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Nanoadhesive layer to prevent protein absorption in a poly(dimethylsiloxane) microfluidic device

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ABSTRACT

Poly(dimethylsiloxane) (PDMS) is widely used as a microfluidics platform material; however, it absorbs various molecules, perturbing specific chemical concentrations in microfluidic channels. We present a simple solution to prevent adsorption into a PDMS microfluidic device. We used a vapor-phase-deposited nanoadhesive layer to seal PDMS microfluidic channels. Absorption of fluorescent molecules into PDMS was efficiently prevented in the nanolayer-treated PDMS device. Importantly, when cultured in a nanolayer-treated PDMS device, yeast cells exhibited the expected concentration-dependent response to a mating pheromone, including mating-specific morphological and gene expression changes, while yeast cultured in an untreated PDMS device did not properly respond to the pheromone. Our method greatly expands microfluidic applications that require precise control of molecule concentrations.

METHOD SUMMARY

A PDMS microfluidic device was bonded using a nanoadhesive layer deposited via initiated chemical vapor deposition (iCVD). The barrier property of the nanoadhesive was tested by measuring the absorption of fluorescent dye into PDMS. The yeast response to pheromone was tested in a PDMS microfluidic device sealed with nanoadhesive and compared with the response in an untreated PDMS device.

KEYWORDS:

device bonding • nanoadhesive layer • PDMS microfluidics • protein absorption • yeast mating

For the last three decades, poly(dimethylsiloxane) (PDMS) has been one of the most widely used materials for the fabrication of microfluidic devices owing to its excellent optical transparency, mechanical flexibility and biological inertness [1,2]. The advantageous characteristics of PDMS-based microfluidic devices have enabled a wide variety of applications including organ-on-a-chip [3,4], sensors [5,6], reactors [7,8] and cell isolators [9,10].

PDMS-based microfluidic devices are fabricated by molding PDMS from a microfluidic channel patterned on silicon (Si) wafer via photolithography, followed by bonding with a glass substrate by plasma treatment [11]. The simplicity of molding PDMS offers researchers a quick way to test many different microfluidic designs patterned on a single Si wafer. Also, PDMS and glass substrates are simple and reliable, enabling the fabrication of many kinds of microfluidic devices in a reproducible manner.

Despite its usefulness, however, the higher permeability of PDMS compared with other materials has been a drawback for many applications. Liquid drying and leaching of small, unreacted molecules into the channel caused by its high permeability can have detrimental effects for biological samples [12,13]. Additionally, certain organic solvents can penetrate through the bulk of PDMS, causing excessive swelling and damaging the channel [14,15]. This is especially important for biological applications involving cell culturing, drug screening or even diagnostics because absorbed reagents, drugs or target analytes may lead to flawed results. Several materials (e.g., polystyrene [16,17], cyclic olefin copolymer [18], poly(methyl methacrylate) [19], Norland Optical Adhesive [20], parylene [21,22] and 3D-printing resins [23]) have thus been developed as alternatives for biological applications, but despite these options, PDMS is still widely used owing to the simplicity in generating various channel designs and ease of handling the microfluidic devices.

To overcome these limitations of PDMS, several approaches for surface passivation have been reported [24]. Some have employed sol–gel chemistry to form a thin inorganic layer [25,26]. However, although the glass-like coating is excellent for blocking the penetration of chemicals into the bulk of PDMS, it alters the channel configuration and can critically impact biological applications requiring microstructures. Others have used liquid-phase growth of polymers on the PDMS channel surface using a 'grafting-from' approach. This approach offers great controllability over the polymer thickness, but requires complicated liquid-phase polymer reaction schemes, such as atom transfer radical polymerization, that must be performed in an oxygen-free environment [27,28]. Vapor-phase parylene coating has also been attempted on fabricated PDMS devices [29] but the coating uniformity is limited by the aspect ratio of the channel.

