A two-compartment microfluidic device for long-term live cell detection based on surface plasmon resonance

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A two-compartment microfluidic device integrated with a surface plasmon resonance (SPR) interferometric imaging system has been developed for long-term and real-time cell detection. The device uses a porous membrane sandwiched between two chambers to obtain an exact medium exchange rate and minimal fluid shear stress for cell culture. The two-compartment device was optimized by COMSOL simulations and fabricated using Poly (dimethylsiloxane) elastomer replica molding methods. To confirm the capability of the microfluidic device to maintain the cell physiological environment over long intervals, HeLa cells were cultured in the device for up to 48 h. The cell proliferation process was monitored by both SPR and microscopic time-lapse imaging. The SPR response showed four phases with different growth rates, and agreed well with the time-lapse imaging. Furthermore, real-time detection of cell behaviors under different doses of Paclitaxel and Cisplatin was performed. The SPR responses revealed dose-dependent inhibitions of cell proliferation, with distinct drug action kinetics. Published by AIP Publishing. [<http://dx.doi.org/10.1063/1.4960487>]

I. INTRODUCTION

Long-term and real-time detection of dynamic cellular behaviors is of great interest in the life sciences; applications range from drug discovery and development, to pathological study, and cancer research.^{1–3} Cellular responses induced by drug stimulations usually persist for a long period. Moreover, the drug action kinetics is meaningful for drug sensitivity tests and personalized cancer treatment.^{4–6} For these reasons, the development of long-term and real-time methods to detect the dynamic cellular behaviors is of great interest.

Many techniques have been reported for cellular analysis. Conventional colorimetric methods involving MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assays obtain the cell viability information by quantifying cell numbers.^{7,8} However, these end-point assays cannot reveal the dynamic cellular response process. Recently, many non-invasive and label-free methods have been proposed to monitor cellular responses in real time.^{9,10} For example, conventional microscopy integrated with a microfluidic cell culture device enables long-term and dynamic visualization of cellular behaviors.^{11–13} Electrochemical impedance spectroscopy (EIS) is another versatile method for real-time and label-free cell detection. Various cellular events such as attachment, adhesion, growth, and apoptosis can be monitored by measuring the impedance changes induced by cellular behaviors on the surface of electrodes.^{14–16} Recently, EIS has been used to monitor drug-induced cellular events for cancer research.^{17–19}

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Surface plasmon resonance (SPR), which is the result of collective charge density oscillation on a metallic surface, is another sensitive approach that has been applied for cell detection. SPR transforms the change in refractive index induced by cellular activities in the SPR evanescence field into the shift of the resonance angle and wavelength, as well as the change of the reflected light intensity and phase. Because of the advantage of sensitive, real-time, and label-free, SPR cell detection has drawn much attention.^{20–22} It has been proved that cellular morphologic change,^{23,24} cell-substrate interactions,^{25,26} and even the ligand-induced intracellular signaling events^{27,28} can be monitored by SPR in real time. Recently, SPR has been combined with other techniques, such as electrochemical measurement, nanoparticles, and microscope imaging, for multi-parametric cell profiling.^{29–31} However, the duration of these SPR-based cell assays was mostly limited to a few hours, not long enough to monitor the cellular phenotypes.

Long-term cell detection mainly depends on the construction of the cellular physiological environment. The cell culture incubator provides a good solution. Therefore, compact sensors, such as the electrochemical impedance spectroscopy (EIS) and quartz crystal microbalance (QCM) sensors, can be placed inside the incubator for long-term cell detection.^{32–35} However, many optical detection platforms, such as microscopy and SPR apparatus, are too bulky and damp-sensitive to be placed into the incubator. These limitations motivated the development of microfluidic cell culture techniques to “minimize” the incubator, where the cell environment was controlled by means of medium perfusion.^{36–38} Integrated with the microfluidic devices, many microscopic imaging and spectroscopy based detection systems have enabled long-term cell monitoring as well.^{39–42} However, to our knowledge, there are very few papers integrating the microfluidic cell culture with SPR for long-term detection of the cell physiological process.

