

# Diffusion of self-assembled monolayers of thiols on the gold surfaces covered with polydimethylsiloxane stamps

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**Abstract** Thiols can diffuse and form self-assembled monolayers (SAMs) on the gold surfaces covered with polydimethylsiloxane (PDMS) stamps. For the first time, with cells as the indicator of how far alkanethiols had diffused to form SAM, we studied the growth dynamics of SAMs of  $\text{HS}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OH}$  ( $\text{EG}_3$ ) and  $\text{HS}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_6\text{OH}$  ( $\text{EG}_6$ ) on the gold surfaces covered with PDMS stamps. The growth of SAMs is well described by one-dimensional diffusion from a line source of concentration, with surface diffusion coefficient of  $193.4 \pm 19.2 \mu\text{m}^2/\text{min}$  ( $\text{EG}_3$ ) and  $95.8 \pm 18.9 \mu\text{m}^2/\text{min}$  ( $\text{EG}_6$ ).

## Introduction

Self-assembled monolayers (SAMs) refer to the formation of monolayers by self-assembly of surfactant molecules at surfaces [1]. In recent years, SAMs found wide applications in chemistry, physics, and especial biology [2–5]

when SAMs combined with microfluidic technology [6–10]; for example, we applied alkanethiolate SAMs on gold surface to study the relationship between the shape of mammalian cells and their migration direction [11]. Recently, when we applied alkanethiolate SAMs combined with microfluidic technology to establish interaction models for multiple types of cells [12], we found that the SAMs diffused on the surfaces of evaporation-coated gold slides which were covered with polydimethylsiloxane (PDMS) stamps [13]. It is necessary to understand the properties of SAMs diffusion in order to precisely fabricate SAMs when applying them in cellular biology and material science [14]. Many groups studied the diffusion dynamics of thiol SAMs on the interface between solid gold and gas in dip-pen nanolithography using atomic force microscopy (AFM) and learned their several properties [15–17]. But, these results are hard to directly apply to the above situation because the interface of SAM diffusion changes from that between solid–gas phases to that gold surfaces covered with PDMS stamps, which gaps in nanoscale should exist between gold surfaces and stamp surfaces considering the nanostructures in the evaporation-coated gold slide, and thiols are able to diffuse in PDMS stamps [18, 19]. Moreover, in general, biological labs are seldom equipped AFM. Therefore, it is still a large challenge for investigation of SAM diffusion in biological labs nowadays. Herein, with mammalian cells as the indicator of how far thiol SAMs diffusing, we report a strategy to study the growth dynamics of SAMs of thiols on the gold surfaces covered with PDMS stamps. Using the property of well-ordered SAMs of polyethylene terminal group of thiols on gold that can effectively resist the adhesion of mammalian cells [20, 21], and uncoated gold surfaces allow mammalian cells to adhere, we designed a simple microfluidic system including a PDMS stamp embedded a microchannel in contact

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with a gold covered slide to determine the distances of the diffusion of SAMs along with diffusion time.

## Materials and methods

### Materials

$\text{HS}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OH}$  (EG<sub>3</sub>) and  $\text{HS}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_6\text{OH}$  (EG<sub>6</sub>) were from Prochimia. Medium DMEM and fetal bovine serum (FBS) were from Gibco, and penicillin/streptomycin was from Hyclone. Cell culture dishes were from Corning.

### Cell culture

3T6 fibroblasts were provided by the Institute of Biophysics, Chinese Academy of Science. The 3T6 cells were in medium DMEM with 10 % v/v FBS and penicillin/streptomycin (100 U/mL). The cultures were maintained on 60 mm cell culture dishes under 5 % CO<sub>2</sub>, at 37 °C.

### Preparation of gold slides

Preparation of gold slides was accomplished using established methods [21]. Gold slides were prepared by firstly evaporating a layer of 5-nm-thick titanium, followed by a 20 nm of gold, both with a vacuum electron-beam evaporator (Edward Auto 500), on acid-rinsed glass cover slides.

