MICROFLUIDIC SYSTEM FOR PULSED STIMULATION AND TIME COURSE ANALYSIS OF MAMMALIAN CELLS: IDENTIFICATION OF THE MINIMAL TNF-ALPHA PULSE DURATION FOR NF-KAPPAB ACTIVATION IN HELA CELLS

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ABSTRACT

We present a simple hydrostatic-driven microfluidic system for spatiotemporal cell stimulation. We used the developed system to culture HeLa cancer cells and apply stimulation pulses of Tumor necrosis factor (TNF) as short as 10 s. Using this system, we established the concentration-dependent temporal limit of TNF-stimulated NF-kB activation in HeLa cells, and determined that a 30-seconds pulse is sufficient for full activation of NF-kB with 100 ng/ml TNF, and that a 1-min pulse is needed with 10 ng/ml TNF.

KEYWORDS: Pulsatile stimulation, Hydrostatic-driven flow, Multiple laminar streams, HeLa cells, Tumor necrosis factor

INTRODUCTION

Microfluidic devices provide unique functionalities for cell biology and allow precise handling of samples. To alter cellular microenvironments in a spatiotemporally controlled manner, multiple streams of laminar flow can be used [1]. Nonetheless, controlling these streams and their interfaces often relies on expensive liquid handling equipment, a barrier to widespread use of these devices in biology laboratories. Here, we present a simple T-junction microfluidic chip for spatiotemporal cell stimulation, powered by manually controlling the hydrostatic pressure at each inlet. Using this setup, we stimulated cancer cells with TNF for varying intervals.

TNF is a cytokine with multiple roles that can induce cell differentiation and proliferation, and cell death at the same time. TNF promotes cell survival by triggering nuclear translocation of the nuclear factor kB (NF-kB) resulting in its activation [2]. NF-kB is a transcription factor that is involved in cellular response to stimuli such as stress and cytokines. When cells are treated with TNF, survival and apoptotic pathways get activated, and consequently some cells die while others survive. The non-genetic source of variability in different cell responses and how each cell makes its own decision remains unknown [3].

To better understand TNF signaling, we used this simple microfluidic system to find the minimal duration of TNF stimulation required to activate NF-kB in HeLa cells, and to establish the relationship between concentration and stimulation time.

METHODS

A microfluidic chip with two inlets and three chambers for cell culture was made in PDMS and bonded to a coverslip (Figure 1a). Spatiotemporal control over cell stimulation was achieved by shifting laminar streams laterally within the chambers. In default mode, Figure 1b, most cells are exposed to culture medium. By raising the TNF solution reservoir, all cells are exposed to TNF for the desired duration (Figure 1c).

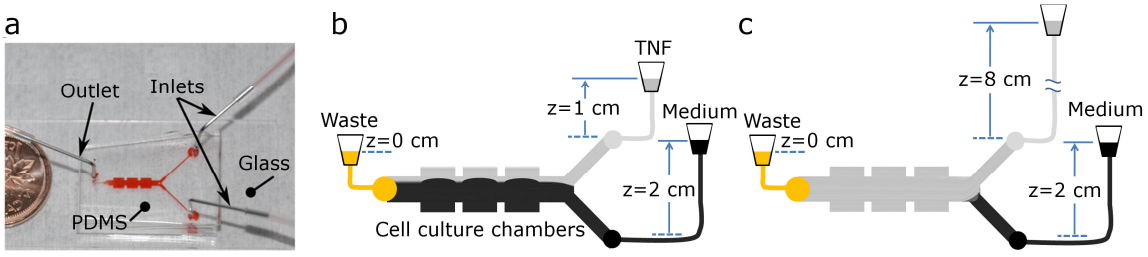

Figure 1: The microfluidic system with hydrostatic flow control used to apply pulses of TNF to cancer cells cultured within chambers. (a) A photograph of the microfluidic chip with tubings connecting it to reservoirs filled with reagents. (b) Default mode with cell media placed 2 cm above chip. Most cells in the chambers are exposed to cell culture medium but a thin band of cells serving as positive controls are constantly exposed to a stream of TNF-solution. (c) Stimulation mode: a pulse of TNF is applied to the whole cell population in chambers by raising the TNF solution to 8 cm for the desired duration.

