Bio-inspired fabrication of superhydrophobic surfaces through peptide self-assembly†

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A vertically aligned peptide nanowire film, prepared by the self-assembly of diphenylalanine upon exposure to fluorinated aniline vapor at high temperature, exhibits a superhydrophobic property due to its nanoscale roughness and low surface free energy. We fabricated a self-cleaning, superhydrophobic surface by hierarchically re-organizing peptide nanowires into a hill-and-valley-like structure using capillary force induced by solvent-evaporation. Our approach provides an alternative way of nanofabrication for superhydrophobic materials, which should broaden the spectrum of applications for peptide self-assembly.

The self-assembly of molecular building blocks into ordered nanostructures is an attractive route for the synthesis of functional materials that are not feasible through conventional techniques. For example, it is well-known that complex hierarchical nanostructures can be constructed, utilizing amphiphilic molecules or polymers, through the self-assembly process. Recently, much attention has been paid to nanofabrication based on peptide self-assembly because of certain unique properties of peptides such as functional flexibility and molecular recognition capability. Among numerous self-assembling peptides reported to date, diphenylalanine is one of the simplest, and can readily form various nanostructures like nanotubes, nanowires, and organogels under different processing conditions. Recently, we reported the growth of vertically well-aligned peptide nanowires by solid-phase self-assembly of diphenylalanine in the presence of aniline vapor at high temperatures.

On the other hand, there is a growing interest in the synthesis of smart materials by mimicking biological systems that have evolved over millions of years. One of the most notable examples is lotus leaves, which have a self-cleaning property that is known as the lotus effect. This phenomenon originates from the low surface free energy of the leaves and their hierarchical nano/microstructures, which imparts superhydrophobic characteristics with a low water-sliding angle (or tilt angle) to the leaves. Superhydrophobic materials are expected to enable various applications, such as self-cleaning smart surfaces, anti-corrosion coatings, and water- and humidity-proof coatings.

Based on the principle of the lotus effect, many efforts have been made to develop an artificial superhydrophobic surface either by fabricating a rough surface with hydrophobic materials or by chemically modifying a rough surface with low surface-free-energy materials. To our knowledge, there has been no report yet of the fabrication of superhydrophobic surfaces through the self-assembly of molecular building blocks.

In this Communication, we report the creation of super-hydrophobic surfaces through the solid-phase self-assembly of diphenylalanine into vertically aligned peptide nanowires upon exposure to pentafluoroaniline vapor at high temperatures. We succeeded in developing a self-cleaning superhydrophobic surface with a static water contact angle of 154.0° and a water-sliding angle lower than 5° by using a solvent evaporation-induced capillary force that induced the formation of hierarchical nano/micro-structures through a reorganization of peptide nanowires. When we investigated the superhydrophobic nanostructured peptide film with multiple analytical tools such as scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and X-ray photoelectron spectroscopy (XPS), we found that both the nanoscale roughness of the peptide nanowire film and the decrease of its surface free energy through the incorporation of pentafluoroaniline molecules into the nanowires are responsible for the superhydrophobic characteristics.

We prepared peptide nanowire thin film through the self-assembly of diphenylalanine peptide at high temperatures in the presence of solvent vapors, as schematically illustrated in Fig. 1a. Briefly, we formed a flat, amorphous, thin peptide film by drying a drop of diphenylalanine solution (50 mg ml⁻¹) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) under anhydrous conditions (step 1). Afterwards, the amorphous thin film was exposed to vapors of aromatic amines such as aniline and pentafluoroaniline (which has a much lower surface free energy than aniline) at 100 °C, to induce the growth of vertically aligned peptide nanowires (step 2). After treating the film in this manner for 12 h, we observed the effect of solvent vapor treatment on the morphology of peptide thin films using SEM and measured surface contact angles. As shown in Fig. 1b, the amorphous peptide thin film exhibited no surface features before the treatment; however, after treatment, we observed well-aligned peptide nanowires having an average diameter of approximately 200 nm growing from the amorphous peptide film treated with aniline (Fig. 1c) or pentafluoroaniline (Fig. 1d) vapors. The structure and morphology of the peptide nanowires formed by the different solvent vapors were almost identical to each other. The vertical growth of nanowires from a flat amorphous peptide film can be explained by surface-initiated nucleation and growth mechanism upon exposure to solvent vapor at high temperature. In terms of thermodynamics, the evaporation of solvent during the formation of amorphous peptide film can generate a gradient of chemical potential along the direction...
perpendicular to the substrate. The potential gradient is responsible for the directional growth of peptide nanowires upon exposure to solvent vapor that interacts with peptide building blocks.