### Preparation of PDMS stamps

Fabrication of the master for microchannels was accomplished using established methods [6]. Patterns used in these experiments were designed by L-edit software and exported as a PS file. We printed the PS file onto a transparency mask on a high resolution printer (up to 3600 dpi). Photoresist film with a height of ~ 60 μm was cast by spin-coating SU 8-2050 (MicroChem, USA) onto silicon wafer at 2000 rpm for 30 s. The photoresist film on the wafer was baked at 65 °C for 3 min and at 95 °C for 9 min and exposed under a UV light source with the transparency mask directly on it. The wafer was post baked at 65 °C for 1 min and at 95 °C for 7 min. The exposed photoresist film was developed using MicroChem's SU-8 Developer for 7 min. The microfeatures for these patterns were visible by the naked eye at this stage. The wafer was silanized with 1H, 1H, 2H, and 2H-perfluorodecyltriethoxysilane (Alfa Aesar, UK) overnight. To generate microfluidic channels, we replica-molded polydimethylsiloxane (PDMS) (Dow Corning, USA) from the masters. The microchannels used

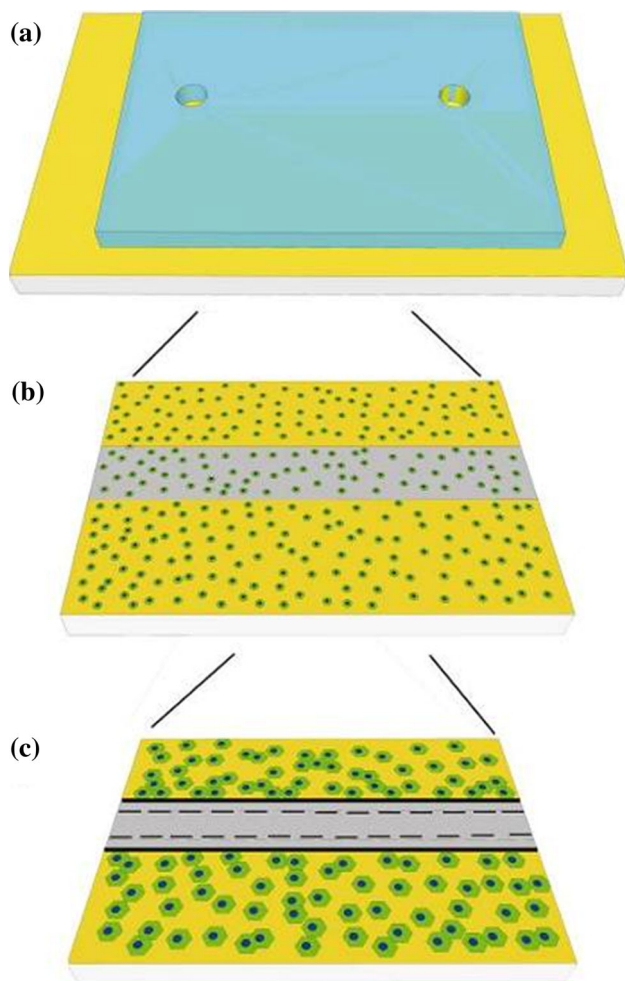
here had the following dimensions: width, 200 μm; depth, 60 μm; length, 1.5 cm. Holes at the inlets and outlets of the microfluidic channels (~ 800 μm in diameter) were generated by a sharpened needle tip.

### Microscopy and imaging

We acquired images on a Leica inverted microscope (DMI 6000) equipped with a digital monochrome camera (Leica DFC 350 FX) and the software provided by Leica (LAS AF). Phase contrast micrographs were obtained with a 10× objective (N PLAN).

