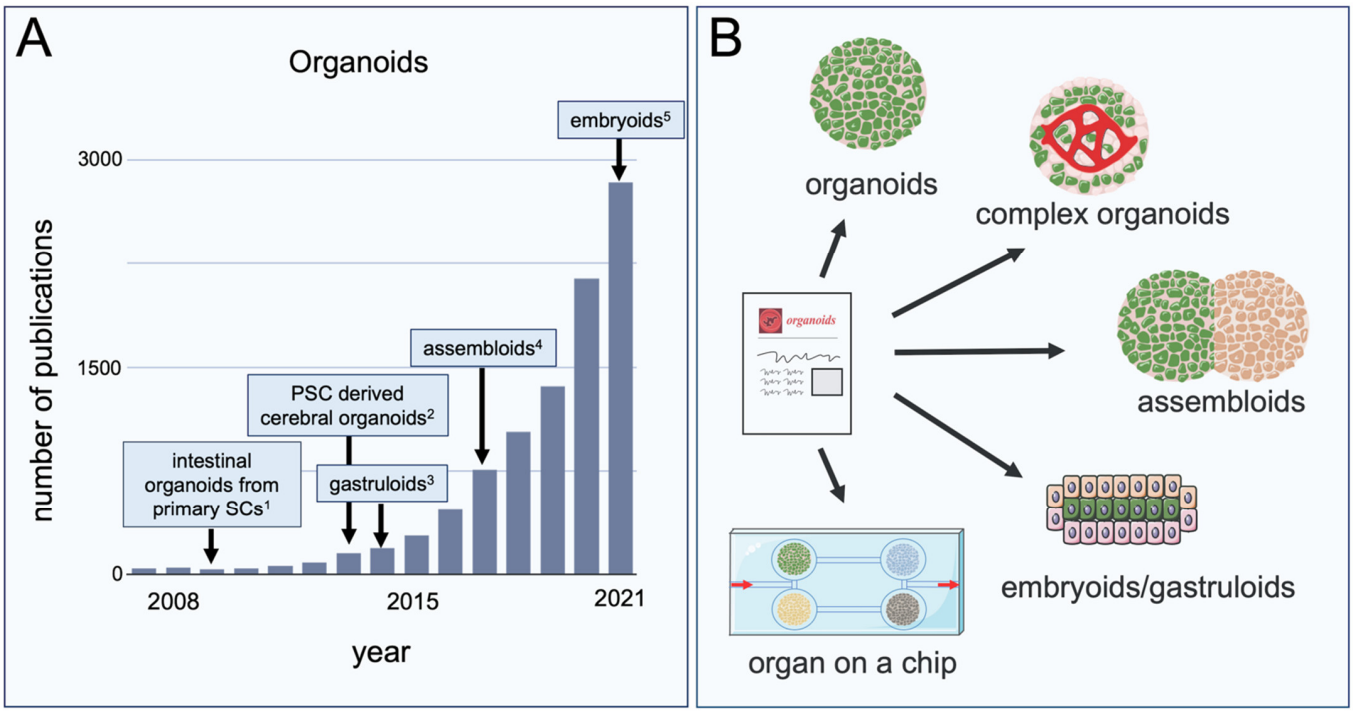

Figure 1. (**A**) Number of publications per year found with the search term "organoid" when performing a *PubMed* search. (**B**) The journal "Organoids" welcomes publications covering all aspects of organoid research such as new organoid models, complex organoids, assembloids, embryoids/gastruloids, or organ-on-a-chip [5,15,17–19].

A more holistic approach seems to be incorporating organ-on-a-chip technology, wherein different types of organoids are brought into a system of microfluidic channels; this allows the investigation of artificial organs that interact with each other as a working system.

Finally, organoids are discussed as building blocks for biofabrication processes. Biofabrication and bioengineering might help to create organoids of a defined geometry, which drives deterministic tissue patterning [21,22].

