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Bioinspired monolithic polymer microsphere arrays as generically anti-adhesive surfaces

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Keywords: Nepenthes, microsphere monolayers, pull-off force, real contact area, anti-fouling

Abstract

Bioinspired surface topographies showing generic anti-adhesive behaviour by minimization of the real contact area not only with rigid, but also with soft and compliant counterpart surfaces recently attracted increasing attention. In the present study, we show that such generic anti-adhesive surfaces, which moreover demonstrate anti-fouling behaviour, can be produced on a large scale by a simple double replication of monolayers of microspheres with diameters of a few 10 \( \mu \text{m} \). Thus, we obtained mechanically stable monolithic arrays of microspheres tightly connected to a support of the same material. Adhesion of these microsphere arrays to sticky and compliant counterpart surfaces was one order of magnitude weaker than that of flat control samples of the same material. The generation of nanorod arrays with nanorod diameters of a few 100 nm as the second hierarchical structure level on monolithic microsphere arrays did not significantly affect the adhesion force. The experimental data on anti-adhesive behaviour were modelled using a modified Johnson–Kendall–Roberts theoretical approach that also provided general design criteria for topographic adhesion minimization to sticky counterpart surfaces.

Introduction

Bioinspired surfaces combining anti-adhesive properties, self-cleaning behaviour and superhydrophobicity are recently attracting increasing interest (Koch \textit{et al} 2009, Bhushan and Jung 2011, Shircliff \textit{et al} 2011, Yan \textit{et al} 2011, Liu and Jiang 2012, Nishimoto and Bhushan 2013, Butt \textit{et al} 2014). Such surfaces typically exhibit hierarchical topographic patterns derived from microsphere monolayers or similar structures. Preparative strategies to access such systems may include transfer of non-contiguously packed microsphere arrays obtained using Langmuir troughs to smooth or topographically patterned substrates (Badge \textit{et al} 2013), particle deposition by spraying methods (Ebert and Bhushan 2012, Mertaniemii \textit{et al} 2012), fabrication of silica microsphere layers by spin-coating (Raza \textit{et al} 2012) as well as pressing polymer microspheres onto gummed tape (Wang \textit{et al} 2014). Other preparative approaches reported so far include the fabrication of thin nanostructured layers on micropatterned substrates (Ho \textit{et al} 2014), two-step moulding combined with initiated chemical vapour deposition (Karaman \textit{et al} 2012), and generation of rough wrinkled surfaces by swelling of polyamide in acetic acid (Zhang \textit{et al} 2010).

Far lesser interest has been directed to the elaboration of design criteria for generically anti-adhesive surfaces that show anti-adhesive behaviour not only on rigid counterpart surfaces, but also on counterpart surfaces that are sticky according to the Dahlquist criterion (Young’s modulus lower than 100 kPa) (Dahlquist 1969). From the geometrical point of view, minimization of adhesion on solid anti-adhesive surfaces is caused by surface topographies that strongly reduce a real contact area. In other words, if the counterpart surface is rigid, microroughness reduces the real contact area and, therefore, adhesion. If the counterpart surface is sticky and compliant, it can adapt to microroughness. In this case, microroughness causes an increase
in the real contact area and, consequently, adhesion (Purtov et al. 2013).

Classical self-cleaning surfaces with their extremely fine surface structures (Barthlott and Neinhuis 1997, Liu and Jiang 2012) sometimes demonstrate even stronger adhesion, if compared to smooth control surfaces (Persson 1998, Purtov et al. 2013). Therefore, they are not necessarily suitable models for the design of stable anti-adhesive surfaces against sticky counterpart surfaces. However, the waxy zone of trapping organs called pitchers of carnivorous (insect-eating) plants from the genus Nepenthes, which is located under the ribbed rim, the peristome, inside the pitcher, shows pronounced anti-adhesive properties (Gorb et al. 2014). Even insects with highly developed adhesive systems cannot adhere to the waxy zone (Gaume et al. 2002, Gaume et al. 2004, Gorb et al. 2005, Scholz et al. 2010, Gorb and Gorb 2011) and slide into the pool of the digestive fluid at the bottom of the pitcher, where they are trapped. Although it is likely that these plants utilize a combination of two effects, such as superhydrophobic physicochemical properties (Gorb and Gorb 2006, Gorb et al. 2007) and the surface roughness (Gorb et al. 2005, Scholz et al. 2010, Gorb et al. 2013), contributing to the anti-adhesive properties of the waxy zone, we consider only the roughness effect in this study.

