Microscopic organs: function and application of organoid models and how to cryopreserve them for future use

Molly Horton and Dr. Zhiquan (Andy) Shu
School of Interdisciplinary Arts and Sciences, University of Washington Tacoma
School of Engineering and Technology, University of Washington Tacoma

ABSTRACT

Organoids are becoming an important solution to further advancements in regenerative medicine, epidemiology, and biomedical research. Using these microscopic models, scientists can study the in vivo function of mammalian organs without performing invasive surgeries or facing complicated ethical barriers. The cultivation and sustainability of such organisms require the proper methods and resources and their further usage in medical settings demands the long-term storage of organoids through stable cryopreservation (biopreservation at low temperatures). However, organoids may be damaged during cryopreservation if improper cryopreservation protocol is applied. By utilization of optimal cryoprotective agents (CPAs), optimal protocols for CPA addition, cooling, rewarming, and CPA removal to avoid osmotic injury and cryoinjury, organoids can be safely cryopreserved for long time for future applications. Having such a valuable organ model on hand for biomedical experimentation and clinical applications will expedite future research compared to studies without organoids.

BACKGROUND

(Q) What is an organoid?
(A) A simplified version of an organ made up of stem cells, operated to function just as a normal organ would (but super tiny!)

(Q) How are they made?
(A) Just like growing bacteria in a dish, organoids are carefully cultivated using a basal medium, growth factors, and tissues from biopsies (heart, liver, etc.). The cells are suspended, plated, and incubated until they're fully grown and ready for use.

(Q) What are they used for?
(A) Organoids provide a unique insight into the routine function of our internal organs. We can study the architecture, cell types, molecular events, and repair mechanisms of structures like human hearts and livers without having to cut anyone open. They can also be used in all kinds of medical research, even tissue regeneration!

(Q) Can they be preserved?
(A) Yes! To extend the shelf life of organoids, we can carefully add cryoprotective agents (CPAs) before cooling and remove them after rewarming due to toxicity. This is a challenging procedure, but if done right the organoids can be safely preserved while maintaining original structure and function.

RESULTS

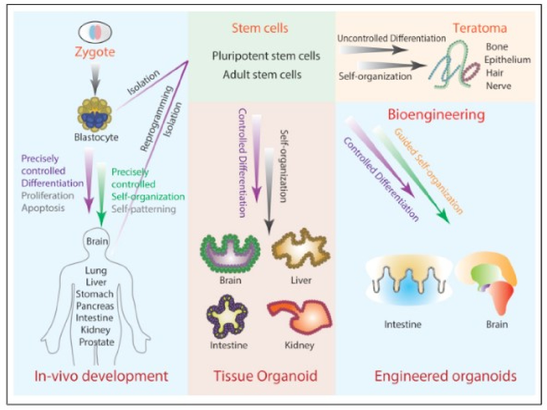

Figure 1 - Just like forming a human, organoid formation is very similar. Through various cellular mechanisms, mature tissues can be formed. Organoids can be derived from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCS), or adult stem cells (ASCs) and can, using similar cellular processes used *in vivo*, become a tissue-specific organoid. However, if the stem cells differentiate and self-organize without control or order, they will produce teratomas (multi-tissue tumors). Further research is being conducted on the bioengineering of organoids and how to more accurately and efficiently guide organization and differentiation in these cell types to produce specific organoid model systems. (Yin et al., 2016)

