Pollenkitt Wetting Mechanism Enables Species-Specific Tunable Pollen Adhesion

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Supporting Information

ABSTRACT: Plant pollens are microscopic particles exhibiting a remarkable breadth of complex solid surface features. In addition, many pollen grains are coated with a viscous liquid, “pollenkitt”, thought to play important roles in pollen dispersion and adhesion. However, there exist no quantitative studies of the effects of solid surface features or pollenkitt on adhesion of pollen grains, and it remains unclear what role these features play in pollen adhesion and transport. We report AFM adhesion measurements of five pollen species with a series of test surfaces in which each pollen has a unique solid surface morphology and pollenkitt volume. The results indicate that the combination of surface morphology (size and shape of echinate or reticulate features) with the pollenkitt volume provides pollens with a remarkably tunable adhesion to surfaces. With pollenkitt removed, pollen grains had relatively low adhesion strengths that were independent of surface chemistry and scalable with the tip radius of the pollen’s ornamentation features, according to the Hamaker model. With the pollenkitt intact, adhesion was up to 3–6 times higher than the dry grains and exhibited strong substrate dependence. The adhesion enhancing effect of pollenkitt was driven by the formation of pollenkitt capillary bridges and was surprisingly species-dependent, with echinate insect-pollinated species (dandelion and sunflower) showing significantly stronger adhesion and higher substrate dependence than wind-pollinated species (ragweed, poplar, and olive). The combination of high pollenkitt volume and large convex, spiny surface features in echinate entomophilous varieties appears to enhance the spreading area of the liquid pollenkitt relative to varieties of pollen with less pollenkitt volume and less pronounced surface features. Measurements of pollenkitt surface energy indicate that the adhesive strength of capillary bridges is primarily dependent on nonpolar van der Waals interactions, with some contribution from the Lewis basic component of surface energy.

INTRODUCTION

The adhesion of particles to surfaces has been of long-standing interest to scientific investigation of many natural and environmental phenomena, including pollination, bacterial, mold and fungal growth, and distribution of airborne pollutants. In addition, particle–surface adhesion is important in engineering applications including filtration, paints and pigments, coatings, sensors, drug delivery, xerography, and semiconductor processing. It is well-recognized that adhesion is influenced by the shape, size, and surface morphology of the particles. Increasingly there is interest in the utilization of particles with complex surface morphologies, such as spiky shapes, in advanced materials. These applications include directed-assembly of cells, conductive metal–polymer composites, Pickering emulsions, zeolite–polymer composite membranes for separations, and xerography. In fact, the fabrication of such complex particles is beginning to become an experimental reality, including techniques such as flow lithography, templated hydrothermal synthesis, and coating processes. However, most of our knowledge of particle adhesion is based upon experiments and models of simple geometries (sphere, cylinder) without organized fine-scale surface physical features.

In contrast, nature provides remarkable examples of evolutionary-optimized microscale biological particles with structures and/or chemistries tailored for effective adhesion to a variety of surfaces under different dynamic and environmental conditions. Prominent among these are pollens, which possess a range of ornamentalizations consisting of combinations of various morphologies and feature sizes making them model bioparticles for evaluating geometry effects on adhesion. For example, pollen microstructures range from smooth, to reticulate (grooves), to extremely spiny echinate varieties, structures that appear qualitatively to be related to dispersal mechanism and to allergenicity. These structures are provided by a unique chemically and mechanically stable outer exine shell, composed predominantly of sporopollenin, a cross-linked complex polymer consisting of long-chain fatty acids and modest levels of oxygenated aromatic and phenylpropanoid residues. Sporopollenin is one of the most chemically resistant naturally occurring materials and has been found intact in fossils that are

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ca. 500 million years old.\textsuperscript{20,21} This stability suggests that pollen could be used directly or as biotemplates to produce microparticles for encapsulation and dispersal (sensors, drug delivery, and agrochemical delivery), promoting stability and delivery of contents over long time and distance spans. However, the exact mechanisms of pollen adhesion to natural and manmade surfaces have remained elusive. A quantitative description of the dependence of adhesion on microstructure is important to our understanding of plant biology, epidemiology of asthma and allergies, and biomimetic materials design, e.g., by mimicking pollen adhesive mechanisms to enable the design of microscale particles with specific tailored adherence.

Pollen grains are additionally coated with an oily liquid that resides on or within cavities in the exine wall.\textsuperscript{22} This coating material (named pollenkitt by Knoll\textsuperscript{22}) is especially prevalent in entomophilous plants, is a mixture composed of saturated and unsaturated lipids and lesser amounts of carotenoids, flavonoids, proteins, and carbohydrates, and is of great import.

