Fabrication of Chemical Gradient Using Space Limited Plasma Oxidation and its Application for Droplet Motion

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A novel, simple, versatile, rapid, and inexpensive method, "space limited oxygen plasma oxidation" is developed to fabricate chemical gradient on both alkylsilane and alkanethiol self-assembled monolayers (SAMs) surface. XPS data confirm that the methyl groups of alkane SAMs are converted into oxidized carbon functional groups. The influences of RF power, O_2 flow rate, and the "wedge" shape on gradient formation are investigated in details. Gradient surfaces with various scales and depths are formed by simply changing the 'wedge' shape or plasma generation parameters. The application of formed chemical gradient on droplet motion has been demonstrated as well.

1. Introduction

A surface having a gradual variation in chemical properties over a certain distance is termed as a surface with chemical gradient. Over the past half century, gradient surfaces have played a pivotal role in numerous aspects of materials and biology research.[1–7] The motivation of making chemical gradients are two-fold: first, they offer the possibility to optimize a desired property by testing a range of surface compositions on one sample in a single high-throughput experiment;[4,7] second, the use of gradients also minimizes sample to sample variation and therefore enhances reliability. Therefore the methods for preparing chemical gradients on substrates have gained considerable attention recently. A number of methods have been used to prepare chemical gradients on various substrates. Many of them involved self-assembled monolayers (SAMs) because they are exquisite systems for introducing functionality to substrates in a chemically well defined way. Ballav et al. prepared alkanethiol gradients on gold substrates using electron-irradiation to tune the exchange reaction between primary SAMs and a potential molecular substituent.[8] Terrill et al. presented a method that relied on the electrochemical desorption of alkanethiols from a fully covered SAM surface upon application of a potential.[9] Direct laser patterning of surface gradients for alkanethiol on gold was demonstrated by Meyyappan et al.[10] Edge spreading lithography was used to generate nanoscale gradient of thiol SAMs.[11] Gradients have also been produced by the oxidation of alkylsilanes using UV - ozone through a density filter,[12] or by microcontact printing technique using curved or tapered poly(dimethylsiloxane) (PDMS).[13,14] However, these forementioned methods rely on either expensive photolithography equipments, or are only suitable for alkanethiol SAMs. Therefore it has great interest in developing a universal and cheap gradient-making method. Recently oxygen plasma was used to convert inert methyl group of SAMs to aldehyde or carboxylic acid functional groups[15–17] for further bio-functionalization of surfaces. Inspired by these results, herein we introduce a novel method named 'space limited plasma oxidation' to fabricate gradients. The advantages of it are fast response (a few seconds), simple and inexpensive (no requirement of additional apparatus except a commercial plasma cleaner), universal (applicable to alkanethiol SAMs, alkylsilane SAMs, and polymer surfaces), and flexible (easy to create variable scales of gradients).

2. Results and Discussion

2.1. Validation of 'Space Limited Plasma Oxidation' Method for Fabricating Chemical Gradient of SAM Surface

The idea of 'space limited plasma oxidation' is illustrated in **Figure 1**. The SAMs modified substrates are covered by a glass slide with an extremely thin spacer at one end to form a 'wedge' shape space (shown in Figure 1a, not to scale). Figure 1b is the side view of Figure 1a. The size of the 'wedge' can be adjusted by varying *L* and *H*. In the following example, the values of *L* and *H* are 20 mm and 100 μm respectively, to form an extremely shallow 'wedge'. When the 'wedge' shaped space is filled with oxygen plasma, the gradient of the number of oxygen ions is determined by the 'wedge' space and increases from position 1 to 2 (Figure 1b). If the exposure time is short enough so that the oxygen plasma reacted with SAM molecules along the dash line region (Figure 1a) can not be refreshed by the oxygen plasma outside of the wedge, the number of oxidized SAM molecules on the surface will be determined by oxygen ion gradient, in other words, the number of oxidized SAM molecules in a unit area will increase along the dash line from position 1 to 2, thus to create a chemical gradient on the surface (Figure 1c).

