High-throughput spheroid printing and toxicity testing using magnetic 3D bioprinting
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Background
Three-dimensional (3D) cell culture can recreate the complex environments of native tissue in vitro. However, technical limitations in speed, handling, and sample retention have prevented its widespread incorporation into high-throughput compound screening processes.

A solution for this unmet need is **magnetic 3D bioprinting**, where cells are magnetized with biocompatible magnetic nanoparticles (NanoShuttle™). Once magnetized, these cells can be rapidly aggregated and formed into spheroids and other shapes using magnetic forces.

These spheroids:
- are rapidly formed (15 min - few hours);
- are viable, with no effect of NanoShuttle™ and/or magnetic fields on cell behavior;
- can be made with most cell types;
- do not require specialized equipment, media;
- fluoresce without interference from NanoShuttle™
- scale down in size for high-throughput formats (384- and 1536-well).

Magnetic 3D Bioprinting

- Cells are magnetized by incubating with NanoShuttle™ overnight.
- The next day, magnetized cells are detached, counted, and resuspended in media.
- Cells are distributed into a low-binding microwell plate (CELLSTAR® Cell Repellent Surface, Greiner Bio-One).
- Plate is then placed on magnetic drive to form spheroids for anywhere between 15 min to overnight.
- Plate can then be removed off magnet to culture and assay.

**High-Throughput Spheroid Printing**

<table>
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<tr>
<th>Number of HepG2s</th>
<th>625</th>
<th>1250</th>
<th>2500</th>
<th>5000</th>
<th>10000</th>
<th>20000</th>
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</thead>
<tbody>
<tr>
<td>Number of spheroids</td>
<td></td>
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<tr>
<td>Scale bar = 500 μm</td>
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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 and viability (CellTiter-Glo, Promega) as a function of cell number. Scale bar = 500 μm.

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

- As NanoShuttle™ is non-specific, spheroids can be printed of any cell types to develop any assay; from organ-specific functional assays, like with cardiomyocytes (Cell®, Cellular Dynamics), co-cultures and invasion assays.

Co-cultures of AS49 lung adenocarcinoma cells (green) and primary human pulmonary fibroblasts (HPF, red) or human thinned smooth muscle cells (HMSMC, red) of different ratios in spheroids of 10,000 cells each.

Viability of spheroids PC3 prostate cancer cells with 2,000, 200, and 20 cells as measured by RealTime-Glo (Promega, left) and live/dead staining (green = live, red = dead, right). Scale bar = 250 μm.

Left: A sphere of cardiomyocytes (Cell®, Cellular Dynamics) with 20,000 cells. Scale bar = 500 μm. Center: These spheroids lead, with their patterns mutating over time. Right: Calcium in cardiomyocyte spheroids 1, 2, 4, and 6 h after exposing the spheroids to various concentrations of isoproterenol.

Viability of spheroids PC3 prostate cancer cells with 2,000, 200, and 500 cells as measured by MTT assay. *: p < 0.05. As expected, with higher, toxic contractions, spheroids contract less.

Spheroid contraction over 10 h. d) Dose response of 3T3s in 3D and in 2D as measured by the MIT assay. *: p < 0.05. As expected, with higher, toxic contractions, spheroids contract less.

Toxicity Screening - BioAssay™

Spheroids will contract immediately (< 24 h) in a manner related to viability, where healthy spheroids will contract while unhealthy ones will not.** We use spheroid size over time as a simple metric for compound toxicity. Contraction is measured in real-time using an iPad (Apple Computer) that captures whole plates of spheroids with sufficient resolution (200 μm) at programmed intervals (< 1 s), forgoing the need to image and measure each individual spheroid under a microscope.

This assay (BioAssay™) works in 96- and 384-well plates, and can also be applied to print 3D rings to mimic scenarios like wound healing, vasoactivity, etc. This assay rapidly and reliably measure compound toxicity in 3D environments.

Acknowledgements

This work was supported by Small Business Innovation Research Grants from the National Institute of Environmental Health Sciences (Phase I - ES024644); and the National Science Foundation (Phase I - 0945954, Phase II 1129551).

References