

Electronic Supporting Information

John H. Day^{1,*}, Tristan M. Nicholson^{1,2,*}, Xiaojing Su¹, Tammi L. van Neel¹, Ivor Clinton¹, Anbarasi Kothandapani³, Jinwoo Lee^{3,4}, Max H. Greenberg⁵, John K. Amory⁶, Thomas J. Walsh², Charles H. Muller^{2,7}, Omar E. Franco⁵, Colin R. Jefcoate⁴, Susan E. Crawford⁵, Joan S. Jorgensen³, Ashleigh B. Theberge^{1,2,**}

¹Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195, United States

²Department of Urology, University of Washington School of Medicine, Seattle, WA 98195, United States

³Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI 53706, United States

⁴Department of Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI 53706, United States

⁵Department of Surgery, NorthShore University Research Institute, Affiliate of University of Chicago Pritzker School of Medicine, Evanston, IL 60201, United States

⁶Department of Medicine, University of Washington, Seattle, WA 98195, United States

⁷Male Fertility Laboratory, Department of Urology, University of Washington School of Medicine, Seattle, WA 98195, United States

*These authors contributed equally to this work.

**Email address for correspondence: Ashleigh B. Theberge, abt1@uw.edu

Table of Contents

Figure S1: Diffusion of fluorescent dyes through collagen and agarose gel walls

Figure S2: Representative images of high resolution imaging workflow

Figure S3: Representative images of sub-optimal device layout in 12-well plate

Figure S4: Monorail1 schematic showing a drafted features.

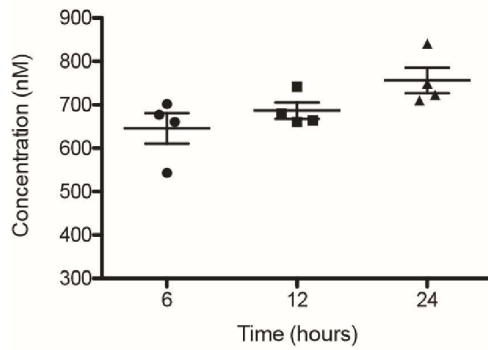
High resolution TIFF images (All)

CAD files

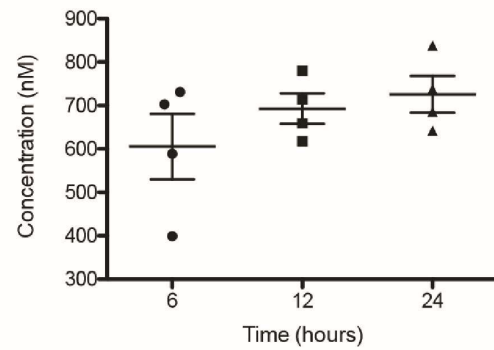
Protocols

Hydrogel loading video

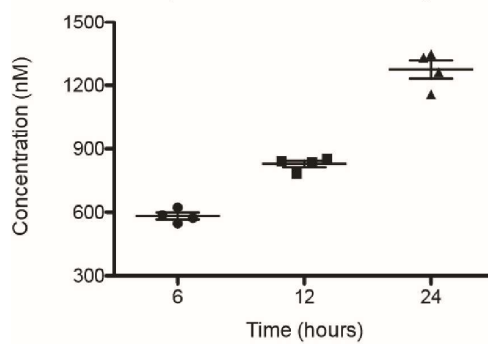
527 Da fluorophore diffusion through collagen



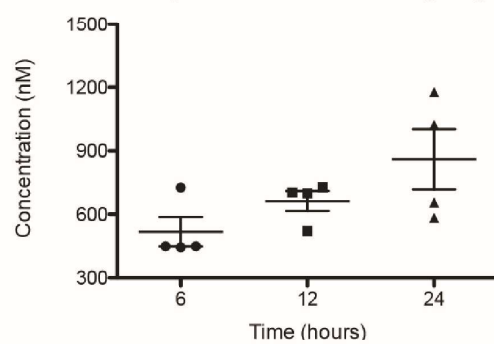
527 Da fluorophore diffusion through agarose



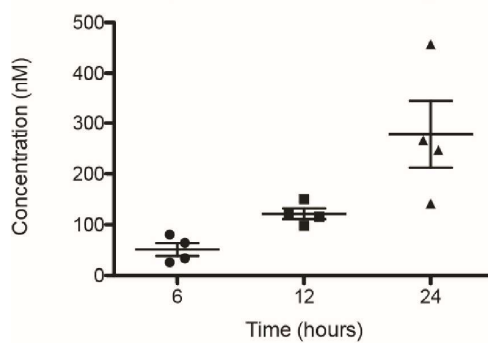
10 kDa fluorophore diffusion through collagen