When we further investigated the hydrophobic characteristics of peptide films, only the peptide nanowire film grown by pentafluoroaniline vapor treatment exhibited superhydrophobicity with the desired contact angle of 151.62° (Fig. 1d). The contact angles of the amorphous thin peptide film without any treatment (Fig. 1b) and peptide nanowire film grown by aniline vapor (Fig. 1c) were 37.05° and 128.05°, respectively. The initial contact angle (i.e., 37.05°) of the amorphous film gradually decreased during measurement due to the spreading and absorption of water droplet by peptide molecules, resulting in the formation of discrete tubular nanostructures later on (data not shown). It was previously reported that the interaction between diphenylalanine and water molecules could lead to the formation of peptide nanostructures. As a control experiment, we tested a pure pentafluoroaniline film prepared on an Si substrate by spin-coating with pentafluoroaniline solution and found that its contact angle was only 67.03°. These results clearly show that the superhydrophobic characteristics of the nanostructured peptide film originate from the increased nanoscale roughness of the film which should allow the trapping of air underneath water droplets as well as from the decreased surface free energy caused by the incorporation of pentafluoroaniline during the peptide self-assembly process.

In order to confirm the incorporation of pentafluoroaniline into the peptide nanowires and to investigate its effect on the superhydrophobic characteristics in more detail, we analyzed thin peptide films with various spectrometric techniques such as XRD, FT-IR, and XPS. Fig. 2a shows the XRD patterns of the thin peptide film

Fig. 1 (a) Experimental scheme for the fabrication of peptide self-assembled nanowire films and (b–d) the relationship between surface morphology and static water contact angle. Amorphous thin peptide film (b) was treated with aniline (c) or pentafluoroaniline (d) vapors at 100 °C to grow peptide nanowires.

Fig. 2 (a) Powder XRD patterns and (b) FT-IR spectra of the diphenylalanine film before and after solvent-vapor (i.e., aniline or pentafluoroaniline) treatment at 100 °C.
before and after treatment with aniline or pentafluoroaniline vapor at 100 °C. Before treatment, the thin peptide film exhibited no characteristic diffraction peaks, indicating its amorphous nature. The diffraction patterns of peptide nanowires grown by aniline or pentafluoroaniline were almost identical to each other except for a slight shift in peaks, which suggest that they have a similar crystal structure and molecular arrangement. Their diffraction patterns were quite different from those of peptide nanotubes having a six-fold symmetry.6 The strongest diffraction peaks of aniline- or pentafluoroaniline-treated thin peptide films appeared at 20 of 7.48° and 7.46°, respectively, and their corresponding d-spacing values, calculated from Bragg's law (\( \sin \theta = \lambda / 2d \)), where \( \lambda = 1.5418 \) Å, were 11.82 Å and 11.85 Å, respectively. The slight difference between the d-spacing values is an indirect evidence for the incorporation of aniline or pentafluoroaniline into the peptide nanowires since it can originate from the difference in the size of the solvent molecules. The incorporation of aniline and pentafluoroaniline into peptide nanowires was further confirmed by FT-IR analysis. As shown in Fig. 2b, the FT-IR spectra of thin peptide film significantly changed after solvent-vapor treatment, indicating the conformational change of thin peptide film. Both hydrophobic (grown by aniline vapor) and superhydrophobic (grown by pentafluoroaniline vapor) peptide nanowire film presented a strong peak at 1656 cm\(^{-1}\), corresponding to \( \beta \)-sheet structure. In the case of the superhydrophobic peptide nanowire film, additional absorption peaks were observed at 1037 and 1226 cm\(^{-1}\), which can be attributed to C–F vibration.13 We also confirmed the incorporation of pentafluoroaniline into peptide nanowires during the self-assembly process by using XPS (Fig. S1, ESI†).