Recently, a polymer thin film-based nanoadhesive layer deposited using the initiated chemical vapor deposition (iCVD) process has been introduced for reliable sealing of microfluidic devices [30]. The iCVD process is a vapor-phase deposition method whereby a thin polymer film can be coated on various substrates [31,32]. Because the entire process is performed in vapor phase, no solvents are required to deposit the polymer thin films, which eliminates solvent-induced damage to the PDMS substrate. Also, the high step coverage preserves the geometry of the PDMS. With a thickness of less than 200 nm, the nanoadhesive layer has been shown to be resistant to various chemicals [30], to sustain a pressure of over 1 MPa [33] and to maintain the flexibility of the substrates [21]. Thus the advantages of fabricating a PDMS microfluidic device using the nanoadhesive are twofold: first, nanoadhesives provide strong, reliable sealing and second, they can minimize the penetration of molecules into PDMS.

Here we demonstrate that a nanoadhesive layer can successfully block the penetration of molecules and drugs into the bulk of a PDMS device. Using fluorescent molecules, we show that the device bonded using nanoadhesive efficiently blocks the absorption of dye by PDMS. More importantly, the nanoadhesive-bonded PDMS device dramatically reduces the absorption of yeast mating pheromone (α-factor), demonstrating its potential for application in quantitative cell biology studies such as signal transduction, chemotaxis or drug screening.

Materials & methods

Fabrication of microfluidic devices & bonding using nanoadhesive

The PDMS-based microfluidic device was fabricated using a standard photolithography process. Briefly, SU-8 2075 photoresist (Microchem) was spin-coated on Si wafer with a thickness of 85 μm and soft-baked at 65°C for 5 min and 95°C for 2 min. The photoresist was subsequently exposed using UV light, post-exposure baked and developed to obtain a Si master. A PDMS device was molded from the Si master by mixing Sylgard 184 (Dow Corning) PDMS base and curing agent at a ratio of 10 to 1. To fabricate a plasma-bonded device, the molded PDMS device and a glass slide were exposed to oxygen plasma for 10 s at 15 W, assembled and cured at 100°C for 1 h. To bond the device using a nanoadhesive layer, poly(glycidyl methacrylate) (PGMA) was deposited using the iCVD process on both molded PDMS and glass slide as reported elsewhere [30]. Growth of the PGMA film was monitored *in situ* using an interferometer [34] collecting reflection signals from a Si wafer placed alongside the PDMS substrates. The thickness on the Si wafer was taken as the thickness of the PDMS substrate. Subsequently, the glass slide was briefly exposed to a vapor of ethylenediamine and assembled face-to-face with PGMA-coated PDMS. The assembly was then cured for 8 h at 75°C to obtain bonding. The static water contact angle of the nanoadhesive-coated surface was measured using a contact angle analyzer (SEO, Inc, Korea).

Dye diffusion experiment

The efficacy of the nanoadhesive layer in preventing absorption of small molecules into PDMS was tested by filling the microchannel with an aqueous solution of Rhodamine 123 (Sigma-Aldrich) at a concentration of 0.1 mg/ml and storing for 14 h with the inlet and outlet of the device closed to prevent evaporation. For comparison, a plasma-bonded PDMS device was tested in the same way. Before imaging, the channel was completely rinsed with water several times and dried with N_2. Also, to test the effect of nanoadhesive thickness in dye absorption, devices bonded with 50, 200 and 500 nm nanoadhesive were filled with the same dye solution and stored for 14 h. In both experiments, the inlet and outlet were sealed to minimize evaporation. The fluorescence intensity profile was analyzed using ImageJ.

Yeast cell experiments

Budding yeast cells were grown overnight to saturation, then allowed to resume exponential growth by diluting them 50-fold into fresh growth medium (Synthetic Defined Media with 2% glucose, $OD_{600} \sim 0.1$) and finally incubated for at least 4 h at 30°C before each experiment in a PDMS-based microfluidic device consisting of a 4-μm gap to trap individual cells. Details of the design have been reported elsewhere [35]. Synthesized α-factor was purchased from GeneScript.

Live cell microscopy & image analysis

The microfluidic device was mounted on the stage of an inverted Nikon Eclipse Ti microscope equipped with a hardware-based automated focusing system (Perfect Focus System) and placed into an incubation chamber set to 30°C. Images were acquired with 40× oil objectives and respective filter cubes, and controlled using micro-manager open source software. A motorized XY-stage was used to capture multiple fields of view per time point. For single cell analysis, automated image analysis was performed using YeastQuant software on raw images running in Matlab® [36].

