AGGREGATION AND LONG-TERM POSITIONING OF CELLS BY ULTRASOUND IN A MULTI-WELL MICROCHIP FOR HIGH-RESOLUTION IMAGING OF THE NATURAL KILLER CELL IMMUNE SYNAPSE

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ABSTRACT

In this study we investigate the ability of Natural Killer (NK) cells to form ultrasound-mediated intercellular contacts with target cells in a multi-well microdevice by high-resolution confocal-microscopy imaging of inhibitory immune synapses. Furthermore, we compare the NK-Target cell cluster migration with and without ultrasound actuation. Experiments indicate that clusters of cells are positioned and maintained centered in the wells for 17 hours when they are exposed continuously to ultrasound. Our system can be used for both screening high numbers of events in low resolution and also for high resolution imaging of long term cell-cell interactions.

KEYWORDS: Natural killer cells**,** Immune synapse, Ultrasound, Multi-well, Microchip

INTRODUCTION

Natural killer cells belong to the innate immune system and their role is critical in host defense and immune regulation. In general the innate immune system reacts rapidly against pathogens and usually it is effective enough to eliminate the invaders or tumor cells before they cause disease. When an NK cell encounters another type of cell during surveillance in the body, it may form a tight intercellular contact and cause accumulation of proteins in a dynamic interface called immune synapse (IS). Observations of the synapse have revealed numerous functions and important communication processes between NK and target cells. However, most of the methods used today in cell biology are based on measurements in bulk solutions containing huge number of cells, displaying only the average cellular properties. Therefore, methods are requested capable of characterizing high numbers of individual cells in order to fully understand the complex heterogeneity of cellular processes and kinetics.

In this paper we demonstrate a platform for dynamic, parallel screening of individual NK– target cell interactions based on ultrasonic aggregation and positioning of cells in a multi-well microchip. Upon ultrasound actuation, clusters containing one or a few NK-target cell conjugates are formed and retained in a precise location synchronously in each of the 100 wells of the microchip for up to 17 h. This platform enables detailed time-lapse monitoring of the immune synapse formed between NK and target cells. Previously, we have demonstrated how frequency-modulated ultrasound can be used for simultaneous, uniform, and parallel trapping of beads or cells in all wells of the microchip. Furthermore, we showed that cells remained viable for up to 3-days during continuous ultrasonic actuation [1].

In the present study, using image analysis we quantify the cell cluster motility with and without retained ultrasound exposure during 17 h and we demonstrate high-resolution confocal-microscopy time-lapse imaging of inhibitory immune synapses formed between NK and target cells. The goal is to use this platform to investigate dynamics of cell-cell interactions, e.g. protein distribution and trafficking at the intercellular contact between NK and target cells as well as effector functions leading to target cell death [2].

EXPERIMENTAL

Device

The microchip consists of a $22 \times 22 \times 0.3$ mm^3 silicon layer bonded to a $22 \times 22 \times 0.175$ mm^3 glass layer. In the center of the microchip there is an array of 10×10 wells etched on the silicon layer with dimensions $0.3 \times 0.3 \times 0.3$ mm^3. The wells are separated by 100 µm thick walls. The chip is glued on a metal holder with a thin cork layer between them to avoid energy loss. A thin polydimethylsiloxane (PDMS) frame is bonded to the silicon layer surrounding the wells in order to contain the required amount of cell culture medium for long term experiments. A new more robust design of wedge transducer is glued on the chip. Upon ultrasound actuation (~2.5 MHz, 10 V_{pp}), synchronized aggregation and positioning of particles or cells in all 100 wells is achieved within seconds, see Fig. 1b.