In this paper, we propose a microfluidic cell culture device which integrates with a SPR interferometric imaging system for long-term and real-time monitoring of cellular behaviors. The microfluidic device features with a porous membrane sandwiched between two chambers, enabling continuous perfusion cell culture with minimal hydrodynamic shear effects. By using Poly(dimethylsiloxane) (PDMS) replica molding method, the two-compartment chamber was fabricated. For the first time, SPR imaging was able to monitor cell proliferation process continuously for up to 48 h. The SPR signatures agreed with the time-lapse imaging of the cell proliferation, confirming the capability of the microfluidic device for long-term cell culture. Behaviors of the HeLa cell under different doses of Paclitaxel were monitored with SPR imaging in real time. The SPR signatures showed dose-dependent cell proliferation inhibitions with distinct action kinetics.

II. DESIGN AND FABRICATION

A. Theory and simulation

Higher medium exchange q rate leads to improved nutrient supply, however it also results in elevated shear stress, τ , which is detrimental to cells.^{9,37} To reduce the shear stress τ while ensuring sufficient nutrient supply, COMSOL was used to simulate the flow characteristics of the cell culture chambers. The simulation model was described specifically in the [supplementary material](#). Figures 1(a) and 1(b) show the distributions of q and τ in the single-compartment and two-compartment chambers. In the two-compartment chamber, only a small portion of the perfused medium diffused through the porous membrane into the bottom culture chamber to replenish nutrients. Thus, the two-compartment chamber can significantly decelerate the medium exchange rate q and reduce the fluid shear stress τ .

The flow characteristics of the two-compartment chamber are determined by the culture chamber height h , as well as the pore diameter d and opening ratio γ of the membrane. To find the optimal parameters, a series of models were simulated. Figures 1(c)–1(e) show the relationship of q and τ with respect to h , d , and γ . It can be seen from Figure 1(c) that q and τ decreased with increasing h . Moreover, the decrease slowed down noticeably when $h > 1$ mm; thus, the culture chamber height h was defined as 1.0 mm. Figure 1(d) shows that q and τ increased with d , and the increase became pronounced when $d > 0.2$ mm; thus the pore diameter d was defined as

FIG. 1. Distributions of perfusion rate q (a) and shear stress τ (b) in single-compartment and two-compartment culture chambers simulated using COMSOL. Relationship of the average perfusion rate q and shear stress τ with respect to the culture chamber height h (c), the porous membrane thickness d (d), and the porous membrane opening ratio γ (e).

0.2 mm. In this case, the surface area to volume (SA/V) of the two-compartment chamber is nearly 2.3 times larger than that of the 90 mm Petri dish, in which the medium is typically replaced every 2 days. Thus, the medium exchange rate q for the two-compartment chamber should be no slower than 0.048 h^{-1} . According to the linear relation of q and τ with respect to γ shown in Figure 1(e), the opening ratio γ was defined as 2% (8 pores of 0.2 mm diameter). In this case, the average shear stress τ was $1.5 \times 10^{-6} \text{ dyn/cm}^2$. Thus, the parameters of the two-compartment chamber were defined as $h_c = 1 \text{ mm}$, $d = 0.2 \text{ mm}$ and $\gamma = 2\%$.

Temperature within the cell culture chamber needs to be maintained within 36–37 °C. Figure 2(a) shows the temperature profile of the two-compartment chamber in a typical thermal insulation configuration, where the chamber is surrounded by an encircling thermal holder, into which the culture medium directly flows. Due to the thermal convection through continuous medium perfusion, there was an obvious temperature gradient (varying from 35.90 to 36.24 °C) along streams in the chamber. To solve the problem, the inflow medium was rearranged to flow over a thermal holder for preheating (Figure 2(b)). Because the medium at the inlet has been preheated closer to the required temperature, the temperature at the bottom of the culture chamber ranges from 36.31 to 36.38 °C, suitable for cell culture.

B. Fabrication and structure

As shown in Figure 3(a), the microfluidic cell culture device was composed of a PDMS two-compartment cell culture chamber and a PMMA thermal isolation shell. The PMMA shell was created out of three pieces of PMMA sheet using dichloromethane (CH_2Cl_2), forming three layers. A PT100 resistance thermometer detector (RTD) and two copper sheets sandwiched with a polyimide heating film were embedded, and a PID temperature controller (TC200, Thorlabs) was used to control the thermal support. The two-compartment chamber was fabricated by one-shot PDMS replica molding and the pores on the membrane were punched by a needle on a xy translation stage. Figure 3(b) shows the key dimensions of the structure. Figure 3(c) illustrates the integration of the microfluidic device with the SPR Prism for cellular analysis. Cellular changes within the evanescent field of the SPR could be obtained. Figure 3(d) shows a photograph of the microfluidic cell culture device.

