Microfluidic stickers for cell- and tissue-based assays in microchannels†

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Difficulties in culturing cells inside microchannels is a major obstacle for the wide use of microfluidic technology in cell biology. Here, we present a simple and versatile method to interface closed microchannels with cellular and multicellular systems. Our approach, based on microfluidic stickers which can adhere to wet glass coverslips, eliminates the need to adapt cell culture conditions to microchannels and greatly facilitates the methods required to position cells into microcircuits. We demonstrate the simplicity and efficiency of the method with HeLa cells, primary cultured neurons and *Drosophila* tissues.

Introduction

Recent studies on bacterial population dynamics,[1] yeast mating,[2] neutrophil chemotaxis[3] or embryonic development[4] have confirmed the potential of microfluidics for quantitative cell biology. However, despite these successful experiments, the use of cell-based microfluidic assays is still limited to a relatively small number of laboratories. This is largely due to the difficulties in culturing cells in microdevices. In particular, cells or tissues have to be loaded into microcircuits and grown for days, often leading to clogging of the channels. Also, culture conditions (especially the gaseous and nutriment exchanges) need to be adapted to a closed microenvironment,[5] with a flow rate sufficient for nutriment transport but slow enough to prevent shear effect on the cellular growth.[6]

Here we propose a new method to overcome these difficulties for short term experiments on live cells. We take advantage of microfluidic devices made of UV-polymerizable material, which can tightly adhere on wet surfaces without any substrate modification. Using these so-called microfluidic stickers,[7] we have developed a simple, rapid and generic approach to interface microcircuits and cell biology assays. Instead of being cultured in closed preformed microcircuits, cells are grown on regular glass coverslips using standard protocols. Subsequently, channels are placed over the cells and tightly sealed onto the coverslip, see Fig. 1. The resulting microdevices are compact and stiff, with a resin thickness ranging between 10 and 200 μm. They are made of an optical adhesive which is transparent and has a very low auto-fluorescence, allowing for imaging techniques with sensitivity down to the single molecule level. By decoupling the cell culture conditions from the constraints of a microfluidic environment, our method markedly simplifies and increases the range of applications of microchannel-based assays in cell biology. In the following, we briefly recall how to make microfluidic stickers. We then describe in details how to stick them on cultured cell substrates. The simplicity and efficiency of the method is demonstrated by imposing controlled microflows over HeLa cells and cultured primary neurons at different stages. We also use microfluidic stickers to position larger samples such as *Drosophilae* tissues in microchannels.

Experimental

Preparation of microfluidic stickers

Microfluidic stickers are made by soft imprint lithography on a PDMS master mold using UV-polymerizable materials.[7–9] We here briefly recall the protocol detailed in ref. 7. A negative

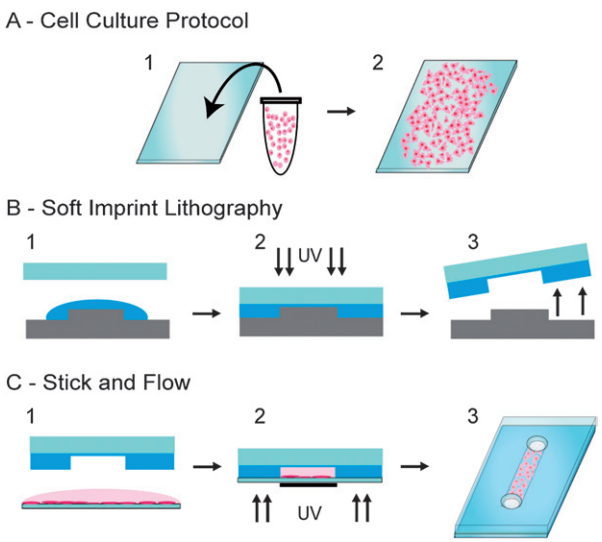

Fig. 1 Cells are grown using standard protocols on a glass coverslip (A1 and A2). The microfabrication of the sticker is made just before experiments (B). A drop of resin (blue) is molded between a glass slide (light blue) and the invert replica of the device (gray, B1), exposed to UV for crosslinking (B2) and eventually detached from the mold (B3). The sticker is aligned on the coverslip (C1) and then sealed with gentle mechanical pressure and short UV exposures (C2). A mask (in black) is placed to block direct UV expose on the cells in the observation channel.