Figure 1. Scanning electron micrographs of AFM colloidal probes of the pollen grains glued to the end of tipless AFM cantilevers. Po, Pp, Pr, Pd, and Psf represent the poplar, olive, ragweed, dandelion, and sunflower pollen grains, respectively; “1” = non-defatted; “2” = CHCl\textsubscript{3}/MeOH washed. The white scale bar represents 10 μm.
importance in pollination ecology. Pollen from land-based plants are largely dispersed by wind or animals (insects, bats, birds). Pollen dispersal is thought to be facilitated by pollenkitt’s ability to keep pollen grains together during transport, to promote adhesion to animals. Pollen germination and fertilization of the ovum are possible because the pollenkitt supports pollen adhesion to the stigma and rehydration. To date, the quantitative dependence of pollen adhesion on solute size and shape has not been reported. Liquid pollenkitt may be expected to contribute to adhesion through capillary mechanisms that differ significantly from those of solvents that contain little or no pollenkitt. The magnitude that each of these potential forces contribute to pollen adhesion has not been determined to date, and reports of quantitative pollenkitt physical properties are limited in the literature. In the present work, five distinct species of pollen were chosen as models, each with a unique surface morphology and pollenkitt volume, and their adhesion with various substrates was determined using AFM. The VDW, Lewis base, and Lewis acid components of pollenkitt surface tension were determined. We demonstrate that adhesion of pollen is affected greatly by the wetting action of pollenkitt, which creates a capillary contribution to adhesion. This contribution is strongly species dependent and is mediated by the geometry of the solid exine, with entomophilous species showing the greatest improvement in adhesion with pollenkitt.

MATERIALS AND METHODS

Sample Preparation. Polystyrene (PS, Mw = 230 000, Sigma-Aldrich), poly(vinyl acetate) (PVAc, Mw = 50 000, Alfa Aesar), and poly(vinyl alcohol) (PVOH, Mw = 89 000–98 000, Sigma-Aldrich) were used as received without further purification. The PS solution was prepared by dissolving 10% by mass in toluene while 5% by mass PVAc and PVOH solutions were prepared in hexafluoroisopropanol (HFIP, TCI America). Polymeric test surfaces were prepared as thin polymer films on piranha-etched (30/70 vol % H2O2/concentrated H2SO4 at 80°C for 2 h) silicon substrates by using a knife-edge coating technique described in detail elsewhere. Films were dried at room temperature for 24 h after coating and then annealed at 80°C under vacuum for 2 h. Film thickness, measured with interferometry, was approximately 1–2 μm, which far exceeds the range of van der Waals interactions (~20 nm) and negates energetic effects of the underlying silicon substrate on the polymer–pollen interactions. The mean (Rg) and root-mean-square (rms) surface roughness of each surface-coating (Table S1) were obtained from topography scans of three random 10 μm × 10 μm areas on each substrate surface using atomic force microscopy (AFM, Veeco Dimension 3100).

Pollen grains from five different species, each with unique ornamentation (as shown in Figure 1), were studied in this work, including olive (Olea europaea), poplar (Populus nigra), ragweed (Ambrosia artemisiifolia), dandelion (Taraxacum officinale), and sunflower (Helianthus annuus). All the native non-defatted pollen grains were purchased from Greer Laboratories (Lenoir, NC) and were used as received without further purification. For comparison of the effects of variations in particle surface topography, pollens representing two morphologies were explored: (1) a nanoscale reticulate (grooved) structure (olive and poplar pollens) and (2) an echinate (spiked) microstructure (ragweed, dandelion, and sunflower). Olive, poplar, and ragweed are widespread anemophilous plants, in which pollen grains are spread primarily by wind and are coated by a thin layer of pollenkitt. In contrast, the pollen grains of dandelion and sunflower (entomophilous plants) are primarily spread by insects and are covered by a relatively thick layer of pollenkitt. In addition, as a control, we obtained non-defatted pollen grains and applied another solvent washing procedure to remove the pollenkitt. Non-defatted pollen samples were washed by us in a mixture of chloroform and methanol (3:1), a solvent for external pollenkitt, but a nonsolvent for the sporopollenin exine for 24 h before being deposited on filter paper (P5, Fisher Scientific, Pittsburgh, PA) supported on a stainless steel 47 mm screen (Kontes Glass, Vineland, NJ).

The separation and collection of pure pollenkitt were accomplished by a chloroform/methanol (3:1) mixture solvent extract, as described in detail elsewhere. Approximately 1 g of each non-defatted pollen was suspended in 10 mL of chloroform/methanol (3:1) solution for 30 s and was then quickly filtered and washed by 5 mL of chloroform/methanol (3:1). This process was repeated three times. Both washed pollen and the filtration solution were collected and dried under vacuum for 1 day. The percentage mass loss of each pollen species was calculated by the measured masses of dried washed pollen and viscous liquid pollenkitt obtained from the filtration after chloroform/methanol removal.

