Does the aquatic invertebrate nipple array prevent bubble adhesion? An experiment using nanopillar sheets

Euichi Hirose1, Hiroyuki Mayama2,† and Akihiro Miyauchi3

1Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan
2Research Institute for Electronic Science, Hokkaido University, N21W10, Sapporo 001-0021, Japan
3Hitachi Research Laboratory, Hitachi Limited, 7-1-1 Omika, Hitachi, Ibaraki 319-1292, Japan

The nipple array is a submicrometre-scale structure found on the cuticle surfaces of various invertebrate taxa. Corneal nipples are an antiglare surface in nocturnal insects, but the functional significance of the nipple array has not been experimentally investigated for aquatic organisms. Using nanopillar sheets as a mimetic model of the nipple array, we demonstrated that significantly fewer bubbles adhered to the nanopillar surface versus a flat surface when the sheets were hydrophilic. Many more bubbles adhered to the hydrophobic surface than the hydrophilic surfaces. Bubbles on the body surface may cause buoyancy problems, movement interference and water flow occlusion. Here, bubble repellence is proposed as a function of the hydrophilic nipple array in aquatic invertebrates and its properties are considered based on bubble adhesion energy.

1. Introduction

The body surface of some invertebrates is covered with an array of submicrometre-scale protuberances. The moth-eye corneal nipple array is one of the most well-known examples, where the refractive index gradient reduces eye glare to decrease visibility to predators [1,2]. Similar surface structures have been described in aquatic invertebrates. Interestingly, these structures have similar dimensions: the protuberances are approximately 0.1 μm in height. For example, the terminal tips of epidermal microvilli form epicuticular projections (sometimes called microvillar caps) in some annelids [3], entoprocts [4,5] and echinoderms [6]. Some tunicates also have a nipple array on the cuticle surface of the tunic, comprising a cellulosic matrix covering the epidermis [7,8] (see electronic supplementary material, S1). The occurrence of the nipple array in various phyla implies its functional significance in both air and water. The primary function of the nipple array for nocturnal insects is eye reflection reduction, but this ‘anti-glare’ is not likely to be a crucial function for sessile marine invertebrates, for instance ascidians. Therefore, the nipple array probably serves another function(s) in aquatic environments. Wettability (hydrophobicity/hydrophilicity) is also an important property; many epipelagic marine larvae have hydrophilic surfaces so they do not become trapped at the water surface [9]. We have observed that water drops usually spread out on the surface of ascidians drawn up from seawater, indicating that the ascidian tunic is usually hydrophilic regardless of the presence or absence of a nipple array.

The recent development of nanoimprint technology has enabled the fabrication of polymer sheets with nanoscale structures, for example nanopillars. Using nanopillar sheets as a mimetic model for the nipple arrays, we examined the properties of hydrophilic and hydrophobic polystyrene sheets with and without nanopillars in water. Here, we report that more bubbles adhere to the hydrophobic surface than the hydrophilic surface. Moreover, the adhesion of
bubbles significantly decreased on the hydrophilic nanopillar surface, indicating another function of the nipple array.

2. Material and methods

Polystyrene sheets (20 × 20 mm) with or without 1-μm-high nanopillars (see electronic supplementary material, S1c/d) were generously provided by Hitachi Ltd. (Hitachi, Japan) [10]. They are unavailable commercially. The polystyrene was originally hydrophobic and some sheets were hydrophilized by plasma etching (EXAM, Shinko Seiki). Abbreviations and specifications of each sheet type are listed in table 1. The hydrophilic nanopillar sheets (0.5-hy and 2-hy) were designated as mimics of the nipple arrays of aquatic invertebrates, such as ascidians and entoprocts, with protuberances approximately 0.1 μm in height [5,7]. The bubble repellences of hydrophilic nanopillar sheets were compared with the hydrophobic nanopillar sheets (0.5-un) to test the effect of the wettability of the nipple array, as well as the hydrophilic sheet lacking the nipple array (0-hy) to test the effect of the presence or absence of the nipple array.

