A NOVEL MICROFLUIDIC DESIGN TO GENERATE MULTIPLEX GRADIENTS OF BIOMOLECULES BY VISULIZED BIOMOLECULE PATTERNING AND DIRECT CELL ADHESION

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ABSTRACT

Here we present a novel microfluidic design to generate multiplex gradients of biomolecules based on hydrodynamic resistances. Chemical gradients were generated within parallel microfluidic channels, in which a range of multiplex concentration gradients with different profile shapes were simultaneously produced. We could also successfully generate covalent surface gradients of biomolecules. IgG antibody conjugated to three different fluorescence dyes was used to demonstrate the resulting multiplex concentration gradients. To demonstrate the applicability of the developed design for cell adhesion and patterning, multiplex concentration gradients of REDV and KRSR peptides were patterned along the width of parallel microfluidic channels and adhesion of primary human umbilical vein endothelial cell (HUVEC) in each channel was investigated.

KEYWORDS

Multiplex gradients, hydrodynamic resistances, surface functionalization, cell adhesion, High-throughput gradients

INTRODUCTION

Over the past decade, microfluidic platforms have increasingly been applied to develop in vitro platforms to study cellular responses to chemical gradients. Generating multiplex gradients of biomolecules with highly resistant surface bonds in a straightforward device, while maintaining a small device footprint, are important factors in designing microfluidic gradient-based devices.

Several recently reported microfluidic devices have implemented gradient-based microfluidic platforms to study biological phenomena. The most commonly used device, known as tree-like design [1], is composed of a series of microfluidic channels dedicated to gradient generation, which results in a large device footprint - reducing the space available for experiments. Recently other microfluidic designs with smaller device foot-prints have been introduced [2-3]. However, generating multiplex gradients of several biomolecules in parallel microfluidic channels has not been reported so far.

In addition, many gradient-dependent biological applications require bio-functional and stable surface gradients. Herein, we introduce a microfluidic platform for generating multiplex solution and covalent surface concentration gradients of biomolecules based on hydrodynamic resistances.

THEORY AND SIMULATION

From the electronic-hydrodynamic analogy, the hydrodynamic resistance (R_h) of a microchannel in a laminar incompressible steady state flow can be defined as:

$$R_h = \nabla P/Q \qquad (1)$$

in which Q is flow rate and ∇P is the pressure drop along the channel length. For a microchannel of length L, hydrodynamic diameter D_h and cross section area A, the hydrodynamic resistance can be defined as:

$$\mathbf{R_h = \frac{(f\ Re)\mu L}{2D_h^2 A}} \qquad (2)$$

in which μ, f and Re are dynamic viscosity, friction factor and Reynolds number respectively. For straight microchannels (in a laminar, steady and incompressible flow) the hydrodynamic resistance depends only on the microchannel geometry. Parallel channels used for gradient generation in our design consist of both straight and curved sections. Therefore CFD analysis was performed to calculate and predict the hydrodynamic resistances at each channel.

Full scale simulation was performed using a 3D model of the microfluidic design. The governing equations used for computational fluid dynamic (CFD) analysis were as follows:

$$\mathbf{\nabla[\eta(\nabla u + (\nabla u)\ ] + \rho(u.\nabla)u + \nabla p = 0} \qquad (3)$$

$$\mathbf{\nabla . u = 0} \qquad (4)$$

Eqns (3) and (4) represent the Navier-Stokes and continuity equations respectively assuming incompressible steady state conditions ($\boldsymbol{u}$**:** velocity vector, p: pressure, η: dynamic viscosity and ρ: liquid density). CFD analysis was performed in COMSOL (COMSOL Inc., Burlington, MA) software.

EXPERIMENTAL

Standard soft lithography using polydimethylsiloxane (PDMS) was employed to create microfluidic channels followed by fabrication of the chip through irreversible binding of a flat glass slide to the PDMS substrate. Detailed

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information about covalent surface functionalization using micro-contact printing protocol can be found in [4]. The microfluidic design (figure 1a) consists of seven inlet channels and seven parallel channels identified as "target channels" in which multiplex gradients are generated. Imposed differences in hydrodynamic resistances of target channels is the most dominant part of the design for gradient generation. For multiplex gradient generation, Cy3 conjugated IgG was introduced from inlets 1, 5 and 7, Cy5 conjugated IgG from 3rd and 6th inlets and FITC conjugated IgG from 2nd and 5th inlets. Outlet of the 3rd target channel was pressurized to block the flow through this channel in order to create the highest hydrodynamic resistance at this channel. Laminar flow streams containing desired biomolecules were applied at a flow rate of 1 mm/s for 60 min. The channels were then washed with PBS.

To demonstrate the applicability of the developed design for guided cell adhesion, three different multiplex concentration gradients of REDV and KRSR peptides were patterned along the width of three parallel channels, and adhesion of HUVEC cells in each channel was investigated.