The organoid field has developed quickly within recent years (Figure 1A). It has now entered a new level of complexity, and has started to play a growing role in medical research and human developmental biology. For that reason, this is the ideal time to set up a new journal dedicated to all aspects of this diverse and rapidly evolving research field. We are curious and excited to see how the field will progress in the next decade. We welcome all scientists with expertise in organoid, assembloid and embryoid technology, as well as those with expertise in combining organoids with bioprinting techniques, to contribute to the development of the newly founded journal "*Organoids*" (Figure 1B). Our aim is to create a specialized platform for the scientific community, to present new and exciting data covering all aspects of basic and translational organoid research.

Funding: This work was funded by the IZKF-Würzburg (Interdisziplinäres Zentrum für Klinische Forschung der Universität Würzburg) (project E-D-410) and the German Research Foundation (DFG) through the Collaborative Research Center SFB/TRR 225 326998133 B04.

Acknowledgments: Parts of the schematic procedure shown in Figure 1 were produced by combining the authors' own drawings with graphics taken from the image bank of Servier Medical Art (www.servier.com, accessed on 1 April 2022), licensed under a Creative Commons Attribution 3.0, Unported License (https://creativecommons.org/licenses/by/3.0, accessed on 1 April 2022).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Low, J.H.; Li, P.; Chew, E.G.Y.; Zhou, B.; Suzuki, K.; Zhang, T.; Lian, M.M.; Liu, M.; Aizawa, E.; Rodriguez Esteban, C.; et al. Generation of Human PSC-Derived Kidney Organoids with Patterned Nephron Segments and a De Novo Vascular Network. *Cell Stem Cell* **2019**, *25*, 373–387. [CrossRef] [PubMed]
2. Moscona, A.; Moscona, H. The dissociation and aggregation of cells from organ rudiments of the early chick embryo. *J. Anat.* **1952**, *86*, 287–301.
3. Weiss, P.; Taylor, A.C. Reconstitution of Complete Organs from Single-Cell Suspensions of Chick Embryos in Advanced Stages of Differentiation. *Proc. Natl. Acad. Sci. USA* **1960**, *46*, 1177–1185. [CrossRef] [PubMed]
4. DeLong, G.R. Histogenesis of fetal mouse isocortex and hippocampus in reaggregating cell cultures. *Dev. Biol.* **1970**, *22*, 563–583. [CrossRef]
5. Sato, T.; Vries, R.G.; Snippert, H.J.; van de Wetering, M.; Barker, N.; Stange, D.E.; van Es, J.H.; Abo, A.; Kujala, P.; Peters, P.J.; et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **2009**, *459*, 262–265. [CrossRef]
6. Martin, G.R.; Evans, M.J. The morphology and growth of a pluripotent teratocarcinoma cell line and its derivatives in tissue culture. *Cell* **1974**, *2*, 163–172. [CrossRef]
7. Martin, G.R.; Evans, M.J. Differentiation of clonal lines of teratocarcinoma cells: Formation of embryoid bodies in vitro. *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 1441–1445. [CrossRef]
8. Martin, G.R. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad Sci USA* **1981**, *78*, 7634–7638. [CrossRef]
9. Evans, M.J.; Kaufman, M.H. Establishment in culture of pluripotential cells from mouse embryos. *Nature* **1981**, *292*, 154–156. [CrossRef]
10. Thomson, J.A.; Itskovitz-Eldor, J.; Shapiro, S.S.; Waknitz, M.A.; Swiergiel, J.J.; Marshall, V.S.; Jones, J.M. Embryonic stem cell lines derived from human blastocysts. *Science* **1998**, *282*, 1145–1147. [CrossRef]
11. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **2007**, *131*, 861–872. [CrossRef]
12. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **2006**, *126*, 663–676. [CrossRef]
13. Yu, J.; Vodyanik, M.A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J.L.; Tian, S.; Nie, J.; Jonsdottir, G.A.; Ruotti, V.; Stewart, R.; et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* **2007**, *318*, 1917–1920. [CrossRef]