Four sheets (0-hy, 0.5-hy, 2-hy and 0.5-un) were adhered to the same glass plate using solvent-free silicone adhesive, which does not affect polystyrene (figure 1). The glass plate was placed in a polypropylene container (8 × 13 cm base) with 200 ml of aerated, ice-cold water, and the container was incubated in a hot bath (e-Thermo Bucket ETB, TAITEC) at 50–80°C for 15 min. Swelling of the polystyrene sheets was not detected during incubation (less than 0.5%). Before incubation, we visually confirmed that the water fully penetrated the surface of the sheets and no bubbles were attached. Air remaining on the sheet was easily detected owing to the marked difference in the reflection indices of air and water. A decrease in the solubility of air owing to the increase in water temperature resulted in the production of bubbles on the sheets as well as the inner surface of the container. The plate was photographed so that the bubbles on

Table 1. Nanopillar sheet specifications.a

<table>
<thead>
<tr>
<th>abbreviation of sheet type</th>
<th>height (μm)</th>
<th>diameter (μm)</th>
<th>interval (μm)</th>
<th>hydrophilization/contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-hy</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>yes/3.8</td>
</tr>
<tr>
<td>0.5-hy</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>yes/2.4</td>
</tr>
<tr>
<td>0.5-un</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>no/105.7</td>
</tr>
<tr>
<td>2-hy</td>
<td>1</td>
<td>2.0</td>
<td>4</td>
<td>yes/4.2</td>
</tr>
</tbody>
</table>

*aSpecifications were provided by the manufacturer. Sheets of 0.5-hy and 2-hy were regarded as mimetic models for the hydrophilic nipple array of aquatic invertebrates.*

![Figure 1. Example of polystyrene sheets with bubbles: 126 bubbles adhered to the 0.5-un sheet (hydrophobic nanopillar sheet), 43 bubbles adhered to the 0-hy sheet (hydrophilic flat sheet) and no bubbles occurred on the 0.5- and 2-hy sheets (hydrophilic nanopillar sheets).](image-url)
3. Results

The numbers of bubbles were significantly different among the sheet types (Friedman test, $n = 25$, $p < 0.001$), as shown in figure 1. Few bubbles were present on the hydrophilized nanopillar sheets (0.5-hy: median 1, rank sum 39.5; 2-hy: median 0, rank sum 36.5) and the difference between the two sheet types was not significant (Dunn's multiple comparison test, $p > 0.05$). The hydrophilized flat sheets (0-hy: median 9, rank sum 74) contained significantly more bubbles than the 0.5- and 2-hy sheets ($p < 0.001$). The hydrophobic nanopillar sheet (0.5-un) contained many more bubbles (median 173, rank sum 100.0), which was significantly different from the other sheet types ($p < 0.05$ versus 2-hy, $p < 0.001$ versus 0.5- and 2-hy). In summary, the number of bubbles and sheet types had the following relationship: hydrophobic nanopillar (0.5-un) > hydrophilic flat (0-hy) > hydrophilic nanopillar (0.5- and 2-hy).

4. Discussion

Bubble adhesion probably causes problems for small aquatic organisms. We have occasionally observed many bubbles attached to organisms in the field, where water can warm up in a short time. Owing to the buoyancy of the bubbles, small organisms float up to the water surface. Suspension feeders often cease feeding when bubbles became trapped on filtering organs. For example, the branchial aperture of some colonial ascidians is less than 1 mm in diameter, and we often found the aperture blocked by a bubble in colonies incubated in warm seawater. Therefore, countermeasures against bubbles would probably be beneficial for small aquatic invertebrates inhabiting shallow areas such as tide pools and reefs, where drastic changes in water temperature and/or breaking waves cause bubble formation.

In this study, the bubbles adhered more to the hydrophobic surface than the hydrophilic surfaces with or without nanopillars, suggesting that the maintenance of a hydrophilic surface is important for small invertebrates. For example, experimentally generated ascidian larvae with a hydrophobic integument were easily trapped at the water surface, whereas normal (hydrophilic) larvae were not [9]. This indicates why aquatic invertebrates, especially epipelagic ones, would have hydrophilic surfaces. Moreover, significantly, fewer bubbles adhered to the hydrophilic nanopillar surface than to the hydrophilic flat surface. Because the contact angles were very similar among the hydrophilized sheets (table 1), the differences in bubble adhesion were caused by different surface structures, specifically the presence or absence of nanopillars. Because the nanopillar surfaces are similar in structure to the nipple array on the cuticle surface of some aquatic invertebrates, the presence of the nipple array probably prevents adhesion of the bubbles.