10 kDa fluorophore diffusion through agarose



70 kDa fluorophore diffusion through collagen



70 kDa fluorophore diffusion through agarose

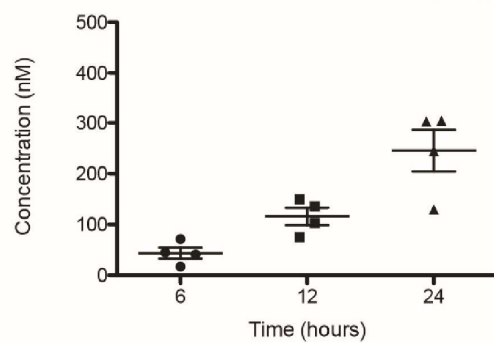


Figure S1: Diffusion of fluorescent dyes through collagen and agarose gel walls in Monorail2 devices. Concentrations of 527 Da (top row), 10 kDa (middle row), or 70 kDa (bottom row) fluorophore in receiving chambers of microculture device after 6, 12, and 24 hours. Gel walls through which diffusion occurred were made of either type I collagen (left column) or agarose (right column). Each plotted point represents a pooled sample from both outer chambers in a Monorail2 device. Error bars represent standard deviation.

Materials and methods for diffusion experiments (Figure S1):

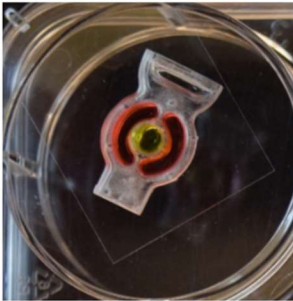
Device preparation

Injection molded Monorail2 devices were sonicated in isopropanol for 1 h, soaked in 70% ethanol for 30 min, and allowed to air-dry overnight. Before use, devices were plasma treated for 5 min at 0.25 mbar and 70 W in a Zepto LC PC Plasma Treater (Diener Electronic GmbH, Ebhausen, Germany) using oxygen. The devices were inserted into the bottom of a tissue culture treated polystyrene 12-well plate (Corning, 07-200-82) and loaded with 40 μL of hydrogel. Once gelled, 1X PBS was loaded into the center chamber (8 μL), side chambers (20 μL /chamber), and sacrificial media reservoir (500 μL) and stored at 6 °C overnight. 1X PBS in the center chamber was replaced with fluorescent dye the following morning ($t = 0$ hour), and the plate was incubated at 37 °C. Solution from the side chambers were collected and pooled at $t = 6$ h, 12 h, and 24 h for each technical replicate. The fluorescence of each sample was measured using a Multiskan Spectrum UV/Visible Microplate Reader (Thermo LabSystems, Waltham, MA) ($n = 4$ for each time point).

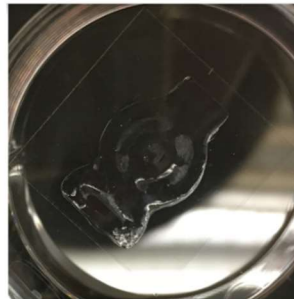
Fluorescent dye preparation

10 μM fluorescent dye solutions for Alexa Fluor 488 (MW 546 Da, Thermo Fisher, A33077), dextran Alexa Fluor 527 (MW 10 kDa, Invitrogen, D22911) and dextran fluorescein (MW 70 kDa, Invitrogen, D1823) were all prepared in 1X PBS.

Culture on coverslip



Remove device



Mount coverslip on slide

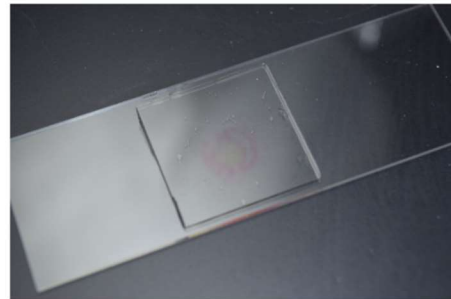
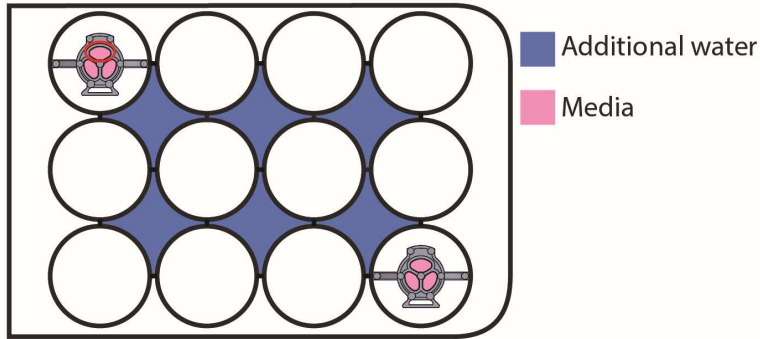


