

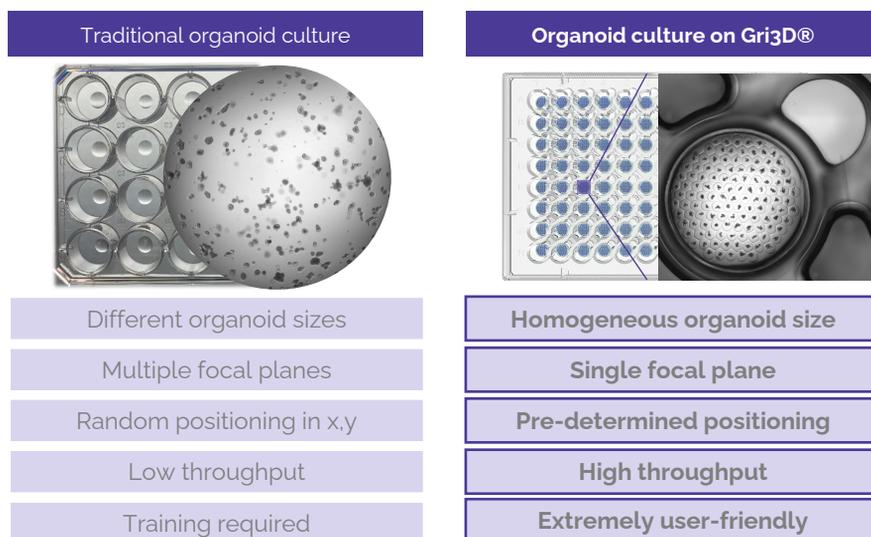
Generation of Homogeneous Mouse Intestinal Organoids on Gri3D®

Mouse intestinal organoids on Gri3D®

Gri3D® is a ready-to-use platform for high-throughput and reproducible organoid culture. We demonstrate that Gri3D® supports the culture of mouse intestinal organoids. The resulting organoids are homogeneously sized and are confined within the microwells, thus facilitating their imaging. After induction of differentiation, the organoids show a typical budding morphology and contain differentiated intestinal cells that express specific markers. Gri3D® provides an ideal tool to standardize organoid cultures.

Overview of organoid culture

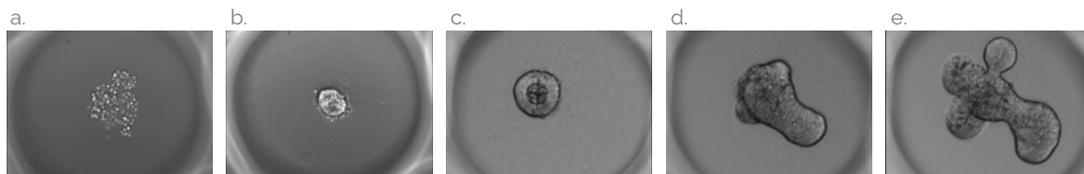
Organoids are three-dimensional, miniaturized and simplified versions of an organ that can be reproduced *in vitro*, mimicking some of the key features of the native tissue [1]. Although promising for multiple applications in biomedical research, traditional organoid culture methods, where organoids are embedded within drops of solidified extracellular matrix (ECM), have an intrinsic lack of reproducibility [2]. The resulting organoids are not only highly heterogeneous in size and shape, but also are randomly placed in multiple focal planes and positions, hampering the generation of robust quantitative data [3]. Gri3D® overcomes the challenges inherent to organoid culture and allows its standardization.



Mouse intestinal organoids generated on solid ECM drops (left) and on Gri3D® (right).

