Microstructured surfaces engineered using biological templates: a facile approach for the fabrication of superhydrophobic surfaces

DUŠAN LOŠIĆ*

University of South Australia, Ian Wark Research Institute, Mawson Lakes Campus, Adelaide, SA 5095, Australia

(Received 2 March, revised 27 May 2008)

Abstract: The fabrication of microstructured surfaces using biological templates was investigated with the aim of exploring of a facile and low cost approach for the fabrication of structured surfaces with superhydrophobic properties. Two soft lithographic techniques, i.e., replica moulding and nano-imprinting, were used to replicate the surfaces of a biological substrate. Leaves of the Agave plant (Agave attenuate), a cost-free biological template, were used as a model of a biosurface with superhydrophobic properties. The replication process was performed using two polymers: an elastomeric polymer, poly(dimethylsiloxane) (PDMS), and a polyurethane (PU) based, UV-curable polymer (NOA 60). In the first replication step, negative polymer replicas of the surface of leaves were fabricated, which were used as masters to fabricate positive polymer replicas by moulding and soft imprinting. The pattern with micro and nanostructures of the surface of the leaf possesses superhydrophobic properties, which was successfully replicated into both polymers. Finally, the positive replicas were coated with a thin gold film and modified with self-assembled monolayers (SAMs) to verify the importance of the surface chemistry on the hydrophobic properties of the fabricated structures. Wetting (contact angle) and structural (light microscopy and scanning electron microscopy) characterisation was performed to confirm the hydrophobic properties of the fabricated surfaces (> 150°), as well as the precision and reproducibility of the replication process.

Keywords: superhydrophobic surfaces; lotus-effect; replica moulding; nano-imprinting; Agave attenuate.

INTRODUCTION

The fabrication of three-dimensional (3-D) nanostructured materials exhibiting well-defined and controllable nanometre-scale features is one of the greatest challenges that has faced chemists and materials scientists in last two decades.¹ A
wide range of very expensive fabrication techniques have been developed in the microelectronics industry, including deep and extreme UV photolithography, phase shift photolithography, electron beam writing, focused ion beam lithography and X-ray lithography. To broaden the accessibility and diversify the capability of nanofabrication techniques, alternative, simple and cost-effective techniques, such as imprint lithography, soft lithography, capillary force lithography and polymer transfer printing, have been developed. In particular, soft lithography, which is based on a soft polymer mould, such as poly(dimethylsiloxane) (PDMS), has been widely adopted for transferring patterns onto various surfaces and for the easy replication of complex nanostructures (replica moulding) with a variety of properties. It has also been applied in the manufacture of electronic and microfluidic devices, optics and biosensors.

Wettability is one of the fundamental properties of solid surfaces and controlling the wetting of surfaces is an important problem relevant to daily life, agriculture, industry and fundamental research. It is generally accepted that superhydrophobic and self-cleaning properties are based on the hierarchical roughness of the surface on a micro and nano scale, combined with the chemistry of low surface energy compounds. Water forms spherical droplets on such surfaces with a high contact angle (> 150°), and can easily roll off the surface taking with it dust particles. Electrochemical oxidation, chemical etching, chemical and electrochemical deposition, plasma etching, plasma deposition, laser ablation, chemical vapour deposition (CVD) and sol–gel processing were the techniques commonly employed. The general approach of these methods was the fabrication of micro- to nano-structured surfaces combined with chemistry to decrease the surface energy. However, processing through many of these methods is expensive, time consuming, with problems of structural instability, difficulty in the control the formation of the structure and complexity of scale up.