14. Kadoshima, T.; Sakaguchi, H.; Nakano, T.; Soen, M.; Ando, S.; Eiraku, M.; Sasai, Y. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 20284–20289. [CrossRef]
15. Lancaster, M.A.; Renner, M.; Martin, C.A.; Wenzel, D.; Bicknell, L.S.; Hurles, M.E.; Homfray, T.; Penninger, J.M.; Jackson, A.P.; Knoblich, J.A. Cerebral organoids model human brain development and microcephaly. *Nature* **2013**, *501*, 373–379. [CrossRef]
16. Worsdorfer, P.; Dalda, N.; Kern, A.; Kruger, S.; Wagner, N.; Kwok, C.K.; Henke, E.; Ergun, S. Generation of complex human organoid models including vascular networks by incorporation of mesodermal progenitor cells. *Sci. Rep.* **2019**, *9*, 15663. [CrossRef]
17. Birey, F.; Andersen, J.; Makinson, C.D.; Islam, S.; Wei, W.; Huber, N.; Fan, H.C.; Metzler, K.R.C.; Panagiotakos, G.; Thom, N.; et al. Assembly of functionally integrated human forebrain spheroids. *Nature* **2017**, *545*, 54–59. [CrossRef]
18. Van den Brink, S.C.; Baillie-Johnson, P.; Balayo, T.; Hadjantonakis, A.K.; Nowotschin, S.; Turner, D.A.; Martinez Arias, A. Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells. *Development* **2014**, *141*, 4231–4242. [CrossRef]
19. Xu, P.F.; Borges, R.M.; Fillatre, J.; de Oliveira-Melo, M.; Cheng, T.; Thisse, B.; Thisse, C. Construction of a mammalian embryo model from stem cells organized by a morphogen signalling centre. *Nat. Commun.* **2021**, *12*, 3277. [CrossRef]
20. Van den Brink, S.C.; van Oudenaarden, A. 3D gastruloids: A novel frontier in stem cell-based in vitro modeling of mammalian gastrulation. *Trends Cell Biol.* **2021**, *31*, 747–759. [CrossRef]
21. Brassard, J.A.; Nikolaev, M.; Hubscher, T.; Hofer, M.; Lutolf, M.P. Recapitulating macro-scale tissue self-organization through organoid bioprinting. *Nat. Mater.* **2021**, *20*, 22–29. [CrossRef]
22. Gjorevski, N.; Nikolaev, M.; Brown, T.E.; Mitrofanova, O.; Brandenberg, N.; DelRio, F.W.; Yavitt, F.M.; Liberali, P.; Anseth, K.S.; Lutolf, M.P. Tissue geometry drives deterministic organoid patterning. *Science* **2022**, *375*, eaaw9021. [CrossRef]

Short Biography of Authors

Süleyman Ergün is a full Professor at the Institute of Anatomy and Cell Biology at Julius Maximilians University, Würzburg, Germany. Prior to his current position, he completed his doctoral thesis (1993) and his "Habilitation" (1998) at the Institute of Anatomy, Hamburg–Eppendorf University Hospital. He undertook a post-doctoral fellowship at Harvard Medical School (Judah Folkman's Lab) (1997) and was a member of the executive board of the Hamburg "Ärztekammer", Germany (2002–2003). In 2006, he was appointed Chair of the Institute of Anatomy at Essen University Hospital, Germany. Since 2011, he has been Chair of the Institute of Anatomy and Cell Biology at Julius Maximilians University, Würzburg, Germany. He was a member of the executive board of the Anatomical Society (2014–2019) and has been elected to the review panel for medicine at the "Deutsche Forschungsgemeinschaft, DFG (German Research Foundation) (since 2020). His research covers vascular biology and endothelial barrier function; cardiovascular regeneration; angiogenesis and vasculogenesis, including tumor vascularization, stem and progenitor cell biology; vascular bioprinting; and organoids. He received the Gordon Research Conference (GRC) Award for Vascular Cell Biology and the Konjetzny Award of the "Hamburger Krebsgesellschaft" (Cancer Society of Hamburg) for his research on processes of the structural formation of the vessel wall in angiogenesis and the growth of tumors, among other awards.