Cells were cultured in 200 (M-200-500) media with low serum growth supplement (LSSG), and 1% PS. Cells were grown in an incubator (at 37 °C and 5% CO2), trypsinized with 0.25% trypsin-EDTA (Gibco, USA) and used in the experiments.

After generating surface gradients of peptides, outlets of the target channels were used as inlets to flow cells into the channels. This ensures that an equal number of cells with equal flow rates will pass through each channel. Target channel`s surfaces were washed by flowing sterile PBS. To avoid non-specific cell adhesion, BSA was passed through the channels and incubated for 30min. Cells were then introduced into the channels at a flow rate of 0.25 µl/min for 40 min.

RESULTS AND DISCUSSION

Concentration gradients of biomolecules were generated within parallel channels by varying hydrodynamic resistances for each channel. Diffusion of biomolecules between different laminar flow streams occurs within the main channel at the onset of the gradient generation process. However, in our design this effect is later offset by advection forces, which is the dominant part of the design that leads to several different gradient profiles on the same device (figure 1c-f). The proposed design also successfully demonstrated the ability to produce different profile shapes of surface concentration gradients (figure 1g-j).

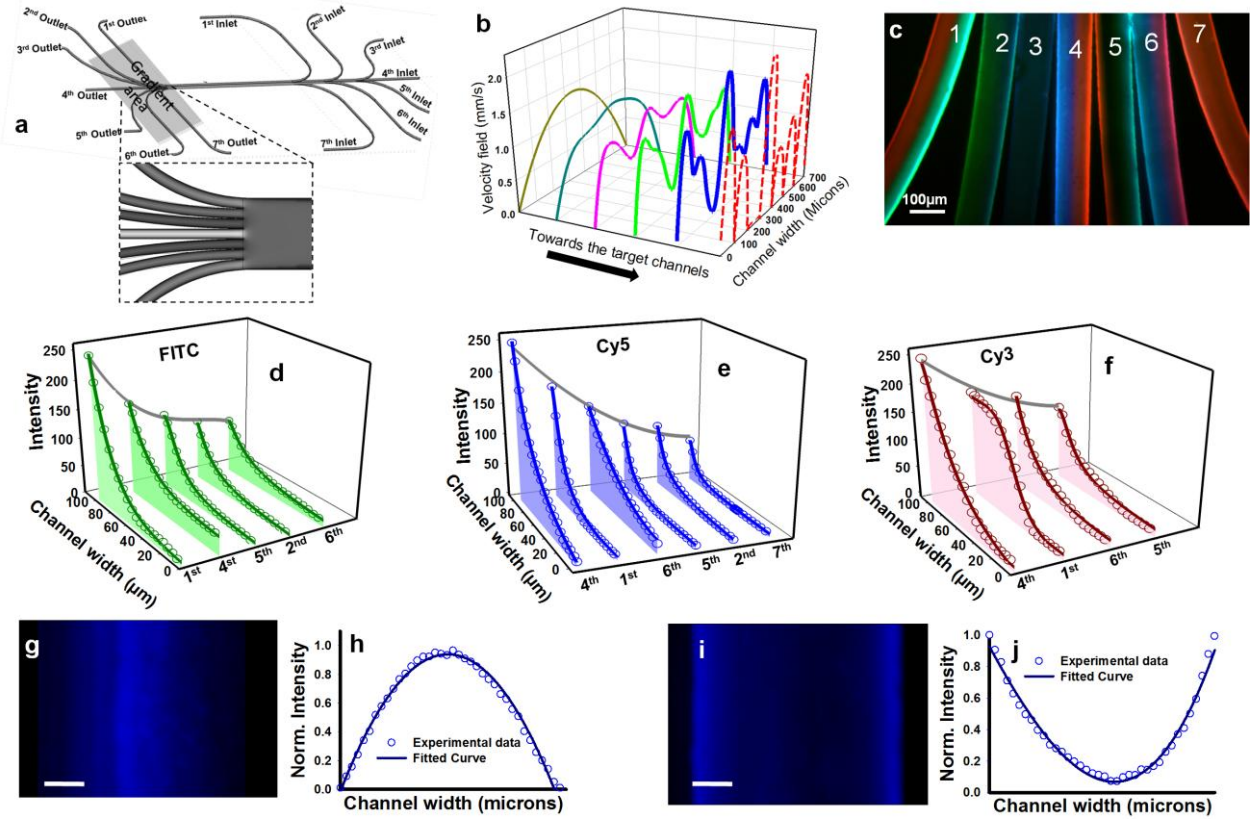

Figure 1. a) Schematic representation of the microfluidic design, b) simulation results for the velocity field before and after entering the target channels. c) superimposed fluorescence microscopy image of the generated multiplex surface gradients of FITC, Cy3 and Cy5 labeled IgG antibody, d-f) gradient profiles and intensities of FITC, Cy5 and Cy3 conjugated IgG antibody shown in c, The fitted polynomial lines in gray color, shows variations in the maximum concentration of each dye in different target channels, g-j) demonstrating possibility of generating nonlinear parabolic shape surface gradients using Cy5 labeled IgG antibody.

