BIOPHOTONIC LAB ON A CHIP WITH INTEGRATED SIZE-EXCLUSION MICROFILTERS FOR CELL PROLIFERATION MONITORING

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ABSTRACT

The extracellular pH monitoring on culture media is indicative of the metabolic activity of the cells. However, their precise determination is commonly limited by the presence of cells that interfere on the measurement. A biophotonic lab-on-a-chip (bioPhLoC) for the accurate monitoring of the extracellular medium pH without interference of attached/detached cells is here presented. The bioPhLoC comprises two identical microbioreactors (MBR) fluidically connected: (i) the retention MBR (RMBR), integrating 21 size-exclusion microfilters (3 μm high, 200 μm wide) regularly distributed and capable to efficiently entrap vascular smooth muscle cells (VSMC) (90% retention) and (ii) the monitoring MBR (MMBR), where the pH changes due to cell metabolism are determined at real time. With this configuration, the metabolic activity of VSMC, determined by measuring the acidification rate in the extracellular environment, was accurately determined without interference of attached/detached cells.

KEYWORD

biophotonic Lab on a Chip, cell proliferation monitoring, size-exclusion microfilters, pH control

INTRODUCTION

The accurate control of the extracellular medium pH has been demonstrated of key importance for cell culture. Extracellular pH changes are indicative of the metabolic activity of cells and the monitoring of this parameter has been already used to detect the perturbation of biological function of cells (e.g: ischemia/reperfusion injury) [1] or to evaluate the effects of drugs or toxins [2]. Besides, even short-time pH changes (minutes) have been proved to affect cell proliferation, for example modifying the synthesis of matrix macromolecules [3]. For this reason, extracellular pH should be continuously and accurately monitored during cell culture.

Electrochemical, ion-sensitive field effect transistor (ISFET) and optical-based systems aimed to measure pH changes have been developed last years. Electrochemical systems for pH sensing have been found to rely on complicated fabrication processes, chemical cross talk between sensors as well as flow-dependence and medium components fouling [4]. On the other hand, the electrical field generated by ISFET based systems has been claimed to affect cell physiology [5]. Optical systems represent the best alternative for being low cost, feasible for miniaturization, non-invasive and immune to electrical interference. In some of them, pH changes are measured by considering the presence of phenol red in the cell culture media (as could be Dulbecco's Modified Eagle Medium, DMEM), since it provides with colorimetric variations when the pH media shifts from the protonated (450 nm) to the deprotonated form (550 nm) [6]. Nevertheless, it was also demonstrated that the presence of the cell culture at the same region where the pH was determined caused significant errors [7]. To tackle this issue, an optical-based system with an architecture that allows the uncoupling and the simultaneous real time monitoring of the cell culture and the extracellular medium pH is here presented.

BIOPHOTONIC LoC DESIGN AND FABRICATION

The design of the bioPhLoC is illustrated in Figure 1A. It consists of two identical microbioreactors (MBR) connected by a common fluidic channel (interconnecting channel, 200 μm wide, 1.6 cm long) and containing optical elements (microlenses, air mirrors, self-alignment channels, etc.) for the continuous optical interrogation of the samples present in both reactors. The sole difference between them is the monolithic integration of 21 size-exclusion microfilters (3 μm high, 200 μm wide) homogeneously distributed around the retention MBR (RMBR) for cell entrapment.

The bioPhLoC was fabricated following a lithographic protocol already reported [8-9]. This protocol involves the fabrication of a two-level SU-8 master (one level for the size-exclusion microfilters and another implementing MBRs, microfluidic and microoptical elements), the replication of the structures with poly(dimethylsiloxane) (PDMS) and their bonding on a glass substrate. A final image of the system filled with crystal violet (only for validation purposes) is shown in Figure 1B.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