In order to confirm this hypothesis, octadecyltrichlorosilane (OTS) SAM modified surface was used to generate gradient using this method. The values of *L*, *H*, and *W* are 20 mm,

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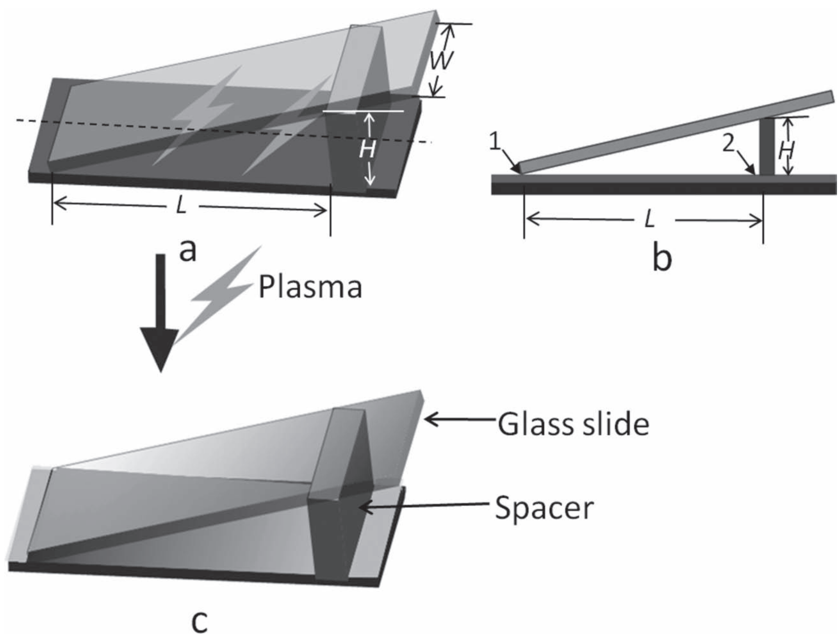

Figure 1. The schematic of SAMs gradient fabrication using space limited plasma oxidation technique (Not to scale). a) The sample block including a SAM modified bottom substrate. b) The side view of a). c) The grayscale gradient from left to right on the sample represents chemical gradient after plasma oxidation.

100 μm, and 10 mm respectively. The built sample block was loaded in a vacuum chamber of a radio frequency (RF) plasma system (Diener), and exposed to oxygen plasma under 50 W *RF* power, 80 cm^3 min^{-1} O_2 *flow* rate for 3s. The contact angle of the resultant surface was measured from position 1 to 2 along the middle line (dash line shown in Figure 1a). The results are shown in **Figure 2**a. It is clear to see that a gradient surface has been created by this way. The initially hydrophobic surface (115°)[18,19] became gradually hydrophilic from position of 0 mm to 10 mm (15°). The depth of the gradient is ~ 100°. In the area between position 10 to 20 mm, the contact angles are constant (data between 16 to 20 mm are not shown). The images of water droplet in different position, shown in Figure 2a, also confirmed the formation of gradient surface.

The wettingability gradient of SAM surface are caused by the conversion of methyl group (–CH_3) of organosilane SAMs into polar functional groups such as a carboxyl (–COOH), aldehyde (–CHO) and hydroxyl (–C-OH) group under oxygen plasma, which was confirmed both by literature results[15–17] and our own X-ray photoelectron spectroscopy (XPS) data, shown in **Figure 3**. Figure 3a shows the C1s peak of OTS SAM before treating with oxygen plasma. It is mainly composed of C–C bond with atomic area percentage of 96%. After 4s oxygen plasma oxidation (*RF power* of 37 W, O_2 *flow rate* of 54 cm^3 min^{-1}) in a wedge shaped space (*L* of 20 mm, *H* of 100 μm, and *W* of 10 mm), the gradient surface was characterized using XPS along the dash line (Figure 1a) from position 1 to 2. The C1s XPS data of position 2 mm, 6 mm, and 10 mm are shown in Figure 3b, c, and d, respectively. Peak 1, 2, 3, and 4 are assigned to C–C (284.5 eV), C–OR (286.5 eV), C=O (287.9 eV), O–C=O (289.1 eV) respectively. It is clear to see from Figure 3a and b that after plasma treatment the oxidized carbon peaks (peak 2, 3, 4) increase, meanwhile C–C peaks decrease. It indicates the conversion of methyl group into polar functional groups. According to the peak area, the surface atomic ratios for those four peaks are listed in **Table 1**. Figure 3e shows the sum of atomic ratios of peak 2, 3, 4 as a function of positions on the gradient surface. It can be seen that the atomic ratios of oxidized peaks become more and more pronounced from position 2 to 10 mm and reach a plateau after 10 mm.

