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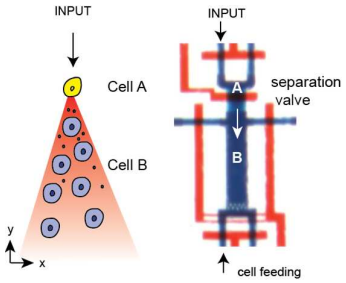

We present an automated microfluidic co-culture system that allows us studying the spatiotemporal signaling propagation from a single activated cell to a population of cells.

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Automated co-culture system for spatiotemporal analysis of cell-to-cell communication

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We present a microfluidic co-culture system that generates localized and precisely formulated immune signals among a population of cells, enabling spatiotemporal analysis of paracrine signal transmission between different cell types. The automated system allows us to create temporally modulated chemical inputs that can be delivered to single signal-transmitting and receiving cells in a highly controlled way. Using this system we stimulated a single macrophage with brief pulses of bacterial LPS and observed the macrophage transmitted TNF signal propagating in a population of fibroblasts via NF-κB activation. The signal receiving fibroblasts transformed the TNF signal into a spatiotemporally distributed NF-κB output, recapitulating the initiation of immune response to bacterial infection.

Introduction

Global response to a local stimulus is ubiquitous in biological systems. A single cancer cell, for example, can kill an organism by forming a tumour [1], localised enzyme production can create gradients of diffusive retinoic acid that patterns a growing organism [2], the global heart pumping rate is controlled by a few local pacemaker cells [3], single bacteria can sense each other in close proximity and build a colony by quorum sensing [4] and localized invading bacteria and viruses activate a global inflammatory response [5]. Common to these examples is a spatially and temporally localized environmental input acting on a small group of cells, from where information spreads via cell-cell interactions to tissue by activating genetically encoded signalling pathways.

Many biological processes involve interactions between different cell types and subpopulations. A typical signalling scenario is presented in Figure 1A. First, an immediate responder like a macrophage (cell type A) senses an environmental input like LPS from invading bacteria or an environmentally derived signalling molecule like TNF. This input is then processed using gene regulatory networks like NF-κB (an important immune pathway [6]), and the appropriate response is computed by the first responder cell. In order to pass this information to neighbouring tissue cells, signalling mediators like inflammatory cytokines are secreted into the extracellular space. These cytokines are sensed by nearby responding cells, become processed and finally turned into a spatially distributed tissue level gene expression output (Figure 1A). Mediators can be transmitted via diffusion, gap-junctions or contact dependent signalling [7]. The type, amplitude, duration and time course of the original signalling input determines the characteristics of cytokine secretion and cellular response. This information flow can lead to local reprogramming, differentiation, migration, apoptosis, proliferation of cells, or lead to further secretion of defined cytokines to elicit a global response. Importantly, these response characteristics may not only appear in a temporally modulated way [8,9], but they are also spatially organised. Spatial effects like tissue patterning or reorganisation is of immense importance in understanding biological phenomena like inflammation, and in modelling of signalling pathways.

Despite their importance, studies aimed at understanding the spatial aspects of cell signalling, especially with single-cell resolution, are few and far between. Traditionally used population assays are often obscured by "biological noise", i.e. the naturally occurring cell-to-cell variability in isolate cell responses. Creating relevant signalling scenarios *in vitro* where single-cells can be manipulated, tracked and quantitatively analysed is a technically challenging task [10]. Many traditional methods are designed for population measurements in homogeneous (well-mixed) environments. For example, many insights on communication between pacemaker cells has been realised with an input delivered to the whole population [11]. The influence of positive feedback in yeast paracrine signalling was studied in a well-mixed environment [12], thus ignoring the spatially inhomogeneous nature of signalling. To distinguish sender from receiver cells in bulk culture conditions, genetic modification had to be introduced, since defined spatial inputs have been lacking [13]. There is consensus that heterogeneous cell environments like morphogen or cytokine gradients play major roles in developmental biology [14] and in inflammatory reaction to

recruit more defender cells, [15] or to conduct successful immune response [16]. How signals evolve and propagate in such environments has not been studied in a spatiotemporally controlled manner. There is immediate need for technical platforms that enable investigating spatiotemporal signal transduction to understand and model how signalling pathways control and process local information transfer, and how collective population behaviour emerges [17].

Chemical stimulation of a single-cell without stimulating the neighbouring cells is a necessary task when studying the common cell-to-cell communication scenario summarized in Figure 1. To date, specific spatial stimulation was achieved by the use of optical or magnetic tweezers [18,19] or photo-caged inducers [20], which require advanced optical systems and their integration with standard cell culture experiments. Robotically controlled micropipettes can deliver local signals to cells, but it is difficult to prevent exposure to the neighbouring cells [21]. Three-dimensional microfluidic systems can be used to bring localized signals to cells, but these systems require non-standard and complex fabrication methods [22]. The complexity of the above-mentioned approaches may limit their application to high-throughput and controlled study of cell-cell communication with single-cell resolution. Exclusive stimulation of single and subpopulations of cells using microfluidic parallel laminar flow has been previously shown [23,24]. However, *in vivo* cell-cell communication is typically mediated by diffusion, and secreted signalling factors are quickly washed away when flow-based systems are used.