The waxy zone in many Nepenthes species has a hierarchical structure comprising lunate cells (N. alata: height ∼9.5 μm, length ∼35 μm, width ∼7 μm) (Gaume et al. 2002, Wang and Zhou 2010, Gorb and Gorb 2011) as hierarchical level 1 as well as superimposed lower and upper epicuticular wax layers as hierarchical levels 2 and 3 (Juniper and Burras 1962, Juniper et al. 1989, Gorb et al. 2005, Gorb and Gorb 2009, Gorb et al. 2013). Relatively large prominent lunate cells, scattered between tabular epidermal cells, occur in a great number (ca. 480 cells per mm²) and are regularly distributed singly over the surface, whereas microscopic wax crystals on top of both cell types form a continuous coverage. Lunate cells are responsible for the surface relief of 10 and more microns in height, whereas the wax coverage creates an additional roughness in the range of about 2 μm ($R_{\alpha} = 1.909 \mu m$, $rms = 2.378 \mu m$) (Gorb et al. 2014).

Here, we show that mimicking the topography of lunate cells constituting hierarchical level 1 of the above mentioned waxy zone yields artificial surface topographies with pronounced anti-adhesive properties to rigid as well as to sticky and compliant counterpart surfaces. Moreover, cell culturing experiments suggest that such surface topographies promote anti-fouling properties. We prepared mechanically stable monolithic arrays of polystyrene (PS) microspheres having similar dimensions and regular distribution as level 1 lunate cells (a few 10 μm) as large-area bioinspired anti-adhesive surfaces. The topography of the monolithic PS microsphere arrays reduced adhesion to sticky counterpart surfaces by one order of magnitude as compared to smooth (called thereafter as ‘flat’) PS films; neither the anisotropy of the lunate cells, nor lower and upper wax layers as hierarchical levels 2 and 3 need to be mimicked to achieve this effect.

Methods

Preparation of self-ordered nanoporous anodic aluminium oxide (AAO)

Self-ordered nanoporous AAO membranes were produced using an established two-step anodization process (Masuda et al. 1998). Al discs with a diameter of 2 cm (Al content >99.99%) were annealed for 3 h under argon at 500 °C and then electropolished at room temperature with a mixture of 25 vol% 60% HClO₄ and 75 vol% C₂H₅OH for 20 min at 25 V under stirring. All anodization steps were carried out with 1 wt% aqueous H₃PO₄ at 195 V and at a temperature of 0 °C–1 °C. The first anodization was carried out for 14 h. The formed first alumina layer was etched with an aqueous solution containing 1.8 g Cr₂O₃ and 7.1 g 85% H₃PO₄ per 100 ml at 30 °C for ∼20 h. The second anodization was stopped as soon as the desired pore depth was reached assuming that the pores grow 500 nm per 6 min. The AAO growth rates are well known; the AAO layer thickness as a function of the anodization duration was investigated by a scanning electron microscopy and capacitive sensing. The nanopores of the self-ordered AAO membranes had a diameter of 180 nm and were widened to the desired diameter by isotropic etching with 10 wt% H₃PO₄ solution at 30 °C.

Preparation of monolithic arrays of PS microspheres

Mechanically stable monolithic arrays of PS microspheres tightly connected to underlying PS substrates that extended 20 mm × 20 mm were prepared by refining previously reported double replication procedures of PS microsphere monolayers (Nam et al. 2006, Rengarajan et al. 2012).