Results & discussion

In order to assess the barrier performance of the iCVD nanoadhesive layer on PDMS, we fabricated a single-channel microfluidic device using PDMS and bonded it to the glass substrate by either plasma or nanoadhesive bonding (Figure 1A). Both methods achieved reliable bonding between the substrates. However, unlike the plasma bonding method, where activated PDMS and glass participate directly in the bonding reaction, the nanoadhesive approach utilizes the high crosslinking of a thin polymer layer that conformally covers the surface of PDMS and glass. Epoxy-containing PGMA and ethylenediamine deposited on both the PDMS and glass substrates undergo a ring opening curing reaction between the epoxides and amines to form a highly crosslinked polymer network. The thickness of the nanoadhesive

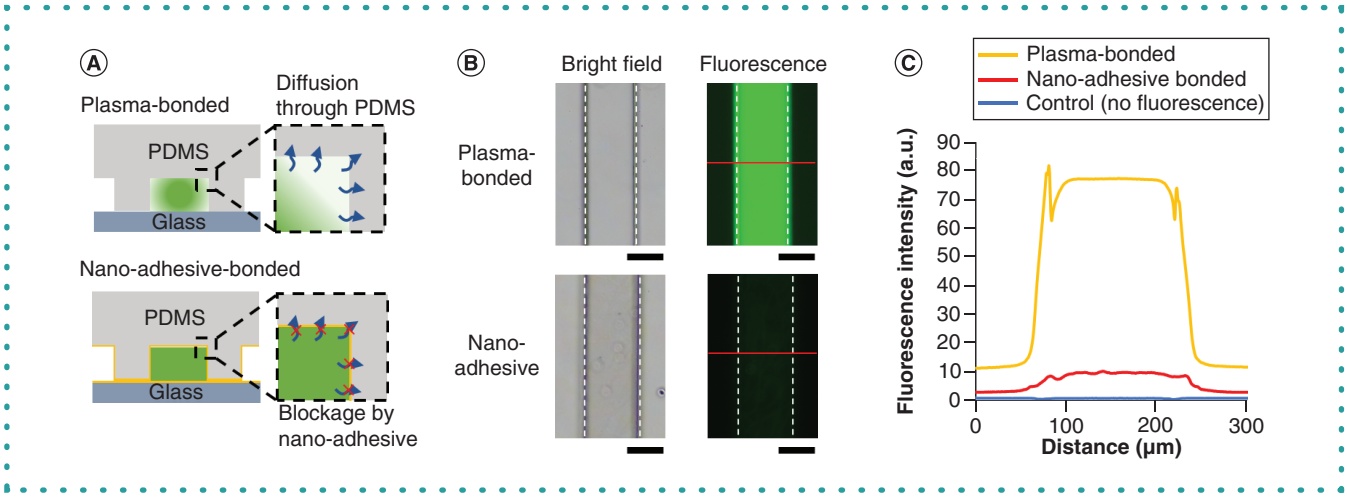

Figure 1. Barrier performance of nano-adhesive-bonded device compared with plasma-bonded device. (A) Schematic drawing showing that in contrast to the nanoadhesive-bonded device, rhodamine dye can diffuse through PDMS in the plasma-bonded device. **(B)** Bright-field and fluorescence images of plasma-bonded (top) and nanoadhesive-bonded device (bottom) after 14 h exposure to a Rhodamine 123 solution. The white dotted lines indicate the channel while the red lines denote the path of the fluorescence intensity profiles. **(C)** The intensity profiles across the channel width. Scale bar = 100 μm.

layer is less than 200 nm, so the geometry of the microfluidic channel is fully retained (Figure 1B, left). The iCVD process is known to possess high deposition conformality and preserves geometrical features, as demonstrated by several previous reports [37–39]. The excellent conformality of the nanoadhesive layer is therefore highly advantageous considering the vast difference between nanoadhesive thickness (0.05–0.5 μm) and typical microfluidic channel dimensions (10–1000 μm). Therefore, unless the channel dimension is of the same order of magnitude as the nanoadhesive thickness, the bonding method shown here does not alter the geometry of the channel.