Mouse intestinal organoids on Gri3D®

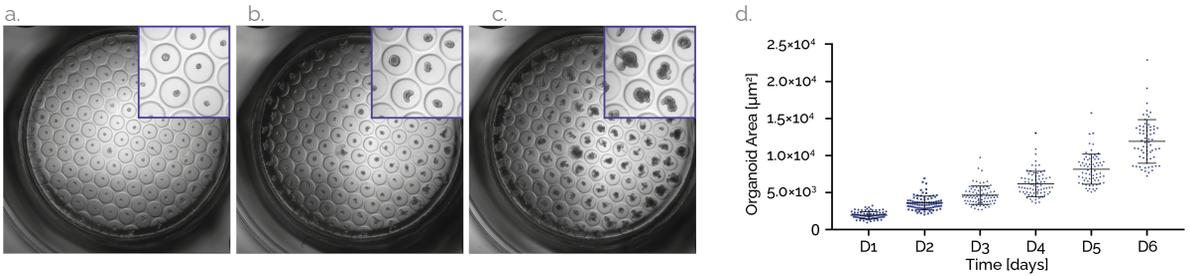
Mouse intestinal organoids were among the first organoid systems to be described [4]. These mouse intestinal organoids displayed below are generated on Gri3D® g6WP glass-bottom 400 µm microwells. Cells are seeded and 121 single organoids develop over time in one well of a Gri3D® g6WP.



Representative brightfield images of a mouse intestinal organoid growing on a Gri3D® microwell. **a.** 1 hour, **b.** 10 hours, **c.** 2 days, **d.** 3 days and **e.** 4 days after seeding.

Mouse intestinal organoid development

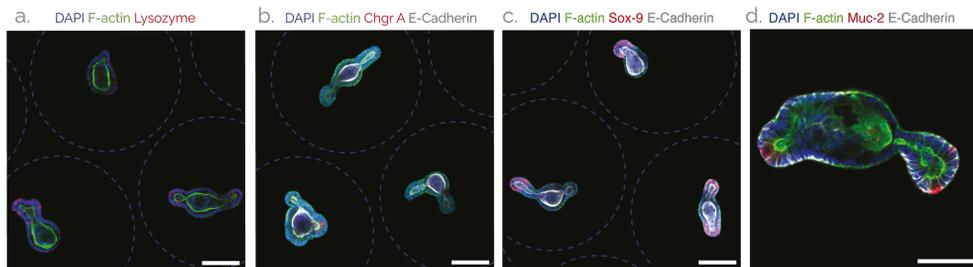
Up to 122 homogeneously sized mouse intestinal organoids grow in each well. The confinement of each organoid to a specific microcavity allows imaging over time and area quantification. Organoid size distribution is homogeneous on days 1-3 and broadens as budding occurs.



Representative examples of mouse intestinal organoids grown on Gri3D® on days a. 3, b. 5 and c. 8. d. Area variability over time.

Mouse intestinal organoid characterization

Mouse intestinal organoids cultured on Gri3D® present a budding morphology typical of intestinal organoids, and contain differentiated intestinal cells such as enterocytes, goblet, Paneth, or enteroendocrine cells. The presence of specific markers of the different cell types, as well as epithelial polarity, are revealed directly on Gri3D® by immunofluorescence.



Protein expression of a. lysozyme, b. chromogranin A, c. SOX-9 and d. mucin-2 highlighting the presence of Paneth cells, enteroendocrine cells, cells comprised in the crypt domain and Goblet cells, respectively, in organoids grown in microwell arrays.

Highlights of the model

- **Homogeneous:** organoid generation is robust and the resulting organoids have similar sizes
- **Scalable:** using Gri3D® 400 µm microwells, 121 organoids are formed in a single well
- **Optimal imaging:** with glass-bottom Gri3D®, organoids are imaged at single-organoid resolution
- **Automatable:** the organoid generation procedure is compatible with automatic liquid handling systems

How can we help you?

Organoid Generation Service

SUN bioscience generates intestinal organoids from different sources (mouse, dog, human), and commercializes Gri3D® for research use.

Contact us for more information at enquiries@sunbioscience.ch

References

The data reported were generated by SUN bioscience.

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- [2] Fatehullah, A., Tan, S. H. & Barker, N. Organoids as an in vitro model of human development and disease. *Nature Cell Biology* 18. 246–254 (2016). doi:10.1038/ncb3312
- [3] Schneeberger, K. et al. Converging biofabrication and organoid technologies: The next frontier in hepatic and intestinal tissue engineering? *Biofabrication* 9. (2017). doi:10.1088/1758-5090/aa6121
- [4] Sato, T. et al. Long-term Expansion of Epithelial Organoids From Human Colon, Adenoma, Adenocarcinoma, and Barrett's Epithelium. *Gastroenterology* 141. 1762–1772 (2011). doi:10.1053/j.gastro.2011.07.050

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