TUNABLE DELIVERY OF CHEMICAL GRADIENTS OVER LARGE CELL CULTURE SUBSTRATES USING STACKED FLOWS

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ABSTRACT

This paper reports a novel microfluidic device for generating tunable biochemical gradients over large areas on cell culture surfaces. The gradient requires flow, but the inter-diffusing laminar streams are stacked above the surface to generate a steady-state gradient via diffusion in the direction orthogonal to the flow and orthogonal to the surface. Finite-element modelling was used to predict negligible shear forces at the range of gradients possible by tuning flow rates. The surface gradients were characterized with fluorescence microscopy; image analysis verified the presence of a uniform, mono-directional gradient across a 2x2 mm area.

KEYWORDS: Steady-state, Diffusion, Polydimethylsiloxane, 3-D Micro-Plumbing

INTRODUCTION

The generation of precise biochemical gradients plays an important role in studying various biological phenomena such as development, cancer, inflammation, and wound healing [1]. Microfluidic gradient generation methods offer greater precision, quantification, and spatiotemporal control over traditional *in-vitro* techniques such as micropipettes or the Dunn and Boyden chambers [1]. Several microfluidic gradient generators have been developed that utilize flow to establish a steady-state gradient such as parallel flow devices [2] and a "microjets" device [3]. While these designs have shown a significant advantage over traditional methods for generating complex gradients and dynamic control, there are certain drawbacks. Parallel flow devices are limited to analyzing a small region of interest because the gradient changes downstream due to diffusion in the orthogonal direction; increasing the area of interest by increasing the flow rate can adversely affect the cells with harmful shear forces. The microjets design overcomes this limitation, but the device is prone to clogging and sensitive to vibrations which cause disruption of the gradient. The design presented here utilizes the inter-diffusion of stacked laminar flows to generate a dynamically tunable steady-state gradient across an arbitrarily wide area.

THEORY

A schematic of the "Stacked-Flow" gradient generator device, fabricated in polydimethylsiloxane (PDMS) by multi-layer exclusion molding [4], is shown in Fig. 1. Finite-element modelling (COMSOL, Burlington, MA) of the device was used to simulate the gradients and predict the shear stress applied at the surface (Fig. 1c). At low flow rates, the two inlet streams become homogenized at the outlets. In practice, for most proteins which diffuse slowly (e.g. the diffusivity of albumin is D $\sim 6.4\times10^{-7}$ cm^2/s), this happens at extremely small flow rates, so the shear stresses involved are negligible over >90% of the gradient area, two orders of magnitude

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smaller than the Dertinger device's threshold for inducing a migration bias in neutrophil cultures (~0.7 dyn/cm^2) [5]. To be extremely conservative the simulation was run assuming a small molecule, fluorescein (MW: 332.31 and D ~ 6.4×10^{-6} cm^2/s), which predicted shear forces of ~6×10^{-3} dyn/cm^2. Since a protein gradient of the same profile as the fluorescein gradient would require much slower flow rates, the shear stresses would be at least an order of magnitude smaller.

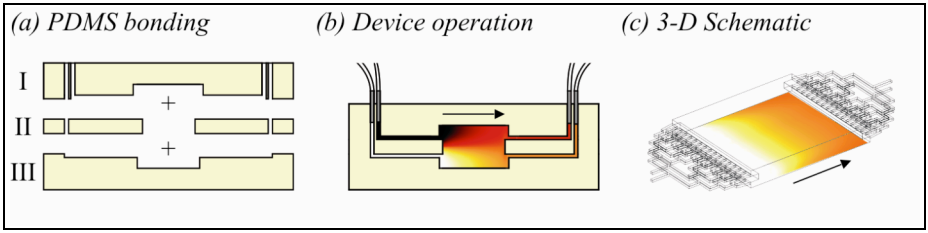

Figure 1. Device assembly, operation, and finite-element modelling.