In this paper, we present an automated microfluidic co-culture device and integrated live-cell imaging system that can induce local signals on single cells and allow them to communicate with a second population of signal receiving cells in a precisely controlled way. Using a one-dimensional arrangement between different cell types, we were able to address a given cell type without affecting the others, and be able to study signal transduction between different populations (Fig 1B and 1C). Using this system, we induced TNF secretion in a single macrophage by a temporally modulated local LPS input, and allowed this cell to communicate with a population of 3T3 fibroblasts, each expressing the fluorescent fusion protein p65/DsRed. These cells reacted by activating the canonical NF-κB signalling pathway. The duration of the local LPS input pulse became converted into a spatial signalling range of NF-κB activation among the 3T3 population. We therefore showed how a single immune cell spatially controls a population of tissue cells through NF-κB transcription factor oscillations in a highly realistic infection scenario established *in vitro*.

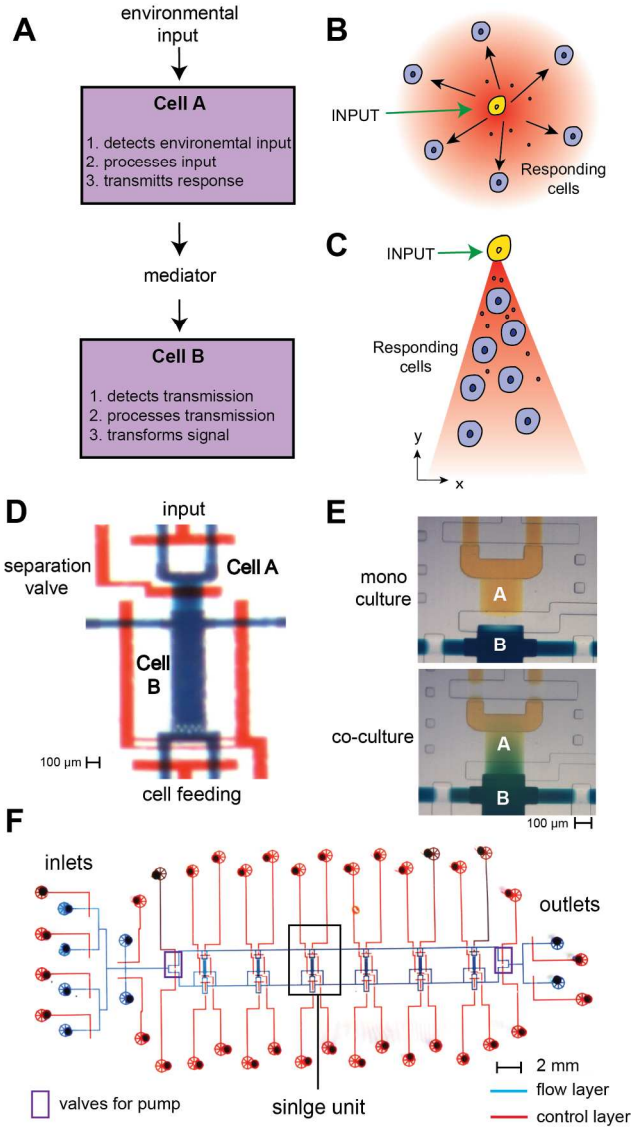

Figure 1:

A) Typical one-way intercellular communication scenario, which we implemented *in vitro* in the current study. An environmental input like a pulse of pathogenic LPS is detected and processed by a first responder Cell A (i.e. a macrophage), and the information is transmitted to resident tissue cells through secreted mediator molecules like cytokines. Cell B transforms this signal into a gene expression program.
(B) First responder cells sense pathogen inputs locally, and transmit signals radially to a dense population of neighbouring cells via secreted molecules.
(C) A one-dimensional geometry was used for technical implementation of one-way signal transmission on chip, which simplified cell manipulation and isolated signal delivery to first responder cells. A single macrophage is cultured in isolation, and responder cells are placed nearby in a linear arrangement.
(D) Drawing of a single experimental unit that establishes the signalling arrangement described in C. First responder cell (Cell A) is cultured in chamber A in isolation. Secondary responder cells (Cell B) are cultured in an adjacent chamber separated by a membrane valve. Each chamber can be addressed independently using different fluid inlets, and cell-cell communication can be established by opening the separation valve.
(E) Different culture modes on chip. In the monoculture the separation valve is closed. Co-culture mode allows cell-cell communication via secreted factors.
(F) Up-scaled device with 6 independent experimental units. The violet boxes show the four valves for an on chip peristaltic pump (scale-bar= 2 mm).

Materials and Methods

Device design and fabrication

We designed, produced and implemented our device according to our standard protocol, which is reported elsewhere [25]. Briefly, we designed our two layer devices using AutoCAD (Autodesk Inc., San Rafael, CA, USA). The designs were then printed on transparencies at 40 kdpi resolution (Fine Line Imaging, Minneapolis, USA). Moulds for PDMS casting were produced using standard soft-lithography. The channel network of control as well as flow layer was produced with SU-8 3025 (Microchem, Westborough, MA, USA) on silicon wafers. For the flow layer we used AZ-50X (AZ Electronic Materials, Luxembourg) at valve positions. Both resists were spun to a height of 25 µm. For the flow layer, 72 g of PDMS (10:1; polymer:catalyst) was mixed, de-bubbled and poured over a TMCS treated silicon wafer. The PDMS was then cured for 60 min at 80 ºC. Inlet holes were then punched on the cured flow layer, which was then plasma treated and aligned to the flow layer that was spun at 2300 rpm with 5 g of PDMS (10:1). After a 2 h thermal bonding the holes for the control layer were punched and the chip was bonded to a PDMS coated coverslip and cured for 12 h at 80 ºC.

