A microwell platform to standardize human rectal organoid cultures for high-content imaging and phenotypic analyses

Meyer, M.[1], Clapés, M.[1], Sirenko, O.[2], Lim, A.[2], Storm, R.[3], Brandenberg, N.[1]

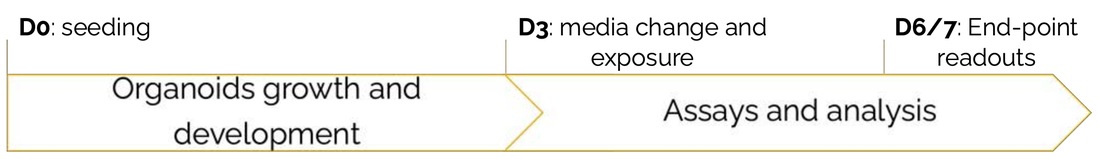
[1]SUN bioscience SA, Lausanne, Switzerland. [2]Molecular Devices LLC, San Jose, United States. [3]Molecular Devices (UK) Ltd, United Kingdom.

INTRODUCTION

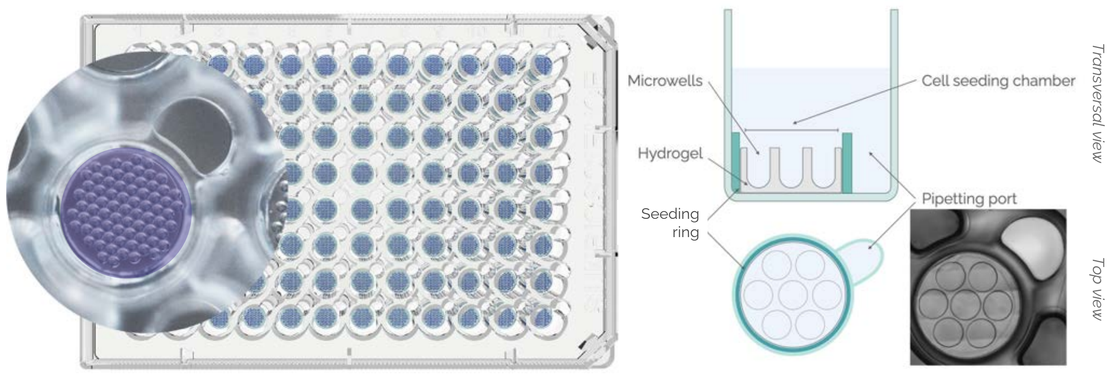
Organoids are three-dimensional (3D), self-organizing *in vitro* cell culture organ models. These 3D structures derive from stem cells and harbor key features of their native organs. Since the development of the first mouse intestinal organoids in 2009, a large variety of organoid models have been established[1]. However, they still heavily rely on the use of solid extracellular matrix (ECM). This conventional culture method introduces a high level of heterogeneity, both in terms of size, shape and distribution of the organoids, which complicates subsequent downstream readouts and image analyses[2]. To overcome these challenges and closely follow organoid development, we use our innovative technology Gri3D®, a ready-to-use platform for high-throughput and reproducible 3D cultures[3]. This platform enables the homogenous generation of a single microtissue in each microcavity in suspension-like conditions, without the need of a solid ECM. The organoids are positioned in predefined locations and on the same focal plane, allowing simultaneous tracking at high resolution. Combined with the ImageXpress® Micro Confocal system, we follow the development and self-organization of human rectal organoids over time and investigate the effects of a small panel of compounds using brightfield image analyses and fluorescence-based readouts.

METHODS

Human rectal organoids are generated in Gri3D® 96WP imaging-bottom 500 µm microwells (SUN bioscience) starting from a single cell suspension and cultured for up to 7 days. Organoid development is followed over time with TL imaging on an ImageXpress® Micro Confocal system (Molecular Devices). In parallel, we compare organoids cultured on Gri3D® or embedded in solid-ECM drops using IN-Carta® Image Analysis Software. Finally, Live/Dead assay is performed on compound-treated organoids and analysed using IN-Carta® and in-house developed image analyses pipelines.



GRI3D® TECHNOLOGY



Gri3D® is a ready-to-use platform for high-throughput and reproducible organoid culture. Based on an array of ultra-dense U-bottom microwells in a hydrogel, single organoids are robustly generated in each microcavity and grown in suspension-like culture without a solid ECM.

