Leverage automated workflows to enable complex organoid assays

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Summary

3D organoids are increasingly popular in drug discovery and disease modeling as they better represent biologically relevant microenvironments, tissue architecture, and functionality. However, the complexity of 3D models remains a hurdle for the wider adoption in research and drug screening.

The aim of our work is to alleviate the hurdles of the labor-intensive manual protocols by using automation. Accordingly, we developed automation protocols for the entire 3D culture workflow and increase the throughput and accuracy of organoid assays.

The automated methods utilize an integrated workcell, providing automated cell plating, culture monitoring, media exchange, and a high-content imaging. In addition, the method allows automation of compound addition, cell staining, and end point assays.

The automated procedures covering two most common organoid workflows:

1. organoids cultured in matrix (Matrigel) that includes primary tissue-derived or iPSC-derived intestinal or colorectal organoids
2. organoids cultured matrix-free using U shape ULA labware, used for spheroids and patient-derived breast cancer tumoroids

Materials and methods

Figure 1. The integrated system includes an automated CO_2 incubator, liquid handler, IXM-C HT.ai confocal imaging system, automation compatible plate reader and centrifuge A Collaborative robot and scheduling software allows automated plate transfer between instruments, and scheduling of different steps.

Cell culture

The methods for generating tumoroids and PDX organoids (PDXO) have been previously described (Matossian, et al 2021). The primary tumor sample was implanted into SCID/Beige mice and exhibited rapid tumor growth, with 14 days to reach maximal tumor volume >1000 mm^3, Then cell line was generated from that sample able to be expanded in 2D culture. Tumoroids were formed from 4IC cells expanded in 2D. 4IC cells were dispensed ~2,000 cells per well (in U-shape low attachment 384 plates, Corning) and incubated for 48 hours until they formed tight tumoroids. 4IC cells were cultured with Advanced DMEM supplemented with glucose, NEAA, 2mM glutamine and insulin 120µg/L, 10% FBS (Gibco 12491-015). For metabolic assays, tumoroids were cultured with DMEM + 10% dialyzed serum (2mM glutamine, 5mM glucose, without phenol red.

3D intestinal organoids were derived from primary mouse intestinal cells or human iPSCs (STEMCELL Technologies). Cells were cultured and differentiated according to the STEMCELL Technologies protocol. IntestiCult™ Organoid Growth Medium (STEMCELL Technologies) was utilized for cell culture. Cells were seeded in 50% growth-factor reduced Matrigel (Corning) domes in a 24-well plate format and were fed every second day with fresh media for 7–10 days. Intestinal organoids were then passaged- dissociated and re-plated into fresh Matrigel domes. Note: project was under the license from HuB Institute.

Cell monitoring and imaging

Transmitted light (TL) of fluorescent images were acquired on the ImageXpress Confocal HT.ai High-Content Imaging System (Molecular Devices) using MetaXpress High-Content Image Analysis Software. Tumoroid images were acquired in TL with approximately 60 µm offset. Z-stack images were acquired with the 10X or 20X objectives using confocal mode. MetaXpress or IN Carta™ Image Analysis Software were used for analysis.

Automation of cell culture and imaging protocols

Automated imaging and analysis of organoids are important for quantitative assessment of phenotypic changes in organoids, and for increasing throughput for experiments and tests. We built an automated, integrated system that allows automated monitoring, maintenance, and characterization of growth and differentiation of organoids and stem cells, as well as testing the effects of various compounds. The automated system includes ImageXpress Confocal HT.ai system and analysis software, automated CO_2 incubator, Biomek i7 or Hamilton liquid handler, collaborative robot and rail. Robotic automation was enabled by Green Button Go solution.