Force Measurements. Adhesion force was measured using atomic force microscopy (AFM, Veeco Dimension 3100). Tipless rectangular cantilevers with nominal spring constants of 0.1–0.6 N/m for cleaned pollen and 1.2–6.4 N/m for non-defatted pollen (Applied NanoStructures, Inc., Santa Clara, CA) were used. Single pollen grains were glued to the tipless cantilevers with a small amount of epoxy resin using a procedure described in detail elsewhere. The actual spring constants for the cantilevers with the attached pollen grains were determined directly by the methods of Burnham and Hutter et al. A series of 20 force–distance curves were measured for each combination of pollen tip–polymer surface, taken on three separate substrate surfaces within three randomly chosen 10 μm × 10 μm areas on each substrate under normal air condition (20°C, 80% humidity 30%). Three separate pollen AFM tips were used for each species. The applied load during force measurements was 2.5 nN.

Contact Angle Measurements. Contact angles were measured at 20°C on the different substrates using a video contact angle system (AST products 2500XE, Billerica, MA). Three standard testing liquids were chosen—two polar liquids (DI water and glycerol, Alfa Aesar >99%) and one nonpolar (diiodomethane, Alfa Aesar, >99%)—to enable the calculation of surface tension components of substrates and pollenkitt from contact angle measurements. Nine 1 μL drops of each liquid were dispensed onto different regions of the sample surfaces. Both the right and left angles between the sample surface and the tangent line to each droplet were measured for each droplet. Surface tension components corresponding to van der Waals (VDW), Lewis acid, and Lewis basic interactions were calculated from measured contact angle data by using van Oss and Good’s van der Waals acid–base theory. According to this theory, the surface energy is given by

\[
\gamma_s = \gamma_{sv} + 2\left(\gamma_{sv}^{\text{pol}}\gamma_{sv}^{\text{w}}\right)^{1/2}
\]

where \(\gamma_s\) is the total surface tension, \(\gamma_{sv}^{\text{pol}}\) is the van der Waals component, \(\gamma_{sv}^{\text{w}}\) is the acid (electron acceptor) component, and \(\gamma_{sv}^{\text{pol}}\) is the base (electron donor) component. The relation between surface energy components, liquid components, and contact angle (θ) is given as

\[
\gamma_L(1 + \cos \theta) = 2\left(\gamma_{sv}^{\text{pol}}\gamma_{sv}^{\text{w}}\right)^{1/2} + 2\left(\gamma_{sv}^{\text{pol}}\gamma_{sv}^{\text{w}}\right)^{1/2} + 2\left(\gamma_{sv}^{\text{pol}}\gamma_{sv}^{\text{w}}\right)^{1/2}
\]

where \(\gamma_L\) represents the surface energy of the testing liquids. Given knowledge of the components of \(\gamma_L\) for three standard liquids, the \(\gamma_{sv}^{\text{pol}}\) components may be determined by regression. The three standard testing liquids for determining surface tension components from contact angles data were selected as deionized water and glycerol (bipolar) and diiodomethane (apolar).

The surface tensions of the test liquids, taken from the literature, are the following: water, \(\gamma = 35.5\) mJ/m²; glycerol, \(\gamma = 21.8\) mJ/m²; dioxane, \(\gamma = 3.92\) mJ/m²; and diiodomethane, \(\gamma = 57.4\) mJ/m². Scanning Electron Microscopy (SEM). The pollen AFM probes were characterized by scanning electron microscopy (SEM) (LEO 1510). After all force measurements were finished, an accelerating potential of 10.0 kV. Probe tips were sputtered with gold and then mounted on metal studs using carbon tapes. We
developed a procedure for estimating the contact area of pollenkitt, based on a technique described in detail elsewhere. Pollen grains were gently deposited onto a clean silicon substrate and then kept for 6 h to allow time for pollenkitt to spread on the substrate. Then the sample was placed perpendicular to a sputtering Au sample holder and was coated with ~50 nm Au while rotating the sample through 360°. Finally, the pollen grains were gently blown off the silicon substrate by N₂ gas. The wetting of pollenkitt acted as a mask blocking the direct adsorption of gold, allowing the noncoated areas to be identified via SEM imaging.

Figure 2. Scanning electron micrograph images showing details of pollen grain surfaces with and without pollenkitt: (A) olive, (B) poplar, (C) ragweed, (D) dandelion, and (E) sunflower. Label “1” indicates non-defatted (used as received), and “2” indicates that pollens were prewashed by CHCl₃/MeOH (3:1) to remove pollenkitt. The black scale bar is 1 μm.
RESULTS AND DISCUSSION

Five different substrates were used to examine the effect of surface chemistry on the adhesion forces of each pollen grain. PS was chosen as a model apolar hydrocarbon substrate, PVOH and PVAc were chosen to represent organic materials with protic and basic characteristics, respectively. The polymer films used herein exhibited a range of water contact angles ($\theta_w$) of 46°, 61°, and 101° for PVOH, PVAc, and PS, respectively. Piranha-etched silicon (Si) is a model inorganic, hydrophilic, and protic surface with $\theta_w \approx 14^\circ$. Piranha-treated Si is known to bear an oxide layer (2–10 nm) consisting of both oxygenated (SiO$_x$) and hydroxylated (SiOH) silicon. To provide a model metallic substrate as well to explore the effect of masking silanol groups, a thin (~8 nm) gold coating was applied to the Si wafer ($\theta_w \approx 84^\circ$) (Au–Si).$^{36}$ One potential concern is variation in surface roughness, which might complicate interpretation of differences in chemistry between the substrates. The measured root-mean-square surface roughnesses of the polymer and silicon surfaces fall within a range of 0.3–2.7 nm (Table S1). Since the variation in roughness is orders of magnitude smaller than the scale of pollen surface microtopography (Figures 1 and 2), the effect of these small differences in roughness is likely to be negligible relative to other effects observed herein.