RESULTS

Human rectal organoids cultured on Gri3D® are imaged over 7 days (Fig.1 A). Organoids rapidly form a lumen and differentiate in the microcavities, showing budding already after 4 days. Using a machine learning image-based approach on TL images, we efficiently detect each single organoid and quantify growth over time (Fig 1. B). When compared to solid-ECM grown organoids, Gri3D® cultures show higher homogeneity in terms of size and positioning (Fig. 2 A and B). Organoid differentiation is promoted only on Gri3D®, and while budding increases the area, it decreases the shape factor (Fig. 2 C and D). Upon exposure to a small panel of compounds known for their intestinal toxicity, Live/Dead assay shows a viability decrease of organoids with increasing concentrations of mitomycin C and doxorubicin (Fig. 3 A). All compounds but cisplatin hamper the development and growth of intestinal organoids, stopping or reducing organoid growth (Fig. 3 B).

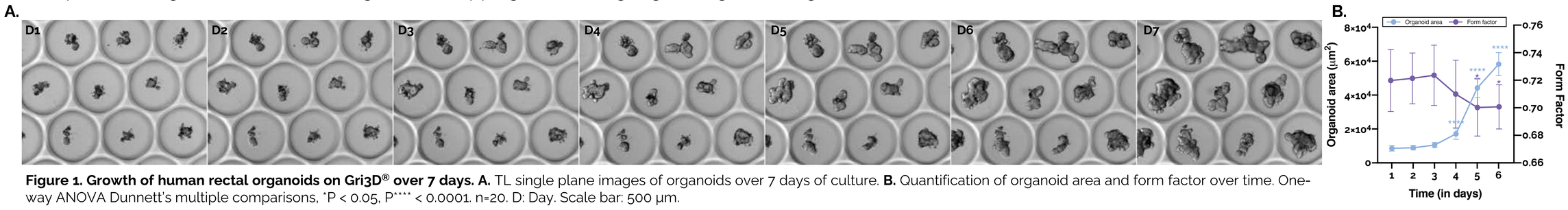


Figure 1. Growth of human rectal organoids on Gri3D® over 7 days. A. TL single plane images of organoids over 7 days of culture. **B.** Quantification of organoid area and form factor over time. One-way ANOVA Dunnett's multiple comparisons, *P < 0.05, P**** < 0.0001. n=20. D: Day. Scale bar: 500 µm.

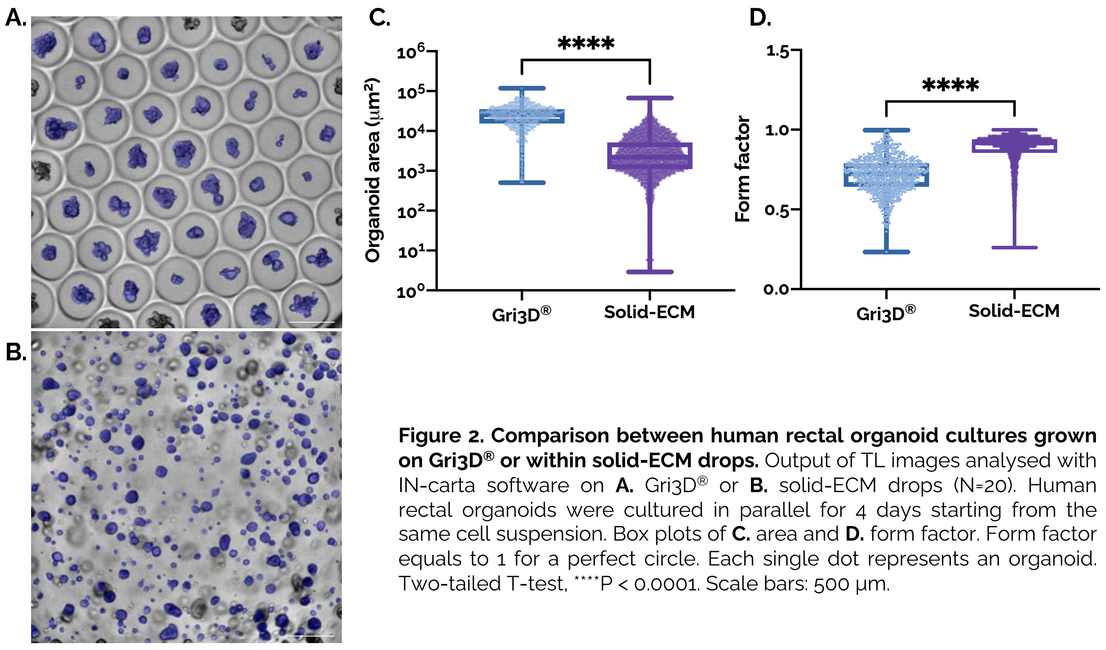


Figure 2. Comparison between human rectal organoid cultures grown on Gri3D® or within solid-ECM drops. Output of TL images analysed with IN-carta software on **A.** Gri3D® or **B.** solid-ECM drops (N=20). Human rectal organoids were cultured in parallel for 4 days starting from the same cell suspension. Box plots of **C.** area and **D.** form factor. Form factor equals to 1 for a perfect circle. Each single dot represents an organoid. Two-tailed T-test, ****P < 0.0001. Scale bars: 500 µm.