To understand the results, we considered the relationships among bubble adhesion, wettability and surface structure (flat/nanopillar) with respect to the adhesion energy of an air bubble onto flat and nanopillar surfaces in water (figure 2a,b). Bubbles can adhere to a nanopillar surface via two possible ways, as shown in figure 2b. The former is the Cassie–Baxter (CB) state in which the bubble does not penetrate the spacing between the pillars, whereas the latter is the Wenzel state with full penetration between the pillars.

Adhesion energy ($E_\text{ad}$) is defined as the work required to detach the bubble from the surface, and the area of interface ($S_\text{air}$ in figure 2a) is a function of the contact angle of the bubble ($\theta_\text{air}$) on the sheet surface [11]. From the energy balance within the $S_\text{air}$ between attachment and detachment,
the following equation is obtained for the relationship between $E_{\text{full}}/S_{\text{air}}$ and $\theta_{\text{air}}$ on the sheet (see the electronic supplementary material, S2 for details).

$$\frac{E_{\text{full}}}{S_{\text{air}}} = \gamma_{\text{L}}(1 + \cos \theta_{\text{air}}),$$

where $\gamma_{\text{L}}$ is surface energy per unit surface area (or surface tension) of the water–air interface. On a more hydrophobic surface, $\theta_{\text{air}}$ approaches to 0, and $\cos \theta_{\text{air}}$ approaches to 1. Therefore, the adhesion energy of air bubbles is larger on a more hydrophobic (i.e. larger $\cos \theta_{\text{air}}$) surface. This equation explains why more bubbles stuck to the hydrophobic surface (0.5-un) than the hydrophilic surfaces (0-hy, 0.5-hy and 2-hy) in the present experiment.

$S_{\text{air}}$ illustrated in figure 2a is the apparent area of interface, and the actual $S_{\text{air}}$ of the bubble on the nanopillar surface is quite different from the $S_{\text{air}}$ on the flat surface. In the CB state, the apparent area of interface is composed of the area between the bubble and pillars, and the area between the bubble and water; thus, the actual $S_{\text{air}}$ contacting the substrate is smaller than the apparent $S_{\text{air}}$. As the adhesion energy is 0 on the water ($\theta_{\text{air}} = 180^\circ$, $\cos \theta_{\text{air}} = -1$) from the equation above, nanopillar surfaces have less adhesion energy than flat surfaces in the CB state (see the electronic supplementary material, S3a for details). This is similar to the anti-contaminating properties of a corneal nipple array in which contaminating particles have a smaller contact area on the nipple arrays than on the flat surface [12]. By contrast, the area of interface between the bubble and substrate is enhanced in the Wenzel state [13] (see electronic supplementary material, S3b). Therefore, nanopillar surfaces in the Wenzel state have more adhesion energy than flat surfaces.

Figure 2c shows the estimates of adhesion energies per apparent area of interface in different adhesion manners. Here, $\gamma_{\text{L}} = 0.072$ (N m$^{-1}$) (the surface tension of water). The values adopted for $\tau$ in the Wenzel state and $f$ in the CB state were based on the surface structures of the nanopillar sheets used here (see the electronic supplementary material, S3c for details). In the present experiments, more bubbles stuck to the hydrophilic flat surface (0-hy) than the hydrophobic nanopillar surfaces (0.5- and 2-hy), indicating CB state adhesion on the hydrophilic nanopillar sheets. Because water fully penetrated the spacing between the pillars before incubation, an air bubble likely nucleated on the tip of pillar owing to heterogeneous nucleation on a physical defect [14].

The function(s) of the nipple array have been experimentally investigated in terrestrial insects but have not previously been studied in aquatic invertebrates, in which the cuticle layer was thought to be involved in defence, such as for protection against mechanical abrasions and microbial infection [6,15]. The present study supports the novel idea that the nipple array prevents bubble adhesion. Because bubble repellency is useless in terrestrial invertebrates, which usually have hydrophobic surfaces, a study considering aquatic habitats was necessary to determine its function. The nipple array is likely to be multifunctional structure, and nanoimprinting technology gives the potential for biomimetically exploiting these functions for human applications. Further investigation of submicrometre structures is also necessary to identify unrevealed functional structures in taxa in various habitats.

Data accessibility. Data available from Dryad repository: doi:10.5061/dryad.9fb1s [16].

References