Rectangular pieces of (100) silicon wafers with edge lengths of ∼2 cm were cleaned with ‘piranha solution’ containing H₂SO₄ and H₂O₂ (30%) at a volume ratio of 7:3 for at least 5 min. Then, we spin-coated a surfactant layer (Triton X-100, CAS 9002-93-1, Sigma Aldrich) at 3500 rpm for 30 s onto the Si wafer pieces. Aqueous suspensions of PS microspheres (Polybead Microspheres, Polysciences Inc., Canada) with radii of 12.5 μm (2.91 × 10⁶ microspheres ml⁻¹), 22.5 μm (4.99 × 10⁵ microspheres ml⁻¹), 37.5 μm (1.08 × 10⁵ microspheres ml⁻¹), and 45.0 μm (6.24 × 10⁶ microspheres ml⁻¹) were at first sonicated for 1–2 min. To prepare PS microsphere monolayers serving as primary templates, 3–4 droplets of the suspensions were deposited onto the surfactant-
treated Si wafer pieces followed by the multistep spin-coating program summarized in table 1.

The deposition/spin-coating cycles were typically repeated 3–4 times until the entire surface of the surfactant–treated Si wafer pieces was covered with dense PS microsphere layers. To prepare PS microsphere monolayers consisting of a mixture of PS microspheres with radii of 12.5 and 22.5 μm, 1–2 droplets of the corresponding PS microsphere suspensions were alternately spin-coated onto the surfactant-treated Si wafer pieces using the multistep spin-coating program summarized in table 1 until the entire surface of the Si wafer pieces was covered by a dense PS microsphere layer. Excess PS microspheres on top of the PS microsphere monolayers were removed by rinsing the surface with distilled water.

Base and curing agent of a poly(dimethylsiloxane) (PDMS) prepolymer mixture (Sylgard 184, Dow Corning, Midland, USA) were mixed at a weight ratio of 10:1 and then kept under ambient conditions until all gas bubbles had vanished. The PDMS mixture was then poured onto the PS microsphere monolayers in such a way that the whole area of the latter was covered by a 0.5–1.0 cm thick layer of the PDMS prepolymer mixture. After curing for 3–4 days under ambient conditions, PDMS moulds containing hexagonal arrays of spherical cavities (negative replicas of the PS microspheres) were obtained. The PDMS moulds were mechanically detached from the Si wafer pieces, and residual PS microspheres were mechanically removed using scalpel.

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Table 1. Multistep spin-coating program applied to prepare PS microsphere monolayers serving as primary templates in the double replication process yielding monolithic PS microsphere arrays.

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The monolithic PS microsphere arrays were imaged with an optical microscope (Di-Li 1027, Kaiserslautern, Germany) in the reflection mode and with a scanning electron microscope (SEM) Zeiss AURIGA (Zeiss, Oberkochen, Germany) operated at an accelerating voltage of 3 kV. To evaluate the roughness of the latter, we carried out atomic force microscopy measurements performed in the semi-contact topography mode using a NT-MDT Ntegra device and cantilever tips VIT_P supplied by NT-MDT (tip curvature 35 nm). Typically, we obtained rms roughness values of 12.3 nm for scanned areas of 625 μm² comprising 256 × 256 pixels.

Adhesion tests

Adhesion on monolithic PS microsphere arrays was tested with a recently developed technique for the measurement of weak adhesion forces (Purtov et al 2013). Force measurements (figure 1) were performed with a microforce tester Basalt-01 (Tetra, Ilmenau, Germany) at a room temperature (20 °C–25 °C) and humidities of 18%–28%. A compliant PDMS half-sphere (radius R₁ = 1.5 mm; elastic modulus ~92 ± 25 kPa) was mounted on a spring with a spring constant of 203.9 N m⁻¹ and brought into contact with the surfaces of tested samples (unmodified and hot-embossed monolithic PS microsphere.
arrays as well as flat PS films). The PDMS half-sphere was retracted immediately after the set loading force \( F_1 = 1 \, \text{mN} \) had been reached. The approach and retraction speeds of the PDMS half-sphere were 50 \( \mu \text{m} \, \text{s}^{-1} \). Using the force–distance curves obtained, we calculated the adhesion force \( F_{\text{ad}} \) which corresponded to the pull-off force (the minimum force in the retraction part of the curve). The elastic modulus of the PDMS half-spheres was determined from force–distance curves measured on flat PS films using the 2-point method (Ebenstein and Wahl 2006).

