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Trapping of Vesicles on Patterned Surfaces by Physisorption for Potential Biosensing Applications*

L. K. Bera, Kian Soo Ong, Zheng Zheng Wong, Zhikang Fu, Madhavan Nallani, Sean O' Shea

Abstract— **The pre-defined selective positioning of a controlled number of vesicles on a rigid substrate is crucial in many potential applications such as diagnostics, biosensors, lab-on-a chip, microanalyses and reaction chambers. In this paper, the vesicles made up of block copolymer using Poly [-(2-methyloxazoline) -poly- (dimethylsiloxane)-poly- (2-methyloxazoline)] (ABA) with dimensions of 100-200 nm are trapped by physisorption on hydrophilic surfaces. We discuss the protocols established for vesicle trapping. The optimum conditions obtained for physisorption is 15 minutes incubation followed by one cycle of DI water rinse. Trapping of 1-10 vesicles in lobe shape micro-wells fabricated by photo lithography using photoresist on UltraStick™ slides was demonstrated. To overcome the issue of amalgamation of emitted light from optically sensitive photoresist and fluorescently tagged vesicles, an alternative approach of Si/SiO_2 microwell array coupled with APTES (3-Amino-PropylTriEthoxySilane) treated bottom surfaces was developed.**

I. Introduction

For the fabrication of biosensors, lab-on-a-chip and micro array assays it is desirable to place intact vesicles at precise locations on solid substrates. There has been considerable interest into the trapping of intact vesicles at precise locations on solid substrates by surface immobilization [1-12]. The optical tweezer technique is widely used to capture single vesicles [13]. However, the optical tweezer technique suffers from several limitations such as difficulty in guiding pre-assigned number of vesicles to be trapped and the high intensity of focused laser beam will cause local heating which can affect the local viscosity of the medium, etc, [14]. Krishnan et al [15] demonstrated geometry-induced electrostatic trapping of nanometric objects in a fluid. However, in this technique the particle or vesicle needs to be electrically charged so that a fluidic slit with appropriately tailored topography can trap such charged objects, hence increasing the complexity of vesicle synthesis and substrate fabrication, and limiting the application where electrical charging is not allowed. Frequently, vesicles made up of lipids have been proposed to be immobilized for such applications. However, these applications have achieved limited success due to their insufficient stability [16]. Block copolymer vesicles or "polymersomes" as an alternative to lipid have attracted increasing scientific research interest based on their excellent stability and the potential to control biological, chemical and physical properties by tailoring block lengths, block chemistry and functionalization [17-20].

In this work, a cost effective and convenient conventional lithography approach has been used to pattern micro wells of various sizes ranging from 5µm×5µm to 25µm×25µm. The aim of such design is to enhance probability of trapping specific quantity of vesicles in the micro well area. In order to achieve this objective, optimization experiments were done including incubation time and DI water rinsing for trapping of 1-10 vesicles with 200nm diameter vesicles by physisorption in a micro well on UltraStick™ (with enhanced silane coating) and APTES (3-Amino-PropylTriEthoxySilane) treated surfaces.

II. Preparation of and Characterization of Polymer Vesicles

Poly [-(2-methyloxazoline) -poly- (dimethylsiloxane)- poly-(2-methyloxazoline)] (PMOXA-PDMS - PMOXA) ABA tri-block copolymer was used to prepared vesicles as described earlier [21].The vesicles were characterized using high resolution TEM and dynamic light scattering (DLS) techniques. The physical structural analysis of vesicles was done using high resolution transmission microscopy (HRTEM). The images of vesicles are shown in Figure 1. The diameter of vesicles from TEM is twice as small as the hydrodynamic radius obtained by DLS [22, 23] because the TEM is undertaken in vacuum. The typical wall thickness is observed ~5-7nm.

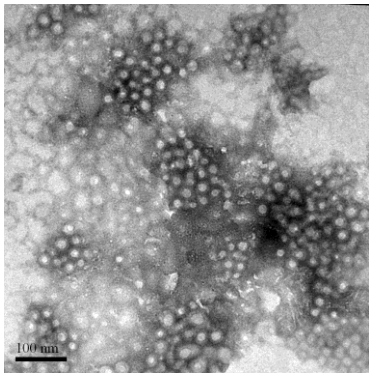

Figure 1. High resolution TEM images of vesicles. The vesicle solution was dispensed on a solid copper grid and images taken at vacuum ambient.