We further investigated the effect of film thickness (Fig. 3a) and aging temperature (Fig. 3b) on the surface hydrophobicity of nanostructured peptide films. The thickness of the amorphous peptide thin film was controlled to be between 300 nm and 4 μm by varying the concentration of diphenylalanine in HFIP solution from 5 to 60 mg ml\(^{-1}\), according to our previous report.8 After treating the amorphous thin peptide films with pentafluoroaniline vapor at 100 °C, we measured their contact angles. According to our analysis, the thickness of amorphous peptide film had a significant effect on the morphology of peptide nanowire film that formed, as well as on the hydrophobic characteristics of the film. When the amorphous film was not sufficiently thick enough (e.g., less than 500 nm), peptide nanowires were aligned almost parallel to their substrate (Fig. S2, ESI†) and did not exhibit any superhydrophobicity (Fig. 3a). The alignment of the peptide nanowires became more perpendicular to their substrate with increased film thickness (Fig. S2†), which resulted in the formation of film with much higher contact angle (Fig. 3a). These results show that we can control the hydrophobicity and the wetting mode of the surface from a Wenzel state (i.e., complete filling of surface grooves by a liquid droplet)16 to a Cassie state (i.e., incomplete filling of surface grooves by trapping of vapor pockets underneath a liquid droplet)16 by simply changing the thickness of the nanostructured peptide film. In contrast, the aging temperature exhibited a negligible effect on the hydrophobic characteristics of thin peptide film (Fig. 3b). Peptide nanowire films exhibited a uniform superhydrophobicity when prepared at the temperatures ranging from 100 °C to 250 °C. When the amorphous peptide film was treated at temperatures lower than 100 °C, the self-assembly of diphenylalanines into nanostructure was depressed and became very slow (Fig. S3, ESI†) and diphenylalanine itself thermally decomposes at around 300 °C (data not shown).

Based on the results, we attempted to fabricate a self-cleaning superhydrophobic surface. According to the literature,16,15 the self-cleaning properties of lotus leaves originate from their hierarchical nano/microstructures, which minimizes the hysteresis between the advancing and receding angles of a liquid droplet. To introduce hierarchical nano/microstructures into our peptide self-assembled superhydrophobic surface, we slightly modified the growth condition for peptide nanowires to induce a slight wetting of the film (Fig. 4a). After growing vertically aligned peptide nanowires at 100 °C, we incubated the peptide nanowire film under supersaturated conditions by rapidly cooling the growth chamber filled with pentafluoroaniline vapor from 100 °C to 50 °C. Then, we completely dried the wetted peptide nanowire film to rearrange nanowires into hierarchical nano/microstructures through an evaporation-induced capillary force.16 As shown in Fig. 4b, peptide nanowires that are treated under a strong capillary force resulted in the formation of hierarchical nano/microstructures with microscale hill-and-valley-like structures. According to a previous report, when one end of nanowires or nanotubes is attached to their substrate, capillary force drives the formation of hierarchically ordered structures similar to ours.16 The hierarchical peptide nanowire film exhibited not only superhydrophobicity with a high contact angle of 154.09° (Fig. 4b, inset), but also self-cleaning properties with a tilt angle lower than 5°. As shown in Fig. 4c, we could observe that a water droplet quickly slides on the peptide.
nanowire film (10 mm × 10 mm) within a very short time (90 ms). With regard to the stability of peptide nanowire film for prolonged time, no significant change was observed in its super-hydrophobic property and morphology even after a week of incubation (Fig. S4, ESI).

In summary, we report successful bio-inspired fabrication of a superhydrophobic surface through peptide self-assembly. The superhydrophobic surface was developed by growing peptide nanowire film in the presence of pentafluoroaniline vapor at high temperatures. We found that superhydrophobic characteristics of the peptide nanowire film originated from an increased nanoscale roughness of the film and the decreased surface free energy through the incorporation of pentafluoroaniline molecules into the peptide nanowires. We could fabricate a self-cleaning superhydrophobic surface by re-organizing vertically aligned peptide nanowires into hierarchical nano/microstructures driven by a solvent evaporation-induced capillary force. Our approach provides an alternative way of nanofabrication for superhydrophobic materials, which should broaden the spectrum of applications for peptide self-assembly.

