FABRICATION OF AN OPTIMIZED MICROLITER CULTURE DEVICE WITH INTEGRATED OPTICAL pH MONITORING

P.P.M.F.A. Mulder1, M.J. Lopez-Martinez1, S. Demming2, S. Büttgenbach2, A. Llobera3, E. Verpoorte1

1Pharmaceutical Analysis, Dept. of Pharmacy, University of Groningen, THE NETHERLANDS
2Technische Universität Braunschweig, Institut für Mikrotechnik, GERMANY
3Instituto de Microelectronica de Barcelona-Centro Nacional de Microelectronica-CSIC, SPAIN

ABSTRACT

The objective of this work is to implement a new integrated microfluidic/microphotonic system for controlled small-volume (6.75 µL) cell culture. pH is monitored directly in the microliter-sized cultivation chamber using an integrated optical approach exploiting multiple internal reflection of light for increased detection sensitivity. Design of the cultivation chamber required special consideration to prevent interference of the adherent cells with the light beam.

KEYWORDS: Optical pH monitoring, Multiple-internal-reflection systems, Endothelial cells, Cell cultivation

INTRODUCTION

Monitoring the pH of cell cultures is key for maintenance of cell viability and metabolism. Phenol red is often added to cell culture medium as a pH indicator [1]. This dye undergoes a visible colour change from yellow (pH 6.4) to red (pH 8), detectable by eye in conventional culture vessels. Phenol red absorbs strongly at 560 nm (deprotonated form) and 450 nm (protonated form), making it suitable for non-invasive optical monitoring of pH in cell and tissue culture [1]. Continuous monitoring and control of microculture parameters is important, as cells can rapidly change their environment in a microchannel. We demonstrated previously that pH changes could be measured in cell medium in a PDMS-glass multiple-internal-reflection device similar to that shown in Figure 1 [2]. The top poly(dimethylsiloxane) (PDMS) layer of this device includes several features, such as a flow cell, grooves for alignment of optical fibers and two curved hollow mirror structures. The latter serve to reflect light twice through the chamber. The optical layer is sealed by a bottom glass chip. See reference [3] for more information about this microphotonic system.

We have used this device to combine cell cultivation and pH monitoring. The cells of interest here are human umbilical vein endothelial cells (HUVEC). Because these cells are adherent, they tend to grow on all surfaces of the flow cell, and can interfere with the propagation of the light beam through the device as a result. To reduce the adverse influence of cells on the optical measurement, two different devices have been considered in this study. The first is the original device with a flat glass bottom, while the second contains a cavity etched into the glass layer to localize cell culture to this region only.

![Figure 1: Top view of a multiple-internal-reflection device. Red circles represent seeded HUVEC. Yellow line shows the light path through the chamber (optical fiber grooves located at bottom left and top right corners) via two curved mirrors [3].](image)

EXPERIMENTAL

To make cavities in glass slides, Pyrex wafers were covered with photoresist AZ4562, exposed to UV light [600 mJ/cm²] and developed to create structures smaller than the flow cell chambers but with the same outer geometry [Figure 1]. The wafers were then etched with HF for 30 minutes, resulting in 50-µm-deep cavity structures.

To determine the effect of cells on the signal, cells were introduced to gelatin-coated glass-PDMS devices using hydrostatic pressure. Gelatin coating in the flat-bottomed device was accomplished by incubating the entire flow cell with 1% gelatin for 45 min. (The PDMS and glass layers were irreversibly bonded using oxygen plasma treatment prior to this step.) Gelatin was then removed and 0.5% glutaraldehyde was added to the device and left to crosslink the gelatin for 15 min. The device

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was then rinsed 5 times with PDMS, and left to stand filled with PBS for 15 min. All surfaces of this device were thus coated with gelatin. Gelatin was coated in the cavity of the second device design in much the same way, except that the coating procedure was carried out on the open glass cavity, by placing a PDMS stencil around the cavity to prevent leakage of gelatin over the whole slide [Figure 2]. Once coated, the PDMS stencil was removed from the slide. The slide was bonded with the PDMS device using oxygen plasma. Both types of devices were filled with medium, and 10 μL of cell suspension [1500 cells/μL] was added to the inlet reservoir. Unattached cells were removed after 1 hour by rinsing with medium.