Biological materials and processes are a relatively new source of inspiration for the design and fabrication of nano-structured materials. Many organisms synthesize inorganic structures into intricate architectures with ordered micro-to-nano scale features, which cannot typically be replicated through laboratory synthesis. Therefore, the use of cheap biological materials as templates for the engineering of structures at the nano scale is a promising and cost effective fabrication strategy. The superhydrophobic and self-cleaning properties induced by surface roughness are widely adopted in nature, including plant leaves, butterfly wings, water strider legs etc. Hundreds of different plants having the ability to completely clean their leaves from contamination (dust particle, spores and pathogens) by a simple rain shower or fog have been reported. Among them, a
most impressive example is the Lotus plant (*Nelumbo nucifera*) the superhydrophobic properties of which were the first documented and named as the “lotus effect”.11

In previous studies it was demonstrated that unicellular algae diatoms, as outstanding examples of micro- and nano-structured materials in nature, could be used as templates for nanofabrication.28–31 The results revealed the possibility of the generation of multiple copies of diatoms based on a replication process that involved the use of the diatom nano-structured silica as a master mould and the transformation of their structure into polymers or metals with unique optical and separation properties.28–30 In the present work, this bio-inspired approach was extended to the fabrication of micro- to nano-structured surfaces with larger dimensions using other biological substrates. The aim was to demonstrate a facile method by applying biological templates, such as plant leaves, for the rapid fabrication of artificial superhydrophobic surfaces. A schematic diagram of the fabrication is outlined in Fig. 1. The leaves of Agave plant (*Agave attenuate*) were used as a model of a structured biological template. The transference of the pattern of the leaf was performed using a soft-lithography approach with two replication steps for the production of negative and positive replicas using two polymers, *i.e.*, poly(dimethylsiloxane) (PDMS) and a UV-curable polyurethane (PU) polymer (NOA 60). The morphological and wettability properties of the surfaces of the plant and fabricated replicas were investigated using light microscopy, field emission scanning electron microscopy (SEM) and contact angle (CA) measurements.
EXPERIMENTAL

Materials

The leaves from Agave plant (Agave attenuate) were freshly collected from local garden (Flinders University Campus, Adelaide, South Australia). To remove possible contaminants, leaf samples were cleaned by running water, followed by rinsing with a stream of nitrogen. A minimum of 50 mm×50 mm square areas of leaf were cut from several different regions and the top side of the plant surface was used as a template for the replications. The poly(dimethylsiloxane) PDMS replicas were prepared from polymer precursor (Sylgard 184 parts A and B) purchased from Dow Corning (USA). The polyurethane based (mercapto ester type) UV-curable polymer (NOA 60) was obtained from Norland Inc. (USA). 1H,1H,2H,2H-Perfluorodecanethiol (PFDT) was supplied from Aldrich (Australia).

Fabrication

The replication process, which combined two soft-lithographic methods, i.e., replica moulding and nano-imprinting, is schematically shown in Fig. 1. In the first step, replica moulding or master processing was performed to replicate the leaf surface (Fig. 1a) into negative replicas using two polymers, i.e., PDMS (Figs. 1b–1c) and UV-curable PU (Figs. 1d–1e). In the second step, both negative replicas were used as masters for the fabrication of the corresponding positive replicas to match the topography of the original plant surface. A replica moulding process was used to prepare a PU positive replica (Figs. 1f–1g) using the PDMS negative replica as the master (Fig. 1c) and a nano-imprinting method was applied to fabricate PDMS positive replicas (Figs. 1h–1j) using a hard PU negative replica as the stamp (Fig. 1e).

The replica moulding fabrication process was adopted from previous studies.28 Briefly, degassed poly(dimethylsiloxane) PDMS prepolymer (Sylgard 184 part A, base) was mixed with a cross-linking catalyst (Sylgard 184 part B, a curing agent which consists of dimethyl, methylhydrogen siloxane) at a 10:1 (w/w) ratio, then poured carefully over leaf samples and cured for at least 6 h at 60 °C. After curing, the cross-linked elastomeric PDMS was peeled from the surface and cleaned by sonication, water, and ethanol to remove any remains of the leaf (Figs. 1b–1c). A second negative replica was prepared by pouring UV-curable prepolymer (NOA 60) over leaf samples and curing under a UV lamp (λ = 365 nm). The precur- ring process was carried out for 20 min followed by postcuring for at least 3 h. The polymer mould was then peeled off the leaf, yielding the negative relief of the initial leaf surface (Figs. 1d–1e). The negative replicas were cleaned and reused for repeated replications.

