

MICROFLUIDIC CONTROL OF STEM CELL DIFFUSIBLE SIGNALING

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ABSTRACT

The microenvironment plays an important role in determining the phenotype of mouse embryonic stem cells (mESCs). We have developed a two-layer PDMS microfluidic device incorporating valves and debubblers that uses perfusion to sweep away cell-secreted factors. With this device we demonstrate that perfusion can modulate diffusible signaling and alter stem cell differentiation.

KEYWORDS: Embryonic stem cells, microfluidic perfusion, diffusible signaling.

INTRODUCTION

A fundamental challenge in stem cell biology is to determine the “recipes” needed to maintain stem cell populations (i.e., self-renewal) or to turn stem cells into other cells (i.e., differentiation). Determining these recipes is challenging in part because the cells themselves contribute to the recipe; they secrete molecules to which they respond. Microfluidics offers a potential solution to this challenge by allowing one to continuously perfuse cells to remove diffusible molecules as they are secreted by cells, creating a “neutral” media environment under which to develop recipes.

While researchers have suggested that microfluidics could indeed alter diffusible signaling in perfused cell cultures [1, 2], to date no group has presented data demonstrating functional biological change due to microfluidic perfusion.

RESULTS

Our microfluidic device contains two sets of triplicate culture chambers, allowing implementation of different culture conditions on the same chip (Figure 1). We have also incorporated a valve architecture modeled after Irimia *et al.* [3], which enables different parts of the device to be fluidically isolated during different stages of the experiment.

We demonstrate microfluidic control of diffusible signaling and differentiation of mESCs into neuronal precursors using our custom developed device. N2B27 is a completely defined serum-free media that is sufficient for differentiating cells into neurons in static cultures in petri dishes [4] (Figure 2a-c). However, when we cultured cells in *perfusion* in N2B27, most cells died and no differentiation was observed (Figure 2d-f). In parallel chambers on the same chip we cultured cells in N2B27 that had been supplemented with media containing cell-secreted factors from a static culture (known as conditioned media, CM). In this N2B27+CM media, we were able to restore growth and differentiation (Figure 2g-i). To further demonstrate that we can modulate differentiation independent of growth, we compared cells grown in our devices in N2B27+CM to cells grown in self-renewal media. Here we see that both conditions are able to support cell growth, while only N2B27+CM is capable of supporting differentiation (Figure 3).

Together, these results show that the loss of growth in perfused N2B27 (Figure 2d-f) was not due to shear effects (as cells under the exact same shear but in N2B27+CM or self-renewal media were able to grow & differentiate) and not due to the perfusion itself (for similar reasons). Instead, these results strongly suggest that a secreted diffusible factor in the conditioned media was responsible for the restoration of growth and differentiation.

CONCLUSIONS

These results present the first clear demonstration that microfluidic perfusion can indeed significantly affect diffusible signaling. We have shown that media that is normally (in static cultures) able to support differentiation is unable to support differentiation when perfused, presumably because one or more cell-secreted factors that are present in a static environment are perfused away in our microfluidic system.

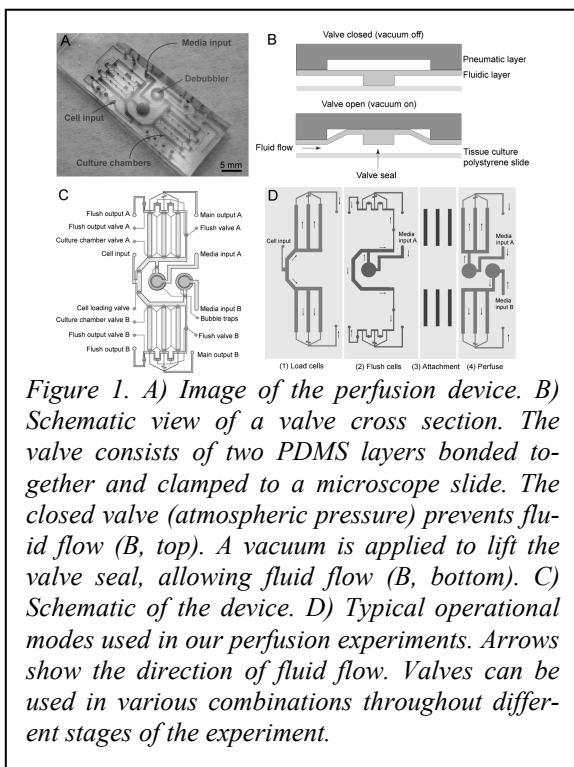
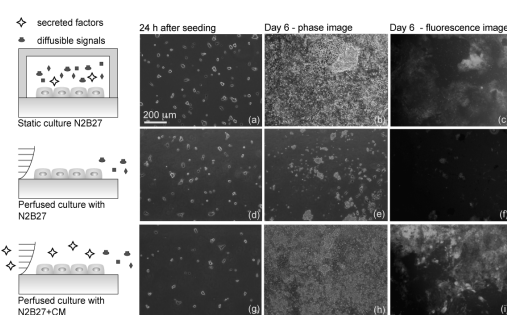