The cantilevers with pollen grains attached are shown in Figure 1, taken after all force measurements were completed. Figure 2 shows high-magnification SEM images for surface ornamentations of each non-defatted (with native pollenkitt) and cleaned pollen (without pollenkitt). It is noteworthy that no damage to pollen grains was observed under typical applied load forces (2.5 nN, approximately the weight of a single pollen). As reported previously, the pollen grains are not subjected to measurable deformation and do not exhibit compliance under the small loading forces used here.$^{27}$ Figures 1 and 2 also show that non-defatted pollens are coated with a thin liquid, which can be seen to fill cavities and pores in the exine as well as form droplets on the ends of some spines. Some of these features are illustrated for example by the arrow marks in Figure 1 Pd1 and Psf1. The pollenkitt contents of the pollen samples ranged from 8 wt % (olive) to 60 wt % (dandelion). Both olive and poplar pollens exhibit a reticulate sporopollenin structure that is characterized by nanoscale raised “bumps” that serve as the outermost protuberances. An example is labeled with an arrow in Figure 2A1. The three other pollens had echinate (spiked) microstructures (ragweed, dandelion, sunflower) and were chosen from the same family (Asteraceae) to minimize deviations in sporopollenin and pollenkitt composition. Table 1 shows the characteristics of each pollen sample, including morphology, size (length and radius determined from SEM) of morphological features (spines or grooves), and amount of pollenkitt recovered by solvent extraction (as mass % of original sample). The radii of the pollen morphological features were not changed significantly after the solvent extraction with chloroform/methanol (3:1).

Figure 3 compares typical force–distance curves for non-defatted (Figure 3A) and cleaned (Figure 3B) dandelion pollen on Si surfaces, which are typical for other substrates investigated herein. As shown in Figure 3A, during the approach for the non-defatted pollen, a large snap-on force of ~125 nN occurs at a relatively large distance of ~30 nm above the surface. “Snap-on” refers to the initial jump to contact, which is caused by attractive forces between the pollen tip and substrate.

In contrast, cleaned pollens did not show any evidence of such large, long-ranged jump to contact forces (Figure 3B), but rather showed short-ranged (a few nanometers) attractions of only several nanonewtons. The magnitude of the snap-on did not vary significantly for different surfaces. During retraction of non-defatted pollens, a large force minimum is reached (~300 nN in Figure 3A), followed by a protracted decay with a shape that is indicative of a liquid bridge being pulled to rupture at a
long-range of separation (~100 nm). In contrast, Figure 3B shows a sudden pull-off event in which the two solids separate suddenly at the maximum adhesion force, which is clearly indicative of a solid—solid short-ranged adhesion. In the case of cleaned pollens, initial contact with flat surfaces is made at the outermost surface protuberances, which are spines (in the case of echinate species) or bumps (in the case of reticulate species).

The clean pollen force—distance data indicate that only one external feature (spine or bump) is making contact in each of the force—distance measurements used herein. The conclusion is made from the AFM force—distance curves (Figure 3B is one example). These curves just displayed a single contact event. For some AFM pollen probes, the pollen is oriented such that more than one spine contacts at one time, and this is evident in the force—distance data. We have not utilized such “multiple contact” probes in this study. The features of Figure 3A, including long-ranged, large-magnitude snap-on and protracted pull-off over a large distance, indicate that non-defatted pollens have adhesion forces that are mediated by formation of a liquid bridge,17 features that are absent for cleaned pollens. In addition, residual pollenkitt was detectable on some substrates after the AFM measurements for non-defatted pollens, but not for cleaned pollens.

An important question is whether or not the liquid contribution comes from liquid pollenkitt or is due in part to condensed water. As in a previous study of ragweed pollen,27 and in the Supporting Information Data to this paper (Figure S1), force—distance data were independent of humidity over a range of 20%–65%, even on the most hydrophilic surface (Si), and features consistent with water condensation were absent in cleaned pollen force—distance data. While we cannot rule out liquid water as a contributor to the non-defatted pollen, the lack of observed capillary forces for clean pollen suggest a negligible contribution of capillary forces from condensed water.