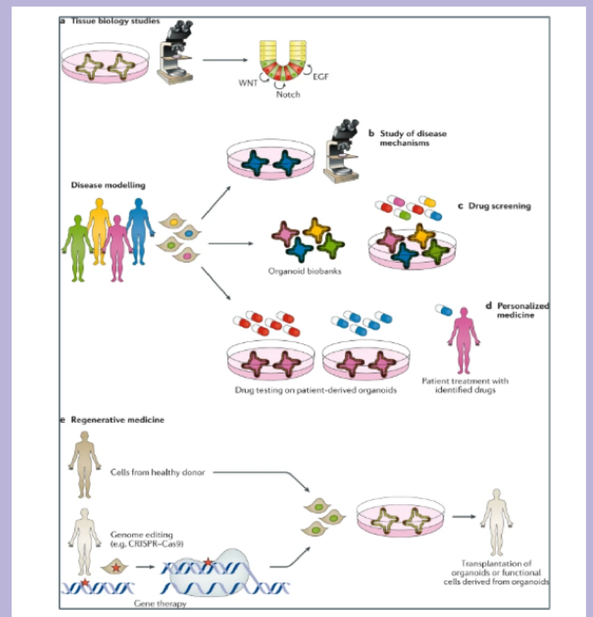

Figure 2 - Simplified visual list of organoid applications. (a) Understand principles of development, homeostasis, and regeneration. (b) Study disease mechanisms because they replicate the complexity of normal organ function. (c) Help identify drugs against broad spectrum of phenotypes. (d) Can identify personalized drugs for patient case by case. (e) Organoids from healthy donor cells can be used as a source for regenerative medicine. (Rossi et al., 2018)

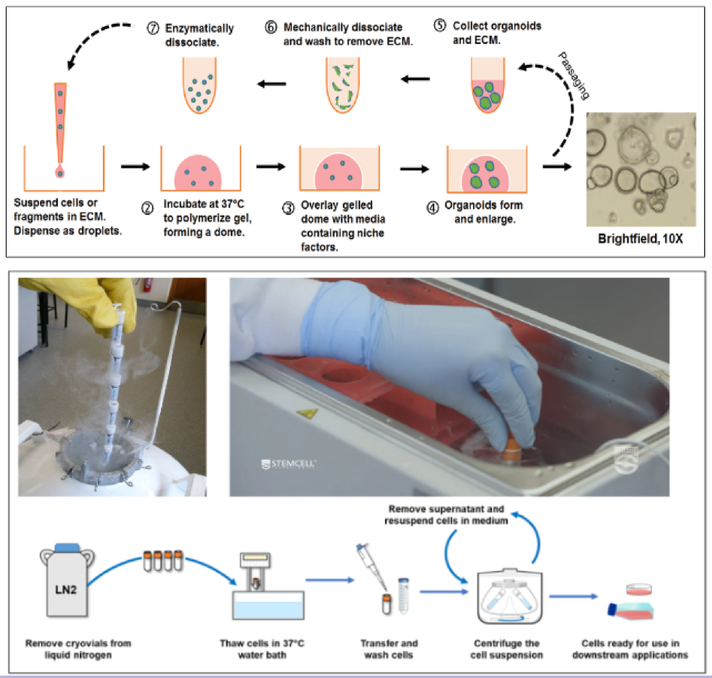

Figure 3 - (TOP) 3-D "dome" organoid culture method. Fragmented organoid cells are combined with a liquid extra-cellular matrix (ECM) and dropped onto a culture vessel. During incubation, the ECM solidifies into a gel-like substance and culture medium can be applied. Within this droplet "dome," organoids will develop as viewable 3-D structures. (Clinton, 2019) (BOTTOM) Visual representation of freezing using tank of liquid nitrogen and thawing using hot water bath. Diagram shows the rewarming and resuspension process of organoid culture after being cryopreserved and freezer stored. CPAs are separated and removed from the suspension at this point. (Cytologics, 2021)

CONCLUSION AND FUTURE DIRECTIONS

- Successful organoid cultures, derived from stem cells and grown using basal medium and growth factors, can be cryopreserved using formulated freezing mediums, which can include DMSO as a strong CPA and FBS (fetal bovine serum) as a rich growth factor supplement to support cell growth and development.
- The cryopreservation protocol for organoids of different types may be different to achieve maximum efficiency. Future research may explore optimization of organoid cryopreservation protocols, including optimal CPAs, cooling temperatures that avoid cryoinjuries, and rewarming temperatures that produce the most positive post-cryostorage evaluations.

ACKNOWLEDGEMENTS

I want to give special thanks to Dr. Zhiquan (Andy) Shu for his mentorship and guidance throughout this journey. His teachings are the foundation of my research.

I would also like to thank Dr. Jutta Heller for supporting me from the very beginning. She's an inspiration as my advisor and a fantastic cheerleader.

Thank you to the University of Washington Tacoma for allowing me to utilize their many tools and resources to complete my project.

SOURCES