Each sample was tested at least at six different spots with different PDMS half-spheres used as probes (1–4 tests per point). The data extracted from the force–displacement curves were statistically analyzed with the SigmaStat 3.1.1 software (SPSS, Chicago, USA).

**Cell culture and fixation**

Unmodified and hot-embossed monolithic PS microsphere arrays with the microsphere radius \( r_m \) of 12.5 \( \mu \text{m} \) as well as the flat PS film were rinsed in deionized water for two days and dried under vacuum. Prior to cell seeding, the samples were sterilized by exposure to UV light (Oriel Apex Illuminator equipped with a 75 W Xenon lamp and a 280–400 nm dichroic mirror, Newport Spectra-Physics, Darmstadt, Germany) for 3 min. The samples were then placed in petri dishes filled with 3 ml MEM medium supplied with 10% fetal bovine serum (FBS) (Biochrom, Berlin, Germany, S0615), 1% MEM non-essential amino acids (PAA Laboratories, Pasching, Austria, M11003), and 1% 2-(4-(2-hydroxyethyl)-1-piperazinyl)-ethansulfonacid (HEPES) buffer. HeLa cells were placed dropwise onto each sample surface to a density of ~50% confluency. Cell culture dishes were subsequently incubated for 48 h at 37 °C in the presence of 5% CO\(_2\). Cell fixation was carried out under standard conditions. The samples were briefly washed with PBS buffer at 37 °C. Then, 2 ml 4% paraformaldehyde in PBS was added for fixation for two days at 4 °C.

Prior to any SEM characterization, the samples were washed with deionized water for three times, dried with absolute ethanol and sputter-coated with platinum. SEM investigations were carried out using the Zeiss Auriga SEM operated at an accelerating voltage of 1 kV.

**Results and discussion**

**Anti-adhesive properties: experimental results**

Extended monolayers of discrete PS microspheres, which are accessible by dip coating (Dimitrov and Nagayama 1996), the use of flow cells (Park et al 1998), isothermal heating evaporation-induced self-assembly (Wong et al 2003), or by spin coating (Jiang and McFarland 2004, Cheung et al 2006), exhibit poor mechanical stability. Therefore, we created mechanically stable monolithic specimens consisting of PS microsphere arrays tightly connected to underlying PS substrates by refining previously reported double replication procedures of PS microsphere monolayers (Nam et al 2006, Rengarajan et al 2012).

The mechanically stable monolithic arrays of PS microspheres (called thereafter ‘PS microsphere arrays’) thus obtained consisted of PS microspheres with radii \( r_m \) of 12.5, 22.5, 37.5, and 45 \( \mu \text{m} \) as well as a mixture of PS microspheres with \( r_m \) values of 12.5 and 22.5 \( \mu \text{m} \) (figure 2).

In hot-embossed specimens, the caps of the PS microspheres were functionalized with arrays of PS nanorods replicating the nanopores of the AAO moulds (figure 3). The PS nanorods had hemispherical tips (inverse replicas of the hemispherical AAO pore bottoms) that were previously reported to show significantly lower adhesion than PS nanorods with flattened terminal contact shapes (Xue et al 2012).