Figure 1. A) Image of the perfusion device. B) Schematic view of a valve cross section. The valve consists of two PDMS layers bonded together and clamped to a microscope slide. The closed valve (atmospheric pressure) prevents fluid flow (B, top). A vacuum is applied to lift the valve seal, allowing fluid flow (B, bottom). C) Schematic of the device. D) Typical operational modes used in our perfusion experiments. Arrows show the direction of fluid flow. Valves can be used in various combinations throughout different stages of the experiment.

Figure 2. To assess the effect of microfluidic perfusion on the biological state of cells we first plated cells in serum-containing media in static culture (a) and in a perfusion device, (d) and (g). One set of chambers was perfused with a defined differentiation media (N2B27). The other three chambers were perfused with N2B27+CM. After 6 days, in static culture cells proliferated (b) and differentiated as assessed by a Sox1-GFP marker (c). N2B27 was not sufficient for cells to proliferate (e) or differentiate in perfusion culture (f). N2B27+CM restored both proliferation (h) and differentiation as indicated by Sox1-GFP in the fluorescence image (i).



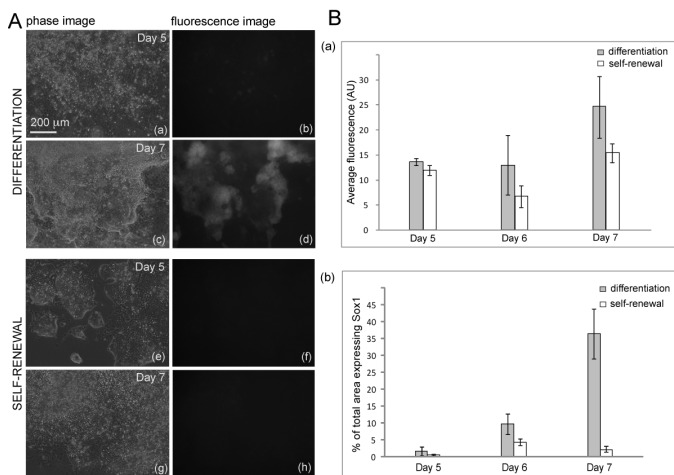


Figure 3. Perfusion modulates on-chip differentiation. One set of chambers was perfused with differentiation media conditioned with cell-secreted factors (N2B27+CM), and the other side with self-renewal media. A) (a) and (c) show phase images of differentiation chambers on days 5 and 7 of perfusion, showing cell growth. (b) and (d) are the corresponding fluorescence images of Sox1-GFP, showing increased differentiation between days 5 and 7. (e)-(f) and (g)-(h) represent phase and fluorescence images of “self-renewal” side, for days 5 and 7 of perfusion, respectively, showing growth but no differentiation in self-renewal media. B) (a) comparison of average fluorescence across the entire device for 3 successive days of perfusion under two different media conditions. A measurable difference in average fluorescence is observed, in particular for day 7 of perfusion, between the two different conditions. (b) indicates increase in total area of cells expressing Sox1 over three days of perfusion. Self-renewal condition is used as a control.

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