FIG. 2. Temperature profile of the microfluidic device. The thermal insulation configuration consists of: 1—the medium outlet, 2—the heating ring, and 3—the SPR prism. The two-compartment chamber consists of: 4—the perfusion chamber; 5—porous membrane; and 6—the cell culture chamber. The color indicates the temperature profile; red color indicates high temperature. (a) A common thermal insulation configuration. (b) The optimized thermal insulation configuration for preheating.

III. EXPERIMENTAL

A. Materials and reagents

11-Mercaptoundecanoic acid (11-MUA, 218.36 Da), N-Hydroxysuccinimide (NHS, 115.09 Da), and N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC, 191.7 Da) were purchased from Sigma Aldrich. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, trypsin, ethylenediaminetetraacetic acid (EDTA), poly-L-lysine (PLL, $\leq 150\,000$ Da), phosphate-buffered saline (PBS), Paclitaxel, Cisplatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Beijing Solarbio Sci & Tech Co. Ltd. Other regular chemical reagents were purchased from Beijing Modern Oriental Fine Chemistry Co. Ltd.

B. Cells and culture medium

The human carcinoma (HeLa) cells were gifts from the Department of Biomedical Engineering, Tsinghua University, and were cultured at 37 °C with 5% CO₂ and 70% relative

FIG. 3. (a) The schematic illustration of the microfluidic device assembly. (b) The key dimensions of the assembly. (c) The integration of the microfluidic device with the SPR sensor. (d) The photograph of the assembled microfluidic device.

humidity in an incubator. The culture was DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin.

C. SPR imaging system and sensing chip preparation

The SPR experiments were performed on a home-built SPR differential interferometric imaging system that has been described in a previous publication.⁴³ Briefly, as shown in Figure 4(a), a p-polarized and collimated light was projected onto a Kretschmann prism-based sensing chip on which cells were seeded. The reflected light was split into two orthogonal-polarized light beams and imaged onto two CCD cameras. The refractive index related factor containing the information on cellular activities was sequentially extracted from the two interferograms. The SPR system is immune to disturbance and light source fluctuation, ensuring long-term cell detection with high stability.

The sensing chip used a Kretschmann prism coated with 2-nm-thick Cr and 32-nm-thick gold films. To facilitate cell attachment, the chip surface was modified with PLL. First, the substrate was immersed in 1 mM 11-MUA ethanol solutions for 24 h at ambient temperature to fabricate a self-assembled monolayer. Then, a 1:1 mixture of 100 mM NHS and 400 mM EDC covered the substrate surface for 30 min. Finally, the film was coated with 35 μ g/ml PLL solution for 1 h. After each step of the process, the chip was rinsed with deionized water and dried with nitrogen.

D. Microfluidic cell culture and detection

To validate the performance of the microfluidic device, HeLa cells were cultured for up to 48 h. Prior to cell culture, the microfluidic system was sterilized by filling with 75% (v/v)

FIG. 4. Photographs of the SPR imaging system, consisting of the SPR optics system (a), the medium injection system (b), and the temperature controller (c). The variations of temperature (d) and SPR responses to the DMEM (e) in the cell unheated and heated cell culture chamber over 24 h.

ethanol for 30 min, and then rinsed with PBS. After sterilization, the two-compartment culture chamber was filled with DMEM. HeLa cell suspension at a concentration of 5×10^4 cells/ml was then injected into the lower chamber using a syringe. After 2 h for cell attachment, DMEM maintained in a 5% CO₂ and 95% air environment was perfused to the microfluidic device at a flow rate of 10 μ l/min. After another 6 h, DMEM with various concentrations (0, 1, 10 μ g/ml) of Paclitaxel and Cisplatin was injected continuously for 40 h. The SPR imaging system or an inverted microscope (XD30A; Sunny Optical Technology (Group) Co. Ltd., Ningbo China) was used to monitor the dynamic cell proliferation process in real time, respectively. The averaged intensity modulation was extracted from the SPR images as the SPR response.

