Journal of Chromatography B 1068–1069 (2017) 105–111

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Different *in vitro* cellular responses to tamoxifen treatment in polydimethylsiloxane-based devices compared to normal cell culture

Lingyu Wang[a,1], Linfen Yu[b,1], Samantha Grist[c], Karen C. Cheung[c,*], David D.Y. Chen[a,*]

[a] Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T 1Z1 Canada
[b] Shenzhen Biorain Biotechnolgy Company, Suit A201, Shenwan 1 Road, Qianhai District, Shenzhen, 518100, China
[c] Department of Electrical and Computer Engineering, University of British Columbia, 2332 Main Mall, Vancouver, BC, V6T 1Z4, Canada

ARTICLE INFO

Keywords:
Cell culture
PDMS device
Parylene-C coated PDMS
Tamoxifen
LC–MS quantification

ABSTRACT

Cell culture systems based on polydimethylsiloxane (PDMS) microfluidic devices offer great flexibility because of their simple fabrication and adaptability. PDMS devices also make it straightforward to set up parallel experiments and can facilitate process automation, potentially speeding up the drug discovery process. However, cells grown in PDMS-based systems can develop in different ways to those grown with conventional culturing systems because of the differences in the containers' surfaces. Despite the growing number of studies on microfluidic cell culture devices, the differences in cellular behavior in PDMS-based devices and normal cell culture systems are poorly characterized. In this work, we investigated the proliferation and autophagy of MCF7 cells cultured in uncoated and Parylene-C coated PDMS wells. Using a quantitative method combining solid phase extraction and liquid chromatography mass spectrometry we developed, we showed that Tamoxifen uptake into the surfaces of uncoated PDMS wells can change the drug's effective concentration in the culture medium, affecting the results of Tamoxifen-induced autophagy and cytotoxicity assays. Such changes must be carefully analyzed before transferring *in vitro* experiments from a traditional culture environment to a PDMS-based microfluidic system. We also found that cells cultured in Parylene-C coated PDMS wells showed similar proliferation and drug response characteristics to cells cultured in standard polystyrene (PS) plates, indicating that Parylene-C deposition offers an easy way of limiting the uptake of small molecules into porous PDMS materials and significantly improves the performance of PDMS-based device for cell related research.

1. Introduction

Microfluidic systems are important and versatile *in vitro* platforms that expand existing tool kits for studying mammalian cells [1,2]. They can have significant advantages over macroscopic culture systems involving cell growth in flasks, dishes, or well plates. Microfluidic devices offer great design flexibility, allowing them to be precisely tailored to mimic specific *in vivo* microenvironments. For example, continuous perfusions and or chemical gradients can be easily established, and they can be used to study single cells or small numbers of cells with high temporal and/or spatial resolution in a way that is compatible with automation, parallelization, on-chip analysis and direct coupling with downstream analytical technologies [2]. Many studies on microfluidic cell culturing systems have been published, including works focused on cell culture models [3,4], cellular microenvironments [5,6], chemotaxis, apoptosis and drug screening [5–7], three dimensional cultures [8,9], tissue engineering [10], and efforts toward organ-on-a chip systems [10,11]. Despite the important advantages of microfluidic cell culture systems, their use also presents some challenges. Among other things, they frequently require the use of non-standard culture protocols and unconventional culturing surfaces such as PDMS.

PDMS has been widely used in microfluidic devices for cell culture and other applications for more than a decade due to the simple fabrication of PDMS devices and the design flexibility it offers [12]. It is optically transparent, gas permeable and biocompatible, which makes it ideal for cell culture and downstream analysis. However, it also has some less favorable properties including high porosity and hydrophobicity, which can result in vapor permeation and absorption of organic solvents and small hydrophobic molecules [13–15]. Additionally, PDMS curing is a time-dependent and temperature-dependent process that does not always result in complete crosslinking. These drawbacks presents two problems for cell culture systems. Firstly, it means that the cured PDMS may retain residual uncrosslinked oligomers that can leach from the bulk polymer into the culture media. Even after extensive

