# Magnetically 3D bioprinted hepatocyte spheroids for *in vitro* metabolic studies

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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.

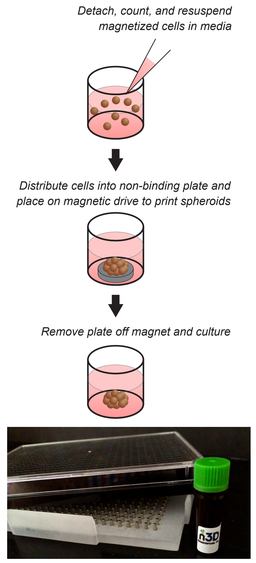
A platform that addresses these issues is **magnetic 3D bioprinting**. The core principle behind this platform is the magnetization of cells with biocompatible magnetic nanoparticles[1,2] (NanoShuttle™), which attaches electrostatically to cell membranes. These cells are then subsequently printed into spheroids with mild magnetic forces.[2] 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** spheroids by holding down with magnets
- **reproducible** in size with fixed magnets
- printed on a **flat surface** for easy imaging
- **viable**, with no effect of NanoShuttle™ and magnetic fields[1,4,5]
- **unlimited** to any cell type
- **fluorescent without interference** from NanoShuttle™
- **scalable in size** for high-throughput formats (**384- and 1536-well**)

In this study we developed a functional assay for CYP450 inhibition/induction using magnetic 3D bioprinting

## Methods

- Primary hepatocytes (BioreclamationIVT) were thawed and magnetized by mixing with NanoShuttle™ (1 µL/10,000 cells) for 2 h with constant shaking
- Magnetized cells are detached, counted, resuspended in media, and distributed into a cell-repellent 384-well plate (1 x $10^4$ cells/well, 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 magnet for 24 h to form a competent spheroid in thawing medium
- Thawing medium was replaced with maintenance medium the next day and the spheroids were cultured off the
- magnet for 24 h
- Maintenance medium was then replaced with serum-free induction media containing a compound
- After 3 d of compound exposure, CYP450 activity was measured (P450-Glo, Promega)



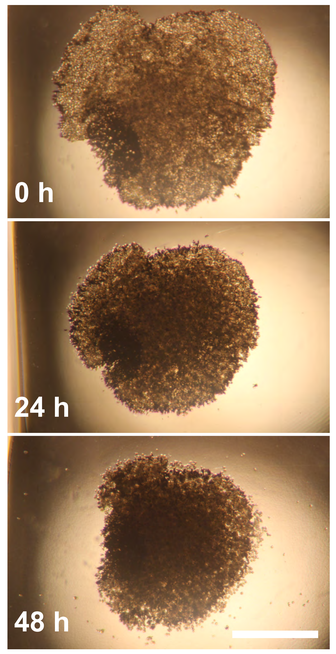

***Top: Schematic of magnetic 3D bioprinting spheroids.[2] Bottom: 384-well Bioprinting Kit with NanoShuttle, spheroid drive, and non-binding microplates***

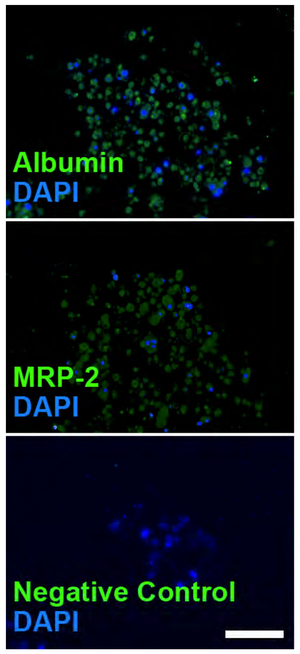
## Results

Primary hepatocytes formed tight, competent spheroids within the magnetic field. We observed a small contraction in size over 48 h of culture, which can be used as an endpoint for viability.[3,6] Competence was maintained after removing the spheroid from the magnet at 24 h.

