

# Surface Energy Induced Patterning of Polymer Nanostructures for Cancer Diagnosis and Therapy

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**Abstract**—We have developed a new simple method to pattern discrete polymer micro and nanostructures. A Si template is patterned by lithography and selective surface treatment to have spatially different surface energies that induce microfluidic self-patterning of a spincoated polymer layer. Biocompatible diblock co-polymer and SU8 are patterned using this method to form monodisperse and shape specific microstructures. After patterning, these particles are lifted off the template surface into aqueous solution. The template is then cleaned and re-used. These freestanding polymer particles with uniform and precise spherical morphology can be used as carriers for drug and imaging agents for biomedical applications.

## I. INTRODUCTION

THROUGH the engineering of bioactive nanostructures and processes, a new wave of medical innovation may be stimulated: for example, functionalized nanoparticles or nanoscale capsules for advanced delivery, targeting, and recognition [1-6]; nano-probes or nanomaterials for early detection of disease and ultra-sensitive imaging [7][8]; nanostructured scaffolds to promote tissue regeneration [9-11]. For these exploitations of nanotechnology, the ability to fabricate structures with nanometer precision is of fundamental importance. Cost effective fabrication methods are highly desired to produce polymeric nanostructures with precise control of geometry, dimensions, and uniformity, but without changing/damaging their active bio-functionality.

Available fabrication methods are mainly divided into “bottom-up” self-assembly and “top-down” nanolithography in conjunction with pattern transfer methods such as etching and liftoff processes. However for self-assembly the control of uniformity, 3D geometry, and transfer to substrate are difficult [5]. For lithography, patterning functional organic materials is a challenge. Pattern transfer using liftoff will involve strong solvents, which is not compatible for organic materials. Pattern

transfer with etching will damage or change functionality of materials as well. Nanoimprint lithography (NIL) [12] and soft lithography [13] are feasible methods to produce polymer structures by thermal or UV induced molding. Other strategies to utilize polymer capillary flow for patterning of micro and nanostructures have also been reported, such as edge lithography [14] [15] and lithography induced self-assembly [16]. However, for NIL and its derivatives, a layer of residue is commonly formed and an invasive etching step is still needed to form separated particles. Recently, DeSimone *et al.* extended soft lithography with perfluoro-polymer molds on non-wetting substrates to fabricate monodispersed particles with controlled morphology [17]. However in order to make discrete polymer structures, liquid precursors must be used, which limits its application to solid phase materials.

Here we present a simple process to pattern discrete polymer micro and nanostructures through discrete surface energy induced microfluidic self-patterning. This method is based on patterning a template to have spatially different surface energies via conventional lithography and selective surface treatment. Then the discrete surface energies on the substrate induce microfluidic self-patterning of materials that are deposited on the surface. The major advantage of this method is that once the template is made, monodispersed particles can be produced by simply putting the sample on a hotplate to induce polymer reflow. After the particles have been harvested, the template can be re-used for “lithography-less” fabrication of particles.

## II. EXPERIMENTS AND RESULTS

### A. SEIP process

Figure 1 illustrates the process of the surface energy induced patterning (SEIP). First, nanoimprint or photolithography is used to define openings in the resist. Then trichlorosilane self-assembled monolayers (SAMS), such as (Heptadecafluoro-1,1,2,2 - tetrahydrodecyl) trichlorosilane (FDTs) is covalently bonded to the hydroxyl groups on the surface of the openings. The resist is removed and the rest of Si is modified with methacryloxypropyl trichlorosilane (MOPTS). A template with spatially different surface energies is formed and then spincoated with polymer. Heating the deposited polymer film above its glass transition temperature ( $T_g$ ) on a hot plate allows the material to re-flow and self-pattern to the

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areas of high surface energy (MOPTS treated Si), leaving empty space at the areas of low surface energy (FDTs treated Si). In this way, discrete polymer structures can be made. For freestanding particles, these structures can be harvested from the template into aqueous solution. Then the template can be cleaned and re-used to produce more.