III. Optimization of Protocol for Vesicle Trapping by Physisorption

Adsorption of vesicles from a colloidal solution onto a solid may proceed by purely physical means (physisorption) or may require the formation of chemical bonds (chemisorption). In this study we are focusing on vesicle attachment by purely physical adsorption process (physisorption). The protocol of vesicle trapping by

*Institute of Materials Research and Engineering, A*STAR (Agency for Science, Technology and Research), 3 Research Link Singapore 117602.

E-mail: wongzz@imre.a-star.edu.sg,
Fax: +65 68720785
Tel: +65 65141446

physisorption consists of optimizing the dispensing volume, incubation time, DI water rinse and subsequent verification by SEM imaging (Figure 2). The experiments were carried out directly on unpatterned UltraStick™ slides. These slides were selected because they have -NH_2 terminated surface which can form hydrogen bonding with the carboxylic functional groups on the surface of ABA vesicles.

The experimental details for protocol optimization are depicted in table-1 as below. Each set of experiments was repeated 5 times.

TABLE I. EXPERIMENT FOR VESICLE TRAPPING PROTOCOL OPTIMIZATION

Samples	Substrate (glass)	V (µL)	Vesicles suspension solution	Incubation time	DI rinse cycle (5 sec dip/cycle)
R3	-NH2 terminated	5	DI	15min	3
R4	-NH2 terminated	5	DI	15min	3
R5	-NH2 terminated	5	DI	15min	3
R6	-NH2 terminated	5	DI	15min	1
R7	-NH2 terminated	5	DI	15min	1
R8	-NH2 terminated	5	DI	15min	1
V17	-NH2 terminated	5	DI	15 min	1
V18	-NH2 terminated	0.1	DI	2 min	1
V10	-NH2 terminated	5	DI	2 min	3
T1	-NH2 terminated	5	DI	1 hr	3
T2	-NH2 terminated	5	DI	1 hr	3
T3	-NH2 terminated	5	DI	1 hr	3
T4	-NH2 terminated	5	DI	2 hr	3
T5	-NH2 terminated	5	DI	2 hr	3
T6	-NH2 terminated	5	DI	2 hr	3

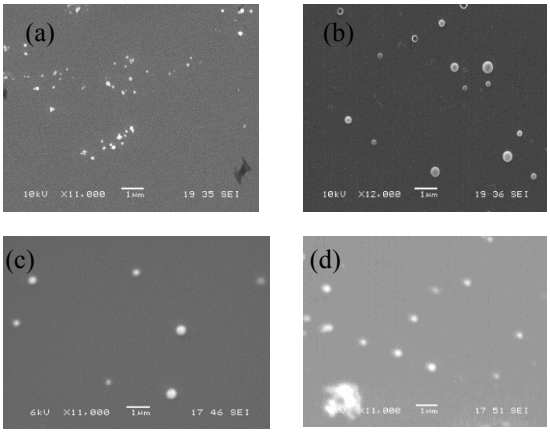

Figure 2. Typical SEM images of vesicles at different incubation times. (a) 2 min incubation time and DI rinse 3 cycles, (b) 15 min incubation time and DI rinse 3 cycles, (c) 60 min incubation time and DI rinse 3 cycles, (d) 120 min incubation time and DI rinse 3 cycles.

To investigate incubation time, solution of 5 µL was dispensed on UltraStick™ slides for physisorption onto the substrate surface. Several incubation times were used as shown in table 1. After the predefined incubation period, the samples were rinsed using 3 cycles (duration of rinsing for each cycle is 5sec) of DI water by dipping. SEM imaging was carried out on rinsed samples after natural drying. The vesicle density was extracted from SEM images using ImageJ analysis. The statistical variation of vesicle density versus incubation time is plotted in figure 3.