Figure S2: Representative photos of crucial steps in high resolution microscopy sample preparation with CNC milled Monorail2 devices designed for use in a 6-well plate. Briefly, the device is placed on a coverslip in a 6 well plate (left), and a coculture experiment is carried out (here, food dye was added to cell culture chambers for visualization). The device is then removed, leaving a faint hydrogel residue (middle). Finally, the coverslip is removed from the well with fine-tip tweezers, inverted, and placed on a glass slide (right).

a) Sub-optimal device layout for evaporation control



b) Live/dead image

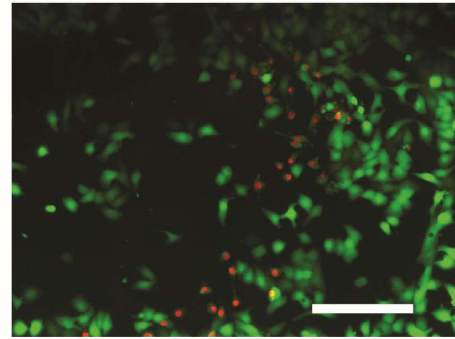


Figure S3: Representative cell viability in sub-optimal conditions for evaporation control. a) Well plate layout that leads to evaporation in cell culture chambers of monorail devices. b) Representative image of cell viability in top culture chamber of upper left monorail device from well plate layout.

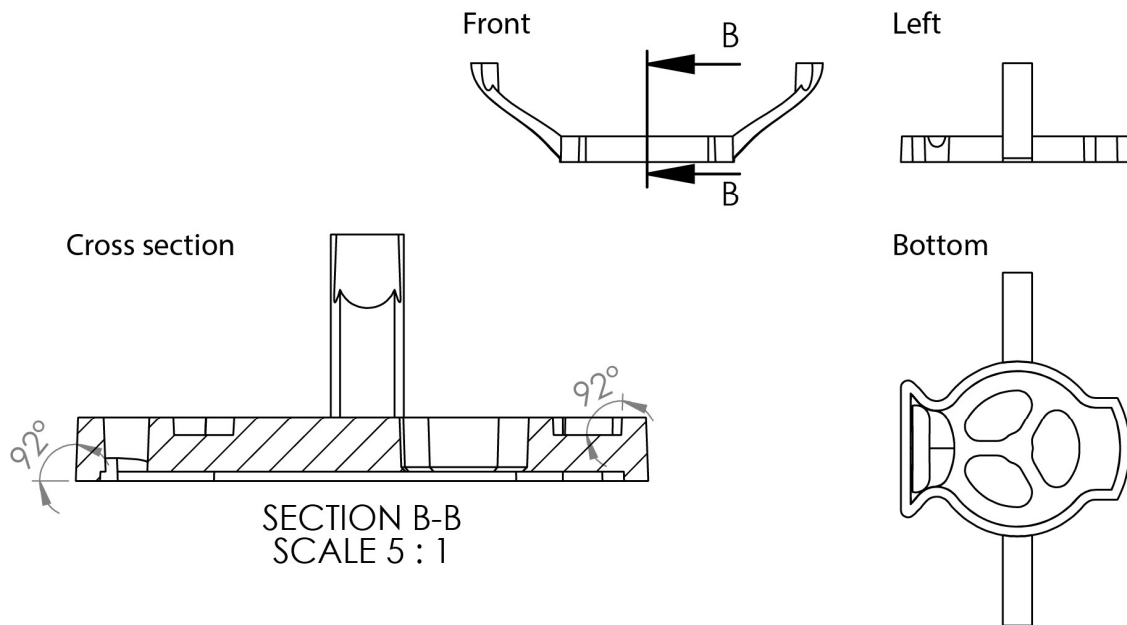


Figure S4: Monorail1 schematic showing a drafted features. All vertical surfaces of both injection molded Monorail devices were angles by at least two degrees to allow for demolding, the last step of the injection molding process. Cross section (left) shows two surfaces that were drafted by two degrees (92° is two degrees greater than the angle that would yield an undrafted surface).