In the results that follow, the pull-off adhesive force is taken as the difference between the minimum force and the long-distance baseline. Figure 4 shows the average pull-off adhesion forces of non-defatted (A) and washed (B) pollen grains with different polymer substrates. Two characteristics distinguish the non-defatted from the washed pollens: adhesion magnitude and dependence on substrate chemistry. The adhesion forces of non-defatted grains are a strong function of the identity of the outermost surface protuberances, which are spines (in the case of echinate species) or bumps (in the case of reticulate species). Non-defatted grains are indicative of a solid short-ranged adhesion. In the case of cleaned pollens, initial contact with flat surfaces is made at the outermost surface protuberances, which are spines (in the case of echinate species) or bumps (in the case of reticulate species).

Mechanism of Clean-Pollen Adhesion. Pollen surface morphology and feature size is expected to affect the adhesion force by altering the contact area between the surface features and other surfaces. If adhesion is governed by VDW forces, then the dependence of adhesion on the size of pollen ornamentations should be approximated well by the Hamaker model:

$$F_{\text{Hamaker}} = \frac{AR}{{6d}^{2}}$$

where $A$ is the material-dependent nonretarded Hamaker constant, $R$ is radius of contact (estimated as the pollen ornamentation radius from Table 1), and $d$ is the cutoff separation distance, taken to be 0.165 nm. According to eq 3, adhesion force is expected to scale proportionately with the exine spine or groove radii. While contact radius at the exact moment of measurement is not directly available from AFM force—distance measurements, the contact radius is estimated as the radius of outermost protuberances determined from SEM and listed in Table 1. Figure 5 shows a plot of $F$ versus $R$ for all five species on silica, indicating an excellent linear fit with an $A$ value of $8.5 \times 10^{-20}$ J. These values are larger than those of organic small molecules, $A \approx (4-6) \times 10^{-20}$ J, e.g., toluene $= 377$ $5.4 \times 10^{-20}$ J and propylene oxide $= 4.0 \times 10^{-20}$ J, and are
Mechanism of Native Pollenkitt-Coated Pollen Adhesion. The SEM image in Figure 6a of dandelion pollen on a Si surface after 6 h, indicating formation of a liquid meniscus. (B) Optical microscopy image showing residual dandelion pollen on Si surface after 6 h, indicating formation of a liquid meniscus.

Figure 6. (A) Scanning electron micrograph of non-defatted dandelion pollen on Si surface after 6 h, indicating formation of a liquid meniscus. (B) Optical microscopy image showing residual dandelion pollen on Si surface after 6 h, indicating formation of a liquid meniscus.

Surface area after 6 h of contact provides clear and unambiguous evidence that a liquid bridge forms. However, a pollenkitt-mediated adhesion could also be due to differences in the composition and hence surface and interfacial tensions at the pollenkitt—solid—air interfaces. Likewise, the species variability in pollenkitt-mediated adhesion could also be due to differences in the wetted area that might result from the large variation in pollenkitt volume or morphological features (Table 1) or potential variations in pollenkitt viscosity. These features could affect flow rate of pollenkitt from the grain during the time scale of the AFM measurements.
To further explore the species and substrate dependence and role of $F_{VDW}$, $F_{tension}$, and $F_{Laplace}$, we determined the pollenkitt surface tension ($\gamma_L$) for dandelion and sunflower pollen species. Pollenkitt from non-defatted dandelion and sunflower pollens through the washing procedure adopted above and films of pollenkitt were prepared by solvent casting. Pollenkitt, although a viscous liquid, spreads and forms a smooth film when cast in this manner. The contact angles of three testing liquids (water, diiodomethane, and glycerol) were then measured on top of the pollenkitt films. For comparison, contact angles were determined also for the test substrates as well, and the results are given in Table 2. Table 3 shows the calculated surface tension components of the selected substrates using eqs 1 and 2, using the van Oss and Good’s method as described in the Materials and Methods section. The results for the solid substrates are consistent with the previous literature values: PVOH ($\gamma^W = 42.0, \gamma^+ = 0.75, \gamma^- = 29, \gamma^\ominus = 51.3$), PVAc ($\gamma^W = 42.6, \gamma^+ = 0.041, \gamma^- = 22.3, \gamma^\ominus = 44.5$), PS ($\gamma^W = 42.0, \gamma^+ = 0, \gamma^- = 1.1, \gamma^\ominus = 42.7$). The pollenkitt has a $\gamma^W$ component that is similar to the organic polymers in Table 3. Its $\gamma^+$ is very small and can be considered negligible. However, pollenkitt from both dandelion and sunflower show a

$$\gamma = \gamma^W + \gamma^+ + \gamma^- + \gamma^\ominus - \gamma_L - \gamma_S$$

(6)
Combining eqs 5, 6, 7, and 8, one can show that the cos(θ2) term in $F_{\text{Laplace}}$ produces a proportionality between adhesion force and the term $(γ_{SL})^{1/2}$, from which dependence on the substrate—liquid interaction appears. From the Hamaker constant measured above, we estimate $γ_{\text{exine}} = A/24πd^2 ≈ 43 \text{ mJ/m}^2$, leading to an estimate of $θ_1 \sim 20°$ from $\cos(θ_1) = (γ_{\text{exine}} − γ_{\text{exine,L}})/γ_L$, where $γ_{\text{exine,L}}$ is estimated from eq 7 with $Φ = 1$ (apolar assumption). For a circular meniscus to have negative meniscus curvature (wetting fluids), $ψ$ must be less than $90°$, and we confine our discussion to $0 < ψ < 90°$.