Chip control and operation

Our general purpose chip set-up and control apparatus is described in detail elsewhere [25]. In brief, our on-chip valves are controlled via external solenoid valves (Festo, Dietikon, Switzerland) that are managed through a custom written LabVIEW software (National Instruments, Austin, USA). The software allows us to automate experiments. To set up a chip, we first connect the PDMS chips to the solenoid valves via DI-water filled TYGON tubing (Milan, Satigny, Switzerland). The pressure is then slowly increased to 1.2 bar. Valve closing is checked visually. Afterwards, the flow layer is connected to waste and a container filled with PBS. Applying pressure (0.5 bar) to the PBS container but not the waste creates a pressure difference and therefore fluid flow. The flow layer gets then fully filled with PBS. In order to debubble the chip, outlet valves are first closed and air is fully pressed out through PDMS while PBS remains in the chip. The supply channels were then flushed with 10mg/mL pluronic acid (Millipore, Zug, Switzerland) for 3 min to prevent cell adhesion, followed by a 30 wash with PBS. Afterwards the cell culture areas are coated with fibronectin (c=50 µg/mL, Millipore, Zug, Switzerland) for 60 min to ensure cell adhesion. PBS or medium then is flown to replace the excess fluid in the cell culture chambers.

Cell culture and live-cell microscopy

We used NIH 3T3 $p65^{-/-}$ cells with a p65-dsRed reporter as well as an H2B-GFP nuclear marker for tracking and analysis of NF-κB nuclear localization. These cells are cultured in DMEM medium. To seed cells into the chip, cells are harvested at 80% confluence with trypsin from a dish. Half of the harvested cells are then resuspended in 1 mL of fresh DMEM and connected to the chip via pressurized vials. The seeding procedure is described in the Result and Discussion section (Figure 2A). RAW 264.7 macrophages $p65^{-/-}$ with a p65-GFP reporter gene as well as H2B-dsRed nuclear marker are used as signal transmitting cells. These cells are harvested at 75 % confluence with Versene. The harvested cells were split, and resuspended (1:10) in fresh DMEM medium. 1 mL of the suspension was connected to chip and seeded in the signal transmitter chamber.

To maintain long term culture on chip, a custom-made cell-culture and incubator system is used (Life Cell Imaging Service GmbH, Basel, Switzerland), consisting of a box surrounding the microscope where the temperature is kept constant at 37 ºC. To maintain 98 % humidity and 5 % CO_2 the PDMS chip is covered with a stage-top-incubator connected to humidifier and gas exchanger. For macrophage stimulation, we used ultrapure LPS (InvivoGen, San Diego, USA) at a concentration of 50 ng/mL in cell culture medium. For blocking secreted TNF, we used anti-TNF antibody (Genwaybio, San Diego, USA) at a concentration of 100 µg/mL.

Image acquisition

For image acquisition a Nikon Ti-ECLIPSE microscope with an automated translation stage and a digital CMOS camera (ORCA-Flash 4.0, Hamamatsu, Japan) was used. The stage and image acquisition was controlled via the microscope's software. (NIS Elements). A 4x objective (NA=0.1, WD=16500 µm) was used for setting up the chip, and image acquisition during experiments was realised by using a Nikon Plan Fluor 20x objective (NA=0.5, WD=82000 µm). The microscopes large image tool was used to stitch images. First a bright field image (it=1 ms) is captured, and then ds-Red channel is captured (it=800 ms) followed by GFP channel (it=300 ms).

Data analysis

Images and data were analysed using MATLAB (Mathworks, Austin, USA). For tracking single cells, a custom written tracking algorithm was applied as described before [8]. The algorithm extracts single traces of nuclear NF-κB localization and reports cell positions as well. In brief, the nuclear area in each image is identified via the fluorescent nuclear marker GFP, and then the nuclear mean intensity of the p65-DsRed marker is measured, and plotted as a function of time.