EXPERIMENTAL

The surface gradient is visualized by flowing a mixture of a non-fluorescent dye that absorbs strongly at the excitation wavelength (490 nm) and weakly at the emission wavelength of fluorescein (540 nm) to collect surface-level fluorescence (~5 µm optical slice) [6]. The gradient shape can be dynamically tuned by the driving pressure applied to the inlets. Fig. 2 shows a typical image of the device highlighting the gradient area (box) and the locations for the linescan analysis (dotted lines).

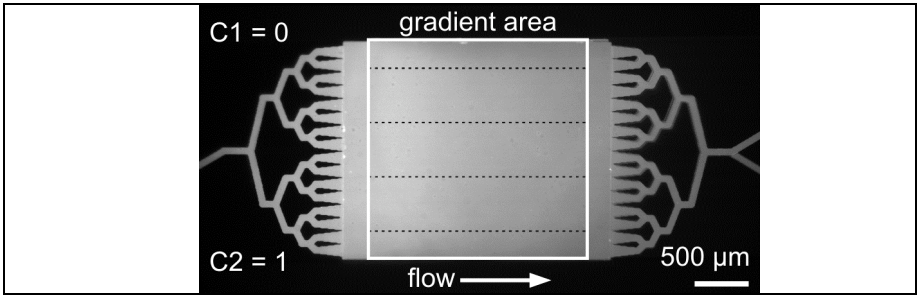

Figure 2. Visualization of gradient with surface-level fluorescence.

RESULTS AND DISCUSSION

Fig. 3 shows a variety of surface gradients obtained simply by changing the inlet configuration and pressure settings. Superimposed onto the images are the linescans taken across 4 different regions of the device which demonstrate lateral uniformity. A unique feature of the device is that the gradient can be set to increase or decrease in the flow direction, thus offering the possibility of exploring whether the fluid shear adversely affects cell function simply by reversing the gradient/force sign. Due to the binary branching of the inlets, the perfused area can be arbitrarily wide (here, 2 mm), which allows for acquiring rich statistics on single-cell variability while analyzing large cell populations in parallel.

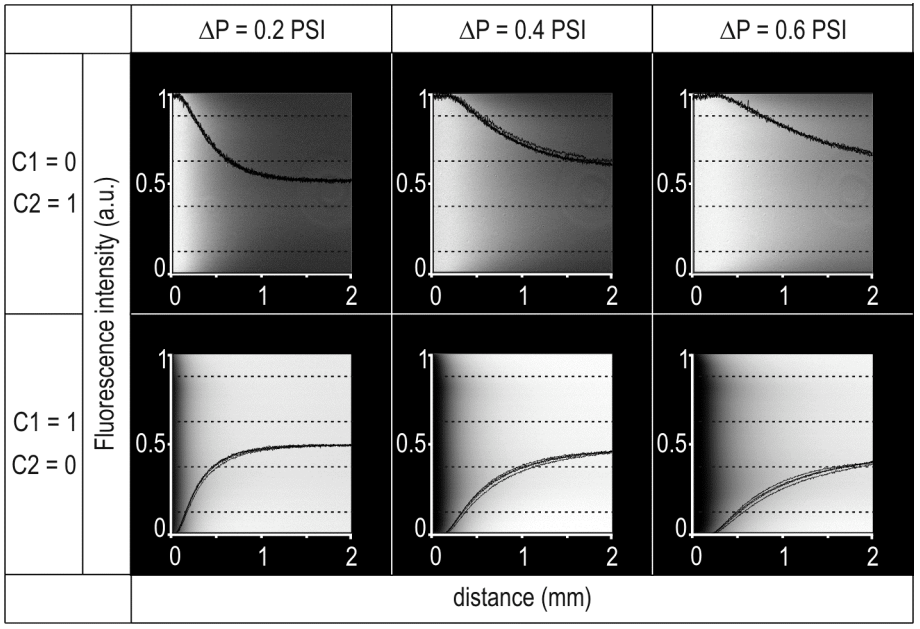

Figure 3. Uniformity analysis of gradients at different operating conditions.

CONCLUSIONS

In comparison to existing methods for generating gradients of biomolecules, the "Stacked-Flow" gradient generator offers improved and dynamic spatiotemporal control of the microenvironment while maintaining stable and reproducible conditions for cell culture over large areas.

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