ABSTRACT

We have designed a PDMS based microfluidic system to provide inflammatory inputs, and measure the cellular immune response of single cells. Cells exist inside a complex, dynamic environment which must be sensed, processed and reacted to in a coordinated fashion. To fully understand the dynamic complexity, and heterogeneous behavior of the immune response, it is necessary to study cellular behavior at the single-cell level by both providing dynamic input and measuring dynamic response. Such stimulation and measurement overcomes problems of population and time averaging which can obscure analysis of complex system behavior.

KEYWORDS: Single-cell, Microfluidics, Immune, Macrophage

INTRODUCTION

The immune system is composed of complex interactions and time dependent behavior. Multiple stimuli such as cytokines, reactive oxygen species, and interactions of diverse cell types exist and vary dynamically. Proper immune function ranging from response to pathogens, tissue repair, and resolution of inflammation rely upon these relationships. Additionally, pathological conditions affecting large segments of the population such as autoimmune disease, persistent infection, and sepsis are the result of improper interactions of these systems. Understanding of these relationships however is lacking due to current investigation methods which do not possess capabilities to measure such dynamic and complex behavior in a noisy environment. Furthermore, individual cells, even clonal populations, react in diverse ways to identical input necessitating single-cell measurement. These challenges impede progress on development of therapies addressing immune dysfunction, as high quality, temporal, quantitative measurements are difficult to obtain. The system we have designed will address these issues by (1) providing a controllable, dynamic input space, (2) obtaining dynamic multiparameter measurements of the cellular response to immune stimulation.

EXPERIMENTAL

In order to study the immune response, we have developed multilayer valve based microfluidic chips (2) (Fig. 1), which will provide complex, dynamic input, as well as allow monitoring the dynamic response of cells. The chips function by first isolating single cells via a fluidic trap (Fig. 1b). Once isolated, the cells will be stimulated with a complex pattern of input ligands. Simultaneously, measurement of the cellular response will be conducted by various means of automated time-lapse microscopy and biochemical assays. A wide variety of assays are compatible with the chips as the device consists of a fully controllable fluidic environment allowing introduction of multiple components. Automated microscopy will be conducted in multiple fluorescent channels for specific assays, as well as with brightfield imaging to measure parameters such as morphology, migration, apoptosis and proliferation. Data gathered will consist of the defined input stimulation profile combined with dynamic biochemical assays of the cell immune response. These data will then be analyzed to uncover specific details of the dynamic nature of the immune reaction.

The chips used were created by standard polydimethylsiloxane (PDMS) soft lithography. Master molds were fabricated via chrome and transparency mask photolithography with SU8-3025 negative photoresist for square channels, and AZ50XT positive resist for rounded channels. Channels were 100 μm wide and 25 μm tall in all areas except for trap structures. Molds were functionalized with TMCS (Chlorotrimethylsilane) to facilitate PDMS removal. RTV615 (Momentive Performance Materials) PDMS was used, and was spun on, or cast onto control and flow wafers respectively before bonding of the two layers. Bonding was achieved with oxygen plasma treatment and chips were aligned with a microscope before being brought into contact. After bonding, chips were incubated at 80°C for one week prior to use. Before introducing cells, chip surfaces were coated with fibronectin for 12 hours.

The chips are controlled with a custom, automated MATLAB graphical user interface. The interface controls timing of solenoid openings and closings which pressurize or depressurize fluidic lines connected to the chip. This causes membrane valves to deform which achieves the on-chip valving function. [3]. With this control system, fluid flow including medium replacement, stimulation, and sampling have fine time resolution and control. This increases controllability and decreases noise in measurements as the overall fluid handling precision is increased. Imaging of the chip was conducted with an Eclipse Ti microscope equipped with an automated stage, inside an environmental chamber which maintained cells at 37°C, 5% CO₂ and 100% humidity. Image acquisition was conducted with Elements software, and a Hamamatsu Orca-Flash 4.0 camera.
RESULTS AND DISCUSSION

We have created a microfluidic system capable of isolating single cells and exposing them to well defined, dynamic input, (Fig. 2). This automated system is compatible with live imaging, and several types of assays such as immunofluorescence, internal calcium imaging, and migration analysis, among others. Stimulation and assays can both be conducted at multiple time points.

The single-cell isolation component is a critical but challenging aspect of the system. This is due to several factors such as easily manufacturing structures compatible with cell size, avoiding cell clumping, and achieving cell viability. The trap which has been used to isolate cells inside our devices consists of a slit structure spanning the height of the microchannel composed of two diamond-like halves. Cells introduced into the microchannel encountering the slit are forced against it and pinned as long as flow is maintained, (Fig. 1b). Once trapped, flow can be stopped and cells can attach to the channel surface for subsequent experiments, (Fig. 1c). This specific geometry was designed to separate single cells from medium containing a high excess of cells and cell clumps both of which must not be trapped in order to achieve single-cell isolation. The design is structured so that once the trap is occupied, additional cells encountering the trapped cell are shed off the sloped sides of the diamond geometry. Cell clumps, being larger, also are more exposed to the flow and can be shed if encountering the slit. The most critical dimension for the trap was seen to be the slit gap, which for the cells used (3T3 fibroblasts and RAW 264.7 macrophages) was most effective when 4 μm wide. With this design, rapid manual and automated loading of single cells was possible in under 15 minutes. A loading efficiency of ~70-80% was possible under automated control and higher efficiencies were possible with manual control. Non single-cell loading usually consisted of multiple cells with empty traps being rarely found.

After trapping of single cells inside the microfluidic chips, fresh medium was given at two hours intervals to maintain viability. The two hour replacement time was found to be necessary for the volume of the single-cell chambers used, which was ~1.3 nl. Trapped cells were next stimulated with various pulses of temporally well-defined stimuli, such as lipopolysaccharide (LPS) which can activate TLR4 receptors. During experiments, cells were imaged at 10 minute intervals which allowed their reaction to be monitored in detail. This system will next be integrated with several biological assays to detect specific components of the immune response, complementing imaging and automated cell stimulation. Analysis and modeling of the dynamic single-cell immune reaction data provided by these chips will lead to
more accurate and complete understanding of immune behavior. This will facilitate uncovering solutions to immune needs such as treatments for diseases, sepsis, and methods for drug screening, which ultimately depend upon understanding the complex, dynamic nature of the immune response.

CONCLUSION

Measuring cell behavior in a high throughput, dynamic, and single-cell manner is crucial if complex biological systems are to be understood. The work presented here has described developments towards a microfluidic system for capturing, culturing and stimulating single cells for long time periods while conducting multiple, diverse measurements. These measurements can consist of dynamic imaging of cell behaviors such as migration and death as well as other biological assays which can be introduced into the microfluidic chip. Detailed, high volume measurements of this type, coupled with the defined input space will present a complete image of the cellular immune response. This will provide data useful for deciphering many areas relating to immunology and biology.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Schweizerische Nationalfonds (S F). The authors would also like to acknowledge imaging assistance provided by Dr. Thomas Horn at ETH Zürich.

REFERENCES


CONTACT
*S.Tay, tel: +41 61 387 31 57; savas.tay@bsse.ethz.ch