Results and Discussion

Device design for spatially localized stimulation of single cells and co-culture

We designed a microfluidic device that allows local stimulation of a single signal-transmitting cell independent of neighbouring cells, which also enables co-culture of the stimulated cell with other cell types. This ensures that each cell type is addressed independently, and signalling dynamics is not disturbed before exposure of cells to each other. In our device, cell-cell communication is mediated by diffusion of cell-secreted factors, and the position of signal sending and receiving cells is

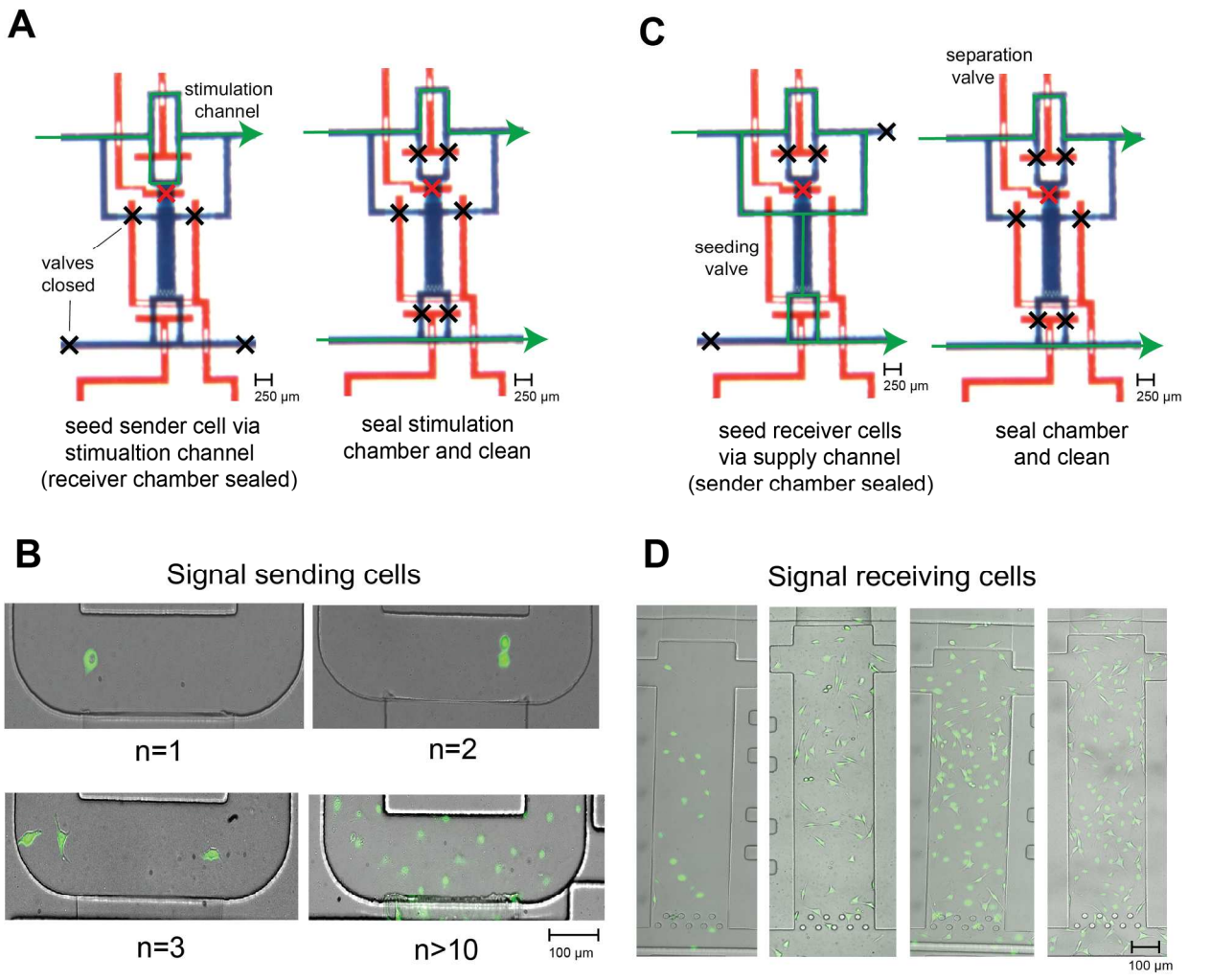

Figure 2:

(A) Sender cells (i.e. macrophages) are seeded via the upper stimulation channel (in green). After trapping cells in the sender chamber, supply channels are cleared from remaining cells by washing with fresh medium for few seconds while the seeding valve is kept closed. Crosses indicate closed valves.
(B) The experimenter can control the number of sender cells. Here different numbers of macrophage cells seeded in sender chamber are shown.
(C) Receiver cells (i.e. fibroblasts) are seeded in the receiver chamber by flowing cells from the upper supply channel through to the lower supply channel. After the desired densities of receiver cells is reached, the cell culture chamber is sealed by closing valves and the supply channels are cleared from remaining cells by flushing with fresh medium.
(D) We can seed different densities of receiver cells. Density can be controlled by input suspension density. Further an iterative seeding process (seed, attach, repeat) allows fine tuning of cell density.

fixed by device geometry. If needed, the secreted factors can be flown over the second population by use of an on-chip peristaltic pump.

In vivo, the signal transmitting cells are surrounded by other tissue cells and transmit their signal radially (Figure 1B). Spatially and temporally controlled stimulation of such a cell in the middle of other cells is a challenge. We reduced this 2-D problem to a 1-D problem by arranging the communication chain in a signalling axis that allows us to stimulate the signal-transmitting cell at will, and to send the signal in one dimension along a signalling axis by opening a separation valve (Figure 1C). The design simplicity of the single experimental unit shown in Figure 1D allowed us to easily up-scale our chip with six independent copies of the same unit design (Figure 1F) in order to run different experiments with various conditions. This design is suitable for further up-scaling by using a denser arrangement of chambers to realize high-throughput devices. Our system can operate with very few number of cells, which is an important advantage if rare cells from primary tissue samples are the object of investigation.