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PDMS stamp is made by replica molding of a photo-lithographied SU-8 mold. A drop of UV curable resin (NOA 81, Norland Optical) is deposited on the PDMS stamp (Fig. 1 B1). Less than 200 μL of resin are sufficient to form a 24 × 36 mm^2 microcircuit. A microscope slide is then gently pressed on the resin. For subsequent fluidic connection we use glass slides with holes drilled with a sandblaster (Texas Airsonics). A uniform 365 nm illumination (LC8 lamp, Hamamatsu) for 30 s at 7.5 mW/cm^2 through the glass slide forms a stiff micropatterned layer of resin (Fig. 1 B2). Since oxygen inhibits the free-radical polymerization leading to the formation of the polymer network, the permeability to gas of the PDMS ensures that an ultra thin superficial layer of liquid remains uncured.[9,10] As a result, the PDMS stamp can be easily removed and the free surface of the resin retains adhesive capabilities. The PDMS stamp can be used to make approximately 20 devices and just needs an isopropanol/acetone wash (90/10 v/v) before a new molding.

Bonding on substrates with adherent cells

Using a culture coverslip as substrate, various adherent cultured cells can be inserted in microchannels without any modification of well established culture protocols (Fig. 1 A). The device is immersed in culture media and aligned on the coverslip. The sealing is completed by gentle mechanical pressure for approximately 10 minutes to enforce the conformal contact between the sticker and the wet glass surface. The two surfaces can then be covalently bonded by a couple of short (~5 s) UV exposures (Fig. 1 C). During this second illumination step, the cells can be locally protected from the UV light by a UV-blocking mask. Finally, the device is connected to external flow or pressure regulators with adapted fluidic connectors (UpChurch Rheodyne). Such a device could support an inlet pressure of typically 1 bar. The last additional UV exposures are not necessary for flow experiments which do not require pressures above 100 mbar. In this case, the method allows for reversible bonding. The entire procedure – sticker fabrication and bonding – takes around 30 minutes. Moreover, our method does not require pretreatments of the glass surface for the bonding step. Consequently conventional coatings (such as fibronectin, poly-lysine or laminin) are preserved during the whole procedure and do not impede the sealing of the device. Note that a few other methods have recently been proposed to interface cell cultures and microdevices.[11,12] However, the associated sealing methods strongly constrain the chemical and physical properties of the channels material. The present method based on microfluidic stickers circumvent these limitations.

Results

Biocompatibility and cell survival

To first demonstrate the biocompatibility of microfluidic stickers and their interest for cell biology, cultured HeLa cells were grown on standard coverslips and placed in a 100 μm high–1mm wide channel. The sealing procedure was done in Leibovitz L-15 media complemented with bovine serum albumin and DNAse I. Standard cell culture media was then flowed in the channels, and the device was placed in a 37 °C, 5% CO_2 incubator. Cells showed good viability in the incubator and remained healthy in the microcircuit over hours of experiments at room temperature. Staining with tryptan blue indicated 80% cell viability after 8 hours, with most of dead cells near the channel walls. Importantly cells could be prepared with confluence conditions ranging between 10 and 90% (Fig. 2 A and B). No clogging of channels was observed, even with channels width down to 50 μm. We also stress the excellent control of the flow over the cultured cells. As shown in Fig. 2 B and supplementary Fig. 1,† subcellular hydrodynamic focusing of chemicals can be achieved in the microfluidic sticker.