## Results and discussion

We investigated how thiols with polyethylene terminal group migrated under the PDMS to form inert SAMs on the gold surfaces. We selected  $\text{HS}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OH}$  (EG<sub>3</sub>) and  $\text{HS}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_6\text{OH}$  (EG<sub>6</sub>) as examples to illustrate our strategy. In general, thiol SAMs growth on gold substrate from solution includes three steps: the first step of the chemisorption of the headgroup (SH), the fastest step; the second step of the straightening of the hydrocarbon chains to form lying-down phase, the slower step; the third and final step of the reorientation of the terminal groups to form functional standing-up phase, the slowest step [22]. SAMs of standing-up phase with the terminal groups (OH) exposure can be detected with cells as the indicator in our experiments. Only well-ordered SAMs with the polyethylene moiety of the EG<sub>3</sub> or EG<sub>6</sub> exposed can effectively resist cell adhesion. We identified the area without cell adhesion covered with thiol SAMs as inert area and the area with cell adhesion covered with gold as active area. To measure the inert areas on surface, we introduced an aqueous solution of EG<sub>3</sub> or EG<sub>6</sub> into a channel with a width of 200 μm and allowed it to incubate at room temperature for 3, 5, 7, 9, 20, 30, and 40 min and then rinsed the channel with vast PBS solution at once to get rid of residual solution of EG<sub>3</sub> or EG<sub>6</sub> in order to avoid residual thiol further diffusing (Fig. 1a, b). We peeled off the PDMS stamp and cultured 3T6 cells on the surface (Fig. 1c). After culturing for 3 h, a length of time that allowed the complete adhesion and spread of 3T6 cells, we measured the width of the areas which prevented cell adhesion. When EG<sub>3</sub> or EG<sub>6</sub> solution incubation lasted for only 3 min, we noticed that there was a stripped area without cell adhesion (inert areas formed). The longer time EG<sub>3</sub> or EG<sub>6</sub> solution incubated, the wider the inert areas became (Fig. 2). The measured width of the areas that resist cell adhesion was greater than that of the channel with which we introduced EG<sub>3</sub> or EG<sub>6</sub>, for example, the width of well-ordered SAMs with EG<sub>3</sub> or



**Fig. 1** Strategy for determination of distance of EG<sub>3</sub> or EG<sub>6</sub> diffusion on the surfaces of a gold slide covered with a stamp of polydimethylsiloxane (PDMS). **a** A PDMS stamp with an embedded microfluidic channel came into contact with a gold-coated glass substrate (the thickness of the gold is only 20 nm to allow optical transparency) to form an enclosed system of the microchannel. EG<sub>3</sub> or EG<sub>6</sub> solution was introduced into the channel and incubated for certain minutes under room temperature to allow EG<sub>3</sub> or EG<sub>6</sub> to diffuse and forms SAMs (gray area in **b** and **c**). **b** After washing, the channel with PBS to get rid of residual solution of EG<sub>3</sub> or EG<sub>6</sub> in order to avoid residual thiol further diffusing, we peeled off the PDMS stamp and plated cell suspensions on the substrate (enlarged the part previously covered with microfluidic channel). **c** After incubation for 3–6 h, cells adhered on the substrate everywhere except for SAM areas (gray area). The areas between two neighboring dash lines were that of the previous microchannels

EG<sub>6</sub> in ethanol in 200- $\mu\text{m}$ -wide microchannels within 9 min was more than 300  $\mu\text{m}$ . These results indicated that EG<sub>3</sub> or EG<sub>6</sub> migrated under the PDMS to form inert SAMs on the gold surface.

To calculate the diffusion coefficient of EG<sub>6</sub> and EG<sub>3</sub> migrated on the gold surfaces under the PDMS, we used diffusion equation in isotropic media as the following to calculate the coefficient of diffusion [23],

$$\frac{\partial C}{\partial t} = \text{div}(D\text{grad}C)$$

where  $D$  is the coefficient of diffusion,  $C$  is the concentration of EG<sub>3</sub> or EG<sub>6</sub>. In our experiments, measurable diffusion mainly occurred perpendicularly to the direction of the channels that introduce EG<sub>3</sub> or EG<sub>6</sub>. As the channels were long in contrast to the diffusion distance, we can approximate the diffusion of EG<sub>3</sub> or EG<sub>6</sub> to form well-ordered SAMs as one-dimensional planar diffusion, and its diffusion equation could be simplified as the following:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

where  $x$  is the distance along the direction of diffusion.

