High Resolution Spatio-Temporal Dosing of Subcellular Targets

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Statement of Purpose: Cells receive cues from the environment that trigger intracellular signaling cascades responsible for regulating processes like differentiation, growth, and death. Although entire culture dishes are commonly dosed with reagents, there is a growing need to exert greater spatio-temporal control over interactions between chemicals and cells. Potential areas that may benefit from this include studies focused on chemotaxis, neuritic pathfinding, and angiogenesis.

One of the techniques employed for spatially localized chemical dosing is the use of parallel laminar-flow streams in a microfluidic environment containing cultured cells (Takayama S. Nature 2001; 411:1016). Although these strategies permit the establishment of stable gradients that makes dosing subcellular features feasible, they are constrained by the initial device design. This makes dosing multiple sites and dynamically targeting individual cellular features challenging. In a previous paper, we have described a cell-dosing strategy to overcome some of these limitations. In this approach, cells are cultured on an ultra-thin polymer membrane that serves as a barrier between two stacked laminar-flow chambers: one containing the cell culture, the other the reagent. By focusing a pulsed laser beam onto one or more selected membrane positions, micron-diameter pores can be ablated upstream of desired cellular targets, through which stable reagent streams enter and dose the targeted regions. We can also shut off reagent streams by photocrosslinking a protein plug over the selected pore (Nielson R. Anal Chem. 2006; 78(17):5987-5993).

In an attempt to increase the spatio-temporal capabilities of this system, we have been working on changing the orientation of the laminar-flow streams on-the-fly. This can be achieved by replacing the single-channel flow cell, used to form the cell culture chamber in the previous configuration, by a multiple-channel asterisk flow cell. In conjunction with computer-activated pinch-valves, the stream orientation can be changed rapidly, which not only allows for greater coverage of cellular targets but also permits very brief reagent exposures.

Methods: The cell dosing set-up consists of two flow cells stacked on top of each other, separated by 2.5 μm thick Mylar™ membrane (SPI Supplies, West Chester, PA), stretched taut on a Delrin frame. PDMS gaskets containing access ports for inserting the feed and drain tubes are used in conjunction with the flow cells to establish flow inside the channels. The entire device, consisting of the membrane, and the two flow cells with their corresponding PDMS gaskets, is assembled on a microscope stage. Channels in both flow cells as well as all the entry and exit ports (of the flow cells and the corresponding gaskets) are aligned and compressed with the membrane sandwiched between the flow cells. A plexiglass plate with two screws is used to hold the mated channels onto the stage, and to reinforce the seal between the flow cells and the gaskets. Feed and drain tubes are connected to both flow cells, and the other end of the feed tubes is connected to syringes hooked to electro-mechanical pumps. Cell medium is flowed in the top chamber, and the reagent in the bottom. 6% bovine serum albumin (BSA) is often added to the reagent for visualization of reagent streams inside the cell chamber.

Pores in Mylar™ membranes are ablated using the output from a pulsed, frequency-doubled (532 nm) ND:YAG laser that is aligned into a Zeiss Axiovert inverted microscope, and is collimated to fill the back aperture of a 40x air objective.

Results: In the basic device configuration, the flow direction is pre-defined, which may restrict access to certain cellular targets. For example, an axon growing parallel to the flow direction would either have to be dosed completely or not at all. Modifications to the basic design have allowed for different flow orientations that can be switched on-the-fly. In the modified setup, the single-channel flow cell used to form the cell chamber is replaced by an asterisk flow cell that consists of four channels that intersect in the center. Solenoid pinch valves controlled by LabView software are used for changing the flow direction. Eight flow orientations are obtained by opening individual valves. Fig. 1 shows two of these orientations. These have been further increased to sixteen by opening two valves simultaneously. Also, sub-second resolutions (i.e., the minimum period of time that a cell can be dosed) can be obtained with this device.

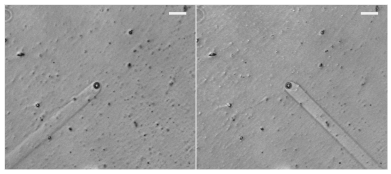

Fig. 1. Flow orientations obtained using an asterisk flow cell. 6% BSA was used as the reagent, and PBS was flowed in the top chamber. Scale bars are 20 μm long.

Ongoing experiments involve fluorescence staining of neutrophils with a mitochondrial dye (e.g. Mitotracker Green), and influencing cell migration using a chemoattractant peptide fMLP. This approach can also be used for studying the role of vascular growth factors involved in angiogenesis.

Conclusions: We have described a membrane-ablation based device here for dosing cells in a spatio-temporally controlled manner. This versatile tool can generate steep gradients that can be established and dissipated easily, can be steered in different directions, can be sustained for hours in one direction or switched under a second, thus providing precise control over cell-reagent interactions, which are critical to cell function and differentiation.