The gradient surface can be visualized by attaching gold nanoparticles[20] or cells.[21] In the following contexts the fluorescent latex beads were attached onto chemical gradient surfaces for visualization. It is well known that NHS/EDC actives –COOH group for attaching –NH_2 functionalized reagents.[22–24] The mixture of NHS/EDC (0.2 mM for each component, molar ratio 5:1) was used to active gradient surface for 1 hour. After thoroughly rinsing with water, the activated gradient surface was incubated in amine functionalized fluorescence beads for 1 hour. The results are shown in Figure 2b. The density of fluorescence beads increases along with the hydrophilicity of the surface. It indicates a –COOH gradient on the surface, which is consistent to –COOH area ratios listed in bottom

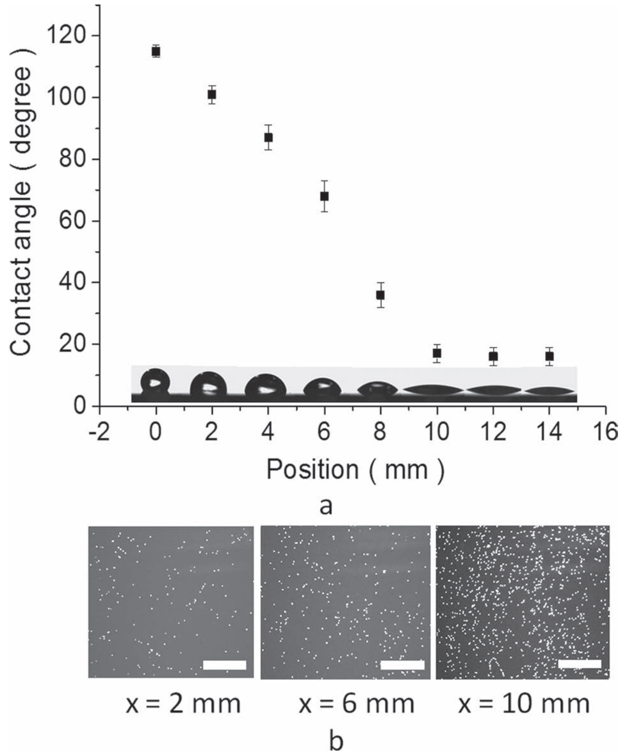

Figure 2. a) The contact angle data and water droplet images (side view) as a function of distance starting from position 1. b) The fluorescence microscopy images of amine functionalized beads attached onto different regions of the chemical gradient surface. The scale bar is 200 μm.

