High-throughput spheroid formation for compound screening using magnetic 3D bioprinting

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Overview

Biomedical research has gravitated towards three-dimensional (3D) cell culture platforms that replicate native tissue environments *in vitro* better than traditional two-dimensional (2D) platforms. These platforms can replicate tissue stiffness, extracellular matrix (ECM) composition, heterogeneity, and access to nutrients and compounds. However, technical issues in handling and speed have so far limited the expansion of 3D cell culture platforms into high-throughput screening.

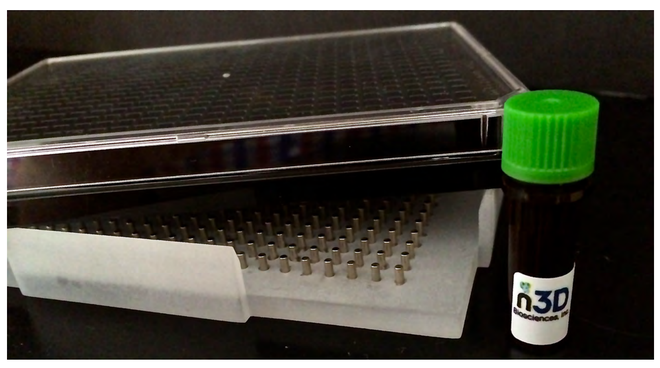
A platform that addresses these issues is **magnetic 3D bioprinting**. The core principle behind this platform is the magnetization of cells with biocompatible magnetic nanoparticles[1] (NanoShuttle™), which attaches electrostatically to cell membranes. These cells are then subsequently printed into spheroids with mild magnetic forces. These spheroids escape the limitations of other platforms in high-throughput screening by being:

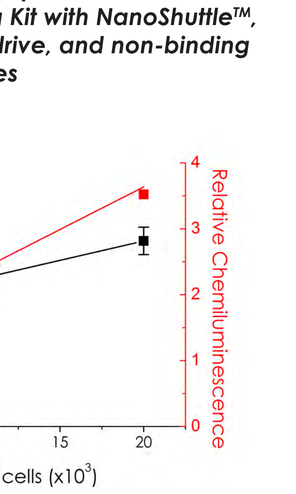
- **rapidly formed** (few hours - overnight)
- **unattached** to any stiff substrate
- **easy to handle**, hold down with magnets to retain spheroids
- **reproducible** in size with fixed magnets
- **viable**, with no effect of NanoShuttle™ and magnetic fields on cell behavior[1]
- **unlimited** to any cell type
- **fluorescent without interference** from NanoShuttle™
- **scalable in size** for high-throughput formats (**384- and 1536-well**)

In this study developed functional assays for cardiotoxicity (beating in cardiomyocytes) and drug-drug interactions (CYP450 inhibition/induction in hepatocytes).

Methods

- Cardiomyocytes (iPSC, Cellular Dynamics) and hepatocytes (primary, BioreclamationIVT) are thawed and magnetized by mixing with NanoShuttle™ (1 µL/10,000 cells) for 2 h
- Magnetized cells are detached, counted, resuspended in media, and distributed into a cell-repellent 384-well plate (CELLSTAR®, Greiner Bio-One)
- Cells are printed into spheroids by placing the plate atop a magnetic spheroid drive of 384 magnets (Nano3D Biosciences), that aggregate cells into spheroids at the bottom of the well
- Spheroids are left on the plate overnight to interact and build a mature spheroid with ECM





Top: Schematic of magnetic 3D bioprinting spheroids.[2] Left: 384-well Bioprinting Kit with NanoShuttle™, spheroid drive, and non-binding microplates

Left: Magnetically 3D bioprinted spheroids of HepG2 hepatocytes in a 384-well plate of various cell numbers after 15 min of printing. Right: Spheroid size (black) and viability (red, CellTiter-Glo, Promega) as a function of cell number. Scale bar = 500 µm.

Magnetic 3D bioprinting rapidly prints viable spheroids by using **magnetic forces to accelerate spheroid aggregation**. With fixed magnet sizes, spheroid size is **reproducible** and **scalable** for high-throughput testing.