The main advantage of the introduced design is in achieving simultaneous multiplex gradient profiles and concentration ranges in parallel microfluidic channels. This is an important benefit compared to the previously proposed designs in which only one concentration profile could be obtained at a specific time. This would be beneficial for high throughput gradient dependent experiments where different concentration profile slopes are needed.

HUVEC cells adhesion along the width of parallel channels in response to the REDV and KRSR multiplex gradients, showed HUVEC adhesion to REDV peptide patterns (figure 2), while under the same experimental conditions, they did not respond to the KRSR surface gradients. In addition, cell adhesion density was proportional to REDV peptide surface concentration. The proposed design and fabrication technique is straightforward, rapid, reliable and cost effective which could facilitate future developments in the field of lab-on-a-chip devices.

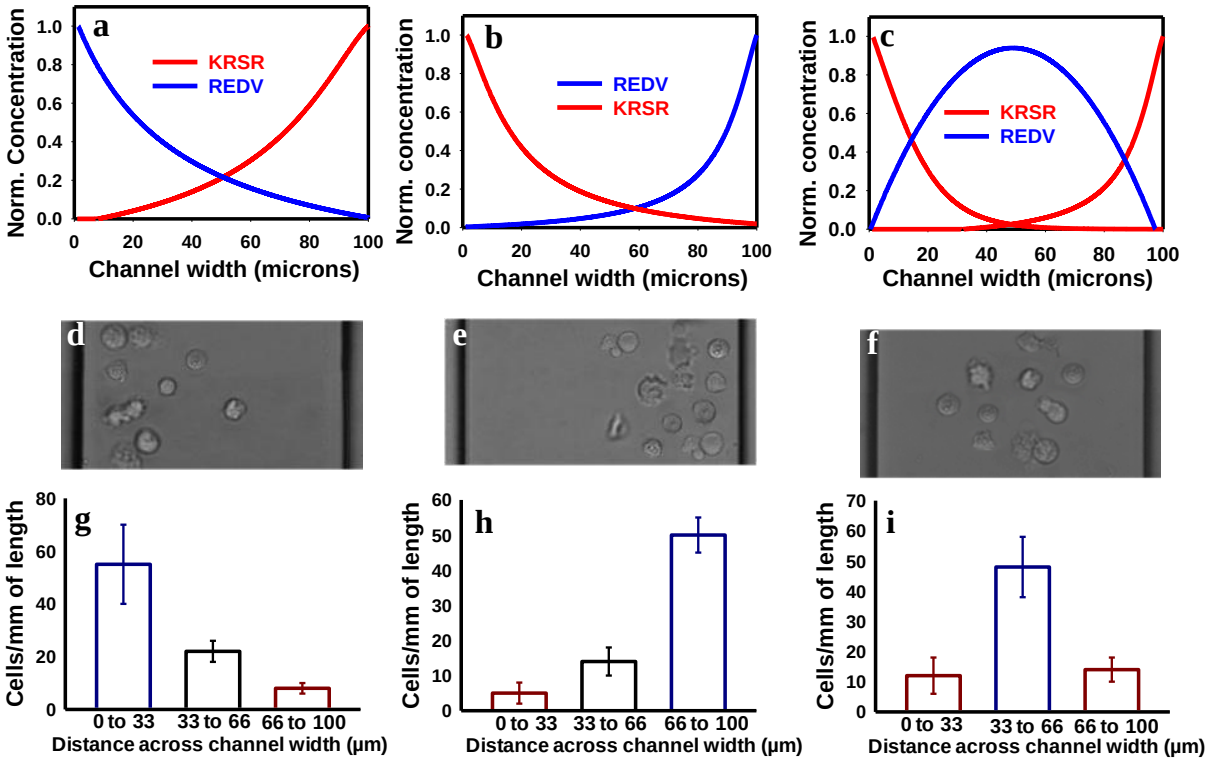

Figure 2. HUVECs adhesion on multiplex gradients of REDV and KRSR peptides. a, b and c) show the generated surface concentration gradients of REDV and KRSR peptides, d, e and f) representative optical microscope images of HUVECs adhesion, g, h and i) Degree of HUVECs adhesion across the width of three parallel channels. Error bars represent standard deviation of analyzing 5 different images for each channel.

CONCLUSION

High throughput chemical gradients of biomolecules were generated by the innovative design of a chip in which parallel microchannels provide different concentrations and varying distributions of biomolecules. Biomolecule gradients were covalently functionalized onto microchannel surfaces. The produced chip was used to study the degree of adhesion of HUVEC cells onto microchannels functionalized with multiplex peptide gradients.

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