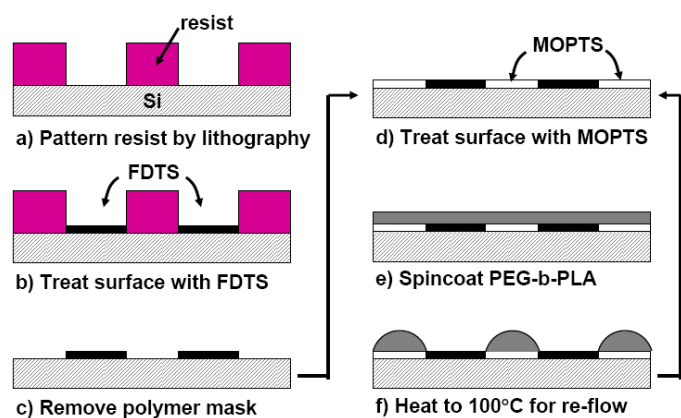


Fig. 1. Schematic of SEIP process flow

### B. Template fabrication

Photolithography and NIIL were used to make the discrete surface energy templates. Templates with 0.5  $\mu\text{m}$  and 1  $\mu\text{m}$  wide chemical lines were produced using nanoimprint lithography into 500 nm thick 950K poly (methyl methacrylate) (PMMA) on an Obducat 2.5 nanoimprinter at a temperature of 180  $^{\circ}\text{C}$  and a pressure of 6 MPa for 10 min. The PMMA residue layer was then removed by reactive ion etching. Exposed Si areas were then soaked in the FDTs ( $\sim 0.1\%$  in n-heptane) solution. PMMA was found to be a good mask material to selectively treat the substrates, keeping the n-heptane solution from penetrating and treating the masked areas. The PMMA was then removed with acetone. The template was then soaked in MOPTS solution in n-heptane to coat the rest of the Si template. With similar process, templates with 2  $\mu\text{m}$  dots were made by photolithography in S1813 resist over over a 1 sq.in.Si Sample. The SAMS treatment was the same as the nanoimprint process. After the templates were made, AFM was used to evaluate their surface conditions. As shown in Fig. 2, AFM image of FDTs treated Si (before MOPTS treatment) shows that the FDTs thickness measured 0.65-0.94 nm. After MOPTS treatment, the surface topography is very small. Topography image shows no significant patterns (Fig. 2b), while phase image shows clear dot patterns (Fig. 2c). The phase image indicates that the friction coefficients in the dots and in the spacing areas are different. Therefore the heterogeneous surface energies were formed on the Si template. Since the maximum topography difference of the template is only about 1 nm, this roughness is not believed to affect the polymer reflow during thermal annealing.

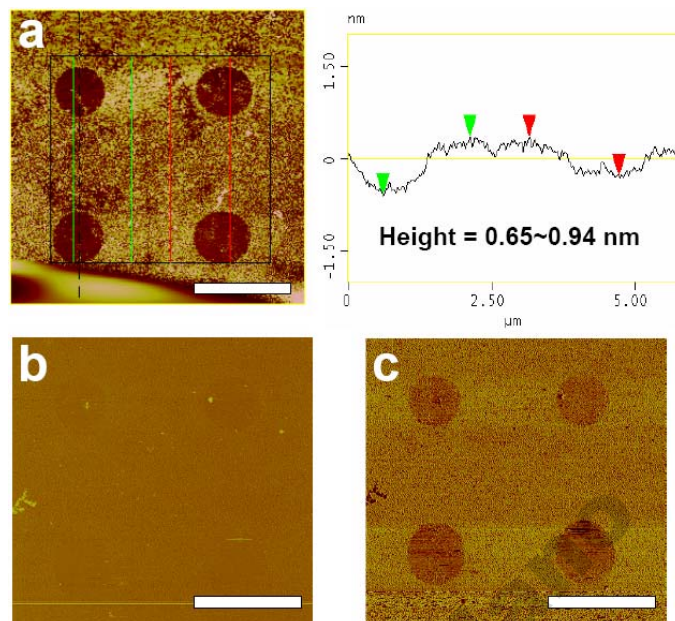


Fig. 2. AFM images of template with heterogeneous surface energies. (a) AFM topography image of FDTs treated template and cross-sectional analysis. (b) AFM topography image and (c) phase image of final template. Scale bar is 4  $\mu\text{m}$ .

To quantify the surface energies of the templates, contact angles of deionized water and acetone on the un-patterned Si treated with only FDTs or MOPTS were measured. Their surface energies were then calculated using a two-liquid method. The surface energy of FDTs and MOPTS treated Si are 17.8  $\text{mJ}/\text{m}^2$  and 50.0  $\text{mJ}/\text{m}^2$ .