The barrier performance of the nanoadhesive layer was investigated by exposing the channel to Rhodamine 123 solution (0.1 mg/ml in deionized water) for 14 h. In the case of the plasma-bonded device, fluorescence signal was detected in the channel even after rinsing the device multiple times with deionized water, suggesting adsorption of rhodamine dye into the PDMS channel wall. Also, the relatively high intensity near the channel shows penetration of the dye into the bulk of PDMS. The nanoadhesive-bonded device showed much weaker fluorescence intensity (~87% weaker) than the plasma-bonded device. Also, the intensity near the channel was much weaker than in the plasma-bonded device, demonstrating the barrier capability of the nanoadhesive layer against the adsorption of the rhodamine dye.

Clearly, the nanoadhesive acts as a physical barrier, preventing the penetration of dye into the bulk of PDMS. The plasma bonding method renders the surface of PDMS hydrophilic by creating silanol functional groups on the surface [24]. Such groups can interact with water molecules to form a thin layer of water, reducing the adsorption of large molecules such as proteins. Still, the intrinsic porosity of the material permits penetration of chemicals. On the other hand, with a static water contact angle of $\sim 68.2 \pm 1.1^{\circ}$ (n = 3), the surface of the nanoadhesive is less hydrophilic compared with that of plasma-treated PDMS. The excellent barrier property of the nanoadhesive despite its lower hydrophilic surface characteristic compared with plasma-treated PDMS is therefore attributed to the physical barrier property arising from the high crosslinking of the polymer network.

After bonding the microfluidic devices with nanoadhesive, unreacted epoxide groups that did not participate in the crosslinking reaction with primary amines from ethylenediamine may remain. Although these groups may be useful for post-bonding functionalization, unexpected epoxy-mediated adsorption is another form of loss that must be minimized when performing drug delivery experiments with cells. We tested the adsorption of Rhodamine 123 using devices bonded with three different thicknesses of nanoadhesive (50, 200 and 500 nm) by exposing the channel to a solution containing Rhodamine 123. As shown in Figure 2, the device bonded with 50 nm showed minimal dye adsorption in the channel, while the device bonded with 500 nm showed significant adsorption. The higher adsorption on thicker nanoadhesion is attributed to a higher proportion of unreacted epoxide groups in the channel. However, it is worth noting that even 50 nm of nanoadhesive can achieve reliable bonding and efficiently blocks dye penetration.

Next, we tested properties of live cells cultured in a PDMS microfluidics device bonded with a nanoadhesive layer. Many cells secrete proteins that serve as mediators of cell–cell signaling and cellular proliferation; thus the secreted signal concentrations play an important role in a wide variety of physiological and pathological processes. For instance, budding yeasts have two types of mating cells, **a** and α cells (genotypes *MAT* **a** and *MAT*α), and the cells secrete mating type-specific pheromones, called **a**- and α-factor, respectively. When a yeast cell is stimulated by pheromones secreted by a nearby cell of the opposite mating type, it undergoes a physiological program including significant changes in the expression of about 200 genes, arrest in the G1 phase of the cell cycle, oriented growth toward