**The contraction and consolidation of NanoShuttle™ within the spheroids demonstrate their viability and competence.**

***Right: Hepatocyte spheroids of 1 x $10^4$ cells each over 48 h. Note the small contraction in size over time, which reflects cell viability within the spheroid. Scale bar = 500 µm***

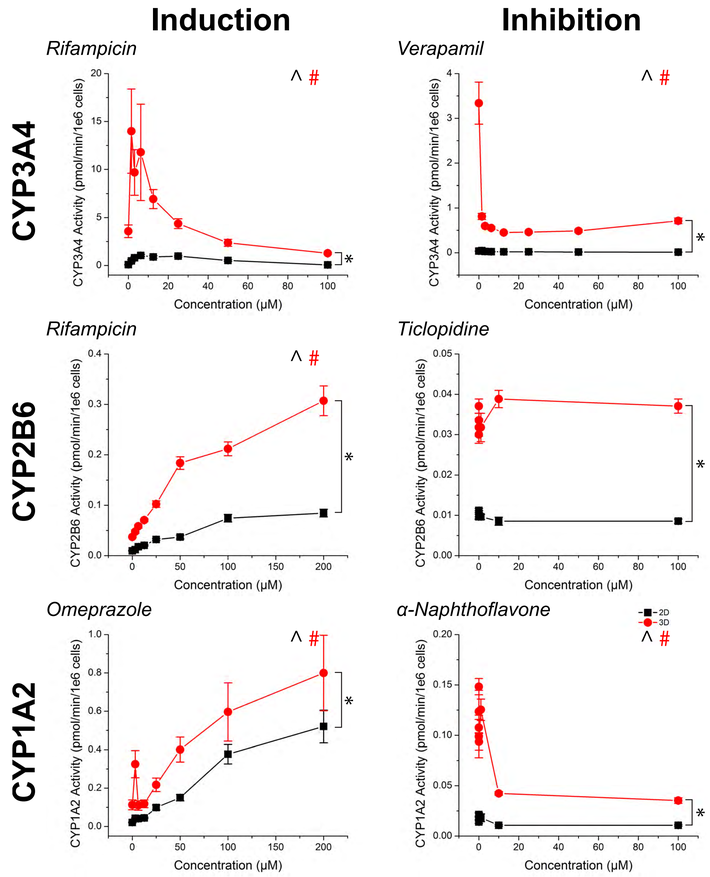



Hepatocytes in spheroids maintained their phenotype as shown by immunohistochemical staining. The cells were loose within the spheroid, showing a very cellularized spheroid with little extracellular matrix (ECM), although damage due to embedding in paraffin, sectioning, and paraffin cannot be ignored.

**Magnetically 3D bioprinted spheroids maintained hepatocyte phenotype**

***Left: Immunohistochemical stains of spheroids primary hepatocytes for albumin and multidrug resistance-associated protein 2 (MRP-2) (green) after 48 h of culture. Nuclei were counterstained with DAPI (blue). Positive staining of these proteins demonstrate the maintenance of hepatocyte phenotype in 3D. Scale bar = 25 µm.***




***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.***

After 72 h exposure to known CYP inhibitors and inducers, CYP activity was altered as expected. These primary hepatocytes showed low values of CYP2B6 and CYP1A2. Luminescence readings were not affected by the presence of NanoShuttle™.

**Primary hepatocyte spheroids printed with magnetic 3D bioprinting are reproducible, maintain their phenotype, and can be used to measure compound effects on CYP activity.**

## Acknowledgements

This project was funded by: a SBIR Phase I award (R43ES024644) from the **National Institute of Environmental Health Sciences (NIEHS)**; and a SBIR Phase II award (1127551) from the **National Science Foundation**. We would like to thank: **BioreclamationIVT** for the primary hepatocytes; **Promega** for the P450-Glo assay; and Drs. Eva Sevick-Muraca, Grace Wu, Nat Wilganowski, Reynolds Brobey, Sheri Skinner, and Robert Amato from the **University of Texas Health Science Center at Houston** for the spectrophotometer and microscope.

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