

Characterization of Ragweed Pollen Adhesion to Polyamides and Polystyrene Using Atomic Force Microscopy

BENG JOO REGINALD THIO,
JUNG-HYUN LEE, AND
J. CARSON MEREDITH*

School of Chemical and Biomolecular Engineering, Georgia
Institute of Technology, 311 Ferst Drive NW,
Atlanta, Georgia 30332-0100

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Pollen is a leading contributor to asthma and allergies, yet pollen adhesion to common indoor surfaces is not well understood. We report the adhesive behavior of short ragweed (*A. artemisiifolia*) pollen grains with Nylon 6 (N6) and Nylon 6,6 (N66), chosen due to their use in synthetic carpet, and three control surfaces: polyamide 12 (PA12), polystyrene (PS), and silicon. The forces were measured by using atomic force microscopy (AFM) under controlled humidity, where single pollen grains were attached to tipless AFM cantilevers. Pollen grains had an average adhesion of 10 ± 3 nN with the surfaces, independent of surface type or relative humidity from 20% to 60%. van der Waals forces are the primary molecular attraction driving pollen adhesion to these surfaces. The results also indicate that ragweed pollen contacts the polymer surface via its exine surface spikes, and the total adhesion force scales with the number of contacts. The pollen surface spikes are strong, resisting fracture and compliance up to a load of 0.5 GPa.

Introduction

Indoor air quality is an important factor in respiratory health, especially asthma, which affects 20 million people at a cost exceeding \$10 billion annually in the U.S. alone (1, 2). Floor coverings and upholstery, with their large surface areas per unit mass, are thought to have a dramatic effect on the indoor accumulation and distribution of biological allergenic particulates (mold, dust mites, pet dander, and pollen). Bioparticle adhesion to fibers is thought to hinder their release back into the air, which may be beneficial to indoor air quality. Airflow models have supported the idea that carpeting can act as a filter in this manner (3–5). However there is also disagreement about this conclusion (6), and there is a significant lack of basic understanding about bioparticle adhesion to materials used in carpet. Included in these unknowns is the adhesive force magnitude and mechanism for the most prevalent bioparticles. Other factors include the effects of humidity, particle origin (species), shape (smooth versus textured, fragments versus whole particles), carpet material (N6, N66, polyester, or polypropylene), cleaning methods, and the presence of soil- and stain-resistant

treatments. Previously we reported measurements of the adhesion of *E. coli* to a series of polymers utilized in carpeting (7). However, while *E. coli* is a good model organism, it does not represent a significant problem with regard to indoor air quality.

Proteins from flowering-plant pollen grains represent the most important source of natural allergens (8, 9), yet very little is known about pollen adhesion to synthetic polymers. Significant allergenic pollens include ragweed (*Ambrosia*), grasses (*Poacea*, *Phleum*), and birch (*Betula*) (10). While exposure to such natural allergens is unavoidable, it is desirable to develop protective and preventive measures to minimize their negative impact on the indoor air environment. Measurement of adhesion forces between pollen grains and polymer surfaces is an important aspect in understanding the phenomena, identifying problems, and designing solutions.

Since the invention of atomic and lateral force microscopy (AFM and LFM) in the 1980s (11), researchers have used both techniques to characterize nanometer-scale surfaces and adhesive interactions in biological systems (12, 13). AFM provides quantitative, real-time, spatially resolved information on the interactions between the scanning probe and the sample surface. In this study, the “colloidal” AFM probe method developed by Ducker et al. (14) was adopted to measure the adhesion forces of ragweed pollen with polymers widely used in carpet: Nylon 6 and Nylon 6,6. Polystyrene, polyamide-12, and silicon were included as controls for comparison. We used a series of control experiments, including varying humidity and investigation of gold-coated surfaces, to determine the adhesion mechanism.

Experimental Section: Materials and Methods

Sample Preparation. Thin polymer films were prepared on Piranha-etched silicon wafers. These included polystyrene, (PS, average molecular mass = 100,000, Avocado Research Chemicals, Lancashire, England; and average molecular mass = 2330, 3680, and 114,200, Aldrich Chemical Co., Inc., Milwaukee, WI), Nylon 6 (N6, average molecular mass = 10,000, Aldrich), Nylon 66 (N66, average molecular mass = 22,000, Aldrich), and polyamide 12 (PA12, average molecular mass = 40,000, Arkema Group, Philadelphia, PA). The PS solution was prepared by dissolving 10% by mass in toluene while 1% by mass N6, N66, and PA12 solutions were prepared in hexafluoroisopropanol (HFIP, TCI America, Portland, OR). Polymer films were made by spin-casting the solutions onto silicon wafers at 1000 rpm for 30 s, followed by annealing in a vacuum oven at 100 °C for 2 h. Film thickness, measured with interferometry, was approximately 1–2 μ m, which far exceeds the range of van der Waals interactions (~50 nm) and negates any effects of the underlying silicon substrate on the polymer–pollen interactions. Short ragweed (*A. artemisiifolia*) nondefatted pollen grains were purchased from Greer Laboratories (Lenoir, NC) and stored at 4 °C prior to use. The pollen grains were first suspended in ethanol before being deposited on filter paper (P5, Fisher Scientific, Pittsburgh, PA) supported on a stainless steel 47 mm screen (Kontes Glass, Vineland, NJ). Ethanol, a nonsolvent for sporopollenin (15), was used to wash the grains on the filter paper prior to their attachment to the AFM cantilevers.