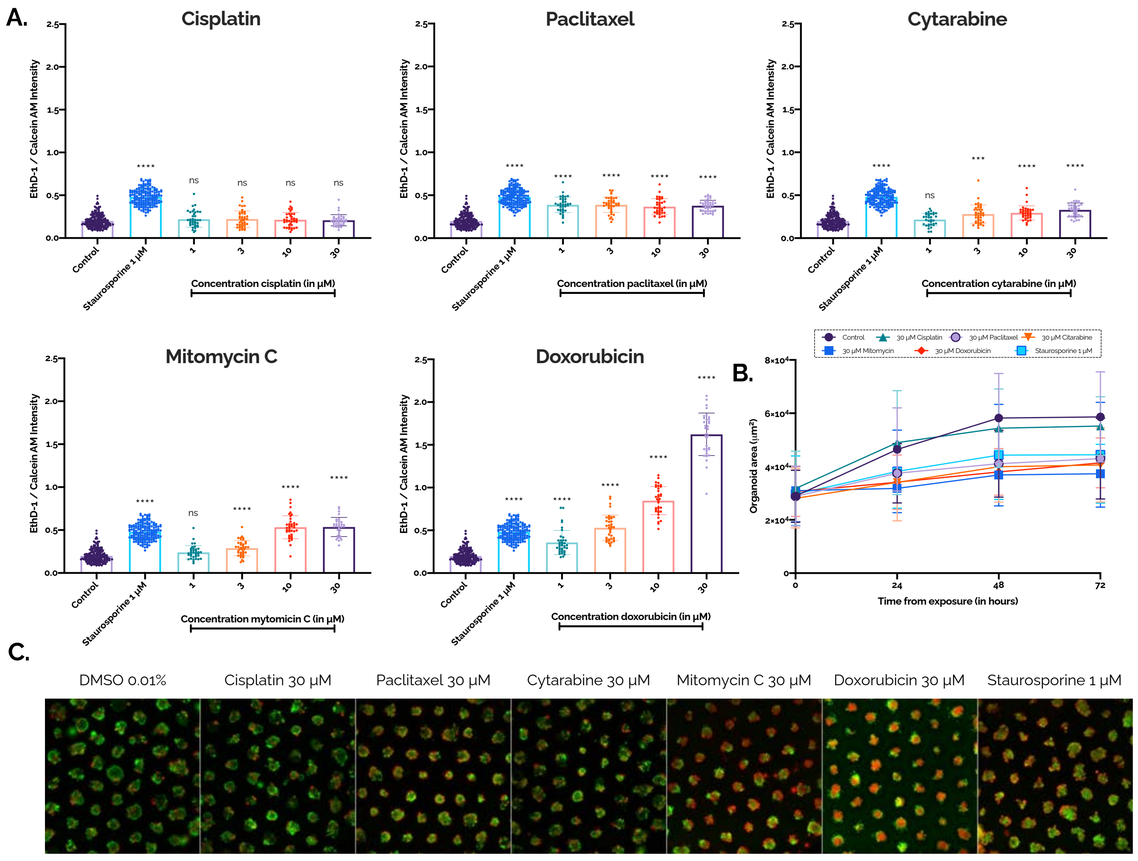


Figure 3. Response of human rectal organoids exposed to a panel of intestinal toxicity compounds. Live/Dead assay is performed on 6-day old organoids grown on Gri3D® after 72 hours exposure to the drugs. **A.** Ethidium homodimer-1 (EthD-1) to Calcein AM intensity ratio. Error bars show standard deviation. Each dot represents an organoid. **B.** Organoid area over time during exposure to the highest concentrations (30 µM) of the compounds. **C.** Maximum projection images of organoids after Live/Dead assay. Green: Calcein AM, live; red: EthD-1, dead. One-way ANOVA Dunnett's multiple comparisons, ***P < 0.001, P**** < 0.0001, ns: non-significant. Scale bar: 500 µm.

CONCLUSIONS

- Based on a 96 well plate format, Gri3D® 500 µm microwells allows the homogenous and robust generation of more than 70 organoids per well.
- Gri3D® is a front-to-end solution for high-content imaging of organoids enabling phenotypic-based screening workflows.
- The combination of Gri3D® technology and a high-content imaging system allows the characterization of single organoids in one plane.
- The reported drug toxicity assessment workflow enables in vitro studies of human rectal organoids in high-throughput.
- Our innovative approach has high potential in solving key challenges related to 3D cultures and compound assessment at large scale using patient-derived samples.

REFERENCES

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