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. However, they still heavily rely on the use of solidified 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. To overcome these challenges and closely follow organoid development, we use our innovative technology Gr3D®, a ready-to-use platform for high-throughput and reproducible 3D cultures. This platform enables the homogenous generation of a single microcavity 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 with transmitted light (TL) and fluorescence imaging.

**METHODS**

Human rectal organoids are generated in Gr3D® 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 and fluorescence imaging on an ImageXpress® Micro Confocal system (Molecular Devices) using live-cell compatible SPY555-FastACT™ (Spirochrome). In parallel, we compare organoids cultured on Gr3D® or embedded in solid-ECM drops using IN-Carta® Image Analysis Software. Finally, Live/Dead assay is performed on staurosporine treated organoids and analysed using a 3D Custom Module Editor on MetaXpress®.

**RESULTS**

Human rectal organoids cultured on Gr3D® are labelled with SPY555-FastACT™ and imaged over 7 days (Fig 1A). 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 1B). When compared to solid-ECM grown organoids, Gr3D® cultures show higher homogeneity in terms of size and positioning (Fig 2 A and B). Organoid differentiation is promoted only on Gr3D®, and while budding increases the area, it decreases the shape factor (Fig 2 C and D). Upon staurosporine exposure, Live/Dead assay shows a viability decrease of organoids with increasing drug concentration (Fig 3).

**CONCLUSIONS**

- Based on a 96 well plate format, Gr3D® 500 μm microwells allows the homogenous and robust generation of more than 70 organoids per well.
- Gr3D® is a front-to-end solution for high-throughput imaging of organoids enabling phenotypic-based screening workflows.
- The combination of Gr3D® technology and a high content imaging system together with machine-learning algorithms allows the characterization of single organoids in one plane.
- 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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