

# LOGARITHMICALLY PERFUSED EMBRYONIC STEM CELL CULTURE ON CHIP

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## ABSTRACT

We present a microfluidic device for culturing adherent cells over a logarithmic range of flow rates to study the effects of continuous media exchange on the soluble microenvironment. The device uses syringe-driven flow and a network of fluidic resistances to set flow rates through four separate 0.32  $\mu\text{L}$  cell-culture chambers. We have demonstrated successful culture of mouse embryonic stem cells (mESCs) perfused for four days at a range of flow rates that span  $>300\times$  across the array. Qualitative results showed variation in colony size and number at different perfusion rates.

**Keywords:** cell arrays, embryonic stem cells, microenvironment, perfusion culture

## 1. INTRODUCTION

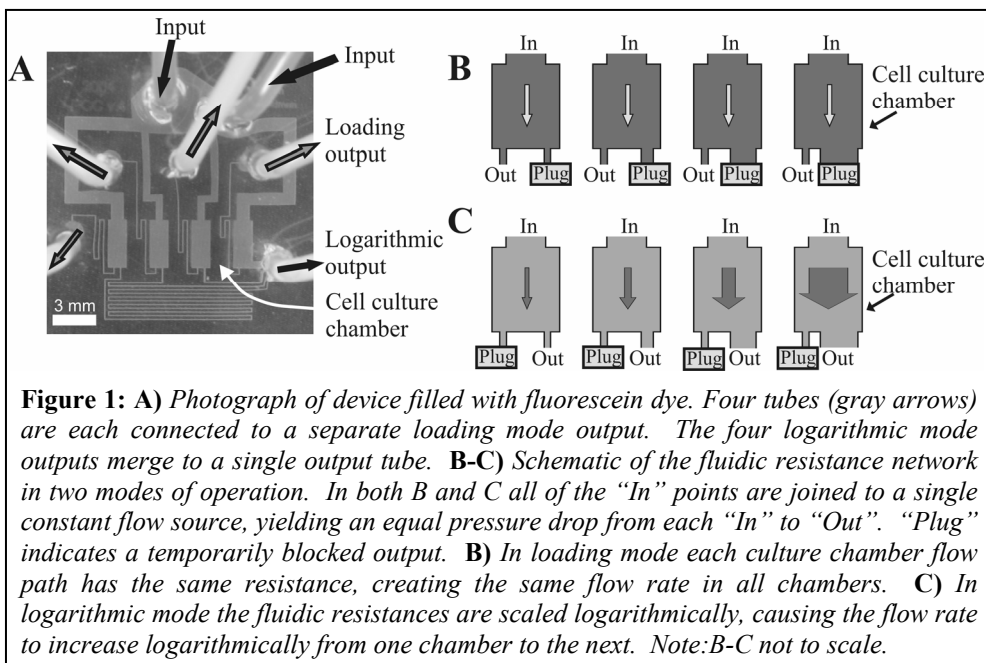
In conventional static cell culture the composition of the soluble microenvironment changes over time as cells deplete the media of nutrients and secrete diffusible signals and waste. By continuously perfusing the culture with new media at various rates, it is possible to regulate the biochemical composition of the media, creating an unprecedented level of control over the cell-culture microenvironment. We are interested in using fluid flow to quantitatively control cell-cell diffusible signaling, particularly for studying ESC biology.

Recently, others have demonstrated microfluidic perfusion culture, including large arrays of culture chambers [1-2], arrays with linearly varying flow rates for studying cell adhesion [3], arrays with programmable flow rates [4], culture with on-chip reagent gradients [5], and culture with cell patterning [6]. Our device focuses instead on simultaneously applying *logarithmically varying* flow rates to cell cultures across a device. Logarithmically varying experiments are commonly used in experimental biology to search a large parameter space, but to-date have been lacking in microfluidic cell-culture devices.

## 2. EXPERIMENTAL

The device (Fig 1A) is a single-level network of 80- $\mu\text{m}$ -high PDMS microfluidic channels plasma-bonded to a glass substrate to which cells adhere. Our current version has four 1.25-mm  $\times$  3.2-mm cell-culture chambers, and increasing the number of chambers is straightforward. During cell loading, uniform seeding requires the same flow rate in all chambers (Fig 1B). However, logarithmic flow rates are desired across the chip during culture (Fig 1C). Thus the culture chambers have one set of outputs used during loading and another set used during logarithmic operation.

