A novel vascular "ring" assay for smooth muscle contractility using magnetic 3D bio-printing

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Background

One of the most common assays for studying pharmacodynamics on vascular smooth muscle is wire myography, in which *ex vivo* vessel segments are exposed to a drug and its contraction and contractile forces are recorded. This assay suffers from high costs and low throughput, limiting its widespread use. Yet *in vitro* alternatives are insufficient, as they are commonly performed on stiff two-dimensional (2D) substrates that poorly mimic the soft vasculature. ***Thus, there is an unmet need for an in vitro assay that mimics the 3D structure of the blood vessel and is high-throughput.***

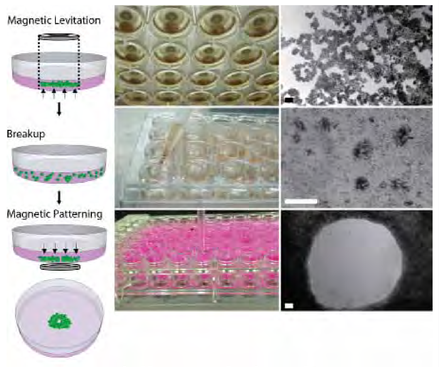
To address this unmet need, we apply magnetic 3D bioprinting towards assaying vascular toxicity for high-throughput screening as an in vitro alternative to wire myography. The basis is the magnetization of cells by binding magnetic nanoparticles to them.[1] These cells can be rapidly printed into rings using magnetic forces in high-throughput formats.[2] These rings visually mimic vessel segments and immediately respond to compounds by contracting or dilating. Contraction is captured using an iPod-based system programmed to image whole plates at specific intervals, thereby forgoing the need to image well-by-well under a microscope.

In this study, we have validated this vascular "ring" assay using A10 rat vascular smooth muscle cells and primary aortic smooth muscle cells (ASMC).

Hypothesis: Magnetic 3D bioprinting can be used to rapidly print cells into vascular "rings" that contract or dilate *in vitro* for high-throughput testing.

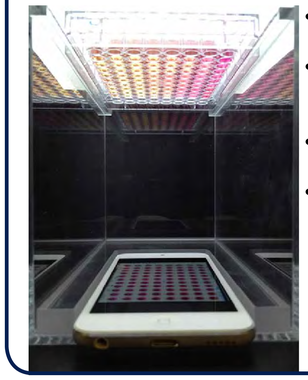
Magnetic 3D Bioprinting

- 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 6 h (200K cells/ring) in 96-well plates →
- Magnetic field removed and cells are allowed to contract



Magnetic 3D bioprinting can rapidly and simultaneously print multiple vascular rings

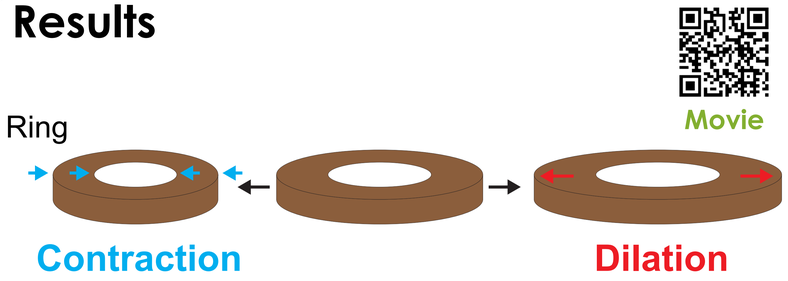
iPod-Based Imaging System



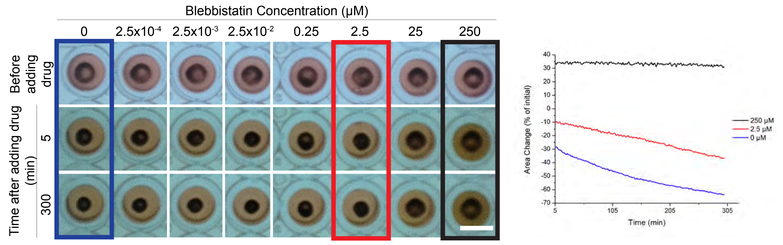
- Images of 3D printed rings are taken with an iPod (Apple Computer)
- iPod is programmed using a freely available app (Experimental Assistant, Nano3D Biosciences) to image in real-time
- ←Imaging setup fits within a standard incubator
- iPod imaging forgoes time-consuming well-by-well imaging with a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