As shown in Figure 1D the single unit consists of two culture chambers (250 µm ×1200 µm × 25 µm, and 250 µm × 400 µm × 25 µm) that are separated by a reversible separation valve that allows quick switching from mono- to co-culture conditions (Figure 1E). Further we can maintain the spatial

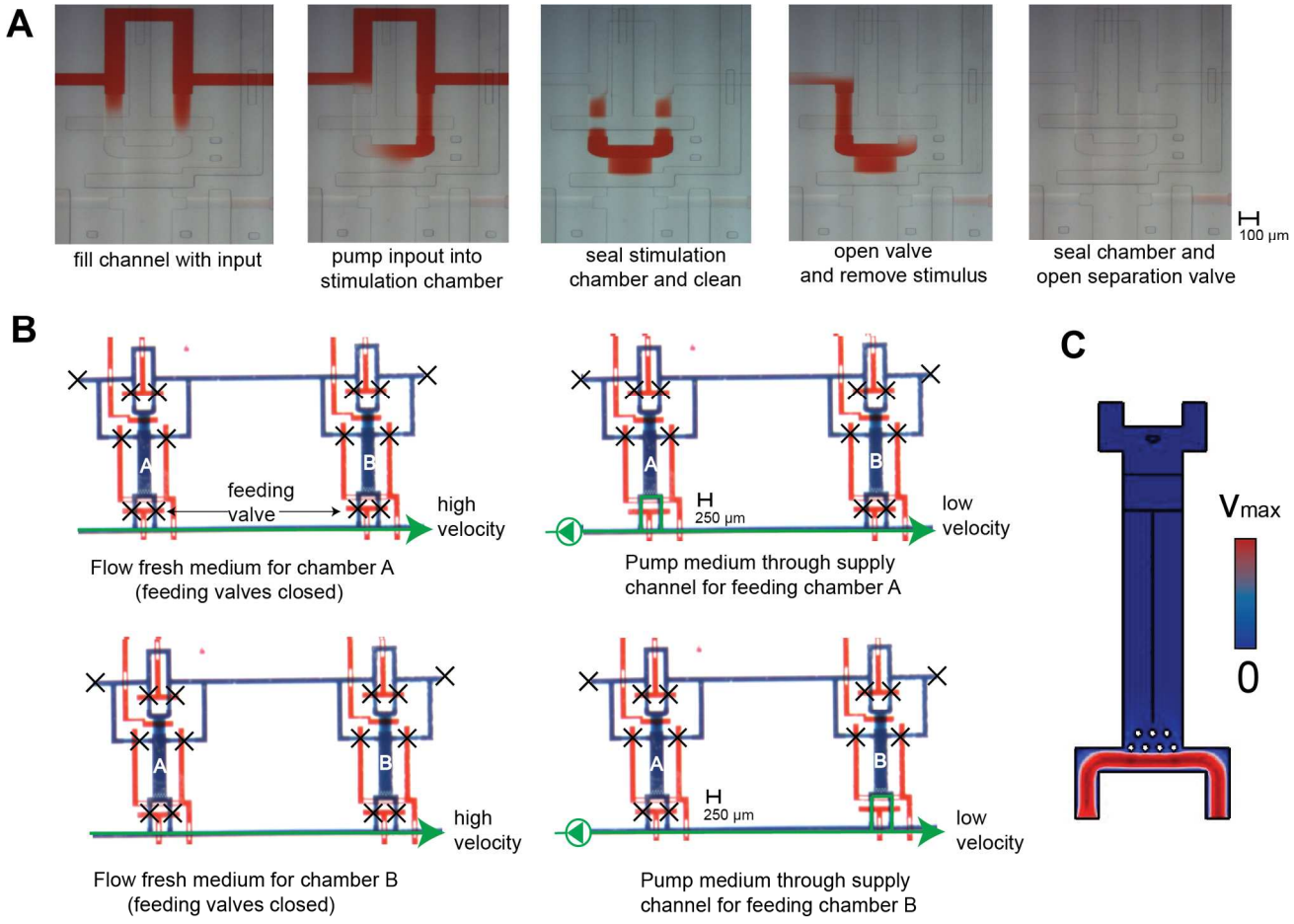

Figure 3

(A) Stimulation starts by filling the upper flow supply channel with the stimulus. Next we gently pump the stimulus into the sender chamber. We then close the stimulation valve and wash away the stimulus in the supply channel. After the stimulation time is over the sender cell chamber is washed until the chamber is cleared from the stimulus.

(B) Cells are fed via diffusion from the lower supply channel. First, we flush the supply channel with fresh medium for 5 seconds while the valves separating cell culture chambers from supply channels are kept closed. We then open a cell culture chamber while the neighbouring chambers are still closed to avoid cross-contamination. We then pump slowly fresh medium to the edge cell culture chamber for 25 seconds at a frequency of 10 Hz. The chamber is then disconnected from the supply channel by closing the valve and waste products are removed by flushing fresh medium for 5 seconds. The next chamber is then fed as before. Once all chambers were addressed, the procedure repeats.