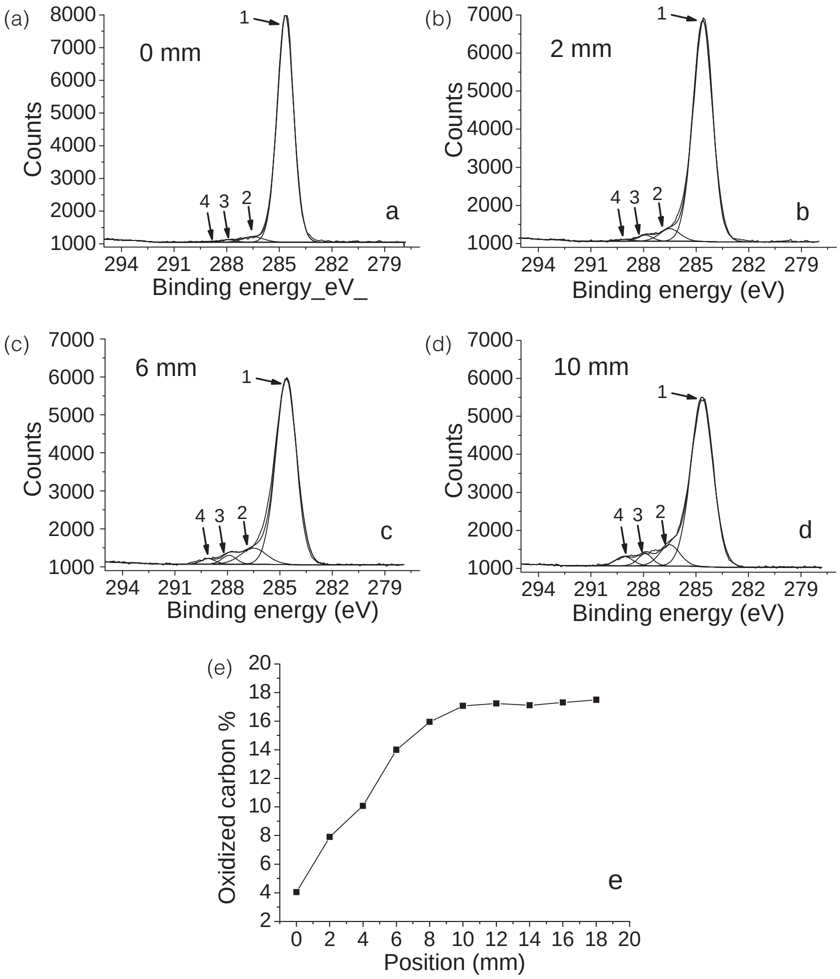

Figure 3. XPS characterization of OTS SAM gradient surface. a) C1s data of OTS SAM surface. C1s data of gradient surface at position of b) 2 mm, c) 6 mm, and d) 10 mm. e) Oxidized carbon area ratio against distance starting from position 1 in figure 1b.

line in Table 1. More importantly, this experiment demonstrates that the gradient surface can be further biofunctionalized by other amine containing materials, such as proteins.

2.2. Influence of *H*, *Plasma Power*, *O_2 Flow Rate*, and *W* on Gradient Fabrication

The influences of parameters of wedge shape, *RF power*, and O_2 *flow rate* on gradient fabrication were investigated in details in the following content.

Table 1. The atomic ratios of C1s XPS data of a chemical gradient.

Bands	Chemical species	Banding energy [eV]	0 mm Atomic%	2 mm Atomic %	6 mm Atomic %	10 mm Atomic %
1	C–C	284.5	95.96	92.09	86.00	82.94
2	C–OR	286.5	2.84	5.40	9.30	9.54
3	C=O	287.9	0.88	1.88	2.89	3.92
4	O–C=O	289.1	0.33	0.63	1.80	3.61

In order to study the influence of wedge height *H*, we fixed *RF power*, O_2 *flow rate*, exposure time, *W*, and *L* to be 37 W, 54 cm^3min^{-1}, 2 s, 10 mm, and 20 mm respectively. The *H* values were varied from 100 to 400 µm. The water contact angle data of gradient surfaces are shown in **Figure 4**a. The chemical gradient is very shallow from 0 to 18 mm at *H* of 100 µm (tilt angle 0.29°), while it becomes much steep and deep from 112° to 35° between 0 to 12 mm at *H* of 200 µm (tilt angle 0.57°). When the *H* is 400 µm (tilt angle 1.14°) a short gradient with a slop of 36°/mm between 0 to 2 mm was generated. The left shift of end point of each gradient demonstrates the importance of wedge space in this method. The higher *H* allows more oxygen plasma filled in the wedge, and caused shorter and deeper chemical gradients.

To study the influence of *RF power* on gradient formation, we fixed *O_2 flow rate*, exposure time, *W, L,* and *H* to be 54 cm^3 min^{-1}, 2 s, 10 mm, 20 mm, and 100 µm respectively. From Figure 4b, it can be seen that 18 W *RF power* generates narrow gradient between 112° to 94° from 0 to 4 mm, while 74 W RF power generates steep and wide gradient between 112° to 42° from 0 to 12 mm. The higher *RF power* converts more methyl groups into oxidized carbon groups.

In the study of the influence of *O_2 flow rate* on gradient generation, the *RF power*, exposure time, *W, L,* and *H* were fixed to be 37 W, 2 s, 10 mm, 20 mm, and 100 µm respectively. Four *O_2 flow rates* were studied to generate chemical gradients, as shown in Figure 4c. The higher flow rate generates steep gradients.

