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The Science CRO

3D organoid assays for compound screening and optimization

Norman Liaw, Martin Lange, Benjamin Bader, Skadi Lau, Ralf Lesche, Maike Marczenke, Ekkehard May, Barbara Nicke, Danny Schnerwitzki, Anna Thrun, Nina Zablowsky and Patrick Steigemann

NUVISAN ICB GmbH, Müllerstraße 178, 13353 Berlin, Germany

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3D cell culture models better represent the *in vivo* organ state than traditional 2D cellular systems. The increase in complexity and provision of a more native context can reduce costs and speed up drug development. For pharmaceutical research, reproducible 3D cell culture systems are required to accurately measure compound or modality activity in high throughput. Here, we describe the evolution of high-throughput 3D cell culture systems at Nuvisan: from the BMBF-funded establishment of 3D tumor spheroids, screening-compatible assay systems on 384-well plates to 3D fibroblast invasion. We also developed a simple 3D model of polycystic kidney disease and established patient-derived cancer organoids (IMI funded). Finally, we depict our current efforts in human iPSC-derived 3D organoid models on 384-well plates to enable compound screening and characterization. These model systems are compatible with a variety of readouts including high-content imaging, calcium-flux analysis, (single cell) RNA sequencing, proteomics, flow cytometry, immunohistochemistry, and multielectrode array to enable full exploration and exploitation of these more complex models.

Hit Identification
High Throughput

Lead Optimization
Medium Throughput

(Pre-)clinical Validation
Low Throughput

Multiplexed Tumor Spheroid-based Models

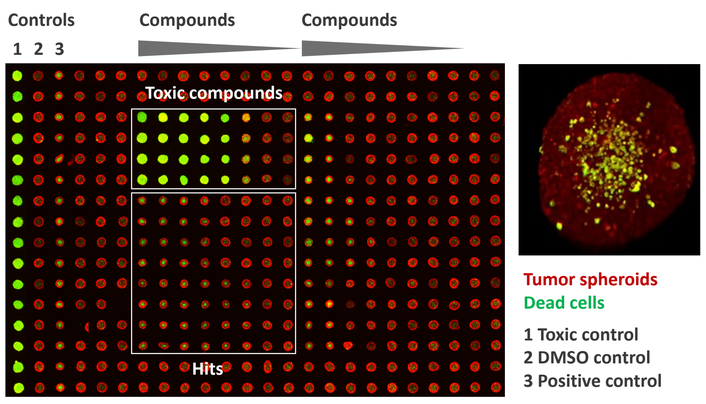


Figure 1. High throughput screening in 384-well format (2x 400k compound libraries) for the identification of compounds that induce cell death specifically in inner tumor spheroid regions. Approximate diameter of tumor spheroids is 500 µm.

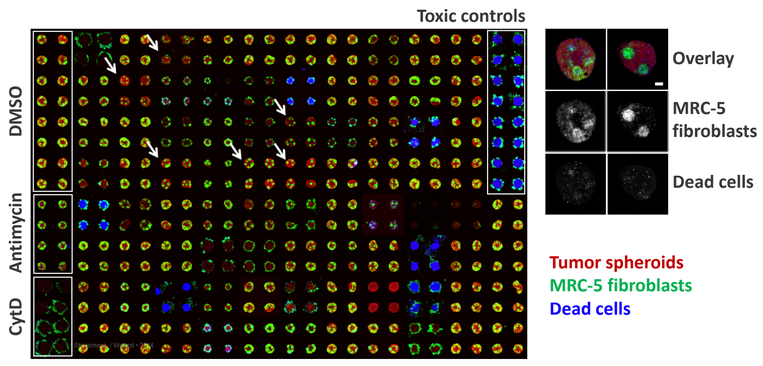


Figure 2. High throughput screening for the identification of compounds that prevent fibroblast invasion into tumor spheroids. 384-well screening plate with n = 4 per compound. Arrows indicate compounds that prevent fibroblast invasion.

Multiplexed Basic Cyst Formation Models

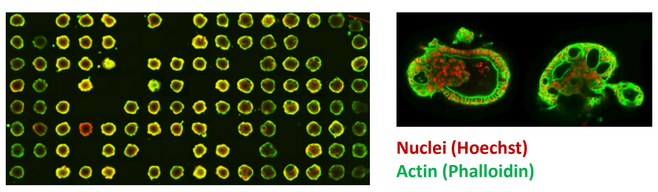
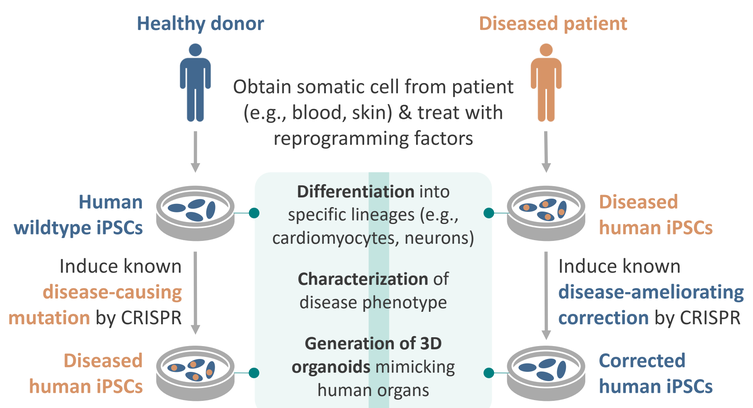


Figure 3. MDCK cells on agarose overlay plates. Alternatively, Matrigel-embedded cysts can be used.