### C. SEIP patterning of PEG-b-PLA and SU8

Diblock copolymer of maleimide-terminated poly(ethylene glycol)-b-poly (D,L-lactide) (MAL-PEG-b-PLA) was used in SEIP. MAL-PEG-PLA was synthesized by ring opening polymerization of D,L-lactide at 110  $^{\circ}\text{C}$ . Poly(ethylene glycol) monoethyl ether maleimide (HO-PEG-MAL,  $M_n = 3,210$  Da) was used as a macro-initiator. D,L-lactide was added as a monomer and Stannous (II) octoate ( $\text{Sn}(\text{Oct})_2$ ) was added as a catalyst. After reacting for 4 h, the mixture was allowed to cool down to room temperature. MAL-PEG-PLA was purified by redissolving in THF and precipitating in hexane 3 times. The degree of polymerization of the PLA was calculated by comparing integral intensity of characteristic resonance of the PLA at 5.2 ppm ( $-\text{C}(=\text{O})-\text{CH}(-\text{CH}_3)-$ ) and PEG resonance at 3.64 ppm ( $-\text{OCH}_2\text{CH}_2-$ ) in the  $^1\text{H}$  NMR spectrum (Figure S1). The amount of maleimide proton was calculated by comparing integral intensity of characteristic resonance at 6.70 ppm and PEG resonance at 3.64 ppm ( $-\text{OCH}_2\text{CH}_2-$ ). The molecular weight and polydispersity of MAL-PEG-PLA were also characterized by gel permeation chromatography (THF as eluent) and the results were found to be consistent with  $^1\text{H}$  NMR data. MAL-PEG-PLA ( $M_n = 7.2$  kDa) were synthesized

with 95% yield and used in this study. It is noteworthy that the commercially available MAL-PEG-OH polymer has 76% of all PEG chains terminated with maleimide groups. This percentage is retained in the MAL-PEG-PLA copolymer, as demonstrated by  $^1\text{H}$  NMR. This class of amphiphilic block copolymers has shown great potential as a multifunctional nanomedicine platform for loading drugs and superparamagnetic iron oxide nanoparticles (SPIO). By further attaching cell-specific ligands on the surface of the copolymer, targeted cancer detection and therapy can be achieved. Recently, co-author Gao *et al.* has demonstrated the self assembly of cyclic Arg-Gly-Asp peptides (cRGD) -encoded, SPIO-loaded micelles as ultra-sensitive MRI T2 agents and drug delivery systems [8], as shown in Fig 3.

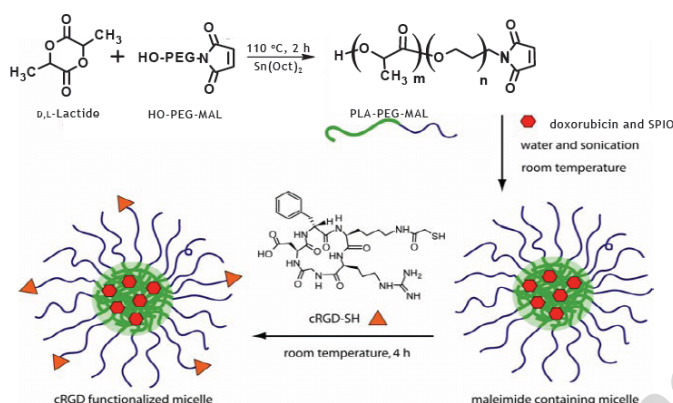


Fig. 3. Schematic diagram of synthesis of MAL-PEG-PLA copolymer and production of cRGD-functionalized, SPIO-loaded micelles.

We intend to use the SEIP process to produce PEG-b-PLA nanostructures to maximize the SPIO and/or drug loading capacity. SEIP will help better control the particle morphology and size distribution. For this purpose, PEG-b-PLA is dissolved in THF or toluene (1~1.5% by weight) and then spincoated onto the template. The film thickness ranges from 100 nm to 250 nm depending on polymer concentration and spincoating conditions, as indicated by profiler measurements. SU8, a UV curable epoxy-based photoresist which is a popular material for microelectronic-mechanic systems (MEMS), was also used in the SEIP process. Since SU8 has well controlled spincoat uniformity and is robust after UV radiation for consequent imaging in AFM and SEM, it is used to study and optimize the SEIP process. The polymer coated template was heated to 100 °C on the hot plate or in a nanoimprinter for about 10 mins. For some experiments, a gas pressure of 0.8 MPa was applied above the film during the thermal annealing, which was found to improve the microfluidic flow of the polymer film. Figure 4 shows the patterning of PEG-PLA copolymers into residue-free discrete micro-structures. Circles 2  $\mu\text{m}$  in diameter and lines with 500 nm in width and 500 nm in spacing are well formed.

