Non-lithographic patterning of phage-displayed peptides with wrinkled elastomers

Swathi Swaminathan, Mitchell Bullough, Qifei Li, Anhong Zhou and Yue Cui

Department of Biological Engineering, Utah State University, Logan, UT 84322, USA

The development of controlled patterning of phage (viruses) could expand opportunities for both fundamental studies and creating various materials platforms. Inducing the elastomeric instability of PDMS film provides a non-lithographic, tuneable, controlled method for generating micro/nanoscale wrinkle patterns. Phage display has emerged as a powerful method for selecting peptides that possess enhanced selectivity and binding affinity toward a variety of targets. In this report, we demonstrate the non-lithographic patterning of phage-displayed peptides with wrinkled elastomers. Our results show that the phage-displayed peptides can be patterned on specific locations in controlled and tuneable ways, be transferred to other substrates and induce the self-assembly of hybrid materials. We anticipate that these results could open up exciting opportunities in fundamental studies and in applications ranging from sensors, hybrid materials, self-assembly, surface and interface, to micro/nanoelectronics.

1. Introduction

Oligopeptides are specific, robust biological recognition molecules with broad chemical diversity. They can be chemically engineered to bind specific targets, including inorganic materials [1], nanotubes [2] and small molecules [3]. Phage display has emerged as a powerful method for identifying specific peptide motifs toward a variety of targets, including metals [1], semiconductors [4], polymers [5] and small molecules [6]. In phage display, a library of approximately a billion (10^9) peptide variants is displayed on the phage (virus), which allows for rapid, combinatorial screening of sequences displaying high affinities toward specific targets.

The spatial control of phage-displayed peptides has attracted great interest, which can open up new opportunities in self-assembly, selective sensors, and nano- and microelectronic devices. Photolithography [7] and e-beam lithography [8] are widely used for generating microscale and nanoscale patterns. Recently, we have demonstrated the patterning of phage-displayed peptides on specific locations with photolithography and soft lithography [9].

Spontaneous formations of textured surface patterns [10–12] via inducing the surface instability of elastomer have attracted great attention and have been investigated under a variety of methodologies. This method can result in patterns ranging from approximately 50 nm up to several micrometres, without involving lithography processes. Owing to the elastomeric property, the geometry of wrinkle patterns can be tuneably changed with a stretching strain, which opens up opportunities for developing tuneable hybrid materials. Recently, we have also demonstrated a flame oxidation method for the generation of tuneable wrinkle patterns [13].

In this report, we explore alternative approaches for the patterning of phage-displayed peptides. The patterning is based on inducing the surface instability of elastomers to generate micro/nanoscale wrinkle patterns non-lithographically. The phage-displayed peptides are shown to be patterned onto uniaxial wrinkle patterns. Tuning the wrinkle patterns with stretching strains can change the geometries of the patterned phage-displayed peptides.
Phage-displayed peptides can also be transferred to other host substrates (SiO$_2$). These advances further allow for the self-assembly of silver nanoparticles onto the wrinkles to develop new hybrid materials.

2. Material and methods

2.1. Screening of phage-displayed peptides

The 7-mer phage-displayed kit (New England Biolabs, Ipswich, MA, USA) was incubated with SiO$_2$ in Tris-buffered saline containing 0.1–0.8% Tween-20 (TBST) for 1 h at room temperature. The substrates were then washed several times with TBST buffer. The phages were eluted from the particles by the addition of glycine–HCl (pH 2.2) for 15 min, neutralized with tris–HCl, pH 9.1, amplified and subjected to additional pannings. Eluted phages were then amplified in E. coli, and the process repeated for up to five rounds of biopanning, under increasingly stringent conditions, to obtain phage-expressing peptides having the highest binding affinities to the graphene/graphite samples. After the final round of panning, DNA sequence analysis yielded heptamic or dodecameric graphene-binding peptides. The PDMS-binding phage-displayed peptide (LSNNNLR) was identified and isolated using the 7-mer phage-displayed kit [14].

2.2. Generation of PDMS wrinkles

PDMS prepolymer and curing agent (Sylgard 184, from Dow Corning, MI, USA) were thoroughly mixed at a weight ratio of 10 : 1, followed by degassing and curing at 80°C for 2 h. A PDMS film (0.5 mm thick) was stretched by 25% with a uniaxial stretching strain, followed by exposure to oxygen plasma (Plasma Etcher Inc., NV, USA) at 80 W for 10–30 min to generate consistent one-directional wrinkles, proportional to the direction of the strain with different sizes based on the duration of etching. By using a 20% biaxial stretching strain with plasma oxidation at 80 W for 20 min, herringbone-structured PDMS wrinkles could be generated.