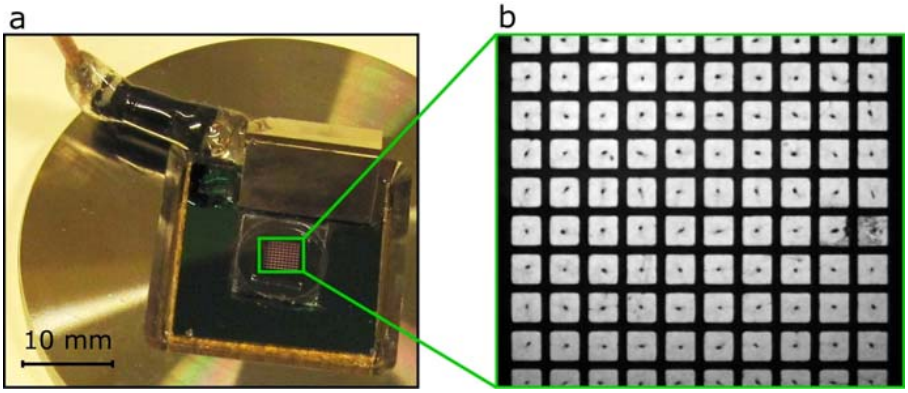

Figure 1: (a): Picture of the 100-well microchip with the ultrasonic wedge transducer attached. (b): Demonstration of the uniform trapping of 5 µm beads in the center of each well after ultrasonic actuation at ~2.5 MHz and 10 V_{pp} (experiment by Otto Manneberg).

Cell culture

As effector cells we have used either primary polyclonal human NK cells or a human NK cell line (YTS) transfected to express the inhibitory NK cell receptor KIR2DL1. Polyclonal primary human NK cells were harvested from blood of healthy donors by centrifugation and separation of peripheral blood mononuclear cells followed by negative magnetic bead sorting. Primary NK cells were cultivated in IMDM supplemented with 10% human serum, 2mM L-glutamine, 100 U/ml Penicillin-Streptomycin, 1× non-essential amino acids, 1 mM sodium pyruvate and 200 U/ml human interleukin-2 (IL-2). As target cells we used the human B cell line 721.221, deficient in endogenous surface expression of major histocompatibility complex (MHC) class I proteins and transfected to express MHC/HLA-Cw6 (cognate ligand to KIR2DL1) coupled or not to green-fluorescent protein (GFP). All cell lines were cultivated in RPMI-1640 supplemented with 10% fetal bovine serum, 50 U/ml Penicillin-Streptomycin, 1× non-essential amino acids and1 mM sodium pyruvate. All cells were incubated at 37 °C and 5% CO_2.

RESULTS AND DISCUSSION

Clusters of primary NK cells and target cells were used to study the movement of cell aggregates formed by ultrasound, both during short-term actuation followed by release, and during long-term actuation, see Fig. 2a. As seen in Fig. 2b, the average cluster position changed less than 5 µm during 17 hours of continuous ultrasonic actuation ("trap and retain"), compared to >50 µm average position change when ultrasound was employed only initially during cell cluster formation ("trap and release"). We used the ability of the ultrasound to retain cells for matching the cluster position with the field of view of the microscope (cf. green boxes in Figs. 2a and 2c) when performing time-lapse studies at high resolution (100× objective). Finally, we demonstrate time-lapse characterization of the immune synapse formed between different combinations of NK and target cells, see Fig. 2c-h. The lower panel (Fig. 2f-h) shows in false-color coding the green fluorescence from HLA-Cw6-GFP clustering at the intercellular contact, indicating the formation of one (Fig. 2f) or two (Figs. 2g-h) inhibitory immune synapses. In the future we will use this method to investigate how NK cells respond when engaged in multiple, spatially separated activating or inhibitory immune synapses.

CONCLUSIONS

Previously the multi-well platform have been characterized in terms of temperature stability, cell aggregation efficiency and cell viability [1]. This study is a follow up investigating whether NK-cell functions are conserved during ultrasound exposure, such as the ability of NK cells to form immune synapses with target cells. Although the system is continuously exposed to ultrasound, we have observed several events of inhibitory immune synapse formation. Furthermore, it is shown that with ultrasound actuation, we can secure the conjugate of interest locked in the field of view in order to perform long-term high resolution imaging with small field of view. Additional studies of immune cell function upon ultrasound exposure are under investigation, such as calcium signaling and NK cytotoxic killing.

