NESTED HYDRODYNAMIC FLOW CONFINEMENT AND LIQUID RECIRCULATION: MICROSCALE PROBING AND PATTERNING OF BIOLOGICAL SURFACES

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ABSTRACT

To perform biochemistry at the microscale efficiently, we developed strategies to utilize one or more chemicals simultaneously with the microfluidic probe (MFP). The MFP allows for the manipulation and the "shaping" of nL of multiple liquids, to bring them in contact with a surface or to retrieve a biochemical compound from a surface. Here, we showed the possibility of using multiples flows to shape fluids in the vicinity of surfaces to improve biochemical reagent consumption and perform reconcentration of retrieved analytes by recirculating liquid.

KEYWORDS: surface processing, surface probing, hydrodynamic flow confinement, microfluidic probe.

INTRODUCTION

Microscale probing and patterning of biological surfaces plays a significant role in fields ranging from stimulation of adherent cells, microperfusion of brain slices, engineering cellular architectures, modulating stem-cell microenvironments to dispensing chemicals on cells for pharmacology studies [1,2,3]. In view of such applications, we developed the microfluidic probe (MFP), which is a non-contact, scanning microfluidic technology operating in the "open space", i.e. to directly operate on microscope slides and Petri dishes without the need for sealed channels/chambers [4]. The MFP is based on the hydrodynamic flow confinement (HFC) of nanoliter volumes of liquids over tens of micrometers of a surface. An important aspect is to minimize the dilution of the liquid that is in contact with the surface, and improve the efficiency of utilization of the processing liquid.

RESULTS AND DISCUSSION

To exploit the opportunities of HFC, we recently developed nested HFC, wherein multiple layers of liquids are shaped to interact with a surface [5]. In the classical HFC, the asymmetry of injection to aspiration flow rates between two apertures is responsible for the dilution of the liquid of interest (processing liquid) by the immersion liquid, Fig.1a. In the nested HFC, we use two extra apertures to "nest" the processing liquid inside a shaping liquid, Fig.1b.

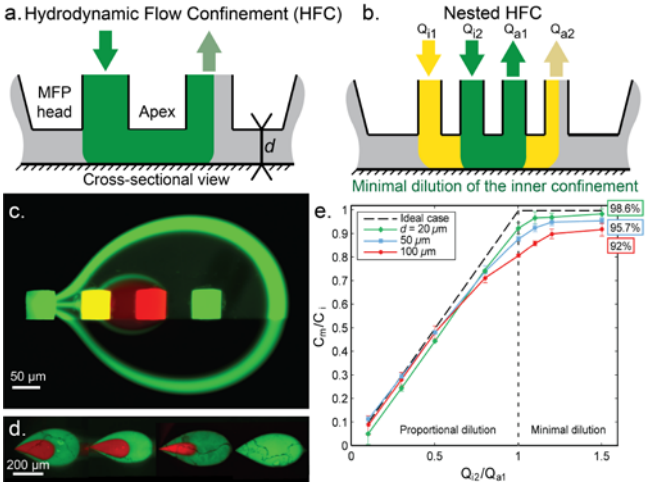

***Figure 1**: Principle and demonstration of HFC and nested HFC. **a.** In the classical HFC, a processing liquid (green) is confined, in the presence of an immersion liquid (grey), between the apex of the MFP*

head and the surface. ***b.*** *The addition of a shaping liquid (yellow) enables the minimal dilution of the nested HFC.* ***c.*** *Fluorescence microscope images of a nested HFC comprising red labeled antibodies in the nested liquid (inner) and green-labeled antibodies in the shaping liquid (outer).* ***d.*** *Assembly of images showing the simultaneous patterning of antibodies on a surface.* ***e.*** *Dilution of a dye (initial concentration C_i and concentration in the aspiration aperture C_m) in a nested liquid as a function of ratio of flow rates (Q_{i2}/Q_{a1}) for various apex-to-surface distances (d = 20, 50, and 100 µm).*

We illustrated the use of nested HFC by efficiently patterning multiple antibodies on a surface simultaneously, with 5 µm resolution and a 100-fold decrease of reagent consumption compared to microcontact printing. Nested HFC not only minimizes the usage of chemicals but also permits efficient retrieval of analytes from a surface, as demonstrated by the minimal dilution (below 2%) of the processing liquid in the inner flow confinement, Fig.1e.

Furthermore, we show here a strategy to repeatedly use and circulate a defined volume of processing liquid within the MFP head. Leveraging the minimal dilution inherent to the nesting of the inner flow confinement, we efficiently recirculate a volume of liquid back and forth within the MFP head. We recirculate in total 1 µL of liquid by switching the pressure of the reservoirs using four valves while shielding it using the shaping liquid, Fig.2a & 3a.