Results

Automated culturing 3D organoids in Matrigel

Culture 3D organoids in Matrigel or other matrix includes several steps of the process: seeding primary or iPSC-derived cells mixed with Martigel into the “domes”, followed by 8–10 days of culture with media exchange every second day. The process of growth and maturation of organoids was be monitored by automated imaging in transmitted light. Then organoids were harvested after re-suspension of domes, and organoids got dis-integrated by either mechanical repeated pipetting, or by enzymatic treatment. After that, cell pellets purified by centrifugation were mixed with Matrigel, and re-plated into new plates. Using the automated liquid handling system allowed for automated seeding of intestinal, colorectal, or other cells in Matrigel droplets into the plates of different formats, followed by automated media exchanges, passaging, and compound addition.

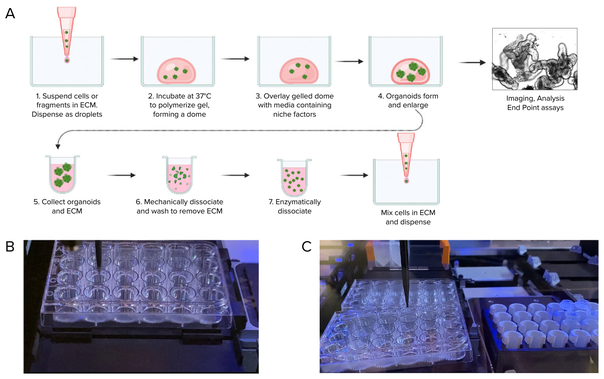


Figure 2. A. Schematic diagram of organoid culture workflow. B. Automated seeding organoids in domes; C. Cell collection by Hamilton liquid handling system.

Monitoring development of 3D organoids and high content imaging assays

Developing organoids were monitored using automated imaging in transmitted light. Then InCarta SW was used to find organoids, then define size and complexity of organoids. Organoids grown in Matrigel self-organize and develop complex structures, crypts and lumens. Machine learning-based image analysis allowed measurements of organoid size, diameter, density, and characterize complexity by counting lumens or crypts.

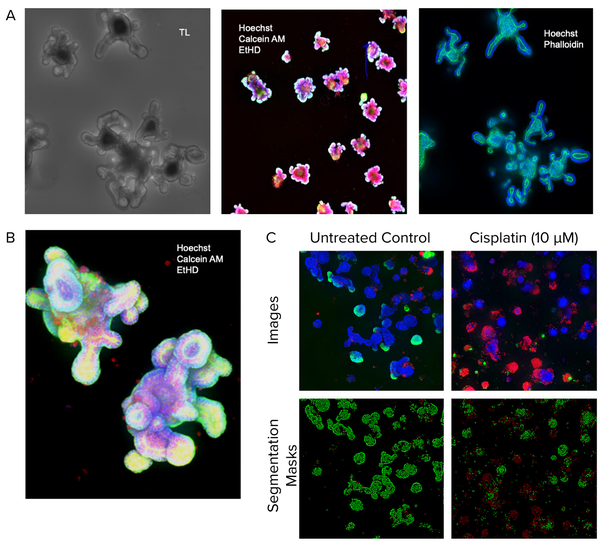


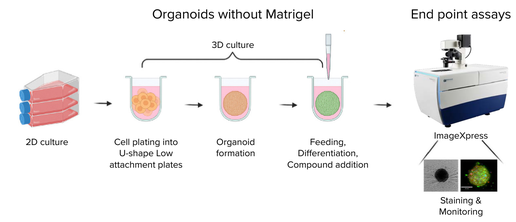
Figure 3. A. Monitored organoid development in transmitted light; live staining of organoids with viability dyes; fixed organoids stained with phalloidin. B. Organoids cultured for 10 days, stained with viability dyes. C. Control and treated organoids stained with viability dyes, live-dead analysis provided by MetaXpress analysis software.

For endpoint measurements organoids were stained for differentiation or viability markers. Automated confocal imaging and image analysis allowed complex phenotypic evaluation of organoid structures in 3D. Characterization of organoid phenotypes included organoids number, size distribution, organoid complexity (number of crypts or cavities), also cell content per organoid, cell viability, as well as nuclei, mitochondria, and cytoskeleton.