A PU positive replica (Figs. 1f–1g) was prepared from a PDMS negative replica (Fig. 1c), which was the mould (master). A PDMS positive replica was prepared by nano-imprinting using a PU negative replica (Fig. 1e) as the stamp employing a previously described procedure.32 Briefly, the precured PDMS was uniformly dispersed on glass or another supporting substrate. A small pressure of the stamp was applied on the PDMS film after an initial curing for 2 h at room temperature. This was followed by complete curing for at least 8 h at 40 °C (Figs. 1b–1j). When the curing process was completed, the PDMS film was separated from the stamp (Fig. 1j). A thin gold film (∼ 10 nm) was deposited on the positive polymer replica made of PU using the sputter deposition system (Anatech, USA). The gold-coated replica samples were modified with a self-assembled monolayer of 1H,1H,2H,2H-perfluorodecanethiol (PFDT) by keeping the samples in 5 mM PFDT solution for 30 min, followed by gentle rinsing with ethanol and drying with a stream of nitrogen.33
Characterisations

The static contact angles of water drops on the samples of the leaf surfaces and their replicas were determined with a custom-built contact angle goniometer. Cut samples of 1 cm² were affixed to a glass slide and a 5 µl droplet of high purity water (resistivity 18 MΩ cm) was applied to the surface. After 30 s, the droplets had equilibrated; digital images of the drop profile (624×580 pixels, 8-bit monochrome) were captured with a progressive scan CCD camera (JAI CVM10BX). The contact angle was determined by in-house edge detection software by drawing a tangent close to the edge of the droplet. The mean value of the obtained contact angles was calculated from at least 10 individual measurements taken from different locations on the examined substrates. Measurements were made at 22 °C and 40–50 % RH.

An optical microscope (Nikon) with a colour CCD camera and colour monitor, as part of the Nanoscope IV, Multi Mode AFM system (Veeco, USA), was used for primary surface observation of all the fabricated samples. Photographic images were taken by a camera connected to the microscope and a PC. More details of the surface morphology of the prepared samples were obtained by field-emission scanning electron microscopy, Philips XL 30 microscope. The samples were mounted on microscopy stubs with a carbon sticky tape and coated with a thin sputtered platinum layer (3–5 nm) to provide a conducting surface. The SEM images were acquired with accelerating voltages from 5–10 kV.

RESULTS AND DISCUSSION

Morphological and hydrophobic characteristics of Agave plant surface

*Agave attenuate*, a succulent plant from the large *Agavaceae* family, known as “lion’s tail”, “swan’s neck”, or “foxtail” is one of the most attractive ornamental plants in indoor and outdoor gardens (Fig. 2a). The plant produces large giant rosettes with smooth and pale green leaves of average size of about 10–20 cm in width and 50–80 cm in length. Water droplets in contact with the leaf surface form nearly spherical beads, as shown in Fig. 2b (inset). The static contact angle of a water drop placed on the leaf surface was measured as 155±3°, which confirms their superhydrophobic properties. The water drops easily roll off the surface and the leaf remains completely dry even during rain, suggesting a very low sliding angle and excellent water-repellence. Experiments with powder dirt particles dispersed on leaf surface demonstrated that particulate contaminants could be easily picked up by these water droplets and removed from the leaves when the water droplets roll off, thus showing their self-cleaning properties. The wetting properties of *Agave attenuate* plants have not hitherto been investigated. Hence, this study for the first time provides evidence of their superhydrophobic and self-cleaning properties.

The surface of *Agave attenuate* leaves were characterised by SEM and typical images are presented in Fig. 2c. The images from both sides of the leaf show the same characteristic hill-like topography with structures that represent papillose epidermal cells responsible for the formation of the micro- and nano-structures of the cells. The cells have a hexagonal geometry of irregular size, in range of 20–50 µm. A circular structure, called papillae, with a diameter of 10–15 µm
and an elongated shape was observed on the middle of the each cell (Fig. 2c, inset 1). These structures were surrounded with numerous, irregularly oriented, smaller crystal-like structures (short ribbons) that were dispersed as brushes across the cell surface (Fig. 2c, inset 2). Their average size was estimated to be several hundred nm wide and a few µm long. They most likely represent wax nanocrystals, which in many plants are made from highly hydrophobic organic compounds. The results presented here confirm that the Agava leaf possesses a two-scale structured surface, suggesting their ability to act as a good substrate for replication and the fabrication of artificial superhydrophobic surfaces.