The average values of the adhesion force \( F_{\text{ad}} \) measured on the unmodified PS microsphere arrays reached only 6%–15% of the average adhesion force \( F_{\text{flat}} \) measured on the flat PS film (figure 4). On unmodified PS microsphere arrays with \( r_m \) values of 12.5, 22.5, 37.5, and 45 \( \mu \text{m} \), we obtained \( F_{\text{ad}} \) values of 294 ± 111 \( \mu \text{N} \), 246 ± 104 \( \mu \text{N} \), 196 ± 124 \( \mu \text{N} \), and 224 ± 112 \( \mu \text{N} \), respectively, while \( F_{\text{flat}} \) amounted to 1825 ± 138 \( \mu \text{N} \).

\( F_{\text{ad}} \) was most efficiently reduced on the PS microsphere array containing microspheres with two
different radii of 12.5 and 22.5 μm (figures 2(c) and 4), which were prepared by a double replication of a PS microsphere monolayer containing both PS microsphere species (see Methods section). Here, F_{ad} value amounted to 110 ± 68 μN, corresponding to 6% of F_{flat}. Moreover, statistical analysis indicated that the F_{ad} on the mixed array containing PS microspheres with radii of 12.5 and 22.5 μm was significantly lower than those on PS microsphere arrays consisting of only one PS microsphere species (Kruskal–Wallis one way ANOVA on ranks; H_{4,39} = 12.473; P = 0.014). In contrast, there were no significant differences in F_{ad} values between PS microsphere arrays consisting of only one PS microsphere species within the investigated r_s range. We interpret the topography of arrays of PS microspheres with both radii of 12.5 and 22.5 μm as a hierarchical defect structure, on which the real contact area with the PDMS half-sphere is even more efficiently minimized than that on PS microsphere arrays consisting of only one PS microsphere species.

Adhesion tests also showed that the functionalization of the caps of the PS microspheres with PS nanorod arrays (i.e., the second hierarchical structure level) had almost no effect on the anti-adhesive properties and resulted in a slight increase of F_{ad} values on hot-embossed specimens compared to the corresponding unmodified specimens. F_{ad} reached 12%–20%
of $F_{\text{flat}}$. Hot-embossed mixed PS microsphere arrays with $r_c$ of 12.5 and 22.5 $\mu$m demonstrated the $F_{\text{ad}}$ value of 217 $\pm$ 58 $\mu$N. For $r_c$ of 12.5, 22.5, 37.5, and 45 $\mu$m, we obtained $F_{\text{ad}}$ values of 374 $\pm$ 67 $\mu$N, 336 $\pm$ 68 $\mu$N, 266 $\pm$ 65 $\mu$N, and 306 $\pm$ 109 $\mu$N, respectively. Statistical analysis showed no significant difference between $F_{\text{ad}}$ values obtained on unmodified PS microsphere arrays versus those on the corresponding hot-embossed specimens ($t$-test; $P > 0.05$). The only exception thereof was found for the mixed arrays of PS microspheres with radii of 12.5 and 22.5 $\mu$m ($t$-test; $P = 0.006$).

Estimation of adhesion forces on arrays of microspheres by a modified Johnson–Kendall–Roberts (JKR) model

We examined adhesion of a sticky PDMS half-sphere with radius $R_1$ to arrays of PS microspheres, which represent nominally flat surfaces. We estimated the dependence of the adhesion force $F_{\text{ad}}$ on the radius $r_c$ of microspheres composing this nominally flat surface using a modified JKR theory (Johnson et al 1971), which predicts the real contact area and adhesion between two solid spheres. According to the JKR theory, adhesion force between two solid spheres with radii $r_c$ and $R_1$ equals:

$$F_{\text{ad}} = -\frac{3}{2} \pi \Delta \gamma R_c$$

where $R$ is an effective radius ($1/R = 1/R_1 + 1/r_c$) and $\Delta \gamma$ is the surface energy of interacting surfaces. The radius of the contact area at pull-off $a_p$ equals (Johnson et al 1971):

$$a_p = \left(\frac{9 \pi R^2 \Delta \gamma}{8 E}\right)^{1/3},$$

where the effective elastic modulus $E$ is determined by elastic moduli of contacting surfaces $E_{\text{PDMS}}$ and $E_{\text{PS}}$:

$$\frac{1}{E} = \frac{1 - \nu_{\text{PDMS}}^2}{E_{\text{PDMS}}} + \frac{1 - \nu_{\text{PS}}^2}{E_{\text{PS}}}$$

($\nu_{\text{PDMS}}$ and $\nu_{\text{PS}}$ are corresponding Poisson ratios).