RESULTS AND DISCUSSION

Using a fluorescence microscope, the stimulation duration was visualized by monitoring the TNF stream, which contained fluorophore-conjugated BSA (Figure 2). By manually switching between operating modes (Figure 1b-c), TNF pulses as short as 10 s were reproducibly achieved (Figure 2).

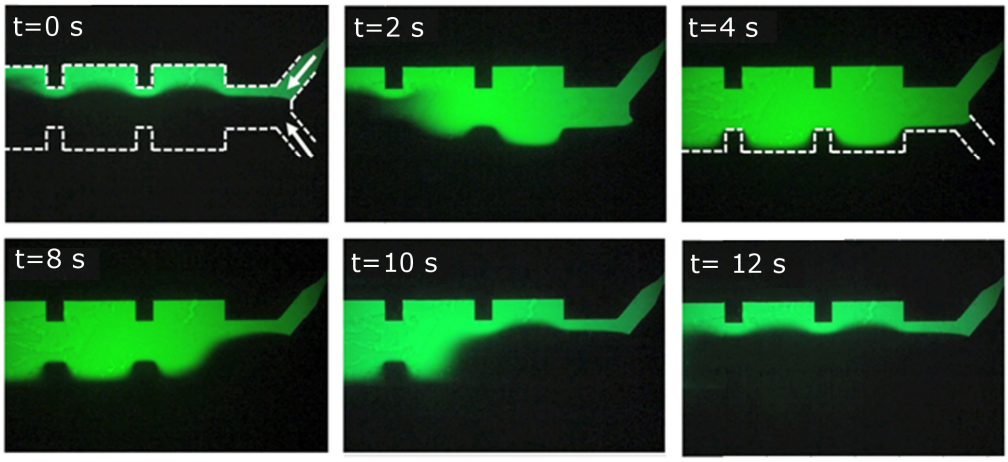

Figure 2: Time-lapse of a 10 s pulse of fluorescein that was added to the TNF reservoir (Figure 1). At t=0 s, the system switched to the stimulation mode by raising the TNF reservoir and then lowered after 8 s back to the default mode. Each chamber has a length side of 2 mm and a depth of 40 µm.

The calculated flow shear stresses applied to cells were in the range of 0 -0.12 Pa during the default mode, and in the range 0-0.4 Pa during the pulse mode (Figure 3a), which has no observable NF-kB activity in negative control experiments.

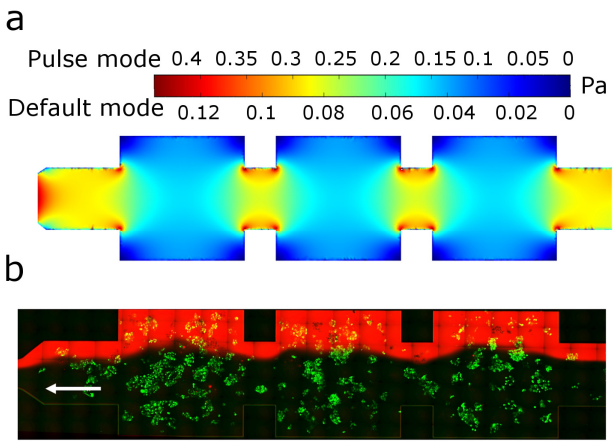

Figure 3: Fluid flow and cell culture in the microfluidic chambers (a) 3D Comsol simulations of the flow shear stress inside the microfluidic chambers during the default and stimulation modes. (b) Fluorescence micrograph showing the GFP-NF-kB tagged HeLa cells (green) cultured in the chambers (around 500 cell per chamber), and the TNF stream (mixed with red fluorescent BSA) operating in the default mode.