(C) We simulated the influence of the feeding flow to the cell chamber in COMSOL. We used the same chamber geometry and 100 [µm/s] as input flow velocity. The heat plot indicates that flow does not reach inside the chamber and cells will not be affected by flow.

position of the cultured cells in a geometrical controlled fashion, which is advantageous for computational model fitting. Since cell-cell communication via paracrine signalling in tissue is a process primarily based on diffusion, we decided to culture our cells in a flow free environment, preventing any active fluid movement to influence the communication environment or wash away secreted signalling molecules. We therefore placed at the bottom as well as at the top of the culture chamber supply channels that allow us to feed cells with fresh medium with diffusion, and remove waste products. These channels act as local source/sink for culture media. They can be seen as a microfluidic analogue of a nearby blood vessel, as suggested by Yde et al [26].

Setting up the co-culture experiment

To run a co-culture experiment, we first coat our device with fibronectin or poly-*L*-lysine. In order to avoid cells sticking to the supply channels, we coat them with pluronic acid (10 mg/mL). Figure 2 shows how cells are seeded for the co-culture experiment and their final arrangement. We flow in the signal transmitting (sender) cells – cells that will see the local external input – through the top supply channel and leave the stimulation valve open. We keep the valves at the bottom supply channel closed. Sender cells only flow through the upper channel. By using low concentration of cells in the input suspension (2000 cells/mL) we can quickly trap the desired number of cells in this small chamber by opening and closing

the stimulation valve (Figure 2A). This manual seeding step enables us to seed different number of sender cells (Figure 2B), from a single cell to tens of cells, without implementing different fluidic designs for each condition. Once we are satisfied with the sender cell(s) seeded, we close the stimulation valve and wash the supply channel with medium to remove all of the remaining cells.