Morphological and hydrophobic characteristics of the replicated films

Low magnification light microscopy images of the leaf surface and the fabricated replicas with their corresponding contact angle measurements are shown in Fig. 3 and Table I, respectively. Images of the plant surface (Fig. 3a) show an array of sculptured microstructures of epidermal cell, which represent papillae structures, seen in greater detail on previous SEM images (Fig. 2c). Images of the negative polymer replicas (PDMS and PU) prepared by replica moulding are presented in Figs. 3b–3c. The structures seen on the images represent the epidermal cells with papillae structures as holes or depressions on the surface, which are not clearly recognised by optical microscopy. No morphological differences were seen
between the two replicas made from different polymers, which was additionally confirmed by SEM. However, a considerable difference is observed in their static CA values. The water drop on the PU polymer replicas shows a lower CA (113±6°) (Fig. 3c, inset) than on the PDMS replicas (132±8°) (Fig. 3b, inset). This disagreement is explained by the difference in the chemical composition and differences in the surface energy of these two polymers.

Fig. 3. Low resolution light microscopy images of Agave leaf surface and the corresponding replicas. Typical topography of the leaf surface with papillae microstructures (a); PDMS (b) and PU (c) negative polymer replicas fabricated by replica moulding process of the leaf; positive PU replica fabricated from PDMS (d); positive PDMS replica fabricated by imprinting using PU negative replica as a stamp (e); positive PFDT/Au/PU replica fabricated by coating with a thin gold film modified with 1H,1H,2H,2H-perfluorodecanethiol (g). The images of water droplets on all surfaces are presented (right bottom on each image) to quantify their hydrophobic properties.

TABLE I. Values of the static contact angles (mean values±SD) measured on Agave leaves, the corresponding negative and positive polymer replicas (PDMS, PU and PFDT/Au/PU) and the corresponding control flat substrates

<table>
<thead>
<tr>
<th></th>
<th>Leaf surface</th>
<th>Negative replica</th>
<th>Positive replica</th>
<th>Control (flat surfaces)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle, deg.</td>
<td>Leaf</td>
<td>PDMS PU PDMS PU</td>
<td>PFDT/Au/PU PDMS PU PFDT/Au/PU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>155±3</td>
<td>132±8</td>
<td>113±6</td>
<td>150±6</td>
</tr>
<tr>
<td></td>
<td>135±6</td>
<td>152±5</td>
<td>120±5</td>
<td>95±4</td>
</tr>
<tr>
<td></td>
<td>118±3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Images of the positive polymer replicas prepared with both polymers (PDMS and PU) are presented in Figs. 3d–3e. The characteristic topography and the cell structures corresponding to the original leaf surface, seen in Fig. 3a, are observed. Again, no morphological differences in these positive replicas were seen between two polymers (Figs. 3d–3e) but a considerable difference was observed in their CA values. The PU positive replica showed a lower CA (135±6°) in comparison with the PDMS positive replica (150±6°), which is caused by the differ-
rences in their surface energies. The very similar CA value of the PDMS replica to that of the original leaf surface (155±3°) clearly demonstrates that it is possible to fabricate a surface with highly hydrophobic properties by a simple and reliable replication of a plant surface. To demonstrate that the hydrophobic properties of the positive replicas are dependent on the surface chemistry, PU replicas were coated with a thin gold film and modified with a self-assembled monolayer of \(1H,1H,2H,2H\)-perfluorodecanethiol. A significant increase in the CA value from 135±6 to 152±5° was observed and the hydrophobic properties matched those of the original plant. These results clearly show that modifications using molecules with a lower surface energy increase the contact angle of the surface, which confirms that surface modification is a practical method to alter the wetting properties of a structured surface to obtain the desired hydrophobic properties.