E. MTT assay

HeLa cells were plated in a 96-well plate at a density of 10 000 cells/well in a final volume of 200 μ l DMEM per well. The plate was incubated for 8 h in a CO₂ incubator. The medium was then removed and replaced with fresh DMEM with various concentrations (0, 1, 10 μ g/ml) of Paclitaxel and Cisplatin. After incubating for another 40 h, the medium in each well was removed and replaced with 90 μ l fresh DMEM and 10 μ l MTT solutions (5 mg/ml in PBS). After incubating for another 4 h, the MTT solutions were removed and replaced with 110 μ l DMSO in each well to dissolve the MTT-Formazan salt and the plate was mechanically shaken for 10 min on a shaker. The optical density of each sample was measured at 490 nm with a spectrophotometer (Model 680, Bio-Rad, Shanghai, China).

IV. RESULTS AND DISCUSSION

A. Characterization of the device

To confirm the capability of the microfluidic device for thermal maintenance, the internal temperature was monitored continuously over 48 h. The chamber was not heated for the first 24 h, and then was heated for the second 24 h. The temperature variations are presented in

Figure 4(d). The maximum temperature variation outside the culture chamber was about 6.8°C . The unheated chamber suppressed the maximum variation to some extent ($\sim 3.7^{\circ}\text{C}$), but not enough for cell culture. The heated chamber, however, maintained the internal temperature to within $37 \pm 0.1^{\circ}\text{C}$ over 24 h, suitable for cell growth. Figure 4(e) showed the variations of SPR response to DMEM in the culture chamber. It can be seen that the SPR response variation was inversely proportional to that of the temperature. Under temperature control, the SPR response fluctuation over 24 h decreases from 154 to 60 RU. These results demonstrate that the microfluidic device is effective in maintaining a long-term stable temperature for cell culture; it can achieve smaller SPR response fluctuations for cell detection.

B. Real-time monitoring of cell proliferation

To validate the capability of the microfluidic device to maintain the normal cell physiological environment, HeLa cells were cultured for up to 48 h. The dynamic cell growth process was monitored by the SPR imaging and the microscopic imaging in real time, respectively. Three individual experiments were performed and the results showed the same trend. Figure 5 shows the representative SPR response and time-lapse imaging. The SPR response (Figure 5(a)) shows that the whole cell growth process can be divided into four phases: the attachment phase (0–2 h), the adjustment phase (2–8 h), the exponential phase (8–36 h), and the stationary phase (36–48 h). In the attachment phase, the round suspended cells rapidly attached to the surface of the SPR prism, leading to a sharp increase of the SPR response. In the adjustment phase, the attached cells gradually spread out on the SPR prism and developed a spindle-shape. The growing contact area gave rise to a continued increase in the SPR response. At the end of the adjustment phase, cells were adapted to the environment and were ready for proliferation. In the exponential phase, the cell number increased significantly because of the continuous cell division. Correspondingly, the SPR response exhibited a nearly linear growth. After about 28 h, cells overwhelmed the substrate and the proliferation slowed down to the stationary phase. The SPR response gradually leveled off with a relative increase of nearly 24 000 RU.

To quantify the cell attachment and to compare microscopic data with SPR detection, the microscopic images were processed by cell edge extraction and binarization: the value of intra-cellular pixels was set as 1 and that of exterior pixels as 0. The total cell attachment area (the cell confluence) was estimated as the ratio between the sum of the pixel values and the pixel number. Fig. 5(a) shows the cell confluencies extracted from the images at different time. The variations of SPR response and cell confluencies extracted from the time-lapse imaging were

FIG. 5. (a) The SPR sensorgram of HeLa cells growth on the Kretschmann prism bearing 32 nm gold films over 48 h, and the cell confluencies extracted from the microscopic images of HeLa cells at different times. (b) The time-lapse imaging (20 $\times$) of HeLa cells grow on a glass substrate bearing 32 nm gold films over 48 h.