Acknowledgements

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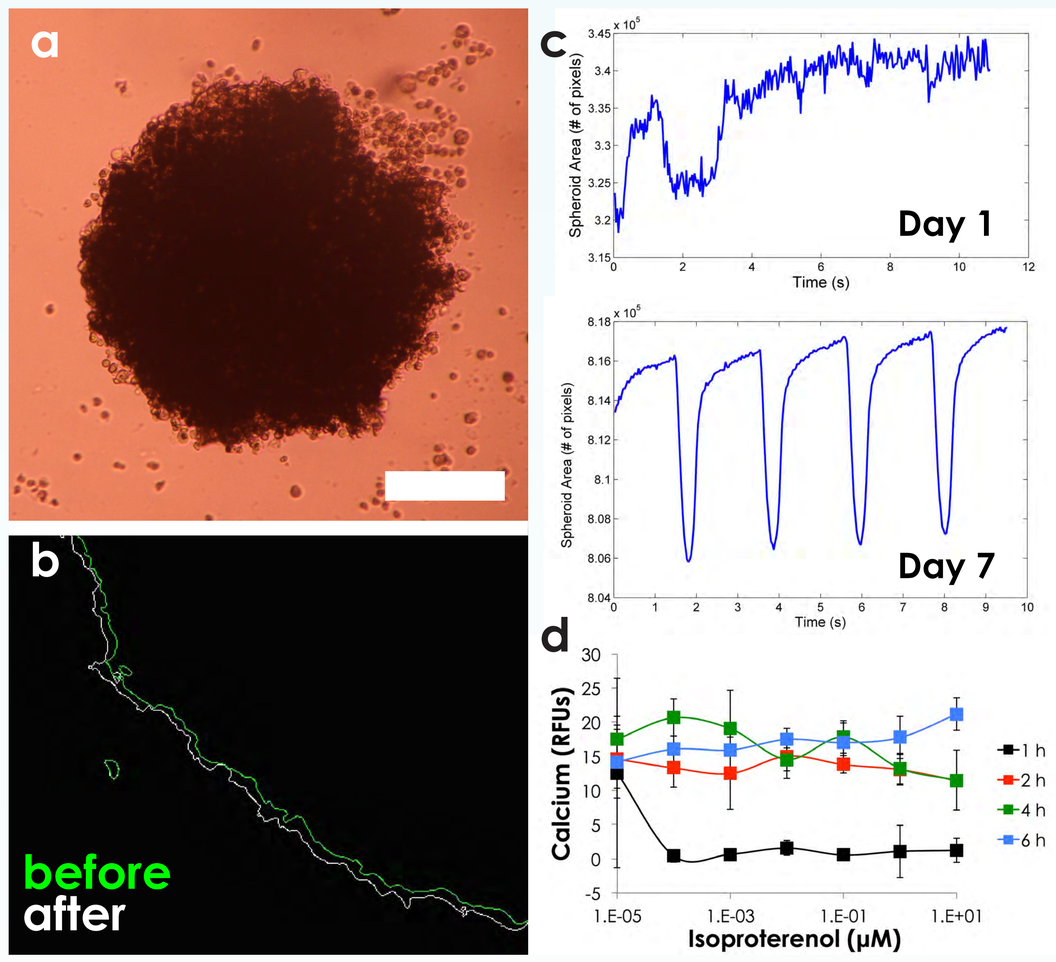
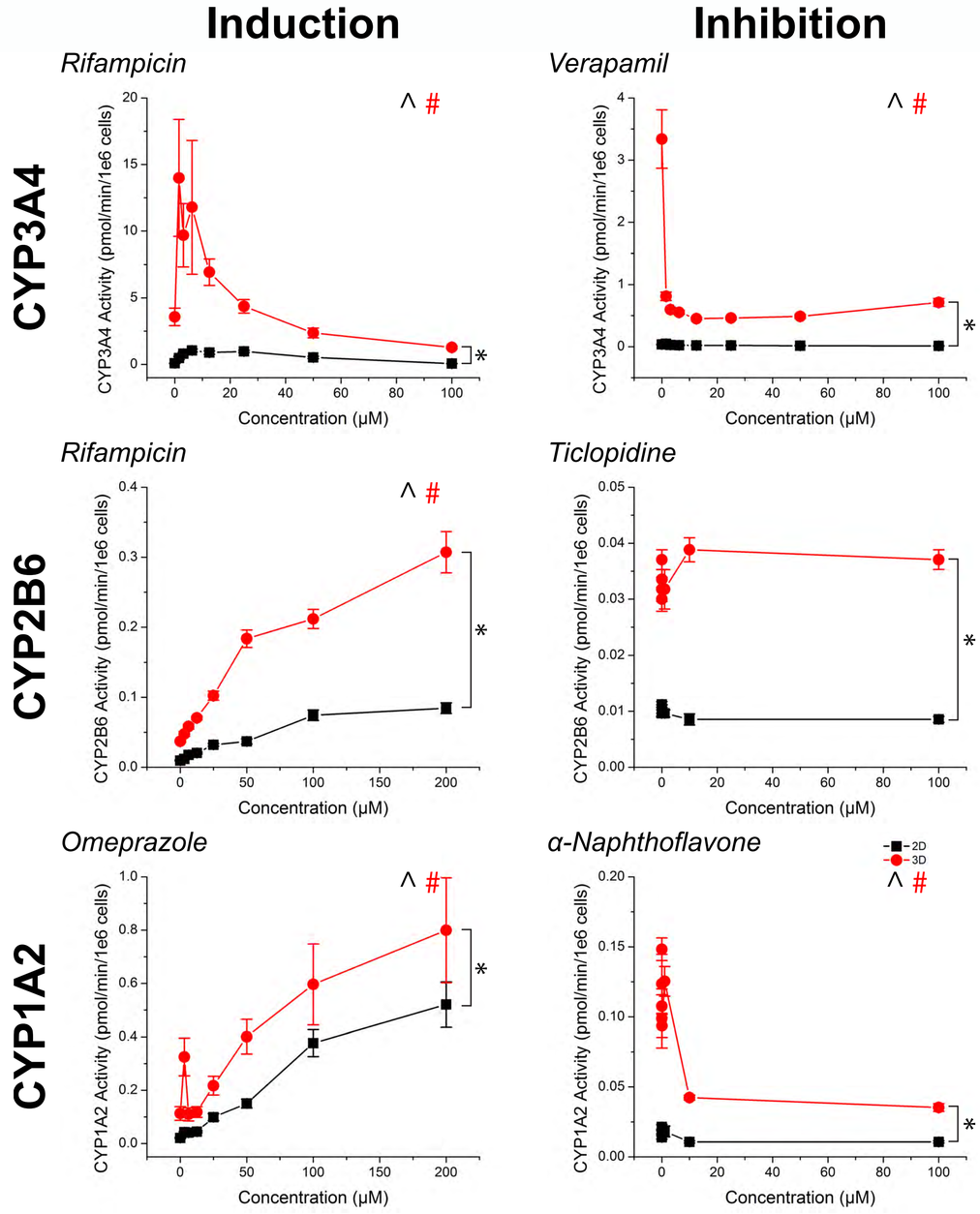
Results



iPS-cardiomyocyte spheroids. (a) iPS-cardiomyocytes (Cellular Dynamics) formed a competent spheroid that beat in the 3D environment, and through (b) image tracking, beating patterns could be recorded. (c) The beating of these spheroids matured into a characteristic beating pattern. (d) Calcium signaling can also be measured. Scale = 500 µm.

We successfully printed cardiomyocyte spheroids that beat to track **functional changes in beating and calcium signaling** in response to cardiotoxic compounds.



Cytochrome P450 (CYP) induction/inhibition in human primary hepatocytes (BioreclamationIVT) in response to known inducers and inhibitors of CYP3A4, CYP2B6, and CYP1A2. Aside from ticlopidine, CYP activities were induced and inhibited as expected. In all cases, there was higher activity in 3D than in 2D. ^,#: $p < 0.05$ effect of concentration on activity. *: $p < 0.05$ difference in activity between 2D and 3D.

Primary hepatocytes were successfully printed into spheroids and exhibited **higher CYP450 activity in 3D** than in 2D, and **more induction and inhibition in 3D**

References

1. Souza GR et al. *Nat. Nanotech.* (2010)
2. Tseng H et al. *Sci. Rep.* (2015)