The receivers – cells that become activated by stimulated

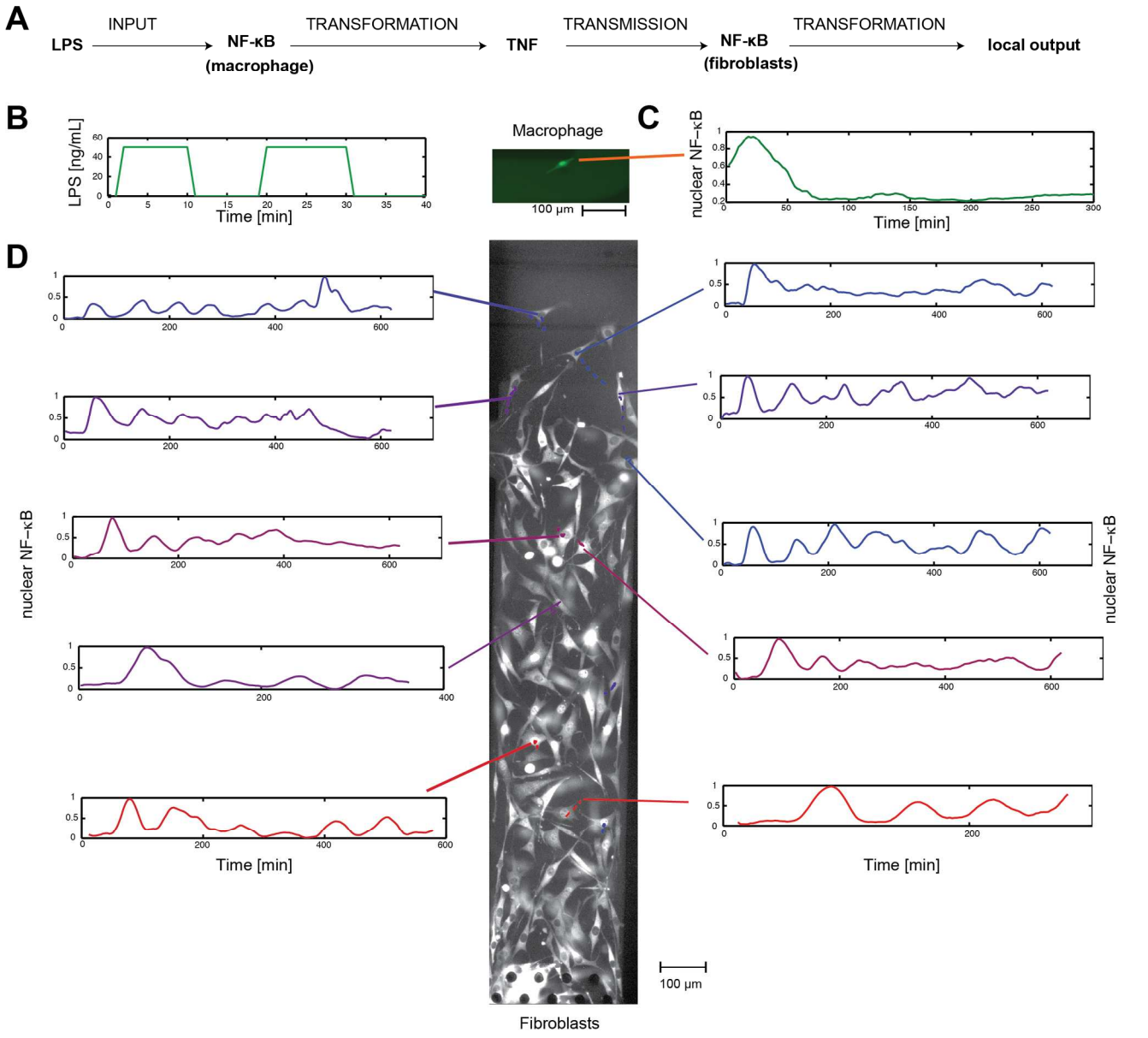

Figure 4

(A) One-way communication chain mimicking an inflammatory process. LPS acts as initiator on macrophages that in turn activate NF-κB signalling. This input is then transformed to a TNF secretion response and transmitted to fibroblasts. These fibroblasts transform TNF information via a NF-κB signal to a local output.
(B) We locally stimulated a macrophage with two 10 minute pulses of 50 ng/mL LPS, with a 10 minutes break in between, before exposing the macrophage to the fibroblasts.
(C) Macrophage NF-κB time course upon LPS stimulation. The picture shows the LPS activated macrophage, since the GFP labelled p65 marker is accumulated in the nucleus.
(D) Single-cell map of fibroblast NF-κB responses with respect of time and space. The secretion product of the macrophage (mainly TNF) initiates sustained and spatially patterned signalling in these cells. The dashed lines represent the nuclear locations of the cells during the experiment. The picture shows the DsRed labelled NF-κB (p65) of fibroblasts. Cells are activated if the p65 accumulated in the nucleus. Otherwise it remains in the cytosol. Mean nuclear p65 intensities are normalized to the maximum of the highest peak.

cells – are flown in from the top supply through the cell culture chamber to the bottom supply channel (Figure 2C). In order to avoid receiver cells sticking in the sender cell area, we implemented a connection from the upper supply channel to the cell culture area that is controlled by the seeding valve. After cells are seeded, we close the seeding valve and flush the supply channels with medium to remove all remaining cells in the supply channel.

The density of the receiver cells in the chamber can be controlled by using different cell densities in the seeding suspension, and in case of adherent cells repeated seeding cycles can be used as well (Figure 2D). Once we have a certain cell number in the chambers, observed by real-time microscopy, we let the cells adhere and apply the seeding cycle a second time, until the desired density is reached. Note that every single unit of this version is independent from the other with respect to conditions. We keep the separation valve closed during the whole process, to avoid cell-cell communication.

Single-cell stimulation and co-culture

We aim to locally stimulate the sender cells without affecting the receiver cells, which is achieved by having the separation valve between the two cultures closed. As shown in Figure 3A and Supplementary movie 1 we fill the top supply channel with the stimulus (i.e. bacterial LPS), then pump the fluid through the sender chamber by opening the stimulation valve and incubating the sender cell in this environment. While the input is acting on the sender cell, we can wash the supply channel or stimulate cells in another chamber in the meantime. Once the stimulus time is over (~10 min), we wash away the stimulus with fresh medium (~1 min), and let the sender cell incubate and secrete its signalling molecules. Most cells secrete minimal amounts of signalling molecules during the stimulation time (for example, TNF secretion peaks several hours after LPS stimulation), and the removal of these molecules has minimal impact in the total amount secreted by the sender cells [27].

As mentioned before, we culture our cells in a flow-free diffusion-based environment. We bring fresh medium via the supply channels to the chamber and fresh nutrients can diffuse from there to the cell culture area. Also waste products and signalling molecules are locally removed from the microenvironment, like in a blood vessel. Since we have six chambers in parallel and these chambers do share the same supply channel, cross-contamination has to be avoided. To fulfil this goal, we apply a flow-switching mode that makes use of fast flow and slow diffusion, as previously reported [28,29]. The flow cycles are shown in Figure 3B. We first replace the fluid in the supply channel with fresh medium. Then we stop the flow and open the valve separating the bottom supply channel with the cell culture chamber. We use four on-chip valves (Figure 1D) to form an on-chip peristaltic pump and pump slowly the fluid into chamber. Typically, we replace the medium for 5 seconds and pump for 25 seconds at a frequency of 10 s^{-1}. This switch between fast replacement of the medium in the channel and bringing fresh medium slowly to cells helps us to avoid flow over the cells. We simulated the influence of the slow flow in the chamber (Figure 3C). These simulations reveal, that the flow remains mainly in the supply channels and does not reach the cell culture area at small flow rates used in this study.

An LPS stimulated macrophage spatiotemporally controls NF-κB activation in fibroblasts

The NF-κB pathway is a major innate immune signalling network that recognizes pathogenic or self-secreted signalling molecules. Upon pathway activation, the inhibitors of NF-κB are degraded, allowing NF-κB transcription factors (like p65) to translocate to the nucleus and activate response genes. These response genes regulate cell physiology, and lead to further secretion of signalling molecules to establish cell-cell communication and ultimate removal of pathogenic cells. Previously, time-dependent analysis of NF-κB activity in single-cells enabled quantitative and accurate understanding of immune signalling and allowed its comprehensive modelling [8,9,30,31]. Prior to this work, however, the spatial aspects of NF-κB signalling have been addressed in very few studies [13,26,32], and one-way communication between signal sending and receiving cells has not been studied in a controlled way due to technical limitations in generating localized signals within co-culture conditions.