In terms of *W* influence, the *RF power*, O_2 *flow rate*, exposure time, *L*, and *H* were fixed to be 37 W, 54 cm^3min^{-1}, 2s, 20 mm, and 100 µm respectively. The water contact angle data of gradient at *W* of 10 mm and 5 mm are shown in Figure 4d. The short *W* causes that the O_2 plasma along the dot line (Figure 1a) is easier to be refreshed. Thus a steeper gradient was generated.

In order to create short scale gradient surface, we shortened *L* to 2 mm and kept the *RF power*, O_2 *flow rate*, exposure time, *W*, and *H* to be 37 W, 80 cm^3min^{-1}, 1s, 10 mm, and 100 µm respectively. An approximate 1 mm gradient from OTS SAMs was created under such experimental conditions. Although its wetting property is difficult to be measured due to small scale of the gradient, it can be visualized by attaching amine functionalized fluorescent beads via NHS/EDC chemistry. The fluorescence image is shown in **Figure 5**. The average fluorescence intensity profile in the white box area clearly shows the gradient on the surface.

2.3. Creating Chemical Gradient Surface on Alkanethiol SAMs

Apart from the alkylsilane SAMs, alkanethiol SAMs on gold surface can also be converted into a gradient surface using this

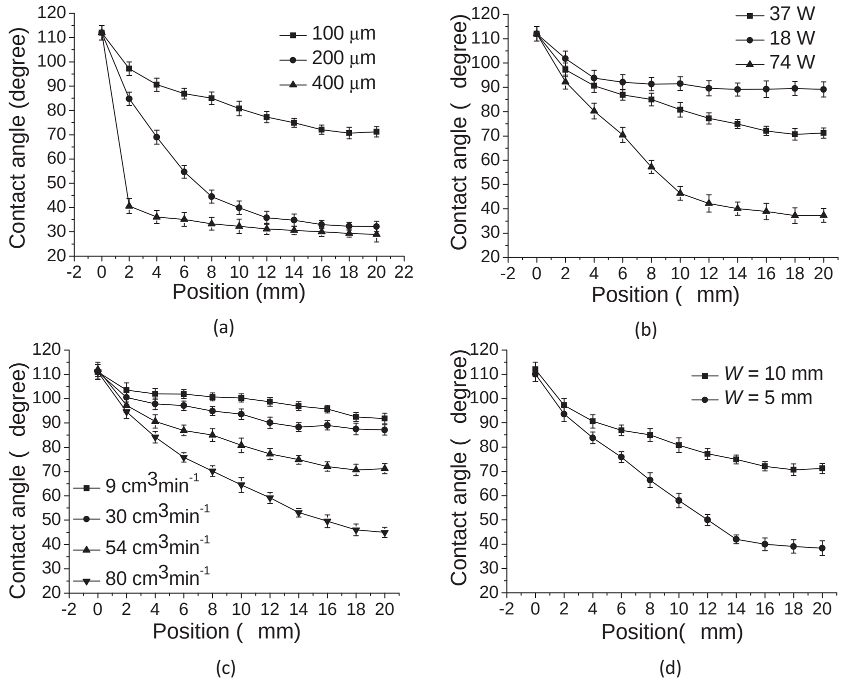

Figure 4. The influence of parameters: a) *H*, b) *RF power*, c) O_2 *flow rate*, d) *W* on gradient generation using space limited plasma oxidation method.

method. The perfluorodecanethiol SAM (wedge shape of L = 20 mm, W = 10 mm, and H = 100 μm respectively) was exposed to oxygen plasma (*RF power* of 37 W, O_2 *flow rate* of 54 cm^3min^{-1}) for 12s. The contact angle data, shown in **Figure 6**, confirm the formation of gradient. Compared with OTS SAMs, the longer exposure time implies that the fluorocarbon is more inert to be converted into polar groups. The gradient surface contains the mixture of fluorodecanethiol molecules and converted polar molecules.