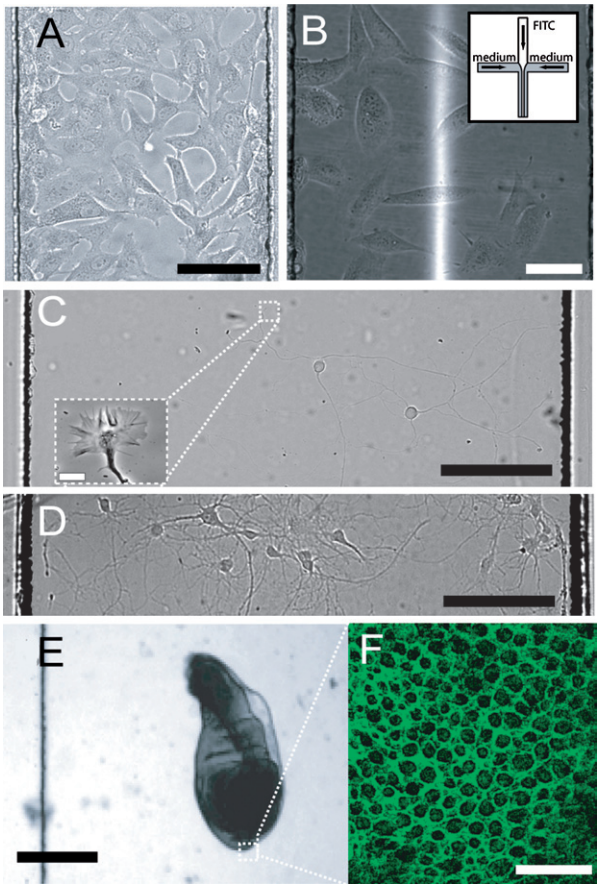

Fig. 2 (A) Microfluidic sticker sealed onto cultured HeLa cells. Cells are preserved in the channel, even at high confluency. Scale bar, 50 μm. (B) Subcellular hydrodynamic focusing of a fluorescent dye (Rhodamine B) over Hela cells. (C) Microfluidic sticker sealed onto dissociated DRG neurons at 1 day *in vitro* (DIV). Scale bar, 200 μm. Close up of an axonal growth cone showing lamellipodial and filopodial motility (see supplementary movie 1†). Scale bar 10 μm. (D) Microfluidic sticker sealed onto cultured dissociated hippocampal neurons at 7 DIV. Scale bar, 200 μm. (E) Dissected wing imaginal disk of a *Drosophila Melanogaster* pupae mutant expressing *myo*II-GFP in microchannel. Scale bar, 200 μm. (F) Confocal images of the myoII-GFP (in green) in the region indicated by a dashed line were taken in the lateral *x*–*y* plane. Scale bar, 10 μm.

We next inserted neurons inside microchannels. Primary cultures of neurons are notoriously difficult to grow and maintain in microchannels. The growth of neurites is strongly affected by the confined linear geometry of the circuit,[13] often leading to abnormal growth or early degeneration. It also requires up to several weeks to structure a mature network with functional synapses. As a result, the study of cultured dissociated neurons in

microcircuits has so far remained very limited to particular channel and chamber designs.[14,15] We have successfully used stickers of various geometries (T junctions or flow focusing, both with a 1 mm width) with primary cultures of mammalian neurons in two distinct regimes of cell density and connections. We first used dorsal root ganglion (DRG) neurons at 1 day *in vitro* (DIV), a developmental stage at which individual neurons are isolated on the coverslip. DRG neurons remained attached to the poly-lysine/laminin coated substrate during the whole procedure and growth cone integrity and motility were maintained in the microchannel (Fig. 2 C and supplementary movie 1†). We also successfully repeated the experiments with cultured hippocampal neurons after 7 DIV (Fig. 2 D). At this stage, neurons are strongly connected and the network architecture was preserved in the channel after sealing the microcircuit. Note that performing this experiments in pre-formed channels would require growth of neurons with controlled culture conditions (nutriments concentration, gas concentration, temperature,…) inside the microchannel for seven days. This requires substantial adjustments of the standard culture protocols.

Implementation with tissues: millifluidic stickers

Our approach also allows for insertion of thick biological samples, such as tissues or explants. In this respect, a particular advantage of the NOA resin is its high Young modulus (~1 GPa) that allows preparation of channels with large aspect ratio and no deformation. Wing imaginal disks were dissected from *Drosophila Melanogaster* pupae and deposited onto the resin substrate. The channel was then closed by sealing a glass coverslip above the resin (Fig. 2 D). More specifically, we used a tissue expressing GFP-tagged myosin II, a molecular motor dominantly localized at the plasma membrane (Fig. 2 E). Confocal images of the tissue in the channel indicate that its multicellular organization has been entirely preserved. These pictures also demonstrate the excellent optical properties of the NOA 81 resin (transparency and low auto-fluorescence).

Conclusions and perspectives

Microfluidic stickers provide a generic approach to routinely position cells into closed microdevices. Stickers are made of a biocompatible resin that tightly adheres on wet glass coverslips. Thereby, they can be placed on cultured cells of various types (immortal cell lines, primary cultures, tissues,…) and allow for a temporal and spatial control of their chemical environment. Compared to conventional techniques using preformed devices, stickers offer several important advantages: (1) they do not require adjustment of established culture protocols; (2) cells could be prepared with confluence conditions up to 90% without any clogging of channels; (3) a large variety of cell biology techniques such as immunolabelling, transfection, microinjection or RNA silencing, can be easily performed before placing the cells in the microcircuit; (4) the bonding of the stickers is compatible with standard surface coating or complex micro-patterning of the glass surface for cell research[16,17] or microarray applications.[18] In addition, the photocurable resin possesses excellent mechanical properties, allowing fast response to pressure change (supplementary Fig. 1 and supplementary movie 2).† It also has a very low auto-fluorescence that makes it compatible with ultra-sensitive fluorescence imaging.

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