Values of $F_{\text{VDW}}$, $F_{\text{tension}}$, and $F_{\text{Laplace}}$ calculated using the parameters estimated above for dandelion-Si and sunflower-Si are presented in Figure 9. $F_{\text{Laplace}}$ is similar to $F_{\text{VDW}}$ in magnitude but is larger than $F_{\text{tension}}$. The influence of Laplace pressure becomes smaller, and the surface tension contribution becomes larger, as the filling angle increases beyond $20°$. The maximum sunflower adhesion force (Figure 9A) is predicted to be $≈ 135 \text{ nN (ψ ≈ 20°)}$, in good agreement with the measured value for sunflower-Si ($≈ 140 \text{ nN}$). The pollenkitt film thickness at the contact line is estimated by $h = a + R(1 − \cos ψ) = 7.5 \text{ nm}$.

In contrast to the good agreement between the model and experiment obtained for sunflower pollen, for dandelion we predict a maximum adhesion force that is only $≈ 100 \text{ nN}$ (Figure 9B), just one-third of the measured value of $≈ 300 \text{ nN}$.

### Table 2. Contact Angles (deg) of Substrate Surfaces and Pollenkitt Films with Three Testing Liquids

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Water</th>
<th>Glycerol</th>
<th>Diiodomethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>13.7 ± 1.3</td>
<td>15.2 ± 0.9</td>
<td>34.8 ± 2.0</td>
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<tr>
<td>PVOH</td>
<td>46.2 ± 1.4</td>
<td>44.0 ± 1.3</td>
<td>40.4 ± 1.4</td>
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<tr>
<td>PVAc</td>
<td>60.7 ± 2.9</td>
<td>70.7 ± 3.0</td>
<td>37.8 ± 3.0</td>
</tr>
<tr>
<td>PS</td>
<td>101.1 ± 3.0</td>
<td>84.2 ± 2.0</td>
<td>33.6 ± 2.0</td>
</tr>
<tr>
<td>Au–Si</td>
<td>84.0 ± 4.0</td>
<td>66.8 ± 2.0</td>
<td>42.8 ± 1.5</td>
</tr>
<tr>
<td>Pd–pollenkitt</td>
<td>63.0 ± 2.0</td>
<td>60.0 ± 1.8</td>
<td>29.0 ± 1.6</td>
</tr>
<tr>
<td>Pd–pollenkitt</td>
<td>54.0 ± 2.0</td>
<td>59.5 ± 1.5</td>
<td>31.4 ± 1.8</td>
</tr>
</tbody>
</table>

### Table 3. Surface Tension Components (mJ/m²) of Substrate and Pollenkitt Surfaces

<table>
<thead>
<tr>
<th>Substrate</th>
<th>γ'</th>
<th>γ''</th>
<th>γ''''</th>
<th>γ''''''</th>
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</thead>
<tbody>
<tr>
<td>Si</td>
<td>2.46 ± 0.04</td>
<td>44.1 ± 1.1</td>
<td>42.1 ± 1.0</td>
<td>63.0 ± 1.2</td>
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<tr>
<td>PVOH</td>
<td>1.06 ± 0.05</td>
<td>28.8 ± 1.1</td>
<td>39.4 ± 1.1</td>
<td>50.4 ± 1.3</td>
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<td>PVAc</td>
<td>0.56 ± 0.09</td>
<td>16.7 ± 1.4</td>
<td>40.7 ± 1.4</td>
<td>46.8 ± 2.2</td>
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<tr>
<td>PS</td>
<td>0.19 ± 0.02</td>
<td>0.42 ± 0.12</td>
<td>42.7 ± 1.3</td>
<td>43.2 ± 1.8</td>
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<tr>
<td>Au–Si</td>
<td>0.54 ± 0.03</td>
<td>2.29 ± 0.50</td>
<td>38.2 ± 1.2</td>
<td>40.4 ± 1.5</td>
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<tr>
<td>Pd–pollenkitt</td>
<td>0.01 ± 0.01</td>
<td>17.7 ± 1.1</td>
<td>44.6 ± 1.3</td>
<td>45.4 ± 1.4</td>
</tr>
<tr>
<td>Pd–pollenkitt</td>
<td>0.02 ± 0.01</td>
<td>26.9 ± 1.2</td>
<td>43.6 ± 1.2</td>
<td>45.2 ± 1.5</td>
</tr>
</tbody>
</table>