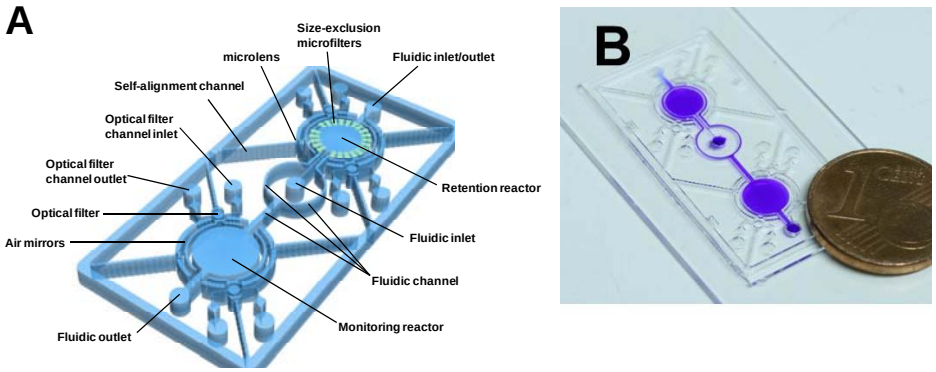

Figure 1: Schematic of the bioPhLoC with integrated size-retention microfilters (A) and photograph of the microchip filled with crystal violet dye (for visual fluidic validation)(B).

CELL TRAPPING

The concept of the bioPhLoC is demonstrated by inoculating rat thoracic aorta vascular smooth muscle cells (VSMC) re-suspended in DMEM under sterile conditions (600 μL containing 10^5 cells/mL) [9]. After the inoculation, the chip was connected to a NEMESYS syringe pump (Cetoni GmbH, Germany) at a constant flow of 100 μL/min to remove non-trapped cells (rinsing step). Cell trapping efficiency was evaluated by measuring the variation in the absorbance magnitude at 600 nm (Abs_{600}) with time. At this wavelength, the absorbance magnitude is directly proportional to the light dispersion produced by cells and thus, to cell concentration.

In the cell inoculation step, Abs_{600} increased in the retention reactor but remained almost constant in the monitoring MBR, MMBR (Figure 2A).

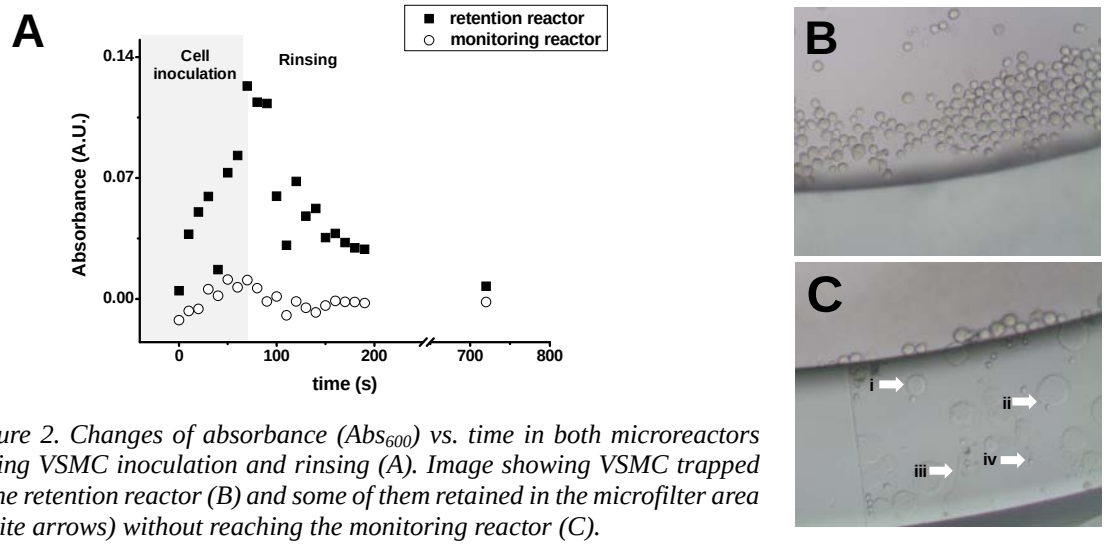

Figure 2. Changes of absorbance (Abs_{600}) vs. time in both microreactors during VSMC inoculation and rinsing (A). Image showing VSMC trapped in the retention reactor (B) and some of them retained in the microfilter area (white arrows) without reaching the monitoring reactor (C).

