A high-throughput three-dimensional cell migration assay for toxicity screening using magnetic levitation with mobile device-based macroscopic image capture

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Background

Purpose: Develop and validate a label-free three-dimensional (3D) in vitro assay for high-throughput drug toxicity screening using magnetic levitation and mobile-device based image analysis. This assay patterns cells into 3D rings that represent native tissue environment and close over time. Ring closure correlates with cell migration and can be tracked at different drug concentrations using a mobile device.

Methods: Confluent flasks of human embryonic kidney cells (HEK293) were incubated overnight with a magnetic nanoparticle assembly, to which they bind.[1] The next day, these cells were detached from the flask, and with the external application of a magnetic field, levitated to the air-liquid interface, where cells aggregated and interact to form larger 3D structures. These 3D structures were levitated for 24 hours to induce extracellular matrix formation. Afterwards, the structures were mechanically disrupted and patterned into rings using ring-shaped magnets. The magnetic field was removed, drugs (ibuprofen, doxorubicin) were added at varying concentrations, and the rings were allowed to close. This assay was validated against a 2D viability assay. A mobile device was programmed to capture the rings at specified timepoints, and image analysis was performed to track ring closure as a function of drug concentration and time. Image analysis using images of the mobile device was compared to a similar analysis using images taken with a microscope.[2]

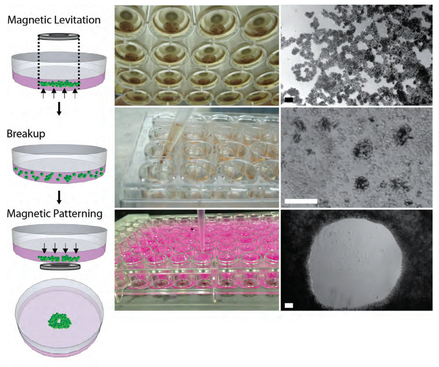
Results: HEK293s were successfully formed into 3D cultures using magnetic levitation, and patterned into rings. Using this assay, we found the IC50 of ibuprofen and doxorubicin to be 1.4 mM and 45 µM, respectively, as compared to the 2D IC50's of 1 mM and 2 µM. Image analysis of images captured with a mobile device of ring closure compared favorably to analysis using images captured with a microscope.

Conclusions: The assay in this study uses the closure of 3D rings as a measure of cell viability and migration under exposure to different drugs. Ring closure was shown to correlate with viability, and varied in rate with drug concentration. This assay is universally applicable and has been used with various combinations of drugs and cell types. Overall, the closure of 3D ring cultures is a simple assay that measures drug toxicity in a 3D environment representative of the native cellular environment.

Hypothesis: This assay, the BiO Assay, can be used to rapidly create rings and dots of cells that close/shrink over time in a fashion dependent on compound concentration

Magnetic Patterning

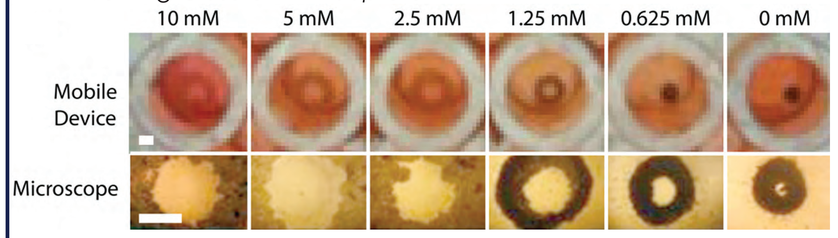
- Cells are incubated with NanoShuttle (Nano3D Biosciences) overnight
- The next day, cells are levitated to synthesize ECM for a few hours to overnight
- Cultures are broken apart and patterned into rings or dots for 15 minutes (150K cells/ring, 75K cells/dot) →
- Magnets are removed and cells are allowed to close for hours to days



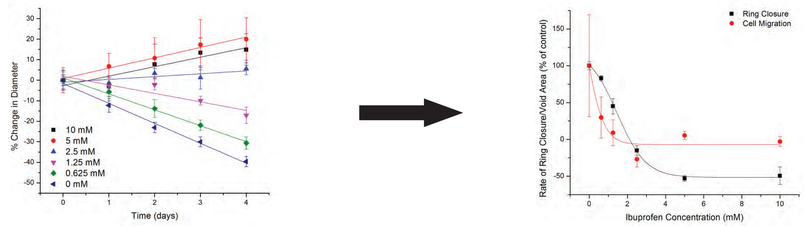
Magnetically levitated cultures can rapidly assemble and pattern 3D cell cultures

BiO Assay

HEK293 Ring Closure with Ibuprofen

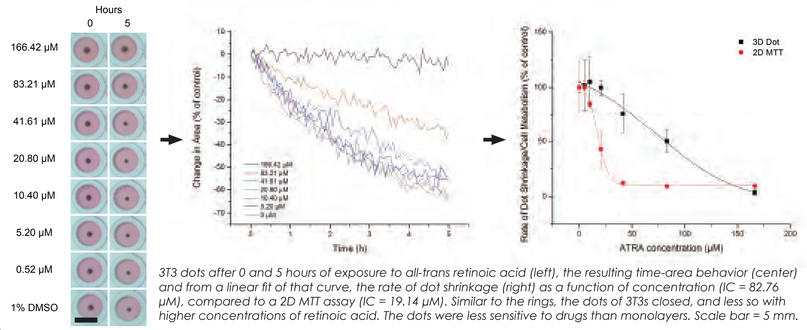


Rings of HEK293s after 4 days of closure taken with the iPod and microscope. With increasing amounts of ibuprofen, the rings are unable to close. Note the resolution of the rings within the well in one image taken by the iPod, compared to the microscope images of individual wells. Scale bar = 1 mm.

