NEXUS Personalized Health Technologies

Miniaturization and automation of organoid assays for drug screening

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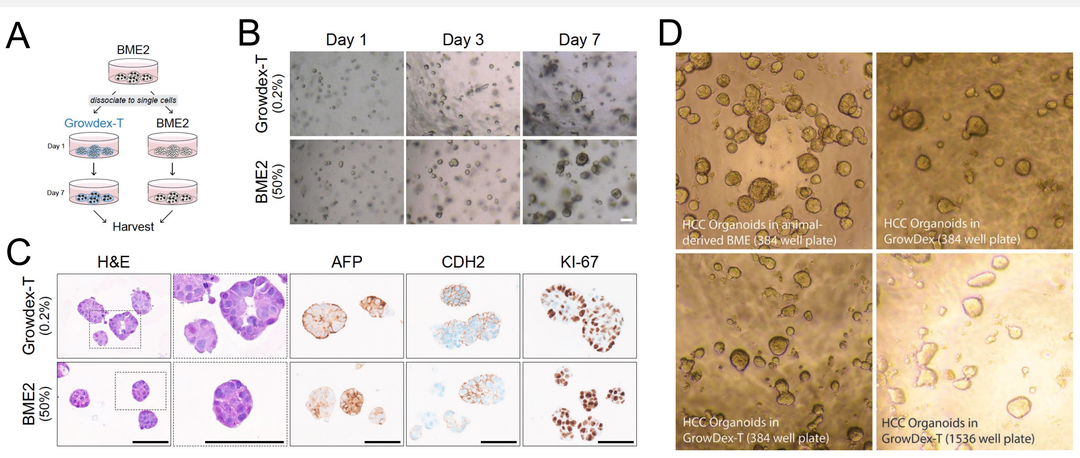
1 Overview

The past decade has shown an enormous increase in the popularity of organoids for in vitro drug evaluation due to their improved physiological relevance. This is in turn expected to reduce the number, and size, of in vivo studies required to select drugs. Due to technological restraints and practical limitations, drug screens with patient-derived organoids have traditionally been performed on a small scale, and these experiments are often used to validate results from earlier in vitro drug screens. To enable large-scale drug screens with hepatocellular carcinoma (HCC) organoids derived from patient biopsies, we aimed to automate and miniaturize the drug screening methodology[1].