2.4. Application of Gradient Surface on Droplet Motion

The chemical gradient surface is of great interest for numerous practical applications, such as in the studies of biomolecular interactions and cell mobility.[7,25,26] A gradient surface can also induce the mass transport of liquid,[1,5] which affords a driving force for droplet based microfluidics.[27] Herein we explored the application of 'space limited plasma oxidation' generated gradient surface (plot 1 in **Figure 7**a) on droplet motion. The sequential pictures in Figure 7b clearly depict the spontaneous motion of a droplet on gradient SAM surface. Plot 2 in Figure 7a is the droplet moving distance as a function of time. The velocity plot 3 in Figure 7b is the differential result of plot 2. The maximum velocity is 0.64 mm s^{-1} at the contact angle of 70°. From 77° to 70°, the droplet was exerted a positive force to drive the droplet moving, while between 70° and 50° a negative force slowed down the droplet.

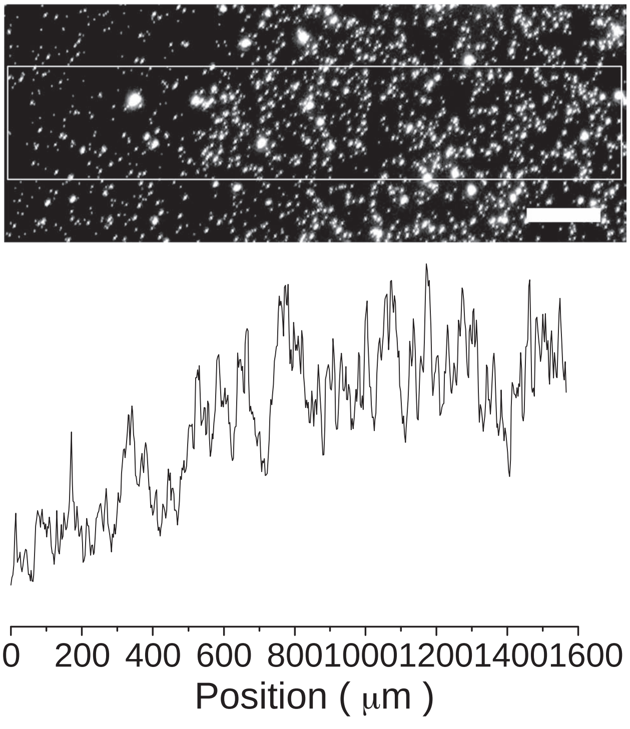

Figure 5. The fluorescence image of amine functionalized polystyrene beads attached on gradient surface generated from OTS SAMs using space limited plasma oxidation method (top), and the average fluorescence intensity of the white box area (bottom). The scale bar is 200 μm.

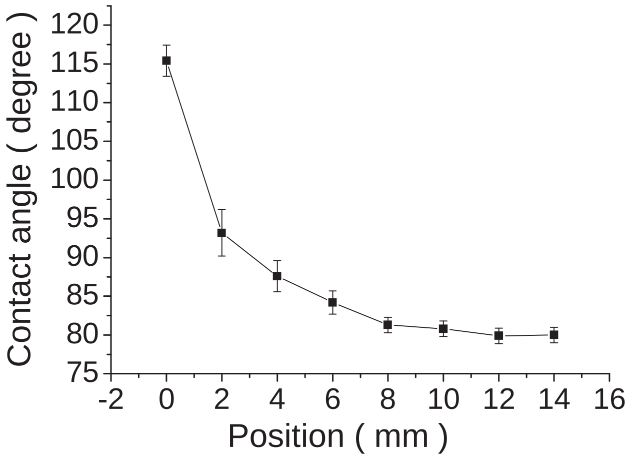

Figure 6. Contact angle data of gradient surface created from perfluorodecanethiol SAMs.