First, the case of a PDMS half-sphere with a nominally flat surface built of microspheres ($r_c \ll R_1$) will be examined further. According to the JKR theory, the highest pulling stress in the contact area is at the edge. Under given force applied to the half-sphere, the equilibrium configuration is governed by the classical equation of fracture mechanics. With a stable system, any change of the loading/pulling force will cause either growth or shrinkage of the contact area in order to achieve a new equilibrium configuration (Greenwood and Johnson 1981). Above some critical value of the pulling force, no stable equilibrium exists and the surfaces pull apart in an unstable manner: a separation between the half-sphere and the substrate on the periphery of the contact area quickly propagates to the direction towards the centre of the contact according to the Griffith criterion (Griffith 1921). To describe the crack propagation between the PDMS half-sphere and an individual substrate microsphere on the periphery of the contact area (figure 5), the JKR theory may be used again. The pull-off force for the microsphere satisfies equation (1). Below, we determine the pull-off force for such microspheres and equate it to the pull-off force estimated from the stress distribution in the contact area of the PDMS half-sphere.

The effective area occupied by one PS microsphere in a hexagonal array corresponds to the area $2\sqrt{3} r_c^2$ of a hexagon circumscribing the contour of the PS microsphere. The effective number of microspheres in the peripheral annulus is
\( \pi (a_p^2 - (a_p - 2r)^2) / (2\sqrt{3} r_f^2) \). The nominal force \( F_{pr}^0 \) required to separate the PDMS half-sphere from the PS microsphere array in the peripheral annulus equals the pull-off force of a single PS microsphere (determined by equation (1), taken into account \( r_s \ll R_1 \)) multiplied by the number of the PS microspheres in the peripheral annulus:

\[
F_{pr}^0 = -\frac{\sqrt{3}\pi^2(a_p^2 - (a_p - 2r_s)^2)}{4r_s^3} \cdot \Delta \gamma. \tag{3}
\]

However, equation (3) overestimates the value of the pull-off force, because many PS microspheres are not completely located within the peripheral annulus, i.e., a fraction of their base areas projected in the plane of the peripheral annulus is located outside the peripheral annulus (figure 5). The interaction of these PS microspheres with the PDMS half-sphere is not adequately described by equation (3). Therefore, \( F_{pr}^0 \) needs to be corrected by a dimensionless parameter \( \alpha < 1 \) to obtain a corrected detachment force \( F_{pr} \):

\[
F_{pr} = \alpha F_{pr}^0 = -\alpha \cdot \frac{\sqrt{3}\pi^2(a_p^2 - (a_p - 2r_s)^2)}{4r_s^3} \cdot \Delta \gamma. \tag{4}
\]

For the sticky PDMS half-sphere, \( F_{pr} \) is the integral of the radial stress distribution \( p(r) \) over the peripheral annulus area:

\[
F_{pr} = \int_{a_p - 2r_s}^{a_p} p(r) 2\pi r dr. \tag{5}
\]