NF-kB is inactive in unstimulated cells and distributed predominantly in the cytoplasm as it is bound to an inhibitor protein, IkB. Upon TNF stimulation, a signalling complex forms that leads to IkB phosphorylation and degradation, allowing translocation of freed NF-kB into the nucleus. Consequently, several genes are activated, including the gene for IkB. Newly synthesized IkB binds NF-kB and shuttles it back to the cytoplasm. NF-kB activation dynamics in each cell were visualized by tracking a GFP-NF-kB reporter (Figure 4).

We discovered that 30-seconds pulse was sufficient to elicit full activation of NF-kB when using 100 ng/ml of TNF (Figure 4a-b), indicating digital activation consistent with prior studies using constant stimulation [4]. However, when reducing the pulse time to 10 s, partial activation was observed. With 10 ng/ml TNF, full activation of NF-kB required a 1-min pulse (Figure 4c-d); only partial activation was observed after a 30-seconds pulse. Full activation is defined as NF-kB translocation levels similar to those observed in cells exposed to the narrow constant stream of TNF at the right side of each chamber (Figure 3b).

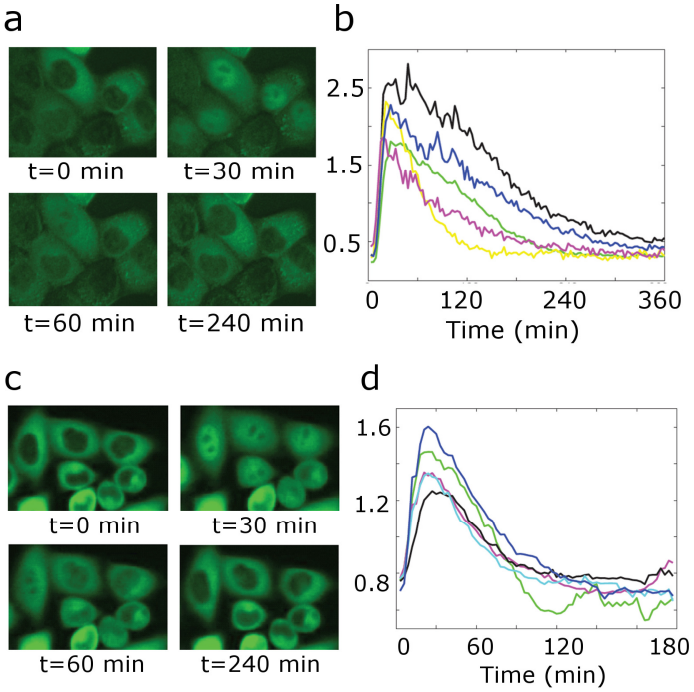

Figure 4: Time course of NF-kB activation in individual HeLa cells visualized by tracking a GFP (green fluorescent protein)-based reporter using a fluorescence microscope. After stimulation with TNF, NF-kB translocates from the cytoplasm to the nucleus and then shuttles back to the cytoplasm in a delayed negative feedback mechanism. (a) Time-lapse fluorescence images after 30 s stimulation (at time=0) with 100 ng/ml TNF showing full NF-kB responses. (c) Time-lapse images after 1 min stimulation with 10 ng/ml TNF showing full NF-kB responses. Charts in (b) and (d) are showing the time course of the ratio of nuclear to cytoplasmic localization of NF-kB (y-axis) in some cells of each case. Single-cell dynamics are shown as colored lines. Each experiment was repeated for 3 times, and more than 500 cells were analyzed for each case.

CONCLUSION

Here we developed a simple microfluidic setup – notably in comparison to others used in prior TNF signaling studies [4] – with high spatiotemporal resolution and used it to establish the concentration-dependent temporal limit of TNF-stimulated NF-kB activation in HeLa Cells. We determined that a 30-seconds pulse is sufficient for full activation of NF-kB with 100 ng/ml TNF, and that a 1-min pulse is needed with 10 ng/ml. This microfluidic system is easy to use and can be replicated in any biology laboratory with no need for sophisticated devices or equipment; the fluids flow was controlled hydrostatically and using off-shelf items, and the imaging system is available in common laboratory. In the future, this device could be easily applied to other stimuli, signaling pathways and cell types.

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