2 Introduction: HCC organoids develop in GrowDex

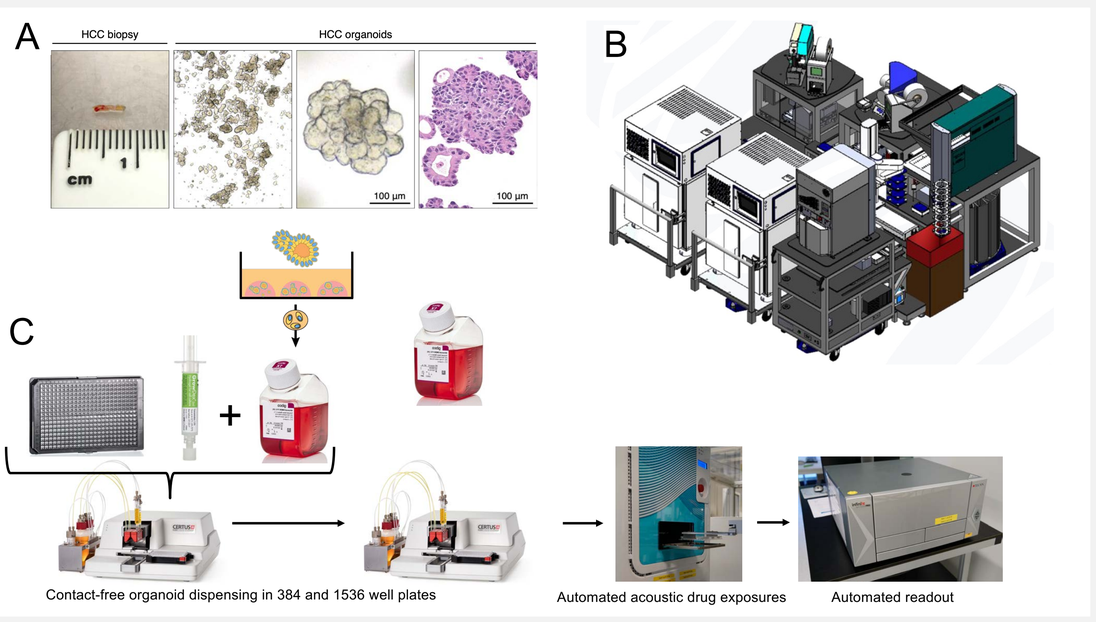


Growth rate of HCC organoids in 50% BME2 or 0.2% GrowDex-T (nanofibrillar cellulose, NFC) is similar over 7 days (A, B).

HCC organoids show similar morphology after 7 days of culturing and no differences in Alpha-FetoProtein (AFP), N-Cadherin (CDH2) and cell proliferation (Ki-67) (C, scale bar 100µm).

Organoids can be cultured in regular 0.4% GrowDex and in 0.2% GrowDex-T both in 384 and 1536 well plates (D).