The radial stress distribution is given by:

\[
p(r) = \rho_0 \left( 1 - \frac{r^2}{a_p^2} \right)^{1/2} + \rho'_0 \left( 1 - \frac{r^2}{a_p^2} \right)^{-1/2}, \tag{6}
\]

where \( \rho_0 = \frac{2E_{ps}}{\pi \gamma} \) and \( \rho'_0 = -\frac{2\pi \gamma}{\pi \rho a_p} \) (Greenwood and Johnson 1981). The effective change in surface energy during pull-off, \( \Delta \gamma_{\text{eff}} \), accounts for the impact of the non-flat topography of the microsphere array and can be expressed in terms of \( a_p \), using equation (2). Substitution of equation (6) into equation (5) and integration yields:

\[
F_{pr} = -2\pi \rho_0 \left( 1 - \frac{(a_p - 2r_s)^2}{a_p^2} \right)^{1/2} \times \left( \frac{4}{3} \rho_0 (a_p - r_s) + \rho'_0 a_p^2 \right). \tag{7}
\]

Equations (4) and (7) deliver approximation of \( F_{pr} \) for a single microsphere and a sticky half-sphere, correspondingly. From these equations, the following equation can be drawn after straightforward simplifications:

\[
\left( \frac{a_p}{r_s} - 1 \right)^2 + 1 = \alpha \frac{\sqrt{3}\pi^2 \Delta \gamma R_1}{16E_{ps}} \sqrt{\frac{a_p}{r_s}} - 1. \tag{8}
\]

From the dependence \( a_p(r_s) \) using equation (2), an effective surface energy for a sticky half-sphere on microsphere array \( \Delta \gamma(r_s) \) can easily be found. Further, \( F_{ad}(r_s) \) profiles can be calculated from \( \Delta \gamma(r_s) \) using equation (1) (figure 6). The value of \( \alpha = 0.55 \) (figure 6(b)) corresponds to the best fit to the experimental data presented in figure 4. The part of the \( F_{ad}(r_s) \) profiles is slightly shifted to larger \( F_{ad} \) values, as \( \alpha \) increases. However, figure 6(b) also reveals that the model is robust with regard to changes in \( \alpha \). The \( F_{ad}(r_s) \) profiles obtained with constant \( \alpha \) values of 0.40 and 0.70 are also in line with our experimental results.

If \( r_s > \frac{a_p}{\sqrt{3}} \), the considerations used to draw equation (8) are not valid and the scenario, in which the apparent contact area contains only a small number of PS microspheres, should be considered. \( F_{pr} \) can be again estimated for a sticky PDMS half-sphere based on equations (1) and (2):

\[
F_{pr} = -\frac{4E_{ps}^3}{3R_0^3}. \tag{9}
\]

On the other hand, \( F_{pr} \) can be estimated for a single microsphere using an approach similar to that yielding equation (4):

\[
F_{pr} = -\alpha \frac{\sqrt{3}\pi^2 a_p^2}{4r_s^3} \Delta \gamma. \tag{10}
\]

Equating equations (9) and (10) yields an expression for \( a_p \):
counterparts are robust with respect to moderate changes in $r_v$. (iv) At $r_v$ values larger than that corresponding to the $F_{ad}$ minimum, $F_{ad}$ increases along with $r_v$. As the number of PS microspheres in the contact as well as the curvature of the PS microspheres decrease, the apparent contact area between PS microspheres and PDMS half-sphere increases. Finally, at $r_v \gg R_i$ the system approaches the JKR limiting case, in which a PDMS half-sphere is in contact with a flat PS surface, and $F_{ad}$ converges to $F_{flat}$.

**Anti-fouling behaviour**

It might be of technological interest if bioinspired artificial surfaces combine anti-adhesive properties (low adhesion on sticky counterpart surfaces) with anti-adhesive behaviour against cells (anti-fouling properties). We cultured HeLa cells for 48 h on unmodified as well as on hot-embossed monolithic PS microsphere arrays ($r_v = 12.5 \mu m$) to evaluate anti-fouling properties of these surface samples. For comparison, HeLa cells were also cultured on flat PS films under the same conditions (figure 7(a)).