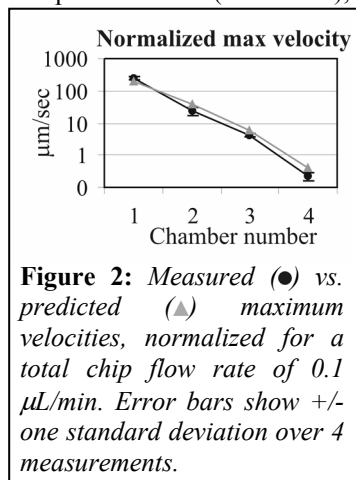
We measured the flow rates across the device using particle velocimetry and calculated the predicted flow rates using measurements of as-fabricated geometries. For the perfusion experiments, ABJ1 mESCs were loaded into the device, allowed to attach for 4 hours in static culture, and then perfused with standard mESC media at flow rates varying  $>300\times$  across the array.



### 3. RESULTS AND DISCUSSION

The measured flow rates are shown in Figure 2 and confirm the logarithmic scaling. Results of logarithmically perfused mESC culture are shown in Figure 3. Colonies at higher flow rates exhibited healthy round morphology and expressed Oct-4 (not shown), a marker of stem cell phenotype, while cells in the slowest flow chamber did not proliferate, suggesting a lack of nutrients.

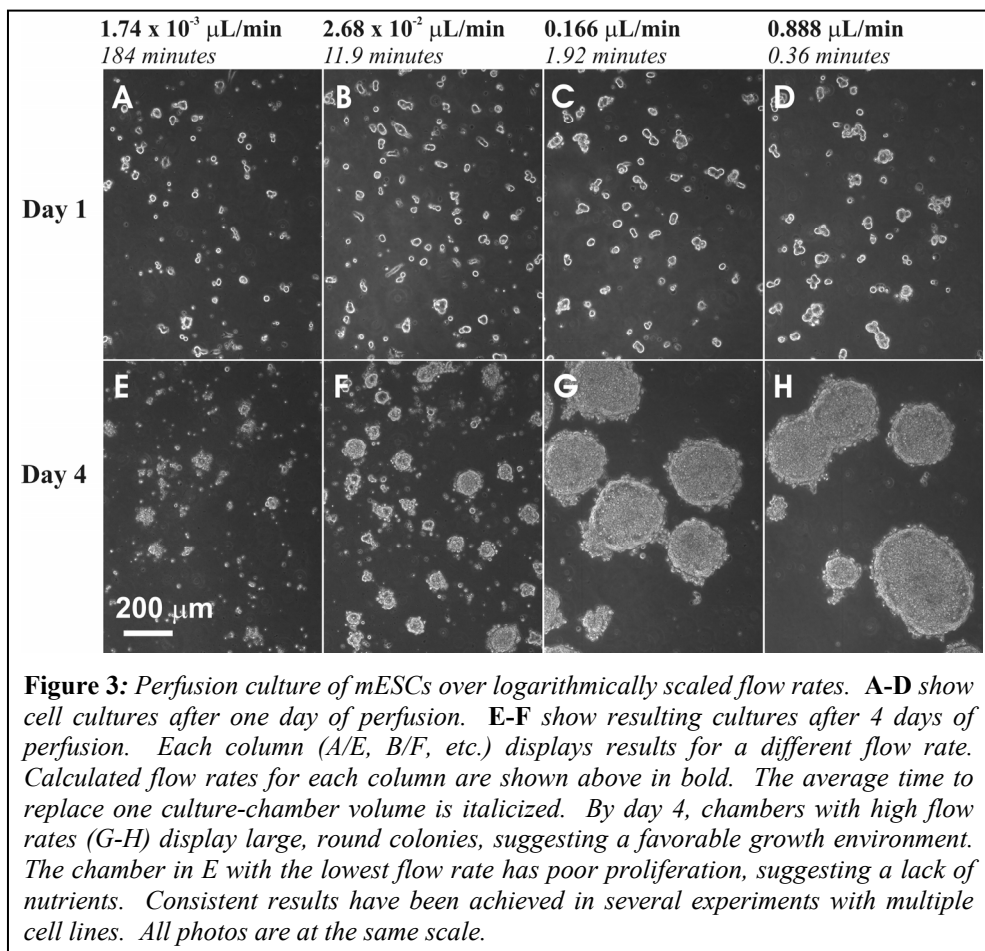
Our approach has several benefits: physical separation between culture chambers ensures that inter-chamber signaling is not a concern, which is important during long-term low-flow-rate assays, and the small chamber volumes can amplify effects of rare diffusible signals if desired. Individual loading outputs enable removal of cells separately from each chamber. The design is easy to fabricate, scalable, and simple to use with no on-chip valves. Our device attains a logarithmic range of flow rates while minimizing shear; even when the culture chamber volume is exchanged once every ~20 seconds, the shear is low at ~0.06 dyne/cm<sup>2</sup>.



### 4. CONCLUSIONS

Improving control over the soluble microenvironment is important for enabling greater precision in cell biology experiments. We have demonstrated for the first time culture of mESCs in continuous, logarithmically scaled perfusion for 4 days, yielding different

biological outcomes at each flow rate. These results prepare us to create large arrays of logarithmically varying culture chambers to systematically explore stem cell biology.



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