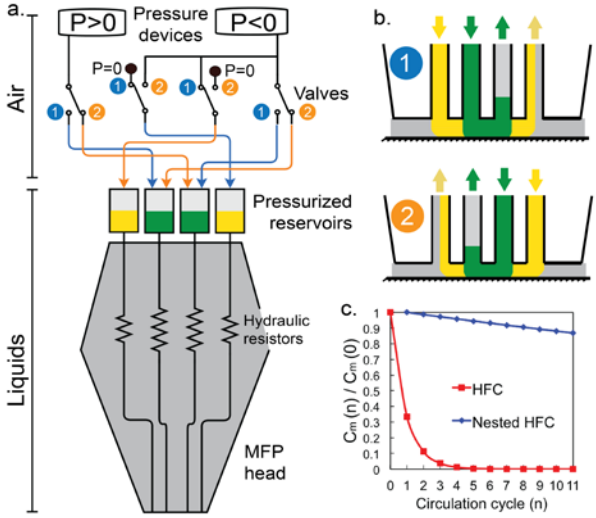

Figure 2: *Scheme for liquid recirculation in the MFP head.* ***a.*** *The liquid recirculation system. Pressure devices coupled to valves control the pressure in reservoirs, in turn controlling the direction of flows in the MFP head.* ***b.*** *The two states of recirculation of the nested liquid (green).* ***c.*** *Estimate of the dilution (ratio of the concentration at cycle n to the initial concentration $C_m(0)$) of an analyte as a function of recirculation cycle. In the nested HFC within two cycles of circulation, the concentration drops to ~10% of the initial concentration of the dye while with the nested HFC, even after n=10 circulation cycles, the concentration of the dye remains ~90% of $C_m(0)$.*

In contrast to classical HFC where the sample is diluted 66% at each circulation cycle, the nested HFC allows for a dilution below 1.4% per circulation cycle, Fig.2c. We designed, microfabricated and validated a MFP head comprising 4 apertures, a storage zone for the 1 µL of processing liquid and hydraulic resistors to accommodate for the asymmetry in flow rates, Fig.3b. Switching of the circulation direction can be achieved in as little as 2 s with negligible loss of processing liquid.

Combining nested HFC with liquid recirculation is a very powerful approach for investigating two critical aspects of microscale surface biochemistry: the efficient removal of analyte, where the concentration is increased steadily at each circulation cycle of the µL volume of the processing liquid, and efficient usage of chemicals on surfaces (e.g. for expensive antibodies) by circulating a defined volume of liquid multiple times over the surface of interest.

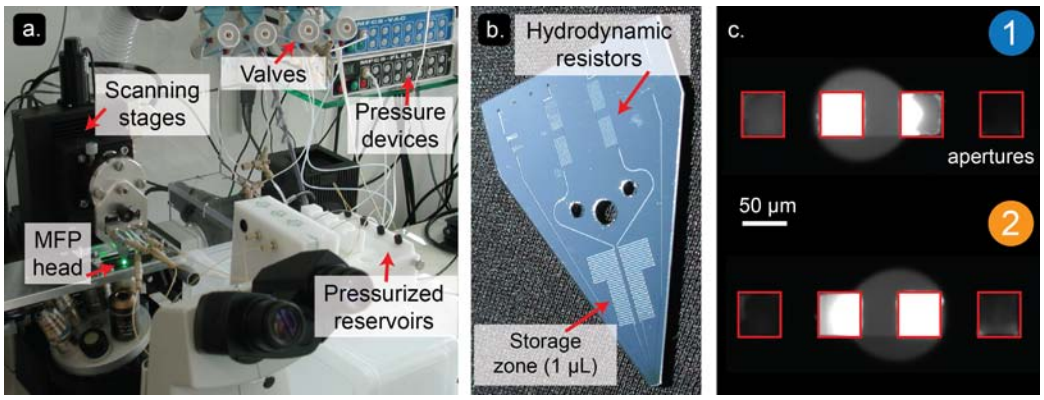

Figure 3: *MFP set-up and head design to implement nested HFC and liquid recirculation. a. Photograph of the experimental set-up with pressure devices and liquid reservoirs. The MFP is placed on a stage of a standard inverted microscope during operation. b. Image of the microfabricated silicon/glass MFP head with hydrodynamic resistors and serpentine channels for recirculating the nested liquid. c. Fluorescence image of two states of the nested HFC. Switching between states is done in 2 sec with minimal loss of processing liquid during this operation.*

CONCLUSION

This new method will allow for new opportunities in microscale surface biochemistry by improving efficiency, reducing costs and allowing for spatial and temporal probing/stimulating of biological surfaces.

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