Figure 2: Cavity glass slide was covered with a thin PDMS slab to prevent gelatin from depositing outside the cavity.

Figure 3: HUVEC grown in the microphoton device for 16 hours at 37°C and 5% CO₂.

For pH monitoring, a 230-μm-diameter fiber connected a light source [Leica KL1500 LCD filter optic illuminator] to the chip. The output fiber was coupled to a spectrophotometer [Ocean Optics HR4000]. The output of the spectrometer was connected to a computer. Medium without phenol red was used as a blank. Cells were introduced to the fully assembled chip. The cells were observed under a microscope after 16 hours of incubation [Zeiss, Axiovert 25]. After cultivation, cells were removed from the chip using trypsin/EDTA and medium was measured for 24 hrs. All measurements were performed at 5% CO₂ and 37°C in an incubator.

RESULTS AND DISCUSSION

Introduction of an adherent HUVEC culture (Figure 3) [4] to a flat-bottomed device resulted in a dramatic decrease in transmitted light compared to a chamber with no cells, a change which was unrelated to pH variation. All surfaces in the chamber had been coated with gelatin prior to cell seeding, and it was confirmed that HUVEC were growing on chamber walls directly in the light path. HUVEC were thus absorbing/scattering light and interfering substantially with the pH monitoring (Figure 4A).

Figure 4: Schematic cross-section of the device. A. Gelatin-coated glass/PDMS device in which cells [red circles] covered the surface and walls of the chip and interfere with the light beam [yellow line]. B. Gelatin-coated glass cavity /PDMS device with cells localized to the cavity surface.

A new culture device was designed with a 50-μm-deep cavity etched into the glass chamber bottom (Figure 4B, Figure 5). Care was taken to coat only this cavity with gelatin, so that the cell culture was restricted to the cavity and thus effectively isolated from the probe beam, so that pH changes could be monitored.

Figure 5: 50-μm-deep cavity etched in a glass slide with hydrofluoric acid.
Transmitted light intensity (560 nm, basic form of dye) was determined during cell cultivation in both the original and newly designed systems and normalized by dividing by intensities at 480 nm (I560/I480 in Figure 6). This latter wavelength is the isosbestic wavelength, where both the acidic and basic forms of phenol red have the same molar absorptivity and absorbance is thus proportional to the formal concentration of this dye (i.e. absorbance is independent of pH at this wavelength). A constant signal was recorded for the medium in contact with cells in the original glass/PDMS device (cell interference). However, the normalized intensity in the new “cavity” chip increases over time, indicating decreasing pH [1]. This is agreement with cultures in conventional flasks, which also exhibit a shift to a more yellowish colour over time, caused by exhaustion of the medium.

![Cultivation of HUVEC in time](Image)

*Figure 6: HUVEC were cultivated in an unmodified gelatin-coated glass/PDMS device [blue line] or gelatin-coated cavity chip [pink line]. There was no change in signal during cell growth in the old glass/PDMS device. However, there was an increase of signal for medium in contact with cells in the cavity chip over time (abnormally high data point at start of experiment is an artifact). Medium was incubated in the same chip after removal of the cells, yielding a constant signal [green line].*

**CONCLUSION**

The results indicate that introduction of a cavity in a glass slide substantially reduces cell interference on the signal compared to a flat glass bottom. Work is ongoing to ensure that the cell culture is truly localized and no longer affects the pH measurement. One concept involves introduction of a filter to horizontally isolate cell culture to a lower compartment while monitoring pH in the upper compartment. Once culture and monitoring conditions have been optimized, we will proceed with e.g. optical monitoring of metabolites.

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**CONTACT**

*P.P.M.F.A. Mulder, tel: +31-50-3633334; p.p.m.f.a.mulder@rug.nl*