Magnetic 3D Bioprinting: A novel high-throughput and high-content assay for toxicity screening

Hubert Tseng[1], Jacob A. Gage[1], William L. Haisler[2], Shane K. Neeley[2], Glauco R. Souza[1], T. C. Killian[3]
[1]Nano3D Biosciences, Houston, TX, [2]Department of Bioengineering, Rice University, Houston, TX, [3]Department of Physics, Rice University, Houston, TX

n3D Biosciences, Inc.

Background

A growing demand exists for three-dimensional (3D) *in vitro* assays for toxicity screening. Animal models are representative of the native tissue but expensive, low-throughput, and ultimately, not human. Currently available *in vitro* assays are rapid but poorly representnative tissues, as they are typically two-dimensional (2D) models on rigid substrates. ***Thus, the choice of assay becomes a tradeoff between efficiency and accuracy, leaving an unmet need for an assay system that is both representative and high-throughput.***

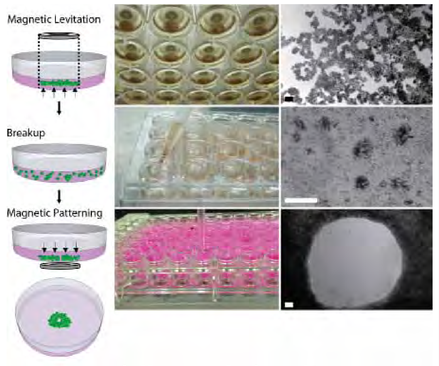
To that end, we introduce magnetic 3D bioprinting for high-throughput screening. The basis of this approach is the magnetization of cells by binding magnetic nanoparticles to them.[1] After resuspension in media, these cells can be rapidly and simultaenously printed into particular shapes, like rings or spheroids, with the use of magnetic forces.[2] These printed structures immediately demonstrate a dose-depdent response, which can be visually monitored. An iPod-based system is used for imaging, which is programmed to image whole plates at specific intervals, thereby forgoing the need to efficiently image well-by-well under a microscope.

In this study, we have applied magnetic 3D bioprinting to generate a basic toxicity model using 3T3 murine embryonic fibroblasts, and a specific model for vascular toxicity using vascular smooth muscle cells.

Hypothesis: Magnetic 3D bioprinting can be used to rapidly print cells into structures that mimic native tissue for high-throughput compound screening

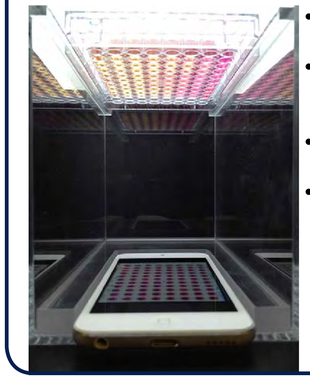
Magnetic 3D Bioprinting

- Cells are incubated with NanoShuttle (Nano3D Biosciences) overnight
- The next day, cells are levitated to induce ECM synthesis for a few hours
- Cultures are then broken apart and printed into rings for 15 min - 6 h (150K cells/ring, 75K cells/spheroid) in 96-well plates →
- Magnetic field removed and cells are allowed to close



Magnetic 3D bioprinting can rapidly and simultaneously print multiple tissue-like structures

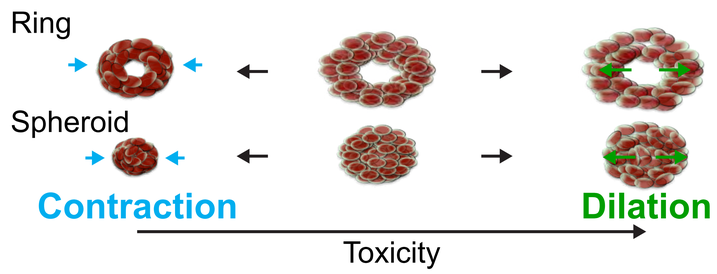
iPod-Based Imaging System



- Images of 3D printed tissues are taken with an iPod (Apple Computer)
- iPod is programmed using a freely available app (Experimental Assistant, Nano3D Biosciences) to image in real-time
- ←Imaging setup fits within a standard incubator
- iPod imaging forgoes time-consuming well-by-well imaging with a microscope

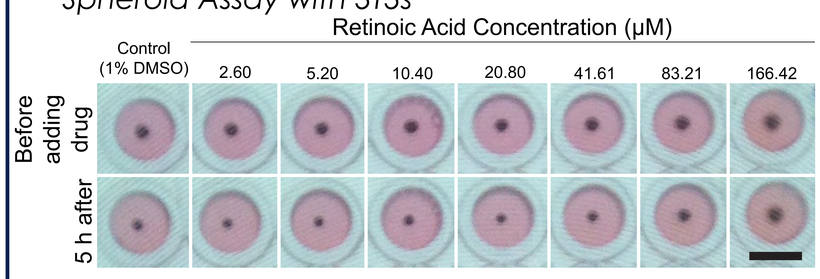
Imaging with an iPod improves throughput and efficiency

Results

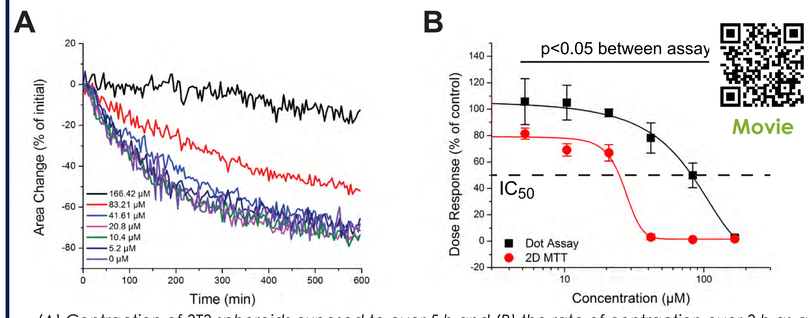


Contraction and dilation in rings and dots after printing and removing the magnetic field