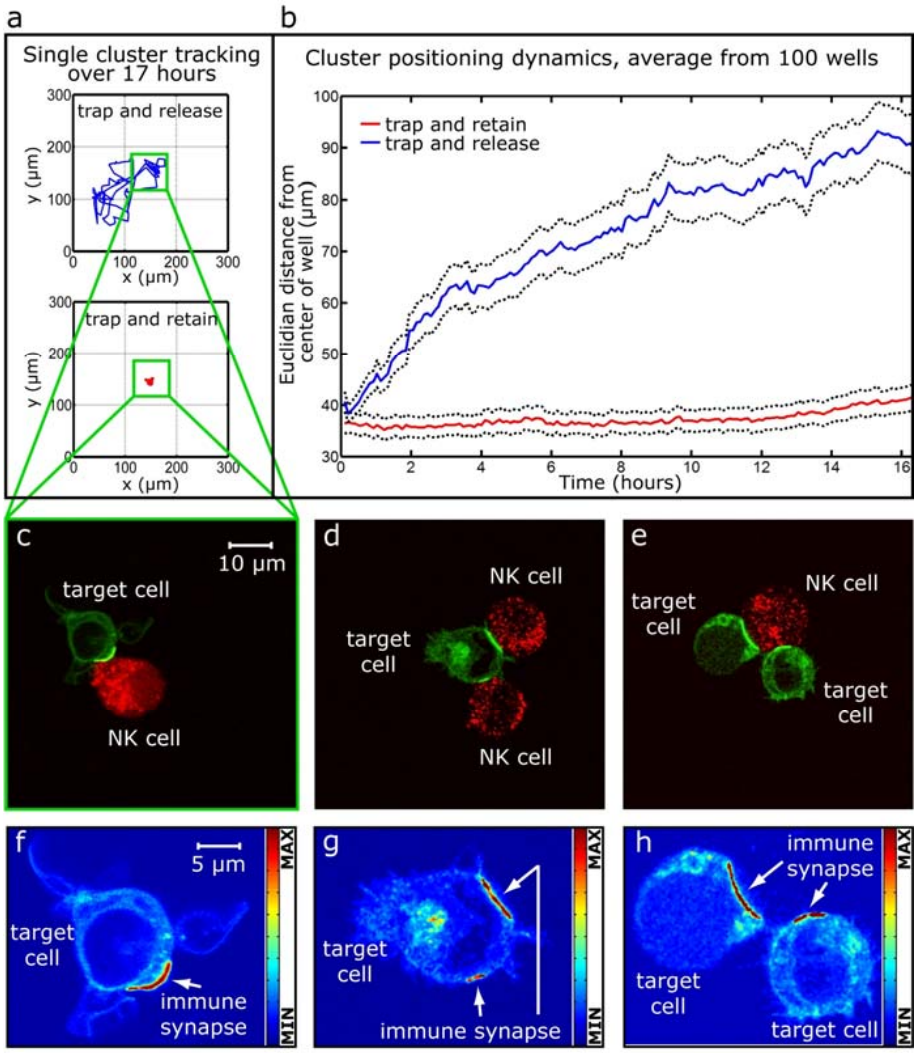

Figure 2: (a): Tracking of the position of a cell cluster in one well without (upper panel) and with retained ultrasonic actuation during 17 hours. (b): Corresponding Euclidian distance from the center of wells to the cell aggregate position, average from all 100 wells. (c-e): High-resolution confocal fluorescence microscopy images of HLA-Cw6-GFP molecules in B cells (target cells) interacting with Far Red-DDAO-SE-labeled NK cells (YTS-KIR2DL1). (f-h): Clustering of the HLA-Cw6-GFP molecules in the B cells (target cells) at the interface between the target cell and the NK cell is displayed by plotting the green fluorescence in (c-e) in a rainbow colormap, revealing the location and shape of the inhibitory immune synapse.

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