Using the measured pollenkitt and substrate surface tensions in the model in Figure 8, we attempted to address two questions: (1) What are the relative magnitudes of $F_{\text{tension}}$ and $F_{\text{Laplace}}$? (2) How can we explain the substrate dependence of the measured pull-off forces for non-defatted pollen? This model predicts a relatively simple dependence of the Laplace capillary force on the substrate and pollenkitt surface tensions, according to the sole cos(θ₂) term in eq 6. Namely, the Young equation, $γ_1 \cos(θ_1) = γ_S - γ_{SL}$, can be substituted into eq 6 if values of $γ_{SL}$ are known. The equation of state of Girifalco and Good can be used to first order here as an expression relating $γ_{SL}$ to $γ_S$ and $γ_1$, and is shown in eq 7.

$$γ_{SL} = γ_S + γ_1 - 2Φ \sqrt{γ_S γ_L}$$  \hspace{1cm} (7)

Combined with the semiempirical parameter $Φ = −γ_S α + β$, where $α$ and $β$ are system-specific constants, this expression can be solved to yield

$$γ_{SL} = γ_S + γ_1 - 2β \frac{γ_S γ_L}{1 - 2α \sqrt{γ_S γ_L}}$$  \hspace{1cm} (8)

In Figure 9, the predicted values of $F_{\text{Laplace}}$, $F_{\text{tension}}$, $F_{\text{VDW}}$, and $F_{\text{total}}$ are presented versus filling angle ($ψ$), for four different pollen—pollenkitt contact angles ($θ_1$). Other parameters utilized for calculations are taken from data measured for the dandelion pollen and Si surfaces: $R = 120 \text{ nm (A, sunflower)}$ and $90 \text{ nm (B, dandelion)}$, $γ_S = 63 \text{ mJ/m}^2$, $γ_L = 45.3 \text{ mJ/m}^2$. For estimation of $γ_{SL}$ nominal parameters for the Girifalco—Good equation of state were taken as $α = 0.008$ and $β = 1$, which are similar to those determined from experimentally determined $γ_{SL}$ values for organic liquids.
The equation of state used to estimate $\gamma_{SL}$ is a potential source of error. For example, $\gamma_{SL}$ is sensitive to the $\alpha$ and $\beta$ values utilized for eq 8, although values of $\beta = 1$ and $\alpha = 0.008$ used here are consistent with literature values obtained from fits to experimental $\gamma_{SL}$ measurements for organic liquids.\textsuperscript{47} Adjusting $\alpha$ to lower values would monotonically raise $F_{\text{Laplace}}$ but this would only lead to a maximum value of $F_{\text{Laplace}} = 60$ nN for $\alpha = 0$, at $\theta_i = 0$ and $\psi = 0$.

The most significant impediment to obtaining quantitative agreement is probably that the model assumes a perfectly hemispherical contact of radius $R_i$ where $R_i$ was taken from an average of features observed in SEM images. Figures 6 and 7 indicated that draining is not complete during the time scale of the AFM measurement, and the effective contact line likely resides further up the conical-like spine than the simple model above depicts. The effective $R$ value at the pollenkitt–pollen contact line is thus likely to be larger than the end tip radius. Any increase in the effective $R$ would have a linear effect on both $F_{\text{tension}}$ and $F_{\text{Laplace}}$. By adjusting the $R$ value used to calculate $F_{\text{tension}}$ and $F_{\text{Laplace}}$ for dandelion, one can arrive at a total adhesive force of 300 nN, equivalent to the AFM-determined value, at $R = 420$ nm. On the basis of the size of the spikes in the SEM images in Figures 1 and 2, this is an entirely reasonable value and is consistent with the measured amounts of pollenkitt on each species; e.g., the dandelion has a significantly larger loading than any other species examined (Table 1).

While the model above does not allow a quantitatively exact prediction without arbitrarily adjusting the parameters, it does provide a motivation for a semiempirical approach to correlating the measured adhesion forces with the independently measured $\gamma_S$ and $\gamma_L$ values: $F_{ad} = a(\gamma_S \gamma_L)^{1/2} + b(\gamma_S \gamma_L)^{1/2}$, where $F_{ad}$ is the experimentally determined adhesion force and $a$ and $b$ are coefficients scaling the VDW and acidic–basic contributions, respectively. The acid contribution from pollenkitt ($\gamma_L^-\gamma_L$) was not included because its magnitude is so small (Table 3). After normalizing the $F_{ad}$ $\gamma_S$ and $\gamma_L$ values to a range of 0–1, by using their minimum and maximum values, the fitted correlation results in a plane, shown in Figure 10, with $r^2 \sim 0.8$ and 0.9 for dandelion and sunflower, respectively. The following fitted parameters resulted: $a = 1.04$, $b = 0.12$ (dandelion) and $a = 1.02$, $b = 0.11$ (sunflower). Since $a \gg b$, we can conclude that the VDW component of surface tension is the main molecular contribution to the pollenkitt-mediated adhesion to the substrates.