2.3. Printing of phage-displayed peptides

A wrinkled PDMS slab was brought into contact with the glass surface coated by PDMS-binding phage-displayed peptide. Then the phage-displayed peptides with the PDMS-binding motif were bound to the PDMS surface, followed by washing and characterization. Similarly, a PDMS stamp with SiO$_2$-binding peptide (HKKPSKS) was brought into contact with an SiO$_2$ surface, followed by washing and characterization.

2.4. Fluorescent characterization

Fluorescent characterization of the binding of phage-displayed peptides was accomplished by incubating the substrates sequentially with (i) the phage-displayed peptides ($5.6 \times 10^{10}$ phage molecules $\mu$L$^{-1}$), (ii) blocking buffer 0.1 M NaHCO$_3$, 1% BSA, (iii) biotin-conjugated antibody M13 anti-phage antibody (1 mg ml$^{-1}$) and (iv) avidin-FITC (2 unit ml$^{-1}$), with TBS buffer washing steps in between to remove non-specific binding. Fluorescent characterization for the binding of bifunctional peptides (Peptide 2.0 Inc., VA, USA) was accomplished by incubating the substrates sequentially in (i) the bifunctional peptide (0.5 $\mu$g ml$^{-1}$ TBS), (ii) blocking buffer 0.1 M NaHCO$_3$, 1% BSA and (iii) streptavidin-FITC (1 mg ml$^{-1}$), with TBS buffer washing steps in between to remove non-specific binding. The colour intensity of the surface was observed through an Olympus IX71 inverted fluorescence microscope equipped with an Olympus DP30BW CCD camera (emission and excitation wavelengths of FITC being 495 and 519 nm, respectively).

2.5. Peptide synthesis of nanoparticles

A bifunctional peptide was designed and synthesized with a binding motif for PDMS (LSNNNLR), a GGGG spacer and a motif for synthesis of silver nanoparticles (NPSLFLYLPSDL) (Peptide 2.0 Inc.). We investigated two conditions for the growth of nanoparticles. First, solutions of the bifunctional peptide (0.5 $\mu$g ml$^{-1}$) and silver nitrate solution (pH 7, 30 mM) were mixed and incubated with PDMS wrinkles and kept in the dark for 3 days [15], resulting in the growth of nanoparticles on the entire PDMS-wrinkled surface. Second, the bifunctional peptides were printed on PDMS wrinkles, followed by incubating with silver nitrate solution to result in the growth of nanoparticles on the wrinkled PDMS surface. Water contact angle analysis was conducted using a goniometer (Sindatek, Taiwan).

3. Results and discussion

We first studied the patterning phage-displayed peptides with uniaxial wrinkle patterns. As shown in figure 1a, a PDMS film was stretched with a uniaxial stretching strain and subjected to plasma oxidation to generate a hard silica layer on its surface (figure 1a, step 1), followed by releasing the strain form wrinkle patterns (figure 1a, step 2). Recently, we identified specific PDMS-binding phage-displayed peptides (LSNNNLR) with phage display bioscreening [14]. Bringing the wrinkled PDMS surface into contact with a glass substrate coated with PDMS-binding phage-displayed peptides resulted in the patterning of phage-displayed peptides on the crests of the wrinkles (figure 1a, step 3). We investigated the fluorescent characterization of the binding of these PDMS-binding phage-displayed peptides to PDMS wrinkles. As shown in figure 1b(i), the crests of the wrinkle patterns showed much higher fluorescence intensity than the
other part of the PDMS, owing to the binding of the phage-displayed peptides or bifunctional peptide. The enhanced binding is attributed to the height of the wrinkles on the surface of the elastomer. This fluorescent differential was much larger than control experiments where we incubated PDMS-binding phage-displayed peptides on the entire surface of the PDMS wrinkles (see the electronic supplementary material, figure S1). In addition, a bifunctional peptide (LSNNLRLGGGHPQ) with a PDMS-binding motif (LSNNNLR) and a streptavidin-binding motif (HPQ) [16] was designed. Similarly, the bifunctional peptides were printed on the crests of the wrinkles. As shown in figure 1b(ii), the bifunctional peptides bind to the wrinkle crests effectively and generate clear fluorescent signals. The results suggest that phage-displayed peptides and the derived bifunctional peptides can be patterned non-lithographically via inducing elastomeric instability.