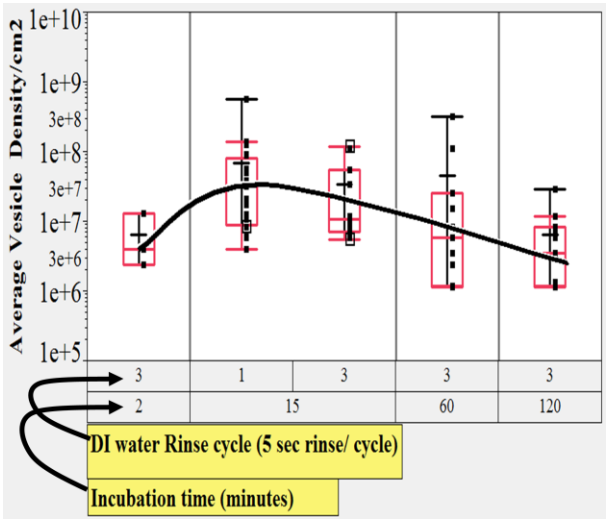

Figure 3. The vesicle density versus incubation time and DI rinse cycles. The vesicle density was extracted from SEM images using ImageJ analysis.

The number of physisorbed vesicles increases with incubation time from 2 minutes to 15 minutes. However, this trend is not observed for longer incubation periods (60 minutes to 120 minutes). In fact the vesicle density reduces for longer incubation time. From this experiment it is clear that adsorption of vesicles on -NH_2 terminated surface occurs in only a very short incubation period. The decay of vesicle density adsorption for longer incubation period is probably due to a fraction of vesicles on the surface undergoing some deformation and forming a monolayer film, as shown in the SEM image in figure 4.

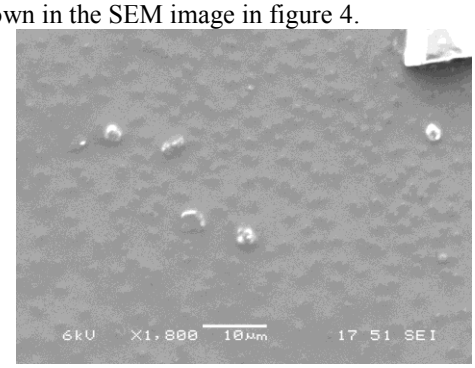

Figure 4. SEM images of vesicles after 120 minutes of incubation time. Longer incubation time shows some vesicle deformation and formation of a monolayer film.

For practical applications it is essential to know the strength of vesicle adhesion on rinsing. Two different DI (de-ionized) water rinsing cycles (1 cycle and 3 cycles) were studied. Each cycle is defined as 5sec DI water rinse. It is observed that 1 cycle rinsing maintains more vesicles than 3 cycles. A reduction of vesicles after prolonged DI rinsing is expected due to the lower physisorption energy as compared to chemical attachment.

IV. VESICLE TRAPPING BY PATTERNED MICRO WELL

A. Photo resist Microwell on UltraStick™ slides

The fabrication of microwells made from resist was carried out using standard lithography process. Resist thickness 1.5µm was used in this experiment. After pattern transfer onto UltraStick™ slides, a 5 µL of as prepared vesicle solution without any dilution was manually dispensed on the patterned slide. Incubation time was fixed at 15 min and 1 cycle of DI rinse. Figure 5 shows the SEM images of a lobe shape micro well pattern. (Note that such architecture was achieved by adjusting the exposure energy during lithography.) Between 1 to 10 vesicles were observed to be trapped in a single well. On drying, the air in the lobe architecture drove the vesicles towards the center of the well as shown in figure 5.

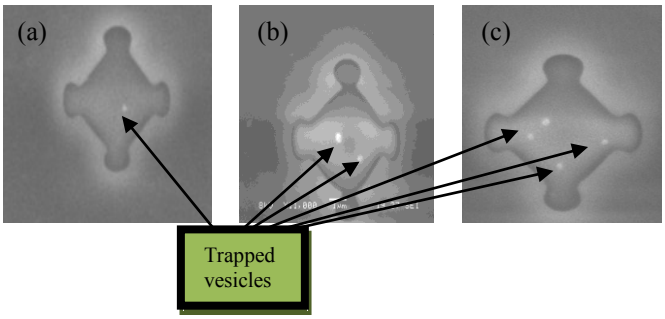

Figure 5. SEM images of lobe shape micro well pattern on UltraStick™ slides using photoresist: (a) one vesicle, (b) two vesicles and (c) four vesicles. Between 1-10 vesicle trapping was observed in a single well. The dimension of each microwell is 5µ×5µ.