This is consistent with the fact that VSMC (around 8-10 μm diameter) remained mainly entrapped in the size-exclusion microfilters (3 μm high) integrated in the RMBR. Considering absorbance data, cell retention was determined to be close to 90 %. However, this value may overestimate the filter efficiency since some of the cells crossing the microfilter structure may not be measured in the MMBR. In Figure 2B, it is proved that a huge amount of cells were deposit in the bioPhLoC bottom after being trapped in the retention reactor. Nevertheless, Figure 2C shows some cells crossing the microfilter structure. These cells were under very stressful conditions and eventually degenerated and died. Cell membrane breakdown and the formation of small lipidic vacuoles are clearly observed in the figure. These cell fragments and vacuoles were too small to be detected in the MMBR thus overestimating the real filtering capacity.

pH MONITORING

After inoculation, the bioPhLoC was introduced in the incubator (37ºC, 5% CO_2) at a constant DMEM flow of 0.5 μL/min [9]. Cell proliferation was indirectly monitored in both MBRs by following the pH changes produced by cell metabolism with the pH indicator phenol red included in the culture medium.

Phenol red is a reversible indicator dye that in acidic conditions (below pH 6.4) absorbs in the blue region of the visible spectra (450 nm). Above pH 8, this dye is deprotonated and, as a result, undergoes a visible colour change to red, absorbing at the green part of the spectrum (550 nm). This behavior of the phenol red makes it suitable for non-invasive optical monitoring of pH at physiological levels in cell and tissue culture. pH changes caused by cell metabolism were simultaneously measured at both MBRs for comparison purposes. Concretely, the absorbance peak of the deprotonated form of phenol red was analyzed (Figure 3).

As shown, in both MBRs the Abs_{550} decreases with time due to the extracellular medium acidification produced by cell metabolism. However, some differences were observed when comparing retention and monitoring reactor. First of all, the absorbance magnitude between 600 and 800 nm randomly oscillated in the RMBR, whereas it was almost constant in the MMBR. Additionally, the variation of the Abs_{550} with time (inset in figure 3A) in the RMBR provided with a non-linear response due to cell attachment/detachment. Concretely, each cell detached from the substrate that crossed the light beam caused a random disturbance on the spectral response. In opposition, the same calibration at the MMBR (inset in figure 3B) presented the expected linear response, confirming the validity of the proposed twin biophotonic reactor.

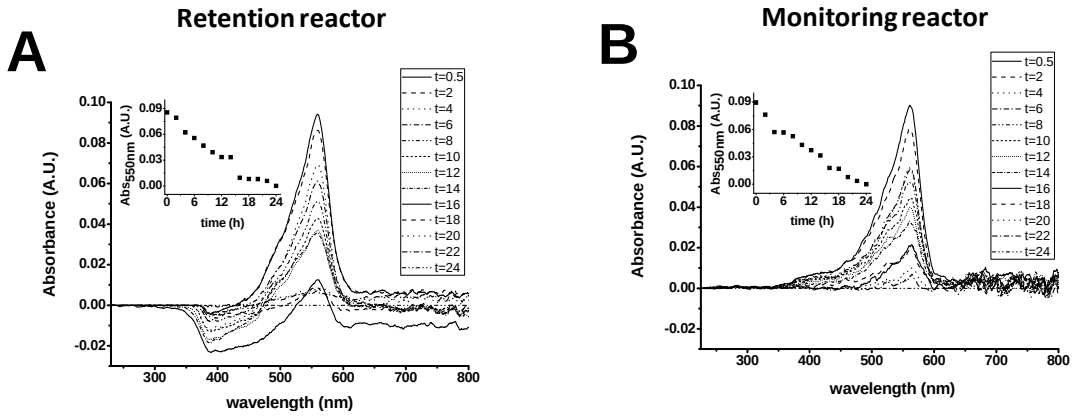

Figure 3. Variation of absorbance as a function of the wavelength in both the retention (A) and monitoring reactors (B). Inset in both figures shows the time variation of the deprotonated absorbance band of phenol red.

CONCLUSIONS

The present bioPhLoC demonstrates high accuracy in the determination of the extracellular pH in culture medium with a non-invasive, simple and fast optical measurement based on the change of colour of the pH indicator phenol red. This accurate pH measurement is performed in the monitoring reactor without interference of attached/detached cells that are retained in the filtering structure (90% trapping efficiency).

ACKNOWLEDGEMENT

The research leading to these results has received funding from the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013) / ERC grant agreement n° 209243. Dr. Xavier Munoz-Berbel was supported by the “Ramón y Cajal” program from the Spanish Government.

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