Patient-derived tumoroids

Development of vascularized 3D tumoroid models using microphysiological systems

Geetika Sahni[1], Hooi Linn Loo[1], Colin Paul[2], Matt Dallas[2], Sei Hien Lim[1], and Kuan Chee Mun[1]
[1]AIM Biotech Pte. Ltd., Singapore; [2]Thermo Fisher Scientific, Frederick, MD, USA

Key Takeaways

- OncoPro™ Tumoroid Cell Lines are standardized, viably cryopreserved 3D cancer organoid models derived from patient tumor cells
- Tumoroids can be readily and consistently expanded in serum- and conditioned medium-free Gibco™ OncoPro™ Tumoroid Culture Medium and used in downstream assays
- Integration of tumoroids with AIM Biotech organiX plate and VasQ Kit results kit results in perfusable, vascularized, patient-derived tumoroid models

Introduction

Patient-derived 3D cancer models (tumoroids or cancer organoids) recapitulate key features of clinical cancer samples (Paul et al., 2025) and can be readily expanded in OncoPro™ Tumoroid Culture Medium (**Fig. 1a**), a serum-free, conditioned medium-free cell culture system. Because derivation of new tumoroid lines from donor samples often takes several months and requires access to tissue, the use of standardized OncoPro™ Tumoroid Cell Lines that are expandable in culture, cryopreservation competent, and genetically and phenotypically stable across multiple passages, culture medium lots, and research sites (**Fig. 1c-d**) may facilitate assay development. However, long-term tumoroid cultures are almost exclusively epithelial in nature and lack stromal, endothelial, and immune compartments. To reconstitute aspects of the tumor microenvironment, notably perfusable vasculature, we describe incorporation of OncoPro™ Tumoroid Cell Lines with AIM Biotech organiX plates and VasQ Kits for reproducible formation of physiologically-relevant cancer models. This method establishes a standardized and human-relevant microphysiological system (MPS) cancer model and could enable mechanistic studies of tumor biology, immune cell trafficking, and therapeutic efficacy.

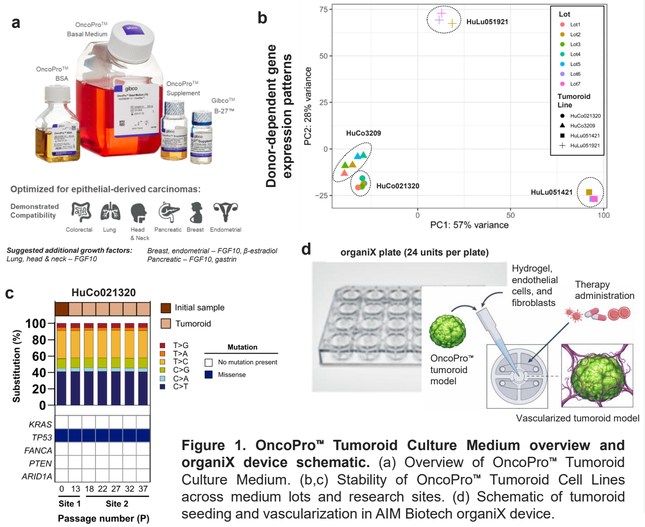

Figure 1. OncoPro™ Tumoroid Culture Medium overview and organiX device schematic. (a) Overview of OncoPro™ Tumoroid Culture Medium. (b,c) Stability of OncoPro™ Tumoroid Cell Lines across medium lots and research sites. (d) Schematic of tumoroid seeding and vascularization in AIM Biotech organiX device.

Results

Optimization of single tumoroid formation protocol

Cell seeding methods were critical for obtaining single tumoroids to seed to the organiX device. In the optimized protocol, 4000 dissociated tumoroid cells were seeded per well of an ultra-low attachment (ULA) U-bottom plate and incubated for 1 hour in the absence of extracellular matrix proteins. Additional culture medium was then added to bring the final concentration of Geltrex™ Flex to 2% (v/v), promoting aggregation and leading to formation of a single tumoroid model in each well by three days post-seeding (**Fig. 2**).