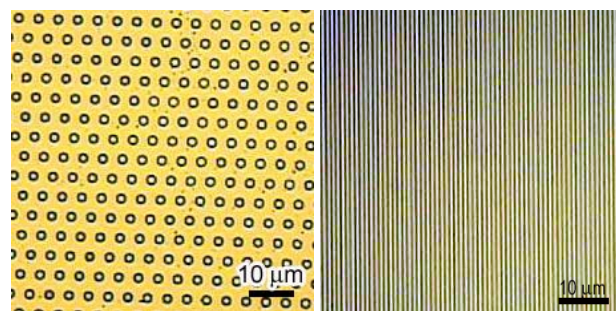


Fig. 4. Optical images of 2  $\mu\text{m}$  PEG-b-PLA dishes (left) and 500 nm wide PEG-b-PLA gratings (right) formed by heating polymer to re-flow to patterned hydrophilic areas.

These periodic polymer patterns were then imaged using AFM and results are shown in Fig. 5. Since PEG-b-PLA polymer easily sticks to the AFM tip, imaging caused material pick-up and re-deposition on the other areas, leading to the formation of shallow bands along the scanning direction. Fig. 4a shows a 5 X 5 dot array with spherical morphology. The dots are about 690 nm in height and 2  $\mu\text{m}$  in diameter, which faithfully matches the underlying chemical patterns. This indicates that the polymer dimensions and shape are precisely defined by the chemical patterns on the template. Some variation in dot height was observed and one possible reason is that the initial thickness of PEG-b-PLA was relatively thick and spincoating was not uniform, which will be discussed in section III. In comparison, SU8 dots show uniform and excellent spherical morphology (Fig. 5b). The width of the particles is the same as PEG-b-PLA and the height is about 380 nm.

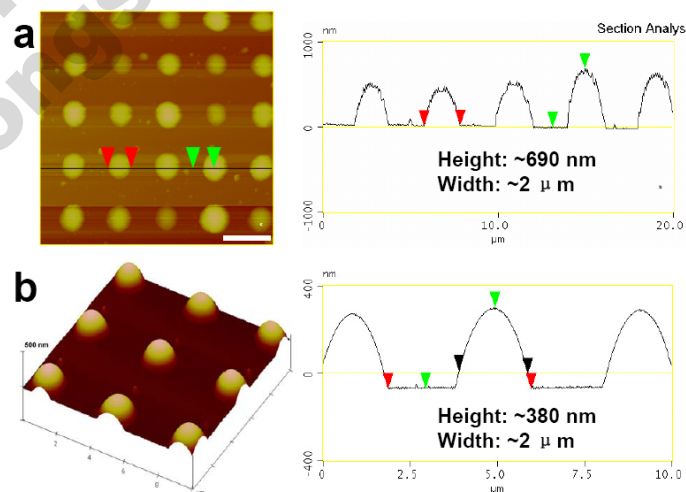


Fig. 5. AFM images of SEIP patterned polymer particles. (a) 5X5 PEG-b-PLA dot arrays and its cross-sectional analysis. (b) 3X3 SU8 dot arrays and cross-sectional analysis.

#### D. Harvesting polymer particles

The periodically patterned polymer micro and nanostructures on the solid surfaces by the SEIP can be used directly for applications in cell adhesion and growth guidance towards



tissue engineering or in microfluidic devices. However for applications in nanomedicine, these structures must be dispersed in aqueous solution. Using a knife edge to collect patterned particles or using polydimethylsiloxane (PDMS) pads to pickup and transfer devices have been reported previously [17][18]. Both methods were tried in this study but were not feasible for the polymers we used. The polymer particles stuck to the knife edge and merged when they were scratched together. On the other hand, PDMS pads were able to pick the particles up, but the particles became stuck on the PDMS and could not be released into an aqueous solution. Instead, a probe-sonicator was used directly to lift these particles off of the Si template into an aqueous solution. As shown in Fig. 6a, the template with patterned polymer was completely submerged in a solution of 50% ethanol in deionized water (DIW). Ethanol in the solution was used to effectively wet the heterogeneous surface of the Si template. An ultrasonic probe tip, partially immersed in the solution, was used to agitate the solution with the template at 11 V for approximately 3 minutes while heating the solution to about 70 °C. The ultrasonic agitation in ethanol solution weakens the interfacial adhesion between the polymer and the MOPTS surface which causes the polymer particles to release from the template when scraped with a blade. After the Si template was taken out of the solution, a carbon film supported by a metal grid was immersed in the solution followed by centrifugation. In this way, the particles were collected on the carbon film and then imaged using SEM, as shown in Figure 6b. Few particles can be seen on the Si template after the harvesting and a large amount of polymer particles were seen on the carbon film. SU8 particles with diameter of 1.6-2  $\mu\text{m}$  have been collected. Some of the particles can be seen on their flat sides, which results from the flat interface between the particle and the template. Currently, we are working on the harvesting of PEG-b-PLA. Since it is softer and stickier than SU8, the probe sonicator approach is more difficult. Moreover for the PEG-b-PLA polymer, we are actually interested in harvesting them into the aqueous solution via template-directed self-assembly to form micellar nanostructures in the future.