To characterise the precision of the replication process, more detailed SEM images of the fabricated replicas were obtained, which are presented in Figs. 4a–4i. The characteristic topography of the Agave leaf surface with the hierarchical organisation of the structures, which includes the circular papillae microstructures (1) and nanostructured wax crystals (2), is presented in Figs. 4a–4c. The SEM images of the corresponding PU polymer replica are shown in Figs. 4d–4f. The epidermal cell and the 3-dimensional papillae structures seen on the leaf surface (Figs. 4a–4c) were replicated as hexagonal depressions with large circular holes in the middle. The large circular holes (Figs. 4e–4f, line 1) represent the replica of the papillae microstructures (Fig. 4c, line 1). The smaller holes and protrusions around the big holes (Fig. 4e, line 2) represent replicas of the wax nanostructures (Fig. 4c, line 2). The images of the corresponding positive replica (PDMS) fabricated by imprinting using a negative PU replica (Figs. 4d–4f) are presented in Figs. 4g–4i. It is evident that both papillae microstructures (Fig. 4i, line 1) and wax nanostructures (Fig. 4i, line 2) from leaves had been successfully and with excellent precision replicated into PDMS. However, there are a few minor variations in comparison with the original leaf surface. The slightly curved shape of epidermal cells seen on original leaves (Figs. 4a–4b) and positive replica (Figs. 4g–4h) was not fully preserved in the positive replica. In addition, the smaller wax crystal structures were replicated with a lower density around the papillae structures than in the original plant surface, which is not surprising because of the limitations of using PDMS polymers to replicate nanostructures smaller than 50 nm. The slightly lower density of these nanostructured replicas from wax structures observed on both replicas may explain the slight disagreement in the CA between the PDMS polymer replicas (150±6°) and the original plant surface (155±3°).

A schematic model of the surface structures of the plant surface and the corresponding replicas based on SEM images is shown in Figs. 5a–5c to understand better their hydrophobic properties. The two scale geometry and double rough-
ness with micron (papillae) and nano-sized (wax) structures from the leaf surface (Fig. 5a) are replicated into the corresponding negative replica (Fig. 5b) and fully preserved within the positive replica (Fig. 5c). According to both Wenzel and Cassie–Baxter theories, a double roughness surface greatly amplifies the apparent contact angle.\textsuperscript{11,12}

Fig. 4. SEM Images of Agave leaf surface and the corresponding replicas showing the replication process in more detail. Leaf surface(a–c), PU negative (d–f) and PDMS positive (g–i) replicas. Papillae structures with micro (1) and nanofeatures (2) are marked.

In the Wenzel approach, it is assumed that liquid drop fills both the peaks and valley of the rough surface (Fig. 5d). From an energy consideration, the apparent contact angle of the drop $\theta_w$ is given by the equation:

$$\cos \theta_w = r \cos \theta$$  \hspace{1cm} (1)

where $r$ is the ratio of the actual area of the liquid–solid contact to the projected area on the horizontal plane and $\theta$ is the equilibrium contact angle of the liquid drop on a flat surface.

According to the Cassie–Baxter theoretical model, the formation of a spherical droplet of water on a surface is explained by the state in which air bubbles are trapped in the surface structures and the water drop sits on the bubbles (Fig. 5e). This model assumed that the water drop settles on the peaks of the roughness geometry with the contact angle given by the equation:

$$\cos \theta_{CB} = f_s \cos \theta + f_s - 1$$  \hspace{1cm} (2)
where $f_s$ is the fraction of projected planar area of the drop in contact with a solid. In the limit of $f_s \to 0$, the contact angle $\theta_{\text{CB}}$ approaches $180^\circ$ leading to super hydrophobic behaviour.

