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

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Background

A growing demand exists for three-dimensional (3D) *in vitro* assays that mimic the tissue of interest to predict the human *in vivo* response with high throughput. Animal models are representative of the native tissue but expensive and low-throughput. Currently available *in vitro* assays are rapid but poor representatives of the native 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.***

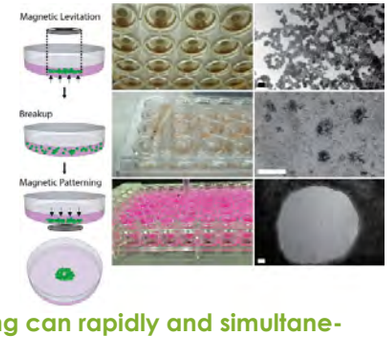
To that end, we introduce magnetic 3D bioprinting for high-throughput screening. The basis is the magnetization of cells by binding magnetic nanoparticles to them. After resuspension in media, these cells can be rapidly printed into particular shapes, like rings or dots/spheroids, with the use of magnetic forces in high-throughput formats. 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 A10 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

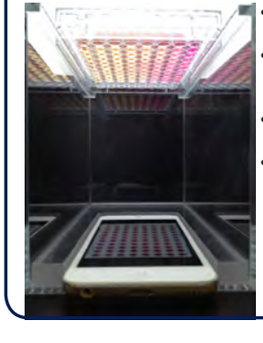
Magnetic 3D Bioprinting

- Cells are incubated with NanoShuttle (Nano3D Biosciences) overnight
- The next day, cells are levitated to induce synthesize ECM for a few hours
- Cultures are then broken apart and printed into rings for 15 min - 1 h (150K cells/ring, 75K cells/dot) 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

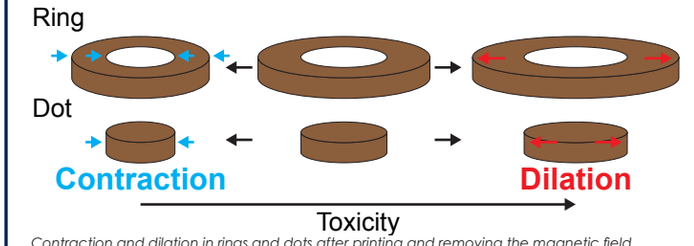
iPod-Based Imaging System



- 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

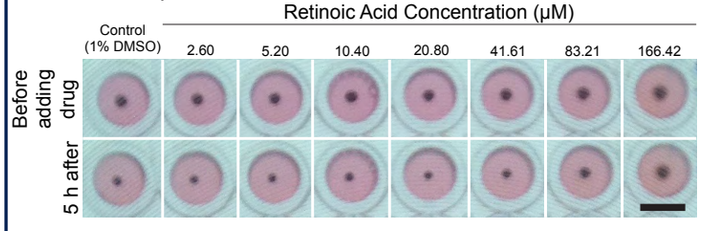
Imaging with an iPod improves throughput and efficiency

Results

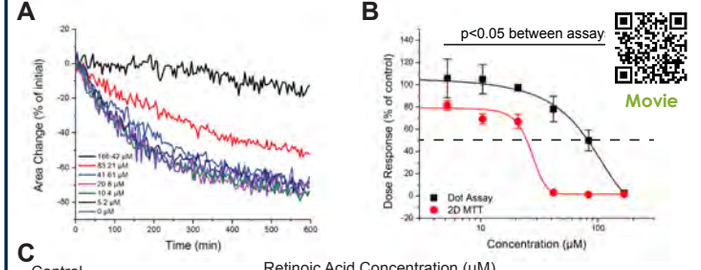


Contraction and dilation in rings and dots after printing and removing the magnetic field

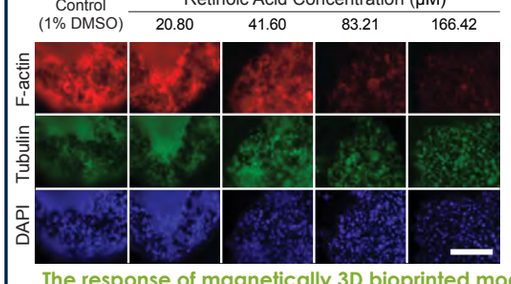
Dot Assay with 3T3s



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



(A) Contraction of 3T3 dots exposed to over 5 h and (B) the rate of shrinkage over 3 h as a function of concentration. (C) Live/dead staining of 3T3 dots. Scale bar = 500 μM. Dot shrinkage demonstrates dose-dependence, and correlates with viability within the dots.

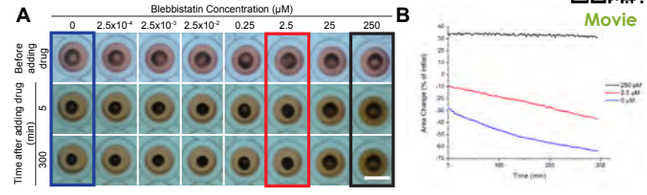


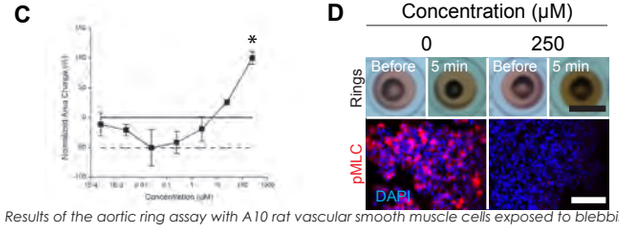
Cytoskeletal staining of 3T3 dots exposed to retinoic acid for F-actin and tubulin with fluorescently tagged phalloidin and paclitaxel. Scale bar = 50 μM. With higher concentrations, cells within the dot become looser and more disorganized.

The response of magnetically 3D bioprinted models correlates with viability and cytoskeletal organization

Results

In Vitro Aortic "Ring" Assay





*Results of the aortic ring assay with A10 rat vascular smooth muscle cells exposed to blebbistatin. (A) A10 rings before and 5 and 300 min after adding blebbistatin to the rings. Scale bar = 5 mm. (B) Ring contraction/dilation over 300 min. (C) Area change after 5 min as a function of concentration. Solid line = 0, dotted line = control. *: p < 0.05 v. control. (D) IHC stains for phosphorylated myosin light chain (pMLC, red) 5 min after adding blebbistatin. Black scale bar = 5 mm, white scale bar = 50 μm. Note with increasing concentrations of blebbistatin, the rings dilate and don't stain for pMLC, which is associated with smooth muscle contraction.[3]*

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

Conclusions

- **Magnetic 3D bioprinting rapidly prints multiple tissue-like structures that respond to compounds within 24 h**
- **Ring/dot shrinkage correlates with viability, cytoskeletal organization, and cell behavior**
- **Magnetic 3D bioprinting can be applied specifically to certain systems, like the cardiovascular system, to create organotypic models for high-throughput screening**
- **iPod-based system improves throughput and efficiency**

References

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