Wrinkle patterns are generated by compressing the hard surface layer that is formed on the stretched PDMS film by plasma oxidation. By tuning the stretching strains imposed on PDMS wrinkles, the geometry of the patterned phage-displayed peptides, which were bound to the crests of the wrinkles, can be tuned accordingly. As shown in figure 2a, we first studied the tuning of the patterned phage-displayed peptides on wrinkles with a 90° stretching strain to the direction of wrinkle lines. The amount and direction of the stretching strain are the same as those of the initial stretching strain. With the stretching strain, the PDMS wrinkles in sinusoidal wave disappeared and the surface became smooth, as shown in the optical image (figure 2a(ii)). The phage-displayed peptides were patterned on the wrinkle crests before stretching. With the stretching strain, the phage-displayed peptides remained on the PDMS surface, confirmed by fluorescent characterization (figure 2a(iii)). The pitches of the fluorescent lines, which were bound with phage-displayed peptides, increased by approximately 25% compared with that of the initial wrinkles. Similarly, with a 0° stretching strain to the wrinkle lines, the width of the wrinkles and the pitch of the fluorescent lines decreased by approximately 18%, as shown in figure 2b. Further, by using biaxial stretching strains on a stretched PDMS film, followed by plasma oxidation and releasing the strain, tunable wrinkles could be generated, and the phage-displayed peptide patterns could be tuned in accordance with the patterns of the wrinkles, as shown in figure 2c. These results indicate that tuning the geometry of the PDMS-wrinkled surface with external stretching strains could change the location of the phage-displayed peptides, and phage-displayed peptides can be patterned tuneably on wrinkled elastomers.

Next, we studied the transfer-printing of phage-displayed peptides with wrinkled elastomers. The phage-displayed peptides for SiO2 were identified (see the electronic supplementary material, table S3), and phage-displayed peptide (HKKPSKS) was chosen for further study. The SiO2-binding peptides did not bind to PDMS and can bind to SiO2 specifically (see the electronic supplementary material, figure S2). The SiO2-binding peptides were brought into contact with PDMS wrinkles, and owing to the height difference of the wrinkles, the SiO2-binding peptides stayed on the crests of the PDMS wrinkles. The peptide-immobilized PDMS wrinkles were further brought into contact with an SiO2 surface, and the SiO2-binding peptides on the crests of the PDMS wrinkles were in contact with and bound to the SiO2 surface. Uniaxial PDMS and biaxial PDMS wrinkles were used as stamps for printing SiO2-binding phage-displayed peptides onto SiO2. As shown in figure 3, the fluorescence characterization showed that the patterning of SiO2-binding phage-displayed peptides was achieved with tunable stretching directions on the SiO2 surface.
peptides on SiO\textsubscript{2} was in accordance with the structure of the PDMS wrinkles. The results indicate that by using the elastomeric wrinkle patterns, phage-displayed peptides can be transferred from PDMS wrinkles to another substrate, and the location of phage-displayed peptides are in accordance with the geometry of the PDMS wrinkle patterns.

Further, we studied the self-assembly of nanoparticles via specific peptides on spatially regulated wrinkled surfaces to develop multi-functional materials, as shown in figure 4\textit{a}. A bifunctional peptide, with a PDMS-binding motif (LSNNNLR) and a silver synthesis motif (NPSSLFRYLPSD), was designed and synthesized. Incubating the PDMS-wrinkled surface with bifunctional peptide and silver nitrate resulted in the growth of silver nanoparticles on the entire PDMS-wrinkled surface in an uncontrolled way (figure 4\textit{b}(i)), which showed an average diameter of 75.0 ± 11.9 nm. Printing bifunctional peptides on the wrinkled surface with microcontact printing, followed by incubating with silver nitrate solution, enabled the formation of uniformly sized silver nanoparticles on the wrinkled surface (figure 4\textit{b}(ii)), which showed an average diameter of 64.8 ± 11.8 nm. Printing bifunctional peptides on the wrinkled surface with microcontact printing, followed by incubating with silver nitrate solution, enabled the formation of uniformly sized silver nanoparticles on the wrinkled surface (figure 4\textit{b}(ii)), which showed an average diameter of 64.8 ± 11.8 nm. Contact angle analyses of PDMS-surfaces were performed, as shown in figure 4\textit{c}. It can be seen that the bifunctional peptide-immobilized PDMS-wrinkled surface showed a hydrophilic water contact angle of 45°. After the self-assembly of nanoparticles, the PDMS wrinkles containing both the bound bifunctional peptide and the synthesized nanoparticles show a hydrophobic water contact angle of 115°. This indicates that the presence of nanoparticles resulted in the change of hydrophobicity. The results suggest that phage-displayed peptides on elastomeric wrinkles can be used for the development of new hybrid materials.

4. Conclusion

In this report, we have demonstrated non-lithographic patterning of phage-displayed peptides via inducing elastomeric instability, which does not involve standard photolithography or e-beam lithography process. The method can result in controlled and tuneable patterning of phage-displayed peptides on specific locations of wrinkled elastomer and other substrates (e.g. SiO\textsubscript{2}), and the development of hybrid materials via peptide enabled self-assembly nanomaterials on wrinkles. The approach we describe here may open new avenues in self-assembly, biological sensors, molecular biology, hybrid materials, energy storage devices, etc. Although these results are promising, further studies are needed to elucidate the effect of selectivity and strength of phage-displayed peptides on non-lithographic patterning and self-assembly.

Funding statement. We acknowledge Utah State for the financial support of this work.
References