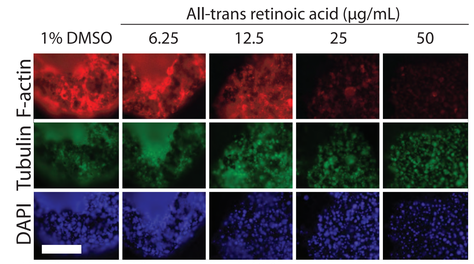


HEK293 ring closure (outer diameter of the ring) as a function of time (left) and the rate of ring closure from that curve as a function of ibuprofen concentration, compared to a scratch assay on 2D monolayers with the same combination (right). Note the difference in sensitivity between ring closure (IC_{50} = 1.21 mM) and the scratch assay (IC_{50} = 0.41 mM)

3T3 Dot Shrinkage with All-trans Retinoic Acid



3T3 dots after 0 and 5 hours of exposure to all-trans retinoic acid (left), the resulting time-area behavior (center) and from a linear fit of that curve, the rate of dot shrinkage (right) as a function of concentration (IC = 82.76 µM), compared to a 2D MTT assay (IC = 19.14 µM). Similar to the rings, the dots of 3T3s closed, and less so with higher concentrations of retinoic acid. The dots were less sensitive to drugs than monolayers. Scale bar = 5 mm.

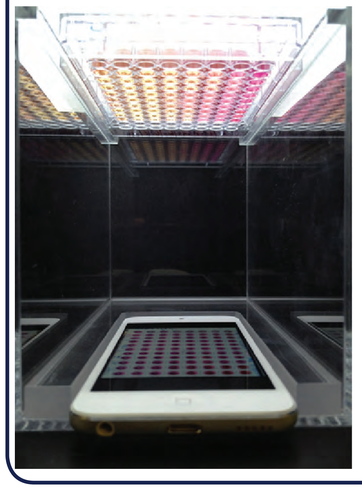


Fluorescent staining for F-actin and tubulin with phalloidin and paclitaxel of 3T3 dots exposed to all-trans retinoic acid. Note with increasing concentrations of all-trans retinoic acid, dots lose cytoskeletal organization and staining intensity, hindering its ability to close the dots. Scale bar = 100 µm.

Cells and Compounds Tested

Cell Types	Compounds	
Cell Lines	Ibuprofen	Adenosine
HEK293 human embryonic kidney	Doxorubicin	U46619
3T3 murine embryonic fibroblasts	Acetaminophen	Acetylcholine
A549 human lung epithelial cells	ATRA	Verapamil
HepG2 human hepatocytes	Norepinephrine	Imidazole
MDA-231 human mammary epithelial cells	Phenylephrine	Dexamethasone
MCF-10A human mammary epithelial cells	Isoproterenol	Paclitaxel
Caki-1 human kidney epithelial cells	Blebbistatin	Xylene
A10 rat vascular smooth muscle cells	Forskolin	SDS
Primary Cells	Endothelin-1	Ascorbic acid
Human pulmonary fibroblasts	Penicillin-G	
Human neonatal dermal fibroblasts		
Human tracheal smooth muscle cells		
Human vascular smooth muscle cells		

iPod-Based Imaging System



- Images of rings closing are taken with an iPod (Apple Computer)
- iPod is programmed using a freely available app (Experimental Assistant, Nano3D Biosciences) to image ring closure in real-time
- ←Imaging setup fits within a standard incubator
- iPod imaging forgoes imaging with a microscope

Imaging with an iPod eliminates imaging under a microscope and improves throughput and efficiency

Conclusions

- Magnetic levitation was used to rapidly pattern 3D rings or dots of cells in 96-well formats
- Ring closure and dot shrinkage are label-free, quantitative metrics that allow for post-assay testing to yield more content per experiment
- iPod imaging system facilitates real-time imaging without imaging each well under a microscope, improving throughput and efficiency
- Future directions include expanding into 384-well formats

The BiO Assay is a complete assay for the creation, imaging, and analysis of 3D cultures for high-throughput and high-content drug screening

References

1. Haisler, W. L. et al. Three-dimensional cell culturing by magnetic levitation. Nat. Protoc. 8, 1940-9 (2013).
2. ***Timm, D. M. et al. A high-throughput three-dimensional cell migration assay for toxicity screening with mobile device-based macroscopic image analysis. Sci. Rep. 3, 3000 (2013).***

Scan the QR-code to see the YouTube video of the image analysis A10 rings exposed to blebbistatin and norepinephrine!

For more information, go to www.n3dbio.com, or email us at info@n3dbio.com

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