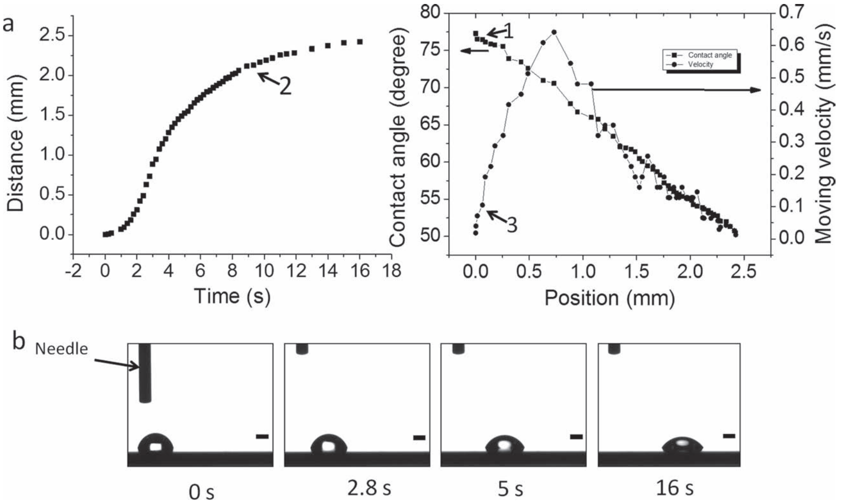

Figure 7. Investigation of droplet motion on chemical gradient surface. a) Plot 1, 2, 3 are contact angle data against distance, droplet moving distance against time, and the differential of plot 2, respectively. b) The sequential images of a droplet motion. The scale bar is 0.5 mm.

3. Conclusions

We have demonstrated a novel method, "space limited plasma oxidation", to fabricate SAMs gradients. SAMs both on silicon and gold substrate were converted into gradient surfaces using this method. The various scales (from 1 to 18 mm) and depths (from 7° to 100°) of the gradient surfaces were fabricated by simply changing the wedge shape or plasma generation parameters. The higher *RF power* and O_2 *flow rate* help the conversion of methyl to oxidized carbon groups. Automatic droplet motion was found in created chemical gradient surface. It can also be applied in the field of liquid crystal displays. This method is highly versatile, rapid, and low cost. Apart from the methyl terminated SAMs, other functional groups terminated SAMs, such as amine, can be converted into the binary component gradient surface. Considering the importance of carboxyl, aldehyde function group, the gradient created by this method has great potential in attaching biomolecules (enzymes, antibodies, DNA, etc.) for investigation of biomolecular interactions, cell-adhesion studies, or diagnostics.[7]

4. Experimental Section

Materials: Methanol, toluene and coverslips (100 μm thick) were supplied by Fisher scientific. Silicon substrates from Rockwood Electronic Materials were cut into pieces with a size of 1 × 2.6 cm. Octadecyltrichlorosilane (OTS), 1H,1H,2H,2H-Perfluorodecanethiol, Hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (NHS), N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and amine functionalized polystyrene beads were purchased from Sigma. Millipore Milli-Q water with a resistivity of 18.2 MΩ cm was used throughout.

Substrate cleaning: Silicon substrates were cleaned by ultrasonication in methanol for 5 min, dried under a stream of nitrogen, and rinsed in Milli-Q water before being immersed in Piranha solution (70:30, v/v, H_2SO_4:H_2O_2; Caution: Piranha solution reacts violently with organic materials and should be treated with great care) for 5 min. The substrates were rinsed in Milli-Q water and dried under nitrogen. Gold substrates (100 nm thick evaporated gold films on glass slide with an adhesion layer of 5 nm chromium) were cleaned in a similar way but only 30s in Piranha solution.

SAMs formation: The clean silicon substrates were immersed into anhydrous toluene solution with 1% OTS under a nitrogen atmosphere for 2 h at room temperature. After silanisation they were washed with toluene and followed drying under nitrogen. In terms of thiol SAMs, the cleaned gold substrates were incubated in 1 mM perfluorodecanethiol in ethanol for 1 hour. The SAMs were used immediately.

Wetting angle measurements: Contact angles were measured on a home-made instrument. The images of droplets were analyzed using Imagej program to obtain contact angle data.

Fluorescence microscopy images: A Nikon Eclipse 80i fluorescence microscope equipped with a Nikon DS-Fi1 digital camera was used to image the fluorescent beads on the gradient surfaces.

X-ray photoelectron spectroscopy (XPS): Spectra were obtained using a Thermo VG ESCALAB MK II with a base pressure maintained below 5 × 10^{-9} mbar during acquisition. All spectra were recorded using Mg Ka source at 15 kV and 10 mA. Spectra were obtained with an electron takeoff angle of 90° (i.e., the angle between the surface of the sample and the photoelectron detector).

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