Track record:
Wenzel et al. Exp Cell Res (2014), Klutzny et al. Cell Death Dis (2017), Vriens et al. Nature (2019), Peirsman et al. Nature Methods (2021), Blondeel et al. Sci Rep (2023, in revision), Wenzel et al. Exp Cell Res (2015)

NUVISAN Human iPSC Platform



3D Brain Organoids

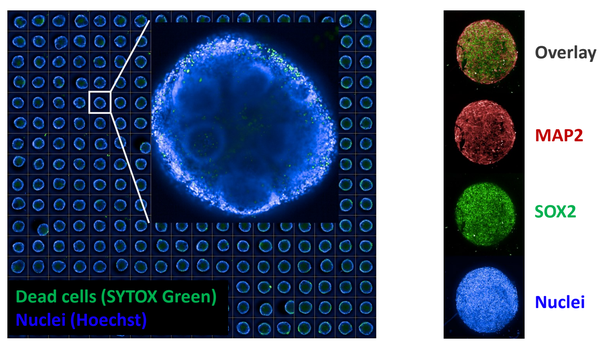


Figure 4. Brain organoids on 384-well agarose-coated plates. SYTOX Green staining shows high viability of 1-month-old brain organoids (*left panel*). Immunofluorescence staining reveals expression of neuronal (MAP2) and stem cell (SOX2) markers (*right panel*). Approximate brain organoid diameter is 500 µm.

Engineered Cardiac Organoids

Figure 5. Engineered cardiac organoids (ECOs) are generated from human iPSC-derived cardiomyocytes, cardiac fibroblasts, and/or endothelial cells. All cellular components arise from the same individual and thus recapitulate the cellular interactions that are unique to the donor. ECOs generate active forces which can be detected in an organ bath and are therefore suitable for modelling genetic-based cardiac diseases and load-related heart failure.

Patient-derived Cancer Organoids

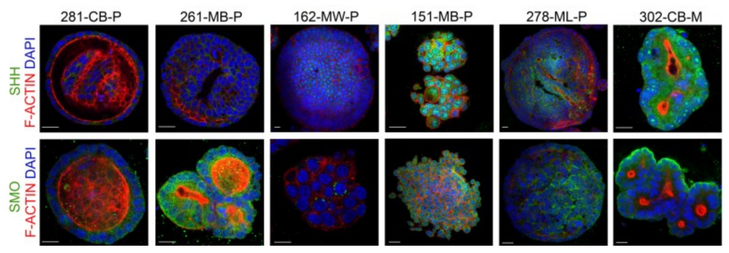
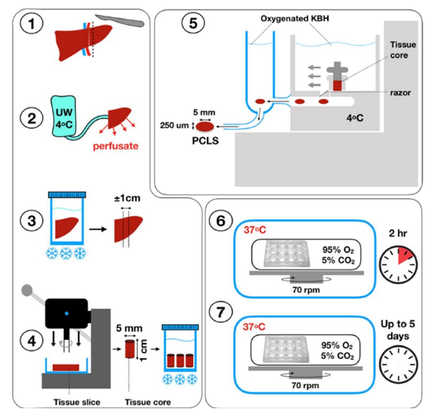


Figure 6. Cancer organoids for drug screening and compound optimization. NUVISAN does not offer patient-derived cancer organoids, but we have the expertise to work with client material.

Track record:
Boehnke et al. J Biomol Screen (2016), Schuette et al. Nature Communications (2017), Regan et al. Cell Reports (2017), Dieter et al. Cell Reports (2021), Regan et al. iScience (2021), Regan et al. iScience (2022)

Precision-cut Liver Slices



Palma et al. Hepatology International (2019)

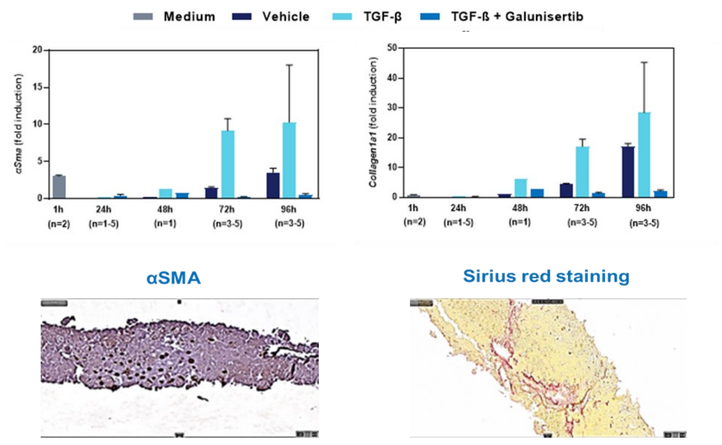


Figure 7. Precision-cut liver slices. Generation and routine use in *ex vivo* fibrosis studies and antifibrotic compound testing applying gene expression analysis and histochemical staining of α-SMA (*left bottom panel*) and collagen (*right bottom panel*).

APPLICATIONS

High Throughput Screening

- Up to 3 million compounds, 800k and 300k subsets, known bioactive compounds/FDA-approved drug libraries
- Accompanied by Life Science Database with access to > 500 million datapoints for compound prioritization

Cell-based Readouts

- Reporter, second messenger, protein-protein interactions, post-translational modifications, ion flux (e.g., Ca^{2+}, Na^{+}, K^{+})
- Broad assay portfolio: Luminescence, TR-FRET, BRET, ALPHA, Radiometric
- Endogenous and genetically engineered organisms (German BSL2)
- Readouts on several multimode detection readers: Pherastar, ViewLux, FLIPR

High Content Analysis (HCA)

- Phenotypic and multiplexed HTS-compatible
- 4 fully automated HCS setups for single timepoint and live cell kinetics
- Ideal to monitor complex 3D cell culture systems: fluorescent stains (e.g., Ca^{2+} imaging by automated time lapse), reporters, immunofluorescence
- Several 3D cell culture screens performed by automated microscopy/high content analysis on subset libraries