The solution of one-dimensional planar diffusion is

$$C = \frac{M}{2(\pi Dt)^{1/2}} \exp(-x^2/4Dt) \quad (1)$$

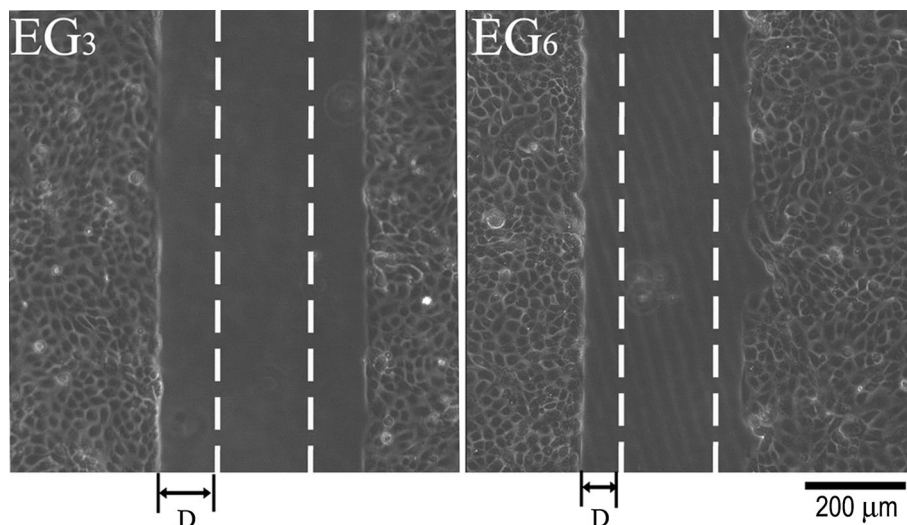
where  $M$  is the total amount of EG<sub>3</sub> or EG<sub>6</sub> in the diffusion process, and it is a constant.

To obtain the diffusion coefficient of EG<sub>3</sub> and EG<sub>6</sub> (that forms well-ordered SAMs), we utilized the least-square regression method. First, take the logarithm of equation [1] on both sides, and we will get

$$\frac{1}{D} \frac{x^2}{4t} + \ln\left(\frac{2C(\pi D)^{1/2}}{M}\right) = -\frac{1}{2} \ln(t) \quad (2)$$

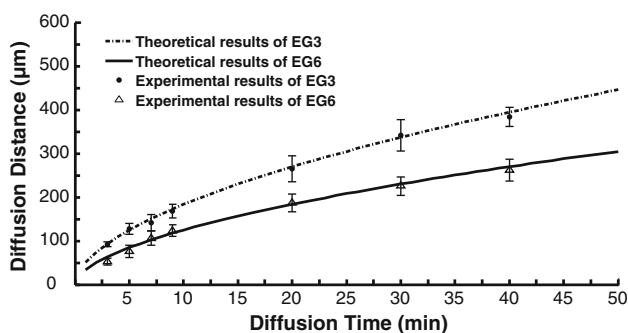
To form a sufficiently well-ordered SAMs to prevent cell adhesion on the surface of substrates, there is a critical concentration of thiol needed,  $C_0$ . Given the total amount  $M$ , diffusion coefficient  $D$ , and critical concentration  $C_0$  are all constants for a certain system, the second term,  $\ln\left(\frac{2C(\pi D)^{1/2}}{M}\right)$ , is also a constant. If we regard  $\frac{x^2}{4t}$  as the independent variable and  $-\frac{1}{2} \ln(t)$  as the functional variable in equation [2], the diffusion coefficient,  $D$ , can be regarded as the inversion of slope in equation [2]. Since the difference between the well-ordered SAMs and EG<sub>3</sub> or EG<sub>6</sub> will only influence the concentration of EG<sub>3</sub> or EG<sub>6</sub>,  $C$ , in equation [2] and will not change the slope in this equation.