Imaging with an iPod improves throughput and efficiency

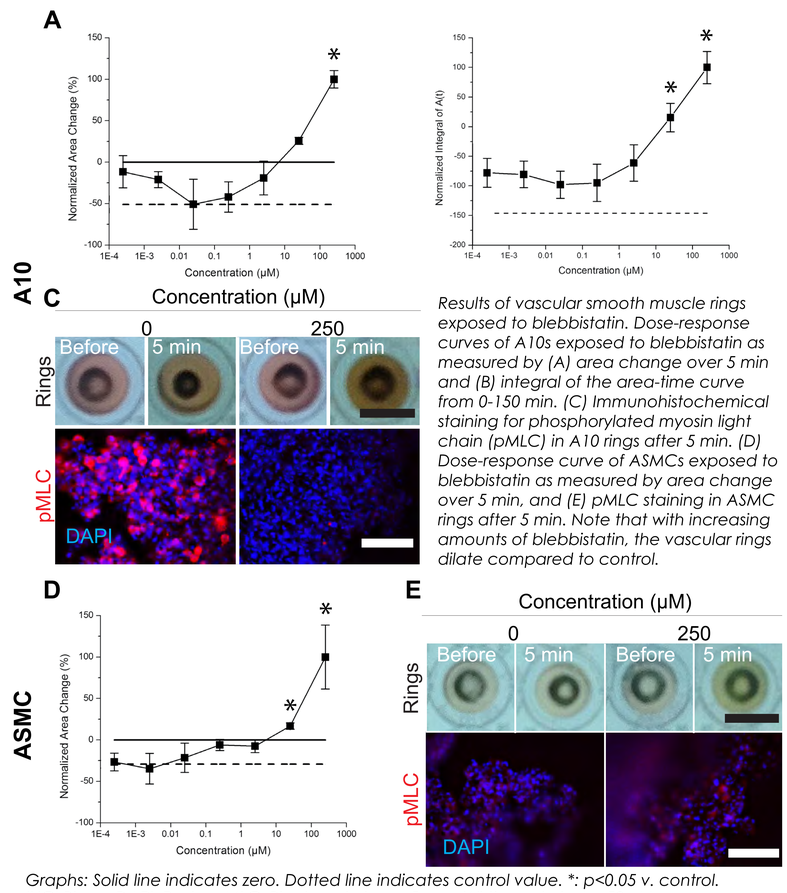
Results



Schematic of contraction and dilation of vascular rings immediately after printing



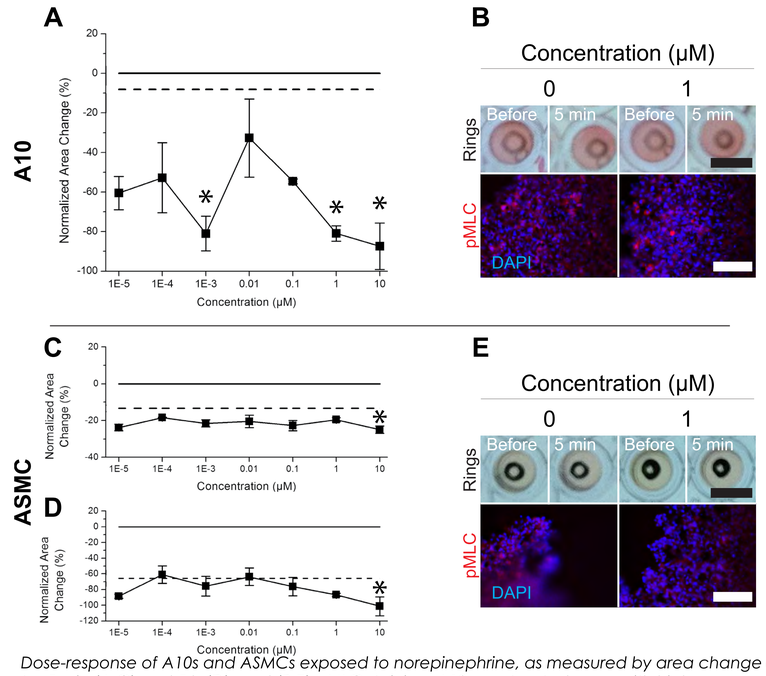
Contraction of A10 rat vascular smooth muscle cells exposed to blebbistatin. (A) A10 rings before and 5 and 300 min after adding blebbistatin to the rings. Scale bar = 5 mm. (B) Ring contraction/dilation as a function of time.



Results of vascular smooth muscle rings exposed to blebbistatin. Dose-response curves of A10s exposed to blebbistatin as measured by (A) area change over 5 min and (B) integral of the area-time curve from 0-150 min. (C) Immunohistochemical staining for phosphorylated myosin light chain (pMLC) in A10 rings after 5 min. (D) Dose-response curve of ASMCs exposed to blebbistatin as measured by area change over 5 min, and (E) pMLC staining in ASMC rings after 5 min. Note that with increasing amounts of blebbistatin, the vascular rings dilate compared to control.

*Graphs: Solid line indicates zero. Dotted line indicates control value. *: p<0.05 v. control. IHC: Blue = DAPI, Red = pMLC, Black scale bar = 5 mm. White scale bar = 50 μM.*

Results



Dose-response of A10s and ASMCs exposed to norepinephrine, as measured by area change after 5 min (A,C) and 5 h (D), and (B,E) pMLC staining. A10s contracted more with higher concentrations, while ASMCs contracted in a non-monotonic manner over a longer time.

Conclusions

- **Magnetic 3D bioprinting rapidly prints multiple vessel-like rings that contract/dilate to compounds within 24 h**
- **Contraction is a label-free metric where rings are available for post-assay experimentation or high-content imaging**
- **iPod-based system improves throughput and efficiency**
- **Results show differences in response between A10s and ASMCs**

Magnetic 3D bioprinting can be applied towards high-throughput vascular toxicity 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).*** →

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