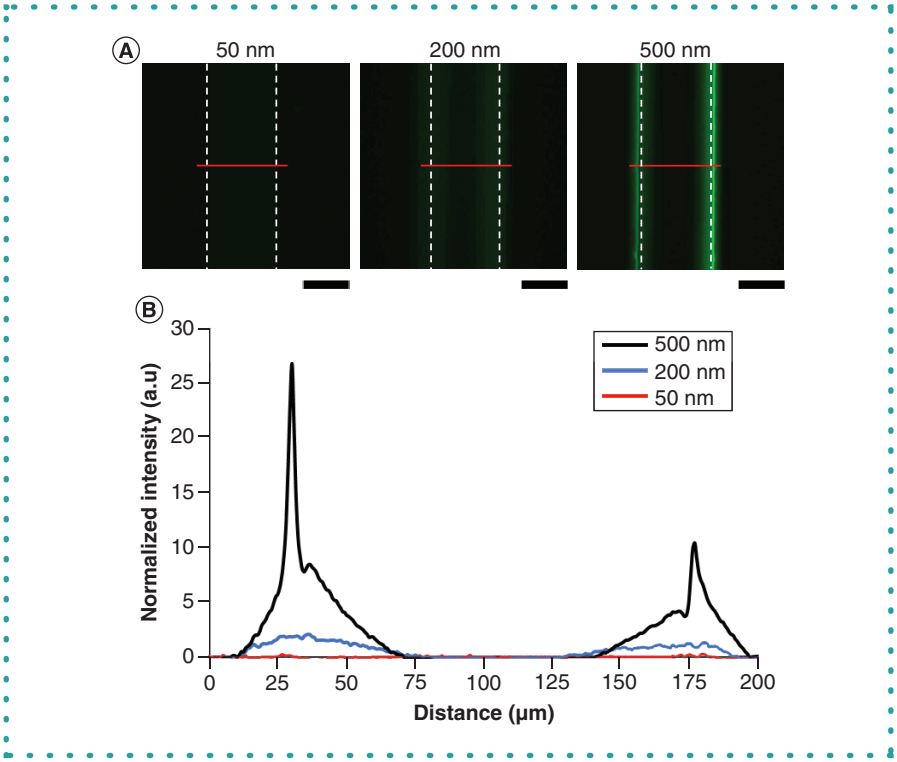

Figure 2. Fluorescence images (top) and intensity profiles (bottom) of microchannels bonded with 50, 200, and 500 nm after exposure to aqueous Rhodamine 123 solution (1 mg/ml). The white dotted lines indicate the channel walls and the red lines indicate the path of the fluorescence intensity profiles. Adsorption of dye is highest when the thickness of nanoadhesive is 500 nm, mainly attributed to the presence of unreacted epoxide groups in the nanoadhesive. Scale bar = 100 μm.

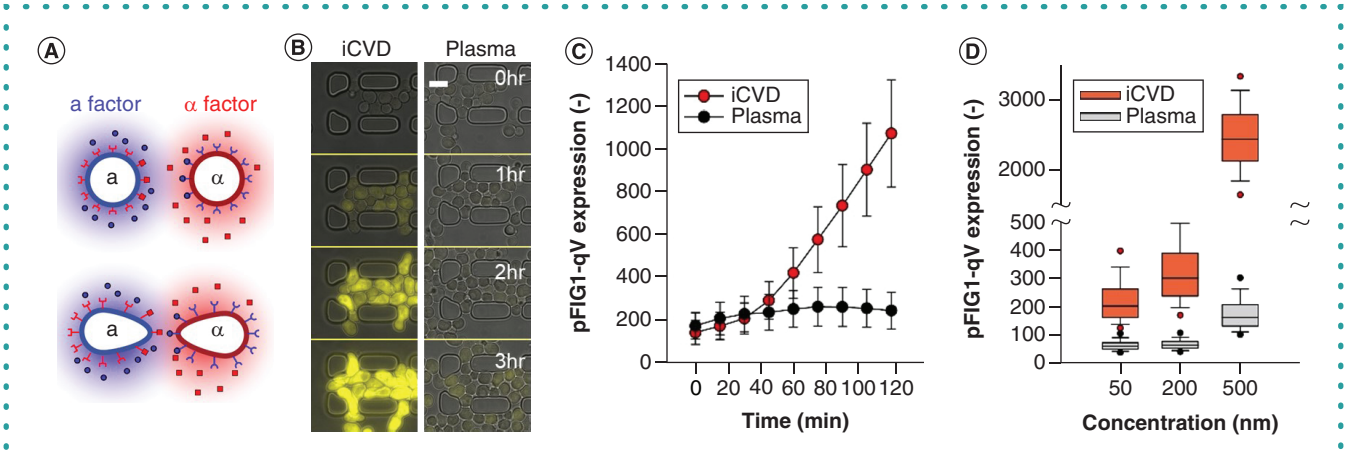

Figure 3. Application of nanoadhesive layer to prevent α-factor absorption. (A) Schematic drawing of the budding yeast mating process. **(B)** The response of MATa-type cells to α-factor (500 nM) in iCVD-bonded (left) and plasma-bonded (right) PDMS microfluidic devices. Scale bar = 10 μm. **(C)** Quantification of α-factor responsive gene expression monitored by the p*FIG1*-qV reporter. **(D)** Single cell analysis of α-factor responsive gene expression after 3 h α-factor treatment. At least 400 cells were analyzed from microscopy images in each experiment.