Results

Culturing and imaging 3D tumoroids

Culturing 3D spheroids or tumoroids matrix-free typically involves steps allowing spheroids to form in U-shape low attachment plates. Automating liquid exchange and compound addition allows to handle cultures of non-attached microtissues without breaking or loosing those during processing. 3D tumoroid culture was started from primary triple negative tumor (see Methods section). Cell line was developed by passaging primary tissues in SCID mice, then adopted for 2D cell culture. Tumoroids were formed by culturing 2,000 cells in 384 well low attachment plates for 48h, then tumoroids were treated with compounds from NCI (National Cancer Institute) library of approved anti-cancer drugs. Five concentrations were used for testing. Biomek automation was used for compound dilutions, cell treatments, and staining. Then tumoroids were cultured and monitored daily by automated imaging.



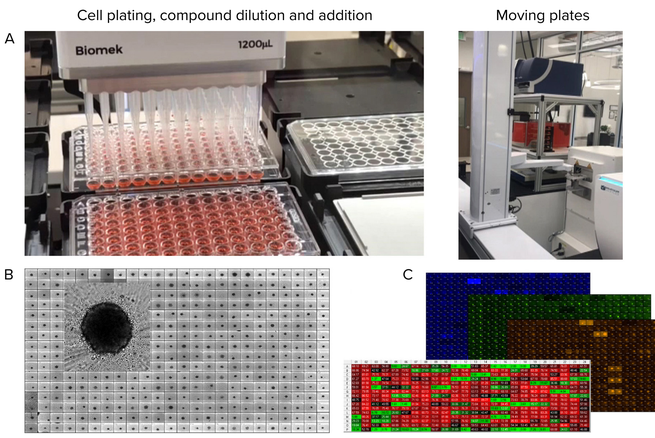


Figure 4. A. Automation of organoid culture, compound addition and staining. B. Tumoroids formed 48h after plating, TL images (10X). B. Tumoroids were treated with compounds for 5 days then stained with Hoechst dye (blue), Calcein AM (green) and EtHD (red), 10X. Organoids were imaged using confocal option, Z-stack of 15 images 10 µm apart,. Image analysis was done using Custom Module Editor (MetaXpress software) for finding organoids, nuclei, live and dead cells.

Automation of cell culture, compound treatment, and imaging

For end point assay cells were stained with combination of Hoechst, Calcein AM and EtHD and analyzed using Custom Module Editor for complex phenotypic analysis. Tumoroid phenotypes, density, and sizes were automatically detected and characterized by AI-based image analysis.

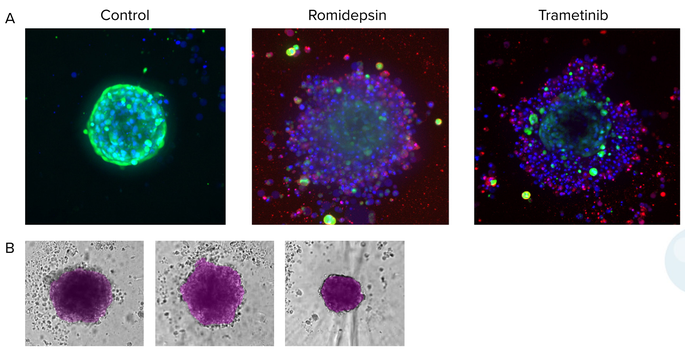


Figure 5. A. End-point analysis of fluorescent images was done using Custom Module Editor ImageXpress software. Images (maximum projection), and analysis masks shown. Multiple measurements were derived for cell scoring and organoid characterization. B. Automated monitoring and image analysis of 3D cancer microtissues was done using transmitted light images (10X) with AI-based image analysis In Carta software (analysis mask shown in purple).

Conclusions

- The processes for organoid assays and compound treatment was automated by integrating several instruments. The instruments allowed automated cell culture, maintenance, and compound treatment of 3D cellular models. The process can be used for automation and compound screening in 3D biology.
- Confocal imaging in combination with 3D analysis allows complex, quantitative analysis of cellular content of organoids as well as count and measurements of cells with different phenotypes. The methods can be used for evaluation of phenotypic changes caused by test compounds and disease modeling.

MOLECULAR DEVICES

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