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Notes and references

Bio-Inspired Fabrication of Superhydrophobic Surfaces through Peptide Self-Assembly

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Experimental Methods

Materials: Diphenylalanine in a lyophilized form was obtained from Bachem AG (Bubendorf, Switzerland). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), aniline, and 2,3,4,5,6-pentafluoroaniline were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Fabrication of superhydrophobic peptide film: Amorphous thin peptide film was prepared by drying a drop of diphenylalanine solution in HFIP under an anhydrous condition in a glove box (model: MOGB 1S, Moisture Oxygen Technology Co., Korea), and then treated with pentafluoroaniline vapor (or aniline vapor for the control experiment) at 100 °C for 12 h. A self-cleaning superhydrophobic surface was prepared by treating as-synthesized superhydrophobic peptide nanowire film by rapidly cooling the growth chamber temperature from 100 °C to 50 °C to induce a slight wetting for the rearrangement of peptide nanowires into hierarchical nano/microstructures. The thickness of amorphous peptide thin film was controlled by varying the concentration of diphenylalanine in HFIP from 5 to 100 mg ml⁻¹. To investigate the effect of aging temperature on the superhydrophobic characteristics of peptide thin films, we prepared the films at a constant temperature of 27, 50, 75, 100, 175, 250, and 300 °C.

Characterization: The morphology of peptide thin films before and after the solvent-vapor treatment was analyzed by an S-4800 field emission SEM (Hitachi Ltd., Japan). The static water contact angle of each surface was measured with a Phoenix 300 contact-angle meter (Surface Electro Optics Co., Ltd., Korea) at five different locations on each film using 6 mg of water droplet. The accuracy of contact
angle measurements is given with a standard deviation (n = 5). The structure of thin peptide films was analyzed with a D/MAX-RC thin-film X-ray diffractometer (Rigaku Co., Japan) under the following conditions: scan range: 2–60°; scan speed: 3° min⁻¹; Cu Kα radiation, λ = 1.5418 Å. The incorporation of pentafluoroaniline into peptide nanowires during the self-assembly process was investigated by measuring their FT-IR and XPS spectra, using a Hyperion 3000 attenuated total reflection-Fourier transform infrared (ATR-FTIR) microscope (Bruker Optics Inc., Germany) and a JPS-9000MX spectrometer (JEOL Ltd., Japan), respectively.

Figure S1. XPS spectra of diphenylalanine film before and after solvent-vapor (i.e., aniline or pentafluoroaniline) treatment at 100 °C. Note that a trace amount of fluorine present in peptide nanowires even without pentafluoroaniline vapor treatment originated from residual HFIP molecules that were used when we prepared the amorphous peptide film.
Figure S2. Electron micrographs of peptide nanowires self-assembled from amorphous diphenylalanine films with four different thicknesses. The thickness of the amorphous peptide film was controlled by changing the concentration of diphenylalanine in HFIP. The final morphology of peptide nanowire film after the solvent-vapor treatment was highly dependent on the thickness of amorphous peptide thin film. When the amorphous peptide film was sufficiently thick (> 1 μm), the solvent-vapor treatment led to the growth of vertical peptide nanowires by the surface-initiated nucleation and growth mechanism. However, peptide nanowires grew parallel to their substrate when the film thickness was not thick enough, implying that the free energy at the interface between the peptide film and the underlying substrate may affect the growth of peptide nanowires.
Figure S3. Electron micrographs of peptide films treated with pentafluoroaniline vapor at 40 °C and 70 °C for 12 h, respectively. At the temperatures lower than 100 °C, the self-assembly of diphenylalanines into nanowire structures was depressed and became very slow.
Figure S4. The stability of superhydrophobic peptide nanowire films. Between each measurement, peptide nanowire films were exposed to atmosphere. There was no significant change in their superhydrophobic characteristics even after a week of incubation. The inset shows the photograph of a water droplet on the peptide nanowire film and the electron micrograph of film morphology at 7th day.