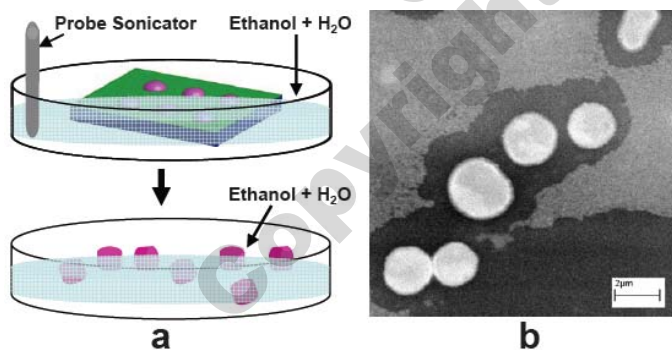


Fig. 6. Harvesting of polymer particles patterned on SEIP substrate: (a) Schematic of probe sonicator method. (b) Freestanding SU8 particles collected on a carbon membrane

from DIW solution.

### III. DISCUSSION

The underlying science of SEIP relates to polymer dynamics on heterogeneous chemical surfaces. Ultrathin films of polymer liquid (typically less than 20 nm) have been intensively studied for their instability on similar surfaces[19-22]. The Van der Waals force in the polymer film and between the polymer film and engineered template surfaces are believed to drive the microfluidic behavior of polymer to re-organize onto the more wettable areas. However, since the film thickness in SEIP is much thicker than the film in previous studies, the pitch of template chemical patterns is much smaller than the spinodal wavelength [20-22]. We believe the polymer microfluidic flow is not driven by the spinodal dewetting phenomena [20-22], but by the strong surface potential gradient at the boundaries of FDOTS and MOPTS treated Si surfaces in a similar way described in ref [19]. The measured height and width in Fig. 5 indicates that the contact angles of PEG-b-PLA and SU8 on the MOPTS-Si surface is about 73° and 60°, which are much higher than their native contact angle on a large homogenous MOPTS surface. Therefore the large contact angles of SEIP particles are not governed purely by the Young's equation but supported by the FDOTS-MOPTS boundary constrains around the particles. More theoretical work and simulation will be carried out in the future to quantify the effects of interface tensions.

The uniformity of initial polymer was found to be a critical factor to determine the yield and uniformity of SEIP fabrication of structures. The spincoating conditions directly affect the film uniformity, which is strongly related to the polar part of surface energy of the trichloro-silane treated Si. For example, FDOTS-Si has a total surface energy of 17.8 mJ/m<sup>2</sup>, where the polar part is nearly zero (0.01 mJ/m<sup>2</sup>). This causes weak adhesion with polymer with high polarity, for example SU8. Actually SU8 cannot be directly spincoated onto the template. For SU8, we used a transfer method to deposit the film onto the template. SU8 was first spincoated onto an oxidized PDMS pad. After the restoration of its low surface energy, the polymer coated PDMS pad was pressed onto the template and slowly removed, releasing the thin polymer film [18]. For PEG-b-PLA, direct spincoating on the template is successful because the polymer has a significant amount of non-polar components that can generate adhesion with the dispersion part of FDOTS-Si surface energy.

### IV. SUMMARY

We have developed a new processing technique utilizing surface energy induced polymer flow to make monodispersed and shape specific polymer structures periodically on solid surfaces. Spherical particles and cylinder lines in micro and nanoscale are formed in diblock copolymers and in UV curable epoxy polymer SU8. A probe-sonicator approach was developed to successfully liftoff structures from a solid surface

into an aqueous solution. The SEIP technique has demonstrated much better pattern uniformity than conventional self-assembly methods and offers excellent flexibility to design particle geometry and dimensions. With these advantages and further work on encapsulation of drug and imaging agents, SEIP is believed to be a promising approach for manufacturing biomaterials for nanomedicine.

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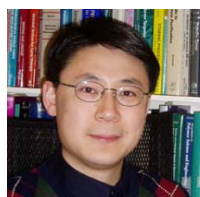
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