To examine the wetting properties of pollenkitt directly, the contact angles of dandelion and sunflower pollenkitt on each substrate are reported in Table 4. The contact angles of each pollenkitt on substrates are increasing with changing the substrate surface wettabilities from Si (16°) to PS (49°). The pollenkitt is the most wetting on the Si surface, also indicating potential acid–base interaction between them. However, although PS exhibits the largest contact angle, the measured adhesion forces of non-defatted dandelion and sunflower pollen on PS substrates (Figure 4A) are higher those on PVAc and PVOH. One possible reason is that pollenkitt is too viscous to be able to spread to an equilibrium contact angle either during contact angle measurement or during the contact time of the AFM force measurement. In addition, the dynamics of pollenkitt flow may vary considerably between substrates. Studies of pollenkitt flow dynamics are beyond the scope of this paper and will be the subject of future studies.

The species dependence of effect of pollenkitt on adhesion observed above may be rationalized based on the size of solid features and volume of pollenkitt. The difference between the magnitudes of adhesion forces of various pollen species (dandelion > sunflower > ragweed, olive, and poplar) may be related to the thickness or volume of pollenkitt on the pollen surface. Figure 1 and Table 1 indicate that dandelion contains the largest volume of pollenkitt and that the pollenkitt is distributed in relatively large pools in between the exine spines. Poplar pollen carries the least amount of pollenkitt. A larger pollenkitt volume is associated with higher contact areas on

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**Table 4. Contact Angles (deg) of Substrate Surfaces with Pollenkitt**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pd-pollenkitt</th>
<th>Psf-pollenkitt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>16.2 ± 1.5</td>
<td>16.8 ± 1.0</td>
</tr>
<tr>
<td>PVOH</td>
<td>26.2 ± 1.2</td>
<td>25.8 ± 1.3</td>
</tr>
<tr>
<td>PVAc</td>
<td>23.4 ± 2.0</td>
<td>23.1 ± 2.0</td>
</tr>
<tr>
<td>PS</td>
<td>42.5 ± 3.0</td>
<td>41.1 ± 2.0</td>
</tr>
<tr>
<td>Au–Si</td>
<td>49.0 ± 4.0</td>
<td>48.8 ± 2.0</td>
</tr>
</tbody>
</table>

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**Figure 10.** Fitting of a planar model of eq 8 to force data for non-defatted dandelion (top) and sunflower (bottom) pollen.
substrates, as was observed in Figure 7, and with higher adhesion forces. The pollens with the highest adhesion magnitudes when pollenkitt is present are dandelion and sunflower, which among those we studied here, are the only two primarily entomophilous species. These also have the largest pollenkitt volumes and the most prominent (longest) spine features. Our results are consistent with previous results that concluded that the variable stickiness of entomophilous pollens depends on the distribution and consistency of the pollenkitt on its surface and is related to the fine structure (size and shape) of the exine.48 The echinate varieties of pollen appear to make the most advantage of pollenkitt in promoting adhesion to surfaces. While a detailed review of pollenation ecology is beyond the scope of this study, we suggest that the enhanced pollenkitt effect in echinate pollens may have been a factor leading to the selection of these shapes in animal-transported pollination, which requires pollen to remain adhered under relatively high shear forces (due to wind of animal motion).

CONCLUSION

We report AFM adhesion measurements of five pollen species with a series of test surfaces representing unique combinations of solid surface morphology and pollenkitt volume. The results indicate that the combination of surface morphology (size and shape of echinate or reticate features) with the pollenkitt volume provides pollens with two adhesion mechanisms, resulting in the ability to tune adhesion over a wide range of magnitude (~30–300 nN). Dry pollen grains (with pollenkitt removed) had relatively low adhesion strengths that were independent of surface chemistry and scalable with the tip radius of the pollen’s ornamentation features, according to the Hamaker model. On the other hand, with the pollenkitt intact, adhesion was up to 3–6 times higher than the dry grains and exhibited strong substrate dependence. The adhesion enhancing effect of pollenkitt was driven by the formation of capillary bridges and was surprisingly species-dependent, with echinate insect-pollinated species (dandelion and sunflower) showing significantly stronger adhesion and higher substrate dependance than either echinate (ragweed) or reticate (poplar and olive) wind-pollinated varieties. The combination of high pollenkitt volume and convex, spiny surface topography in echinate entomophilous varieties appears to enhance the spreading area of the liquid pollenkitt relative to varieties of pollen with less pollenkitt volume and less pronounced surface features. Measurements of pollenkitt surface energy indicate that the adhesive strength of capillary bridges is primarily dependent on nonpolar van der Waals (VDW) interactions, with some contribution from the Lewis basic component of surface energy. We propose that the combination of wet and dry mechanisms observed for natural pollen adhesion may serve as a useful model for the design of novel synthetic microparticle adhesion strategies.

ASSOCIATED CONTENT

Supporting Information
Table S1 and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES
