Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2012

Tunable, pulsatile chemical gradient generation via acoustically driven oscillating bubbles†

Daniel Ahmed,[a] Chung Yu Chan,[a] Sz-Chin Steven Lin,[a] Hari S. Muddanna,[b] Nitesh Nama,[a] Stephen J. Benkovic[c] and Tony Jun Huang*[ab]

[a] *Department of Engineering Science and Mechanics, The Pennsylvania State University, University Park, PA,16802 USA. Fax: 814-865-9974; Tel: 814-863-4209; E-mail: junhuang@psu.edu*
[b] *Department of Bioengineering, The Pennsylvania State University, University Park, PA, 16802 USA*
[c] *Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania, 16802 USA.*

1. Device fabrication

A single-layer polydimethylsiloxane (PDMS) microchannel was fabricated using soft lithography and mold replica technique. In short, a silicon mold for the microchannel was patterned in photoresist (Shipley 1827, MicroChem, Newton, MA) and etched with Deep Reactive Ion Etching (DRIE, Adixen, Hingham, MA). The mold was then coated with 1H,1H,2H,2H-perfluorooctyl-trichlorosilane (Sigma Aldrich, St. Louis, MO) to reduce its surface energy and any subsequent damage to the PDMS channel during the demolding process. Sylgard^{TM} 184 Silicone Elastomer Base and Sylgard^{TM} 184 Silicone Elastomer Curing Agent (Dow Corning, Midland, MI) were mixed at a 10:1 weight ratio and cast onto the silicon mold. The uncured PDMS on the silicon mold was then degassed in a vacuum chamber for 2 h to remove any air bubbles and later cured at 65 ºC for 45 min. After removing the cured PDMS from the mold, the inlets and the outlets were drilled into the PDMS using a silicon carbide drill bit (model 220/395, Dremel). The microfluidic channel was then bonded to a micro cover glass, which had been pre-treated with oxygen plasma. A piezoelectric transducer (model no. 273-073,

RadioShack) was then attached to the glass slide adjacent to the channel using epoxy (Permatex 84101). The large separation between the ladder-like arrangement of horseshoe structures and the site where chemical gradient profiles are analyzed ensures that cells under investigation will experience little shear stress developed by the oscillating bubbles (see Fig. S1).

2. Design and dimensions of the microchannel

Figure S1 shows the design and dimension of the microchannel used for chemical gradient. Figure S2 shows the ladder-like arrangement of the horseshoe structures. Figure S3 shows the SEM images of the horseshoe structures along with their dimensions. The depth of the channel used in all experiments was 60 µm.

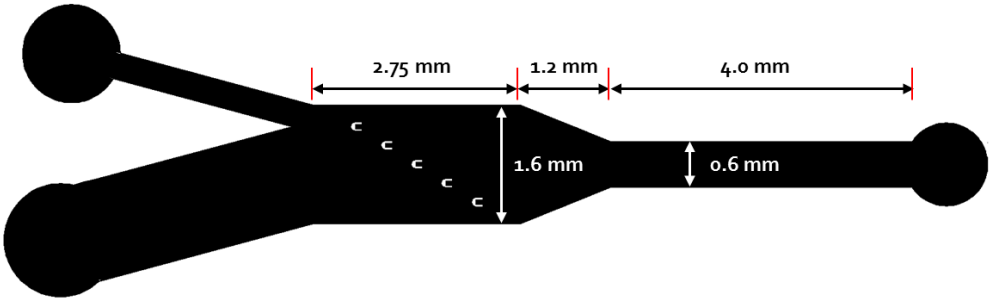

Figure S1 Design and dimensions of the microchannel used for chemical gradient generation.

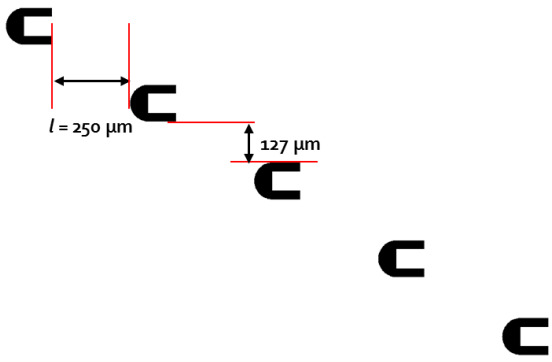

Figure S2 Ladder-like arrangement of the horseshoe structures to establish an exponential decay chemical profile.

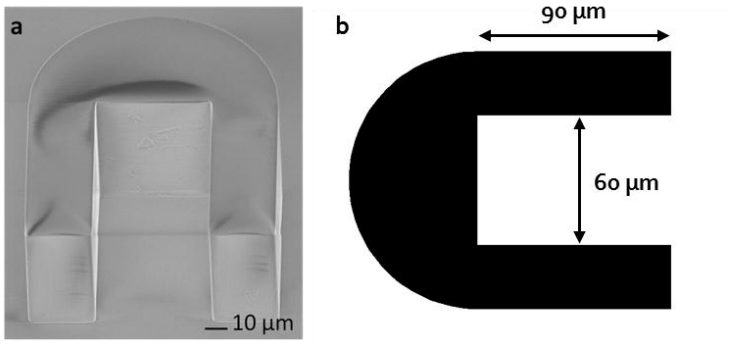

Figure S3 (a) An SEM image of the horseshoe structure within the PDMS microfluidic channel. (b) Dimension of the horseshoe structure.

3. Experimental setup

All the horseshoe structures were designed to be of identical geometry, as a result bubbles trapped are also of same dimensions, so that each trapped bubbles oscillated at a single resonance frequency. For repeatable bubble loading, we infused ink/water into the channel from the outlet at a flow rate of 15 µl/min. The flow rate is optimized to avoid any unwanted bubbles trapped either between adjacent horseshoe structures or between the horseshoe structure and the sidewall.

Gradient characterization was carried out using Dextran-FITC (1 mg/ml, MW = 10 kDa, Sigma, St. Louis, MO) in PBS solution (Life Technology, Carlsbad, CA) as the stimulant in inlet 1 and PBS solution as the buffer in inlet 2. To establish the interface of the co-flowing liquids between the stimulant and the buffer exactly at the middle of the first horseshoe structure, the flow rates of inlet 1 & 2 were adjusted to 0.4 µl/min and 2 µl/min, respectively. The piezoelectric transducer was driven by a function generator (Hewlett Packard 8116A). Experimentally the resonance frequency of bubbles is determined by sweeping the excitation frequency from 10 kHz to 60 kHz in 100 Hz increments while

visually monitoring the oscillation amplitude for a distinct large peak. Once the resonance frequency of the bubbles is set, the amplitude of the bubble oscillation is controlled by the voltage applied to the piezoelectric transducer, which eventually controls the mixing ratio of the stimulant and the buffer and enables the tuning of chemical profile. Fluorescent images of the chemical gradient profiles were analyzed by using Image J (v1.46p, NIH, MD).

4. Video Captions

Video 1: Multiple bubbles trapped within the horseshoe structures

Ink was infused into the channel from the outlet at a flow rate of 15 µl/min by a syringe pump. This flow rate allowed us to repeatedly trap a bubble within the horseshoe structures.

Video 2: Pulsatile gradient generation

Generation of pulsatile gradient at a frequency of 0.1 Hz (the voltage applied to the function generation was 16 Vpp).