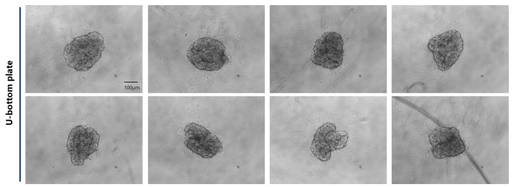

Figure 2. Tumoroid formation for seeding of organiX device. Images of multiple HuCo1044-GFP colorectal tumoroids at 1 day post-seeding of a U-bottom plate, illustrating consistency of size and single tumoroid formation. Intact tumoroids are picked for seeding of the organiX device.

Optimization of hydrogel composition for tumoroid growth in organiX device

3D vascularization assays have traditionally been performed in fibrin hydrogels. However, OncoPro™ Tumoroid Cell Lines are grown either as embedded cultures in Geltrex™ Flex Organoid-Qualified matrix or as suspension cultures in OncoPro Tumoroid Culture Medium supplemented with 2% (v/v) Geltrex™ Flex matrix. Tumoroid growth (as measured by projected area) and metabolic activity (as measured by PrestoBlue™ HS activity) are reduced when tumoroids are cultured for 7 days in the absence of Geltrex™ matrix, suggesting that basement membrane extract is critical for tumoroid health (**Fig. 3a**). Therefore, we compared tumoroid growth within organiX devices in either fibrin gels or in fibrin gels supplemented with 10% (v/v) Geltrex™ Flex (**Fig. 3b,c**). Dead cells were observed around the periphery of HuCo1044-GFP tumoroids cultured in fibrin gels (**Fig. 3c**). In contrast, tumoroid area increased more over time and morphology was maintained in the presence of Geltrex™ Flex matrix (**Fig. 3b,c**), and the composite gel was used in further experiments.

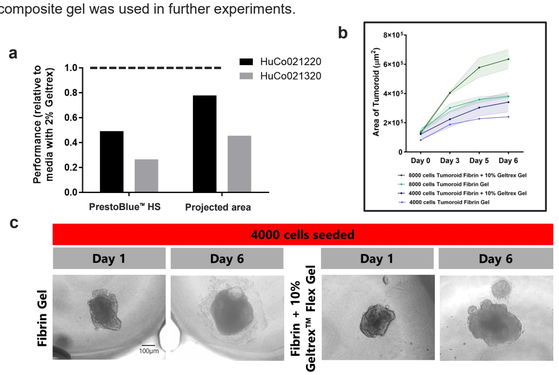

Figure 3. Co-culture matrix optimization. (a) Inhibition of tumoroid size and metabolic activity (by PrestoBlue™ HS readout) in the absence of Geltrex™ matrix. (b) Tumoroid growth over time in organiX device in fibrin gel and fibrin gel supplemented with 10% Geltrex™ Flex matrix. (c) Images of tumoroids within organiX device in different matrix conditions.

Confirmation of vessel perfusion in vascularized tumoroid model

Single tumoroids were formed and mixed with fibrinogen, thrombin, Geltrex™ Flex, endothelial cells (HUVEC/TERT), and fibroblasts (LF/TERT) and plated in the central port of the organiX device. One tumoroid, 300K HUVEC/TERT cells, and 50K LF/TERT cells were seeded per unit of the organiX device. Media was changed daily for the next 7 days, and an intact vascular network formed around the tumoroids. On Day 7, 70 kDa dextran-Texas red, which has a similar hydrodynamic radius to IgG antibodies, was introduced to a side port of the organiX device and flowed through the endothelial cell network, demonstrating intact and perfusable vasculature (**Fig. 4**).

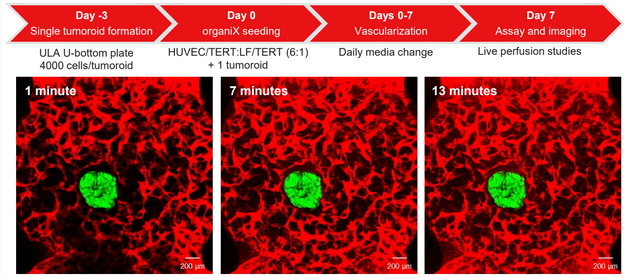

Figure 4. Vascularized tumoroid model formation. Schematic of vascularized tumoroid model formation workflow, and images of dextran-Texas red perfusion through the vascular network on Day 7 post-seeding of the organiX device were acquired every 6 minutes.

