Sequential orthogonal assays for longitudinal and endpoint characterization of three-dimensional spheroids

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Contact person

Eva Blondeel

Organisation

Name of the organisation Ghent University (UGent)

Department Department of Human Structure and Repair

Specific Research Group or Service Laboratory of Experimental Cancer Research

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Other: Characterization techniques
The Method is situated in	Translational - Applied Research
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

Model characterization

Assay optimization

spheroids

Longitudinal analysis

endpoint analysis

phenotyping

automation

Scientific area keywords

3D in vitro models

In vitro analysis

Translational Research

cancer research

Method description

Here we present the self-assembly of single-cell suspensions into spheroids by the liquid overlay method, followed by a modular framework for a multifaceted phenotyping of spheroids. Cell seeding, supernatant handling and compound administration are elaborated by both manual and automated procedures. The phenotyping modules contain a suite of orthogonal assays to analyze spheroids longitudinally and/or at an endpoint. Longitudinal analyses include morphometry with or without spheroid or cell state specific information and supernatant evaluation (nutrient consumption and metabolite/cytokine production). Spheroids can also be used as a starting point to monitor single and collective cell migration and invasion. At an endpoint, spheroids are lysed, fixed or dissociated into single cells. Endpoint analyses allow the investigation of molecular content, single-cell composition and state and architecture with spatial cell and subcellular specific information. Each module addresses time requirements and quality control indicators to support reproducibility. The presented complementary techniques can be readily adopted by researchers experienced in cell culture and basic molecular

biology. We anticipate that this modular protocol will advance the application of three-dimensional biology by providing scalable and complementary methods.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

This protocol describes the self-assembly of single-cell suspensions into spheroids by the liquid overlay method, alongside a modular framework of orthogonal assays to phenotype spheroids both longitudinally and at an endpoint. This method ensures production and long-term culture of spheroids with defined size, morphology and composition. In addition, the workflow addresses a comprehensive number of spheroid metrics including supernatant analysis.

Future & Other applications

The presented pipeline can be readily adopted by researchers working with other 3D models (e.g., patient-derived tissue fragments and organoids) by making small adaptations to fit the model.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

Spheroid characterization.pdf

Links

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