The effect of increasing well size (25×25µm) was also investigated and it was found that vesicles attached randomly inside the well as shown in figure 6. The size of the extended lobe shape structure for this case is small compared to well size and therefore does not influence the drying forces.

Photoresist is optically sensitive material and prone to emit certain wavelength which may blend with emitted light from fluorescent tags during optical imaging. An alternative method is the fabrication of the microarray on Si/SiO_2 substrate.

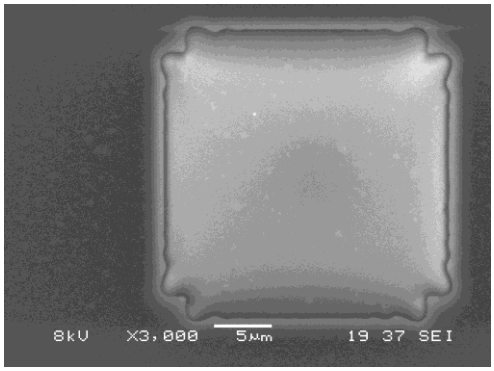

Figure 6. SEM image of 25×25µm well with vesicles. A large number of vesicles are trapped randomly inside the well.

B. APTES coated microwell on Si/SiO_2 surface

The microwell array was fabricated on Si/SiO_2 substrate using standard lithography and dry etching processes. The schematic process flow is shown in figure 7.

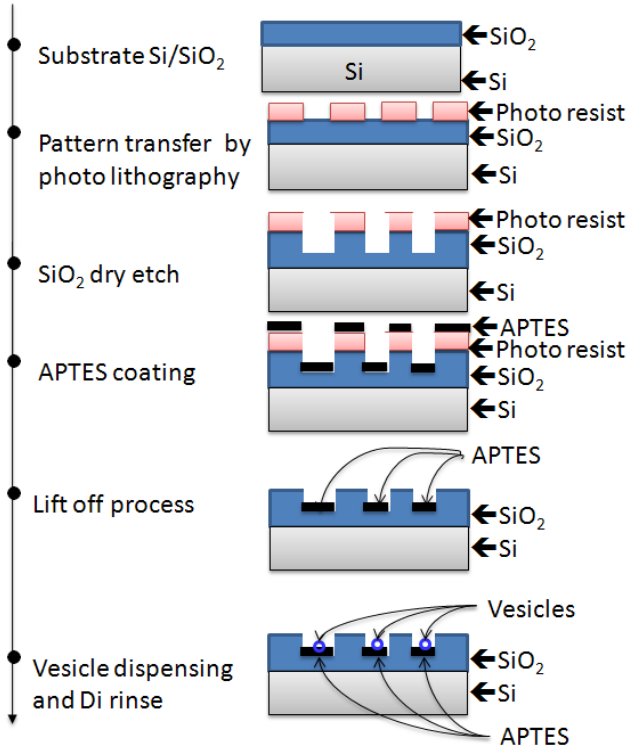

Figure 7. The schematic process flow for fabrication of a microwell array on Si/SiO_2 substrate with amino- silanization at the bottom of the well.

The starting thickness of SiO_2 was 4000A. After transferring the pattern onto the substrate, a partial SiO_2 etch was performed using dry plasma etching. The partial etch was used in order to keep thin oxide at the well region. Typically the silicon dioxide surface is composed of Si-O-Si and Si-OH, where the latter can be deprotonated or chemically modified by reactive silane compounds like APTES [24]. Therefore, the partial etch is essential for providing a suitable surface for the APTES primer. For priming, a desiccator was connected to a vacuum pump for 15 min and sufficient amount of APTES (99%, Sigma-Aldrich) was placed into a small container at the bottom of the desiccator. The patterned samples were placed immediately into the desiccator. The desiccator was pumped down for another 15 minutes and then sealed off, leaving the samples exposed to APTES vapor for ~2 h. After this exposure, the APTES was removed and the desiccator was evacuated to less than 100 mbar pressure. An amino-terminated organosilane layer was formed on the top surface of the substrate and not the side wall during this vapor deposition process. Lift off technology was used after APTES coating and curing at 105°C for 5 minutes. This process flow enables the patterning of amino (–NH2) terminated APTES array at predefined etch pits location. A 5µL vesicle solution was dispensed on the amino terminated microwells which were then incubated for 15 minutes, and one cycle of DI rinse was applied as determined from the protocol optimization. The SEM images of microwells with vesicles are shown in figure 8.