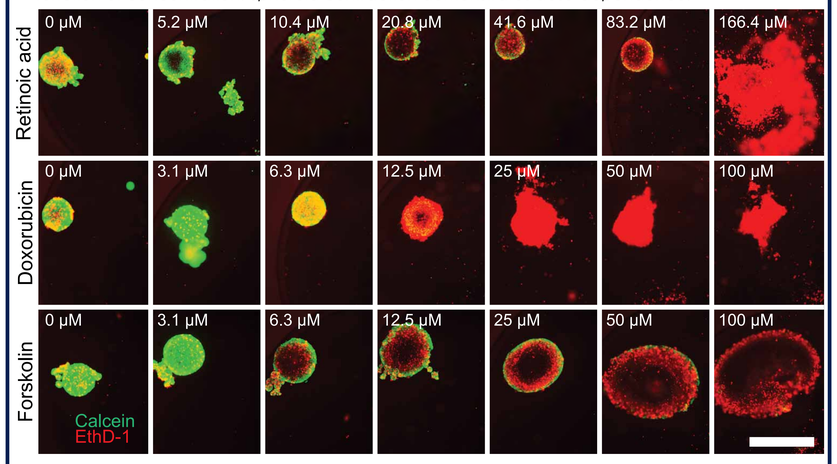
Spheroid Assay with 3T3s



Contraction of spheroids of 3T3 murine embryonic fibroblasts exposed to retinoic acid over 5 h. As retinoic acid concentration increases, the dots are unable to contract as much. Scale bar = 5 mm.



(A) Contraction of 3T3 spheroids exposed to over 5 h and (B) the rate of contraction over 3 h as a function of concentration. Spheroid contraction demonstrates dose-dependence.



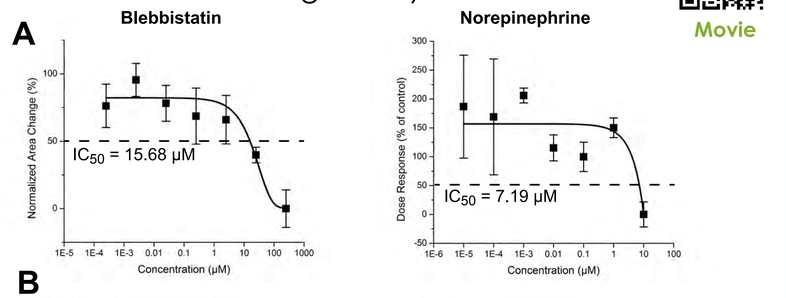
Viability of contracted spheroids exposed to retinoic acid, doxorubicin, and forskolin. As expected, at higher concentrations, the spheroids are less viable, matching spheroid size and the ability to contract. Scale bar = 500 μm

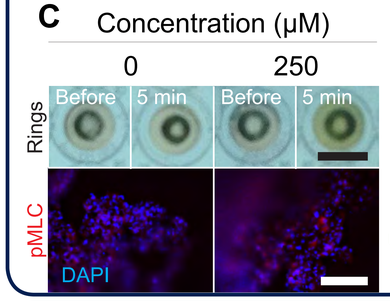
Magnetically 3D bioprinted models measure toxicity in 3D environments, which correlates with viability

Results

In Vitro Aortic "Ring" Assay

Movie





Contraction/dilation of rings of human aortic smooth muscle cells (ASMCs) exposed to blebbistatin and norepinephrine. (A) Dose response of rings to blebbistatin (area change after 1 h) and norepinephrine (slope over 1 h) (B) Gene expression of ASMCs when exposed to 250 μM blebbistatin and 1 μM norepinephrine (green = higher v. control, red = lower v. control). (C) Dilation of rings (top) and IHC stains for phosphorylated myosin light chain (pMLC, red, bottom) after 5 min of exposure to blebbistatin. Black scale bar = 5 mm, white scale bar = 50 μm. With increasing amounts of blebbistatin, the ring dilates, and the cells attempt to produce more pMLC, but is inhibited by blebbistatin binding to MLC.[3] As ASMCs are exposed to more norepinephrine, they appear to downregulate expression of key pathway markers that are activated by the drug.

Magnetic 3D bioprinting can be used to create organ-specific models for high-throughput screening

Conclusions

- **Magnetic 3D bioprinting rapidly prints 3D structures that immediately respond to compounds in a manner related to viability**
- **High-content experimentation can be performed to explore mechanisms of action and yield more data.**
- **Organotypic models, like of vasculature, can be created for high-throughput screening**
- **iPod-based system improves throughput and efficiency**

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).*** →
3. Kovács, M., Tóth, J., Hetényi, C., Málnási-Csizmadia, A. & Sellers, J. R. Mechanism of blebbistatin inhibition of myosin II. J. Biol. Chem. 279, 35557–63 (2004).

Scan the QR-codes in the poster for videos of the printed structures and our publication in Scientific Reports!

Acknowledgements/Funding

This work was supported by the National Science Foundation [Small Business Innovation Research Phase I 0945954 and Phase II 1127551]; and the State of Texas Emerging Technology Fund. The authors would like to thank Ahmed Nasser Salameh, Ph.D., Alexes Daquinag, Ph.D., and Mikhail Kolonin, Ph.D., University of Texas Health Science Center at Houston, for donating the 3T3s