The HeLa cells spread over the entire surface area of flat PS films, which indicates unperturbed cell adhesion. The cell density (number of cells per area) on the flat PS film amounted to $\sim 8 \times 10^5$ cells cm$^{-2}$. Separated HeLa cells growing on the flat PS film exhibited an elongated shape (figure 7(b)) with an average aspect ratio (length divided by width) of 14.3 $\pm$ 2.6 ($n = 10$). The average length of HeLa cells was $64 \pm 12 \mu m$ ($n = 10$). The filopodia protruding from the cell body were distributed homogeneously in all directions. The average diameter of the filopodia amounted to several hundred nanometres, while their maximum length was 11.4 $\mu m$. As compared to the flat PS film, on the unmodified PS microsphere array, the density of HeLa cells was reduced by almost one order of magnitude to $1 \times 10^5$ cells cm$^{-2}$. This is unlikely due to the leaking of cells in the voids between microspheres as the nearest neighbour distance between the microspheres was much smaller than the average length of attached HeLa cells ($25 \mu m$ versus $64 \pm 12 \mu m$) and their surfaces were connected. The topographic roughness introduced by the monolithic PS microsphere array apparently altered the morphology of cells after their attachment. The HeLa cells here were less elongated (average aspect ratio: 2.0 $\pm$ 0.4, $n = 10$) than on flat PS films (figure 7(c)). More strikingly, the density of HeLa cells on the hot-embossed PS microsphere array amounted to only $1.2 \times 10^4$ cells cm$^{-2}$. This value was one order of magnitude lower than that on the unmodified PS microsphere array and nearly two orders of magnitude lower than that on flat PS films. The HeLa cells on the hot-embossed substrates were randomly shaped and preferentially spanned across the grooves and voids between the hot-embossed PS microspheres presumably filled with cell culture medium (figure 7(d)). The hierarchical surface topography
of the hot-embossed surfaces might create physical barriers for direct cell-to-cell contact that has been reported to induce proliferation of muscle cells (Wang and Carrier 2011). The formation of focal adhesion contacts to the surface of the hot-embossed surfaces may be inhibited due to reduced protein adsorption or destabilization of focal adhesion. Cell adhesion requires formation of focal contact sites via serum proteins such as fibronectin adsorbed to the surface (Geiger et al 2001). The PS nanorod arrays on the PS microspheres either interfere with protein adsorption or with the formation of focal adhesion sites, which also depends on surface elasticity.

Conclusions

By mimicking the lunate cell layer of the waxy zone in the pitchers of carnivorous plants from the genus *Nepenthes*, we prepared artificial surfaces that show pronounced anti-adhesive behaviour on sticky and compliant counterpart surfaces. These artificial anti-adhesive systems consist of mechanically stable monolithic arrays of PS microspheres with diameters of a few tens of microns, which are tightly connected to underlying PS substrates. The pull-off (adhesion) force $F_{ad}$ of these monolithic PS microsphere arrays on sticky counterpart surfaces is reduced by one order of magnitude as compared to that on smooth PS films because of the essential reduction of the real contact area caused by the surface topography of the PS microsphere arrays. Modelling the dependence of $F_{ad}$ on the microsphere diameter revealed that for compliant counterpart surfaces, anti-adhesive behaviour is to be expected for a microsphere radius range of several tens of microns, from ca. 20 to 80 μm. If the microsphere radius decreases to values below this range, $F_{ad}$ increases and eventually gets larger than the adhesion force $F_{flat}$ on smooth PS films, because sticky counterpart materials can comply to surface topographies with feature sizes in the micrometre range and below. Hence, the real contact area and, consequently, $F_{ad}$ increase. Our results suggest that adhesion can further be reduced if PS microsphere arrays contain microspheres of different diameters. Moreover, topographic modification of the microsphere caps by hot-embossing is possible without loss of the anti-adhesive properties. Thus obtained PS microsphere arrays with microsphere caps bearing PS nanorod arrays used in cell culturing experiments showed cell densities two orders of magnitude lower than smooth PS films. We envision that the preparative methodology presented here is upscaalable so that large-area technical anti-adhesive surfaces showing also anti-fouling properties are accessible.

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