A significant increase of the CA of fabricated replicas in comparison with flat surface (PDMS, PU and PFDT/Au/PU, Table I) was observed, which clearly demonstrates the central importance of the roughness in the amplification of contact angles. The difference in the CA of the positive replicas with the same topography can be explained by differences in their chemical composition and surface chemistry, which are also important factors defining the CA. The differences in surface energy of PDMS ($\approx 20 \text{ mN m}^{-1}$) and PU polymers ($\approx 40 \text{ mN m}^{-1}$) is confirmed by the differences in the CA of their flat surfaces.\textsuperscript{34,35} However, if it is assumed that the equilibrium CA of water on paraffin waxes is about $110^\circ$, which is lower than that for PDMS ($120^\circ$) and PFDT ($118^\circ$), then their positive replicas should have a higher CA than agave leaf.\textsuperscript{37} This disagreement is explained by the imperfection of the replications of the nano-sized wax structures, previously noted on the SEM images (Fig. 4), which slightly lowered their CA values.

The advantage of this fabrication method in comparison to previous studies where only PDMS was applied is the straightforward separation in both replica-
tion steps (moulding or imprinting), which does not require the application of an anti-stick layer. Experiments with PDMS and an anti-stick agent, could not provide satisfactory and reproducible results, as was also reported in a previous study.

To achieve anti-stick free separation, in this work two polymers with different mechanical (elastic vs. hard) and chemical properties were applied, which makes the separation process simple and easy. Additional benefits obtained when using two polymers is the increased flexibility in fabrication and the ability to combine moulding and imprinting techniques. In comparison with the replication of plant leaf surfaces using the recently reported metal and electroforming process, this process with hard polymers is advantageous because polymer moulding is a cheaper, shorter and simpler process. Regarding the robustness of the PU polymer, the prepared negative replica could be reused as a replication master or stamp many times and was comparable to the metal mould. To investigate their robustness for mass fabrication, the replication process using PU negative replicas was repeated several times and no morphological differences were observed in the SEM images of the fabricated positive replica after the moulding and imprinting process. Therefore, the proposed method has potential for application in the mass-production replication of highly hydrophobic surfaces from plant leaves with dimensions possibly exceeding 10 cm x 10 cm. The hard polymer replica also has potential to serve as a master for the replication of leaves surface to other materials, including metals, metal oxides and carbons.

To demonstrate the self-cleaning properties of the fabricated polymer films, a larger area (150 mm x 150 mm) of a PDMS positive replica was prepared on glass by subsequent imprinting using a PU negative master. The prepared surface was contaminated by alumina particles (size ≈ 1 µm) and then a qualitative self-cleaning test was performed by exposure to artificial rain at an inclination angle of 15°. The water droplets washed away and removed the particles from the surface in a comparable way to natural Agave leaves, which proves the self-cleaning properties of the prepared polymer films. The thin PDMS film could be removed from the underlying glass surface and attached to other substrates which have potential for water repelling applications on specific surfaces (including curved surfaces).

CONCLUSIONS

Two soft lithographic approaches, replica moulding and nano-imprinting were explored for the fabrication of polymer films with microstructured patterns and superhydrophobic properties by replication from plant surfaces (Agave attenuate). The replication process using two polymers with different chemical and mechanical properties, i.e., elastomeric PDMS and hard, UV-curable, polyurethane based (NOA 60) polymer, was demonstrated. It was shown that the fabricated polymer replicas matched the hierarchical topography of the plant surface...
with excellent precision, providing accurate replication of their micro and nano-structures and highly hydrophobic properties, close to those of the original plant. Hydrophobic properties of fabricated replicas were influenced by the surface chemistry of the polymers selected for replication. However, it was shown that these properties could be controlled by further surface modifications of the polymer using self-assembled monolayers based on gold/alkanethiol surface chemistry.

The described method is simple, based on low cost, commercially available materials with the capability to be adapted for rapid and mass fabrication of superhydrophobic and self-cleaning surfaces for use in a variety of applications. The advantages of the method in comparison with existing replication methods were demonstrated. In addition to water repellence, other properties such as transparency, colour, flexibility, anisotropy and breathability could be incorporated into the fabricated superhydrophobic surfaces using these polymers.

Acknowledgments. The financial support from the Australian Research Council (ARC) and the University of South Australia is gratefully acknowledged.

REFERENCES