FIG. 6. SPR sensorgrams (a) and MTT test results (b) of HeLa cell growth with the treatment of 1 and 10 $\mu\text{g/ml}$ Paclitaxel and Cisplatin.

consistent, demonstrating that the microfluidic chip is capable of maintaining the environment for cell growth for a long period.

C. Real-time detection of cell response to drug stimulation

It is necessary to monitor the dynamic cellular response to obtain the kinetics of drug actions. Paclitaxel and Cisplatin show activity against HeLa cells, and was used as the model to validate the capability of SPR to monitor dynamic cellular responses over long intervals. With different concentrations (0, 1, 10 $\mu\text{g/ml}$) of drug treatment, the entire cell proliferation process was monitored by SPR imaging in real time. The experimental results are shown in Figure 6(a). The SPR response revealed that the proliferation of HeLa cells reached distinct equilibrium stages: without drug treatment, the SPR response reached an equilibrium of around 25 570 RU; 1 and 10 $\mu\text{g/ml}$ Paclitaxel treatment results in an suppressed responses of nearly 13 465, 8133 RU, respectively; 1 and 10 $\mu\text{g/ml}$ Cisplatin treatment results in an further suppressed responses of nearly -6021, 2976 RU, respectively. It demonstrated that the treatment of higher drug concentration resulted in a slower cell growth rate, and Cisplatin showed a more severe inhibition than Paclitaxel. The MTT assay results (Figure 6(b)) were consistent with the SPR responses. 1 and 10 $\mu\text{g/ml}$ Paclitaxel and Cisplatin treatment resulted in a 68%, 47%, 45%, and 12% growth rate in comparison with the DMEM group.

The SPR response in Figure 6(a) further showed the dynamic growth process of HeLa cells. The SPR response without Taxol treatment increased throughout the whole cell proliferation process. With the treatment of 1 and 10 $\mu\text{g/ml}$ Paclitaxel, the SPR response reached the peak of 14 875 and 13 111 RU at 34 and 24.1 h, and then decreased gradually due to the detachment of dead cells. The treatment of 1 and 10 $\mu\text{g/ml}$ Cisplatin further accelerated the cell apoptosis; the SPR response peak occurring at 19 and 17 h and reducing to 11 477 and 11 375 RU. The SPR response revealed that the drug action kinetics at higher drug concentrations leads to an earlier and stronger inhibitory effect on HeLa cell growth.

For visualization of the cell response to drug stimulations, HeLa cells were cultured in the CO_2 incubator with the treatment of different concentrations of drugs, and were imaged every 24 h (for figures see the [supplementary material](#)). The cells with no drug treatment grew well and reached a good confluency after 48 h. However, with the treatment of drugs, the cell growth got different levels of inhibitions. The inhibition of 10 $\mu\text{g/ml}$ Cisplatin was the strongest, the inhibition of 10 $\mu\text{g/ml}$ Paclitaxel and 1 $\mu\text{g/ml}$ Cisplatin was similar, and the inhibition of 1 $\mu\text{g/ml}$ Paclitaxel was the weakest. The microscopic images of the HeLa cell growth were consisted with the SPR experiments.

V. CONCLUSION

In this paper, we presented a microfluidic device integrating with a SPR imaging system for long-term and real-time detection of cellular proliferation and chemosensitivity. The microfluidic device features with a porous membrane sandwiched between two chambers to mimic

the *in vivo* environment with an exact medium exchange rate and minimal fluid shear stress for cell culture. HeLa cells were cultured in the microfluidic device for up to 48 h and stimulated with different doses of Paclitaxel and Cisplatin. SPR sensorgrams agreed well the time-lapse imaging of the cellular proliferation. Furthermore, SPR sensorgrams showed dose-dependent inhibition on cellular proliferation with distinct drug action kinetics, consistent with the conventional MTT test results. The experimental results demonstrated a proof of concept that the microfluidic device integrated with SPR imaging could provide great insight into dynamic cellular behaviors over a long period. In the future, more biological experiments will be performed for further confirmation and further improvement on the SPR system, such as increasing the SPR imaging magnification and integrating with a simultaneous microscopic imaging system will be done for direct cell observation during SPR measurement.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the COMSOL simulation conditions and the microscopic images of HeLa cells cultured in the CO₂ incubator with the treatment of different concentrations of Paclitaxel and Cisplatin.

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