Based on a series of diffusion distance versus diffusion time, by utilizing least-square fitting (Fig. 3), we found the diffusion coefficient ( $D$ ) of EG<sub>3</sub> and EG<sub>6</sub> to form functional SAMs to be  $193.4 \pm 19.2$  and  $95.8 \pm 18.9 \mu\text{m}^2/\text{min}$ , respectively. The results have revealed that the speed of EG<sub>6</sub> (longer chain thiols compared to EG<sub>3</sub>) diffusion to form SAMs on gold surfaces under PDMS is slower than that of EG<sub>3</sub>. These results are different from that of thiols to form SAMs on the interface between gold and gas, in which longer chain length thiols covered the gold surface more rapidly than shorter chain length thiols [24]. In



**Fig. 2** Determination of the extent to which EG<sub>3</sub> or EG<sub>6</sub> has migrated on the gold surfaces covered with PDMS stamps. The formation of SAMs by EG<sub>3</sub> or EG<sub>6</sub> can resist the adhesion of cells, which serves as the indicator of the distance EG<sub>3</sub> or EG<sub>6</sub> has migrated and formed SAMs. The area between two neighboring dashed lines

was the channel (width 200 μm) that delivered EG<sub>3</sub> or EG<sub>6</sub>. We note that EG<sub>3</sub> or EG<sub>6</sub> has migrated out of the area defined by the channel and formed SAMs. The incubation time was 9 min. Areas between neighboring *dashed lines* and cellular lines were regions where EG<sub>3</sub> or EG<sub>6</sub> had diffused (“D”)



**Fig. 3** The measured distance *D* in Fig. 2 (a measure of how much EG<sub>3</sub> and EG<sub>6</sub> diffused to form SAMs under the PDMS stamps) as a function of incubation time

previous studies of SAMs on gold surfaces, the longer chain length thiols formed the thicker SAM films [25, 26]. We speculated that the limited space between the gold surface and the PDMS stamp favors short chain length thiols diffusion to form SAMs.

Diffusion of thiol molecules on gold surfaces covered with PDMS stamps is a complex process. A PDMS stamp seals on the surface of an evaporation-coated gold slide to form a microfluidic channel. Considering the nanostructure in the evaporation-coated gold slide, gaps in nanoscale between the gold surface and the PDMS stamp should exist. Moreover, thiol molecules are able to diffuse in PDMS stamps. The media of the diffusion of thiol molecules are possible when the gaps between the gold surfaces and the PDMS stamps or PDMS covered on the gold surfaces or both. In this study, we focused on the SAMs

formed on gold surfaces by the diffusion of thiol molecules and found that using one-dimensional diffusion from a line source of concentration thiols, the experimental and theoretical results were well fitted.

The diffusion distance *x* can also be expressed as an empirical function of diffusion time *t* according to equation [2]:

$$x = \sqrt{4|D|t \ln \left( \frac{2C_0(\pi D)^{1/2}}{M} \right)} + 2|D|t \ln(t) \tag{3}$$

In which the unit of *x* is μm, and the unit of *t* is minute. We applied this coefficient into the equation and obtained a series of data on diffusion distance versus diffusion time. The calculated results are consistent with those from experiments (Fig. 3). Using this equation, it is straightforward to estimate that the distance EG<sub>3</sub> or EG<sub>6</sub> has migrated to form SAMs under the PDMS stamp in any experiment that requires specific distance of separation between different channels of cells or proteins [11, 12]. We believe that this equation will be useful for designing microfluidic channels in experiments for controlling cell adhesion and migration.

**Conclusion**

In summary, for the first time, using the thiol SAMs property of resisting mammalian cell adhesion and cells as the indicator, we have established a technique to determine thiol diffusion coefficients on gold surfaces covered with

PDMS stamps. We have applied the technique to determine the diffusion coefficients of EG<sub>3</sub> and EG<sub>6</sub> to form SAMs on the surface of a gold slide covered with a PDMS stamp. Microscope image data show that shorter chain length EG<sub>3</sub> diffuses more rapid than longer chain length EG<sub>6</sub>, and the diffusion coefficient of EG<sub>3</sub> is bigger than EG<sub>6</sub>. The diffusion equation [3] established in this study is able to calculate the diffusion distance of thiol SAMs and is beneficial to design experimental procedures in the studies using thiol SAMs to modify gold surfaces under PDMS. The manipulation in this system is very simple, and the instruments required are quite common in biological labs, it is hence potentially adaptable to many types of biological labs to studying diffusion on other material surfaces (for example, glass, silver) covered with other polymer materials with more favorable properties than PDMS.

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