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 (NanoShuttleTM), 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 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 NanoShuttleTM and magnetic fields
- unlimited to any cell type
- fluorescent without interference from NanoShuttleTM
- 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 NanoShuttleTM (1 µL/10,000 cells) for 2 h with constant shaking
- Magnetized cells are detached, counted, resuspended in media, and distributed into a cell-repelling 384-well plate (1 x 10^5 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

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. Competence was maintained after removing the spheroid from the magnet at 24 h.

The contraction and consolidation of NanoShuttleTM within the spheroids demonstrate their viability and competence.

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

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References