Here, we used our device to implement a co-culture scenario that mimics the initiation of an inflammatory process induced by bacterial infection. As local environmental input we used LPS, a product emitted from the cell wall of gram-negative bacteria like *E. coli* [33]. This LPS input acts on the macrophage, a specialized first responder cell, that in turn activates NF-κB signalling through binding of LPS to the TLR4 receptor [34]. As a response, the macrophage produces and secretes the inflammatory cytokine TNF, which is sensed by nearby tissue cells, in this case 3T3 fibroblasts. These fibroblasts will then activate NF-κB signalling via TNF/TNFR binding (Figure 4A). Through ELISA measurements we have ensured that our macrophage cells do not respond to self secreted TNF, and that our fibroblast cells do not produce TNF upon NF-κB stimulation (data not shown), which allowed us to create a one-way (macrophage to fibroblast) communication scenario.

In these experiments, we seeded a single macrophage in the sender chamber and placed a dense colony of fibroblasts in the receiver chamber below, each expressing a fluorescent protein (GFP or DsRed) fused to p65, the main subunit of NF-κB in mouse cells. The macrophage was then stimulated with two 10-minute pulses of LPS (c=50 ng/mL) with a 10-minute break in between (Figure 4B), simulating repeated exposure to bacteria. The LPS was then washed away for 1 minute and the separation valve was opened, allowing secreted TNF to diffuse over the fibroblast colony and activate NF-κB. The fluorescent fusion proteins in our cells allowed us to measure NF-κB activity (cytoplasm-nuclear translocation) in real-time using automated live-cell microscopy (Figure 4C). To ensure that the observed

signal is not due to left-over LPS in the top chamber, we filled the top chamber with LPS and washed it away for 1 minute as in signalling experiments. When this chamber was exposed to the fibroblasts no activation was seen, showing that LPS is efficiently washed away from the macrophage chamber (Supplementary Figure 1). To test if TNF is the main secretion product that activates NF- κB in our fibroblasts, we stimulated a macrophage with LPS and co-cultured it with fibroblasts with TNF-antibody added to the culture medium. Compared to the experiment without TNF-antibody, we observed no NF-κB activation in fibroblasts, since the antibody captured all the TNF molecules in the medium (Supplementary Figure 2). This shows that TNF is the main NF-κB signal mediator secreted by the LPS activated RAW macrophage. Further, we have not observed cell cycle dependent oscillations in the fibroblast cells. We also co-cultured one macrophage with a population of fibroblast without LPS stimulation, but did not observe spatially organized signal activation. Nevertheless, one or two cells showed weak NF-κB activation (Supplementary Figure 3), which can arise spontaneously among dense cultures [35].

Our fibroblasts have a nuclear marker (H2B-GFP) that allows us to automatically track them using live-cell-microscopy. Using our chip, we were able to establish the one-way communication scenario described above and measure NF-κB dynamics in both signal sending and receiving cells, and mapped the spatiotemporal distribution of single cell immune response to bacterial infection for the first time. We observed an NF-κB activation pattern propagating from the signal sending macrophage into the fibroblast population (Figure 4D, Supplementary Movie 2). The macrophage emitted immune signal reached most of the fibroblast cells, with farther away cells showing a time-delayed activation profile and fewer NF-κB oscillations. Since NF-κB dynamics control gene expression in fibroblasts, one can expect to see differential target gene expression in the responding fibroblast population depending on their distance from the macrophage [8]. This observed spatiotemporal signalling pattern demonstrates that the cytokine secretion time-course of one single macrophage cell can activate and control over 100 single fibroblast cells that are up to 1 mm away from the secreting cell. The NF-κB oscillations lasted up to 10 hours in some fibroblasts, showing that a brief (20 minute) exposure to a pathogenic signal like LPS can induce a long-term inflammatory response in the nearby tissue cells.

Conclusions

We developed a novel automated microfluidic co-culture device and live-cell imaging system that allows us to address questions in signal transduction with respect to space and time. We can locally induce a chemical signal in single immune cells and measure how secondary signals emitted by activated immune cells propagate in space through a population of signal receiving tissue cells. Traditionally, co-culture experiments are conducted as population-population [36,37,38] or pairwise single-cell interaction [39,40], but we establish a signalling scenario between a single-cell and a dense population. Our microfluidic device does not require the integration of complicated systems like optical or magnetic tweezers for signal delivery [18,19] and can overcome introducing several genetic constructs to conduct spatiotemporal signalling [13].

We established macrophage/fibroblast co-culture experiments on our system and measured spatiotemporal signalling of NF-κB in single-cells. We simulated an inflammatory scenario on chip and found that a local LPS input is converted into spatiotemporal NF-κB activation profile. This device will allow detailed, precise and quantitative investigation of how inflammation is controlled by gene network dynamics, and how gene expression patterns are established in inflamed tissue. Together with mathematical modelling, we will be able to model spatiotemporal aspects of immune signalling and understand innate immunity better, particularly with respect chronic diseases, and confirm model predictions experimentally using this simple yet effective platform.

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† Electronic Supplementary Information (ESI) available: [1 Supplementary Information Document with 3 figures, 2 movies]. See DOI: 10.1039/b000000x/

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