* Corresponding authors.
E-mail addresses: kcheung@ece.ubc.ca (K.C. Cheung), chen@chem.ubc.ca (D.D.Y. Chen).
[1] Lingyu Wang and Linfen Yu contributed equally to this work.

http://dx.doi.org/10.1016/j.jchromb.2017.09.041
Received 14 August 2017; Received in revised form 18 September 2017; Accepted 25 September 2017
Available online 12 October 2017

curing, as much as 5% (w/w) of bulk PDMS can remain uncrosslinked and extractable with organic solvents [16]. Regehr et al. found that uncured PDMS oligomers were detectable in microchannel media and cell membranes [12]. Secondly, the porous hydrophobic PDMS network may sequester small hydrophobic molecules. The uptake of small molecules into PDMS can change their concentration in the microchannels or microchambers of the microfluidic systems, affecting the outcome of cell culture experiments, drug screens, or cytotoxicity assays. Since the recognition of small molecule absorption on to PDMS by Toepke et al. [14], several researchers have quantitated the amount of analyte uptake, such as Wang et al. and Meer et al. [17,18]. In addition, losses of water through PDMS have been found to change the osmolality in PDMS cell culture wells, reducing cell growth [19]. Finally, the adsorption of molecules onto PDMS chamber sidewalls and water losses have been shown to reduce the efficiency of the polymerase chain reaction (PCR) [20,21].

One way to create microfluidic cell culture systems that retain the flexibility and easy prototyping offered by PDMS while avoiding its drawbacks is to coat the PDMS with some surface modifier. Several methods have recently been reported for coating PDMS surfaces with polymers including Teflon [22], polyurethane [23,24], sol-gel silica [25], and poly-p-xylylene (PPX or Parylene) [26]. Parylene is a biocompatible polymer that is deposited from the vapor phase to create a conformal coating. One of the most widely used forms of Parylene is Parylene-C, which is deposited more quickly than Parylene-N [27]. “Parylene-C coating has slightly lower chemical resistance compared to Teflon coating with similar biocompatibility, and its vapor deposition protocol generates less micro pinholes than the spin-coated Teflon [22]. Polyurethane (PU) is a widely used material for disposable medical devices and the PU coating was proven to have high transparency and low protein adsorption, but the protocol was labor-intensive and time-consuming [24]. Parylene-C coating has higher base and acid tolerance than sol-gel silica, which also has a long and difficult coating method [25]. The most significant disadvantages of Parylene-C coating is its cost, as it can only be deposited from vapor phase under vacuum, which requires highly specialized and expensive equipment. Parylene-C has been used to coat PDMS devices to prevent absorption of fluorescent dyes, and to control oxygen transfer inside microfluidic bioreactors and perfused mammalian cell culture channels [28]. It has also been used to coat implantable devices as the structural material in microelectrode arrays for neural applications [29–31]. Microfluidic structures can be coated with Parylene-C to improve PCR performance [20,21] and to reduce moisture losses and changes in solution osmolality in cell culture chambers [19], and to reduce protein adsorption [32].

This paper compares the behavior of MCF7 cells in polystyrene well plates, PDMS wells and Parylene-C coated PDMS wells. Specifically, it investigates the viability and autophagy of MCF7 cells with and without Tamoxifen treatment in three culture systems. Tamoxifen is used as a primary systemic therapy in patients with advanced stage estrogen-receptor positive breast cancers and as an adjuvant therapy for early stage ER+ breast cancers [33]. At the cellular level, Tamoxifen is known to exert its effects by both ER-dependent and independent mechanisms that act to inhibit cell cycling and induce apoptosis [34]. Moreover, it was recently shown to induce macro autophagy in breast cancer cells [35,36]. However, the role of macro autophagy in the treatment response remains largely unclear. In our trials of applying PDMS-based microfluidic devices for cell culture studies with Tamoxifen treatment, abnormal phenomena appeared frequently, such as unexpected cell viability. Despite the growing popularity of microfluidic cell culture devices, we observed that limited attention has been paid to experimental artifacts that may be introduced by microfluidic cell culturing systems that do not occur with traditional culturing methods. Specifically, as far as we know, no related study has been reported about PDMS based cell culture with Tamoxifen treatment. We predicted that such artifacts may mainly arise from the use of different culturing surfaces in microfluidic systems. The key aim of this work was to bridge this knowledge gap and to clarify the differences in cellular behavior between culture devices with PDMS surface and traditional containers. The results obtained show that PDMS surface can lead to uncontrollable decrease of Tamoxifen concentration, and Parylene-C deposition is a promising tool for reducing small molecule uptake into PDMS, making coated PDMS an ideal structural material for cell related research using microfluidic systems.