3 Methodology: Automation setup

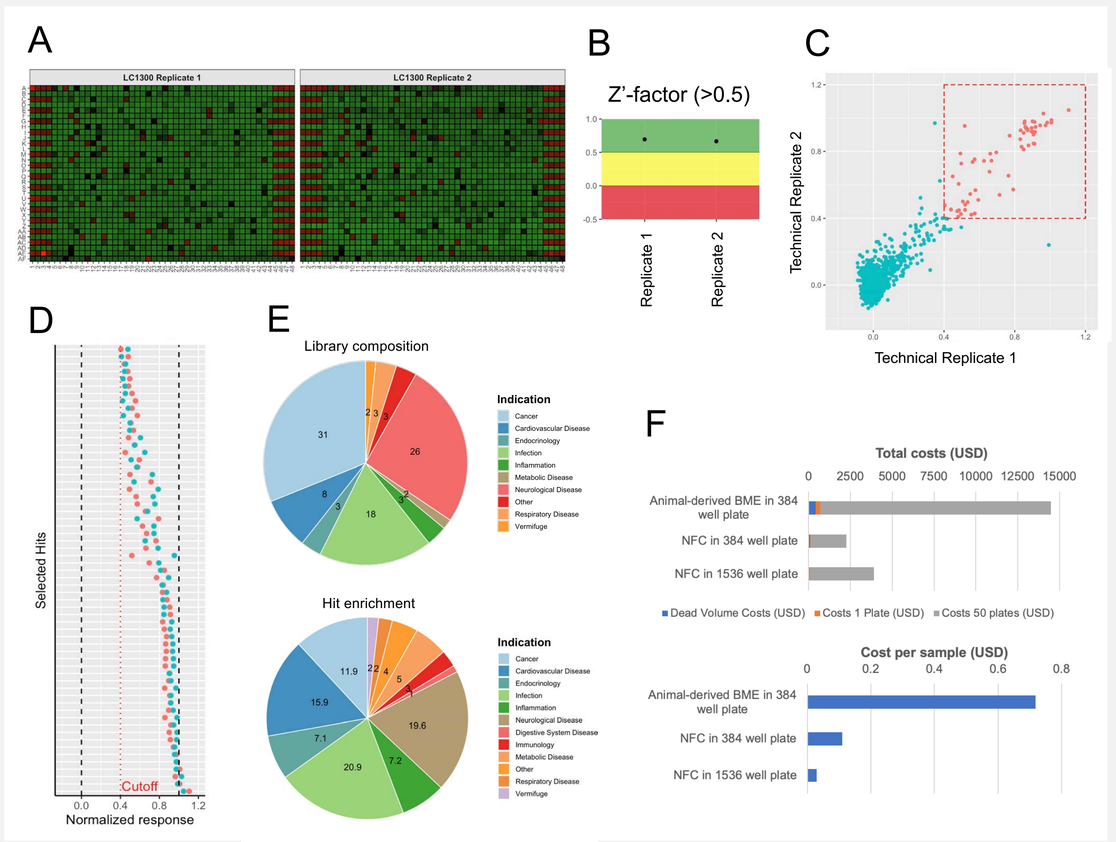


HCC organoids can be derived from needle biopsies[2]. These organoids are embedded in 50% BME2 to develop and can subsequently be used for drug evaluation. (A)

The ETH Zurich core facility NEXUS Personalized Health Technologies operates lab-automation infrastructure designed by HighRes Biosolutions. (B)

Using this platform, we could dispense dissociated organoids suspended in 0.2% GrowDex-T (UPM Biomedicals) directly into 1536 well plates using a Certus Flex (Fritz Gyger AG). With acoustic dispensing technology (Beckman), we treated the wells with compounds in nanoliter range to eliminate the requirement for pipette tips. Drug efficacy was measured using cell viability measurement (CellTiterGlo 3D, Promega). The CellTiterGlo 3D reagents were dispensed to the plates using the Certus Flex (Fritz Gyger AG) and readouts were performed using a M1000 Pro plate reader (Tecan AG). The developed methodology was evaluated for two different patient-derived HCC organoid lines. (C)

4 Results: Screening FDA-approved drugs



We screened 1172 FDA approved drugs (SelleckChem L1300) at 10µM in technical duplicate in 1536 well plate format, performing a readout with CellTiterGlo 3D:

The replicates show near identical performance with high Z'-factor[3] indicating assay quality (A, B, C).

Selected hits reveal a clear enrichment for known anticancer drugs as well as drugs indicated for neurological diseases (D-E).

This screen was, compared to an earlier screen in BME2 performed in 2020, revealing a 77% hit overlap, despite the large differences in assay design. The same screen was performed with a library of 1250 novel compounds and we plan to screen additional libraries (not shown here, manuscript in preparation).

The elimination of animal-derived matrices and removal of pipette tips greatly reduces screening costs. Additionally, the downscaling of the plate format to 1536 meant that lower volumes could be used, further reducing per-sample costs. (F, *figure reproduced from Booij TH and Stirnimann CU[4]*; NFC, nanofibrillar cellulose/GrowDex-T).

5 Conclusion

- We developed a screening platform for two HCC organoid patient lines that uses nanofibrillar cellulose as matrix in 1536 well plates.
- We report high assay quality (Z'-factors[3] above 0.5) and excellent reproducibility of screening results.
- A screen of FDA-approved drugs showed high assay stability and a list of hits containing many anti-cancer drugs.
- The elimination of animal-derived basement membranes and pipette tips caused a 5-10 times reduction in screening costs[4]. This assay is more automation-friendly due to the exclusion of reagents that suffer from batch variation and that are dependent on temperature-controlled liquid handling.
- We expect that the developed methods can be extrapolated to other organoid types, specifically those growing in suspension.

References

1. Booij, Cattaneo and Hirt (2022), Cells, Tumor Organoids as a Research Tool: How to Exploit Them https://doi.org/10.3390/cells11213440
2. Nuciforo S et al. (2018), Cell Reports, Organoid models of human liver cancers derived from tumor needle biopsies, doi: 10.1016/j.celrep.2018.07.001.
3. Zhang JH et al. (1999), J Biomol Screen, A simple statistical parameter for use in evaluation and validation of high throughput screening assays, doi: 10.1177/108705719900400206.
4. Booij and Stirnimann, Drug Target Review (2022)

Partner/Sponsor: GYGER, UPM BIOFORE BEYOND FOSSILS

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