Vesicles are attached at the bottom of each well. Some vesicles are observed to stick there on the rough side wall, as

it is believed more adsorption sites are presented on the rough surface to help anchor the vesicles. In this study the vesicle suspension solution was DI water and the pH is ~6.5. The –NH2 terminated surface at solution with pH value of 6.5 to 7 becomes positive by de-protonation and tends to repel hydrogen ions away from the surface, whereas –OH terminated surface is negatively charged [25]. Thus –OH bonds at the outer surface of vesicles tend to attach at the positively charged –NH2 terminated surface at the bottom of the microwell. A schematic model of vesicle bonding to amino surface is shown in figure 9. The bond energy by de-

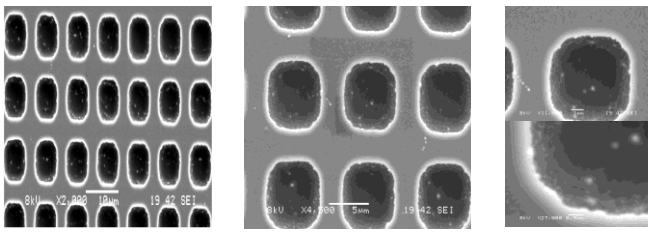

Figure 8. SEM images of microwell arrays fabricated on Si/SiO_2 substrate. Vesicles are attached at the bottom of each well, although the rough side wall also has some vesicles attached.

protonation is ~10 KT at room temperature [26] and thus bonding/attachments by this process falls under physisorption [27]. Excessive DI water rinses will cause detachment of the vesicles from the surface.

Physisorption is less complicated than chemisorption which usually require multiple steps of processing to realize the final device. In potential applications such as biosensors, lab-on-a-chip and micro array assays, vesicles bound with biomolecules can be prepared in solution prior to being attached to substrates via physisorption.

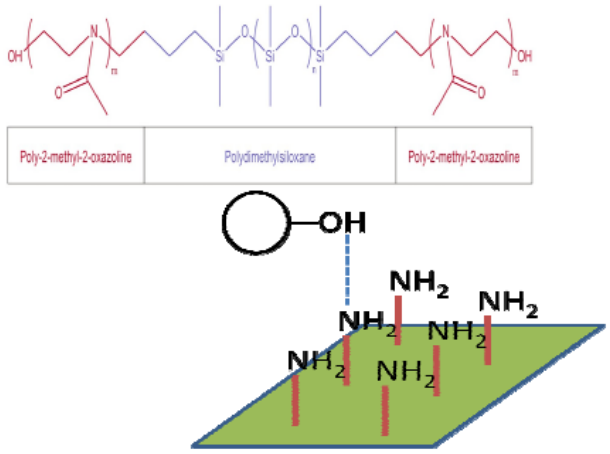

Figure 9. A schematic model of vesicle bonding to an amino terminated surface.

V.CONCLUSIONS

Positioning of vesicles in arrays on predefined locations is important in many potential applications. We demonstrated trapping of vesicles by physisorption on $–NH_2$ terminated surfaces. A protocol optimization for vesicles trapping was established. An incubation time of 15 minutes and one cycle of DI water rinsing is the optimum set of conditions. It was also demonstrated that 1-10 vesicles of 100-200 nm diameter were trapped in a single lobe shape microwell fabricated using photoresist on UltraStick™ slides. An alternative to using photoresist is the fabrication of microarrays on Si/SiO_2 substrate coupled with amino terminated surface at the bottom of the well region. Vesicle attachment at the bottom of these microwells has been successfully shown. The bonding of vesicles at the amino surface is purely by physorption, and aggressive DI water rinses cause detachment of vesicles from the surface. Future studies will target the use of suitably modified vesicles for chemisorptions onto the microarray.

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