Interaction of vasculature and tumoroids within organiX device

Following confirmation that vasculature formed and was perfusable, cells in organiX devices were fixed, stained, and optically cleared. Confocal imaging revealed direct vascular interaction with HuCo1044-GFP tumoroids, including vessels interpenetrating the tumoroids (**Fig. 5a**). Lung tumoroid model HuLu051421 displayed a phenotype in which endothelial cells were excluded from the direct tumor periphery but interfaced with the tumor, opening peri-tumoroid area to dextran (**Fig. 5b**). Models were also stained with CellEvent™ Caspase-3/7 dye.

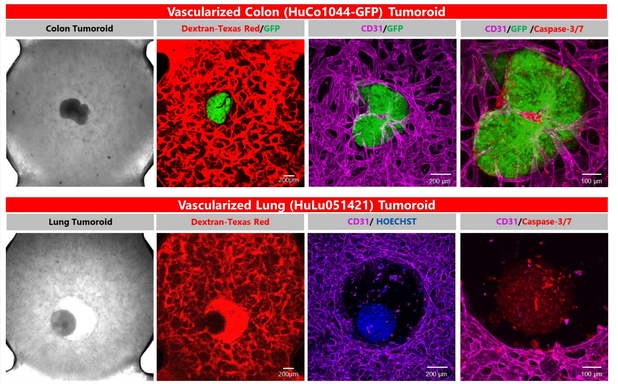

Figure 5. Characterization of colorectal and lung vascularized tumoroid models. (a) Vascularized HuCo1044-GFP colorectal tumoroid model, with CD31-positive endothelial cells interacting with tumoroids. (b) Vascularized HuLu051921 lung tumoroid model, with CD31-positive endothelial cells largely excluded from the direct tumoroid periphery.

Vascular delivery of natural killer (NK) cells in vascularized tumoroids

Following 7 days of tri-culture in the organiX device to enable vascular network formation, CellTracker-stained NK-92 cells were introduced via the media inlet ports at various NK cell:tumoroid cell ratios. NK cells were added in the presence of Hoechst 33342 dye and CellEvent™ Caspase dye, and devices were imaged after 48 hours of NK cell incubation.

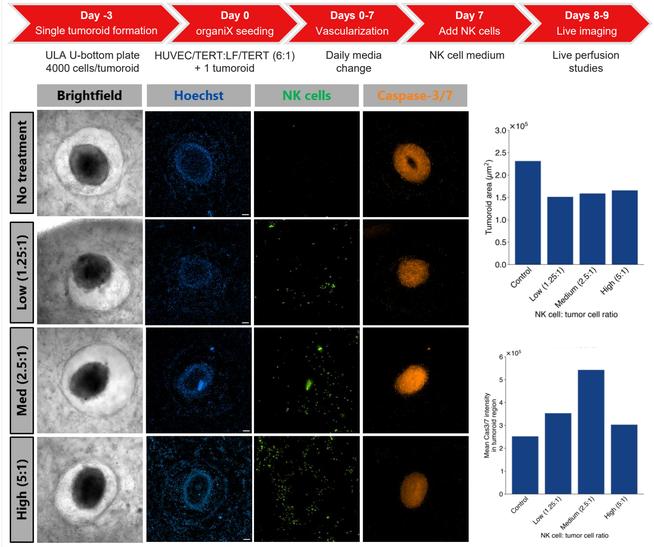

Figure 6. Natural killer cell cytotoxicity testing. Schematic of workflow for NK cell cytotoxicity experiments, representative images of lung (HuLu051421) tumoroids after incubation with NK cells, and quantification of tumoroid area and mean caspase-3/7 staining intensity as a function of NK cell:tumoroid cell ratio.

Acknowledgements

Select panels of Figure 1 modified from Paul et al., Scientific Reports 15: 3933 (2025). Hoechst is a trademark of Hoechst GmbH. Illustration in Figure 1d generated based on image in Adriani, G. and Pavesi, A. Nat Rev Immunology 24: 305-307 (2024).

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at thermofisher.com/oncopro