2. Materials and methods

2.1. Materials

PDMS monomer and curing agent were purchased from Dow Corning (Sylgard184, USA). Parylene-C dimer (di-chloro-di-para-xylylene) was purchased from SCS Coatings (USA). MCF7-GFPLC3 cells was provided by BC Cancer Research Centre (Canada). RPMI cell culture medium, fetal bovine serum, penicillin-streptomycin, Geneticin (G418), Tamoxifen stock solution, Tamoxifen-$^{13}C_2$-^{15}N stable isotope, standard MTS assay kit and chromatographic grade methanol were from Sigma Aldrich Toronto (Canada). Oasis HLB SPE cartridge was purchased from Waters (USA). All of the water used for reagent preparation was obtained from a Mili-Q water purification system. All other common chemicals applied in this study were purchased from Sigma Aldrich Toronto (Canada) with chromatographic grade or higher.

2.2. Instruments

Parylene-C coating procedure was finished with a PDS 2010 Parylene Deposition System (SCS Coatings, USA). LC3-GFP-postive autophagosomes were detected by using InCellAnalyzer1000 from GE Healthcare (Uppsala, Sweden) to analyze high resolution confocal images acquired with a Fluoview FV1000 confocal microscope (Olympus, Japan). SPE procedure was conducted by a 12-Port Vacuum Manifold from Phenomenex (AH0-6023, USA). LC–MS quantitation was performed with a Bonus-RP column (150 mm, 4.5 mm I.D., 3.5 μm particle size, Agilent, USA) together with an Agilent UHPLC 1260 instrument equipped with an auto-sampler and coupled to an Agilent 6130 single quadrupole mass spectrometer.

2.3. Microfabrication and characterization of Parylene-C coating

A 10: 1 wt ratio of PDMS base monomer to curing agent was mixed, degassed under vacuum, poured into a petri dish to a thickness of 6 mm, and cured in an oven at 60 °C for 2 h. The cured bulk PDMS was then peeled off from the template, and 6 mm holes were punched through the elastomer using a stainless-steel puncher to serve as cell culture chambers. A single piece of punched PDMS was then bonded to a flat thin glass substrate by briefly treating the substrate with plasma. A second piece of PDMS was partially coated with Parylene-C by covering the bottom of the PDMS chip with adhesive tape and putting it into a Parylene-C Deposition System. After coating, the tape was peeled off from the PDMS sheet and the uncoated side was bonded to a thin glass substrate by plasma treatment. The bonded PDMS structures (both coated and uncoated) were then baked at 80 °C in an oven overnight to increase the extent of oligomer crosslinking [14].

The structure of the Parylene-C coated PDMS chip is shown in Fig. 1. The vapor phase Parylene-C deposition was performed by placing solid Parylene-C dimer (di-chloro-di-para-xylylene) particles in a Parylene Deposition System and sublimating them under vacuum at 150 °C. The dimer molecules were then pyrolyzed at 680 °C to form free radical monomers, which were condensed and polymerized on the surfaces of the test structures, forming a conformal Parylene-C coating. The manufacturer’s recommended instrument settings were used during polymer deposition: vaporization was performed at 150 °C and 1 Torr, pyrolysis at 650 °C and 0.5 Torr, and deposition at 25 °C and 0.1 Torr. The coating’s thickness was adjusted by changing the quantity of dimer