the mating partner, and, ultimately, fusion with the mating partner (Figure 3A) [40]. This process is a well-known model system for understanding how cells sense and transduce signals; synthesized α-factor peptide is commonly used to control accurate concentration, because **a**-factor is hard to purify and difficult to synthesize. Although yeast cells are often cultivated in liquid culture media or agar pads, PDMS-based microfluidic devices are of interest for live cell imaging because all cells can be imaged in a single focal plane. The softness of PDMS enables the trapping of yeast cells by designing a 4-μm gap between the bottom glass and the PDMS, corresponding roughly to the dimension of budding yeast cells [41,42].

Due to the high permeability of PDMS, α-factor can be absorbed into it. Although the channel walls are often preadsorbed with a solution of nonspecific proteins like bovine serum albumin or casein to minimize loss when the signaling peptide is applied, the stability and uniformity of the treatment is not guaranteed. To test whether the nanoadhesive layer method would prevent α-factor absorption, we cultured yeast cells in PDMS microfluidics devices bonded via either plasma bonding or nanoadhesive bonding, and the devices were used to monitor the cells by microscopy while continuously providing 500 nM of synthetic α-factor solution. Before culturing, the cells were engineered to express a reporter construct based on the *FIG1* promoter which drives the expression of quadruple-Venus fluorescent protein (p*FIG1*-qV), specifically induced upon activation of the MAPK signaling cascade triggered by α-factor mating pheromone [43]. Upon α-factor pheromone treatment, cells cultured in devices with the nanoadhesive layer formed mating projections (shmoos) and induced expression of the transcriptional p*FIG1*-qV reporter (Figure 3B & C). In contrast, these morphological changes and p*FIG1*-qV expression were strongly attenuated in the plasma-bonded microfluidic device. We found a similar trend with lower concentrations of α-factor (Figure 3D). Together, these results demonstrate that the nanoadhesive layer efficiently prevents α-factor adsorption in PDMS microfluidic channels.

The nanoadhesive surface provides an appropriate environment for cells to attach and grow. Previously, Lee *et al.* [44] showed that a titanium surface coated with poly(N-isopropylacrylamide)-grafted PGMA can improve cell adhesion while reducing bacterial attachment. Improved cell attachment on PGMA-coated titanium implants was also reported by Park *et al.* [45]. In addition, Kim *et al.* utilized the epoxide functionalities of PGMA to graft BMP-2 peptide for enhancing the osteogenic differentiation of human mesenchymal stem cells [46]. Therefore we expect our method could be widely used for culturing not only yeast but also animal cells.

In this study, we successfully demonstrate that the nanoadhesive layer efficiently prevents absorption of fluorophore Rhodamine 123 and the yeast mating pheromone α-factor. The thin nanoadhesive layer not only enables reliable sealing of PDMS microfluidic devices, but also acts as a barrier that complements the high porosity of PDMS. In addition, the iCVD process is straightforward and versatile, and can be applied easily to a variety of PDMS microfluidic devices without additional changes to the fabrication protocol. We expect that our method will further strengthen microfluidics as a methodology of quantitative cell biology in cases requiring precise control of solute concentrations, such as during intracellular signaling, chemotaxis studies and drug screening.

Future perspective

PDMS microfluidic approaches in life sciences offer exciting possibilities for investigating cellular behavior. PDMS reproducibly maintains the cellular microenvironment in customized microsystems that allow for microfluidic manipulation. Although we demonstrate in this study that an iCVD nanoadhesive layer is effective in preventing adsorption of hydrophilic materials that dissolved well in aqueous solution like the tested substrates, a large number of cell biological reagents are small hydrophobic molecules; ongoing and future studies will determine whether the iCVD process can be applied broadly to microfluidic-based biology.

Author contributions

J You and S Lee conceived the study. J You, B Lee, Y Choi and S Lee performed experimental work and analyzed data. C-S Lee, M Peter, S Im and S Lee provided supervision. J You and S Lee wrote the manuscript, with contributions from all authors.

Financial & competing interests disclosure

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