Contact Angle Measurement. Static water contact angles were measured on the different polymer films using a video contact angle 2500XE system (AST products, Billerica, MA). A 1 μ L drop was dispensed onto the sample surfaces. Both

* Corresponding author phone: 404.385.2151; e-mail: Carson.meredith@chbe.gatech.edu.

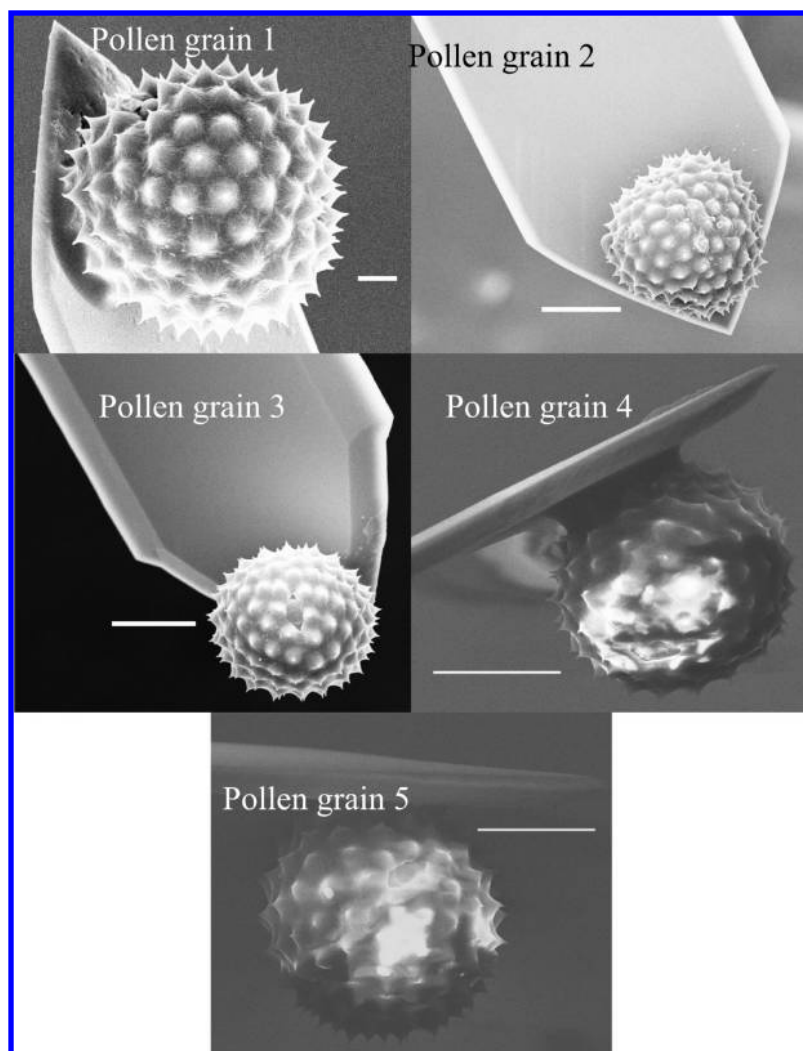


FIGURE 1. Scanning electron micrographs of ragweed pollen grains glued to the end of tipless AFM cantilevers. The white scale bar represents 3 μm for pollen grain 1 and 10 μm for grains 2–5. Pollen grains 4 and 5 were not sputtered with gold prior to the SEM imaging. Note the absence of any visible contamination on all pollen surfaces after repeated force measurements. The SEM images for grains 4 and 5 do not have high contrast due to the lack of a gold coating (to avoid “contaminating” the tips with gold).

the right and left angles between the sample surface and the tangent line to the droplet were measured.

Attachment of Pollen Grains to AFM Probes. Three sets of AFM measurements with the polymer surfaces were conducted on a Pico Plus atomic force microscope (Molecular Imaging Inc., Tempe, AZ). Tipless rectangular cantilevers with nominal spring constants of 0.02–5 N/m (Applied NanoStructures, Inc., Santa Clara, CA) were used. Single pollen grains were glued to the AFM cantilevers with a small amount of epoxy resin using a procedure described in detail elsewhere (14). The cantilevers with pollen grain attached are shown in Figure 1. Five tipless cantilevers, each with a pollen grain attached to the free end, were used for the adhesion force measurements. The actual spring constants for the cantilevers with the attached pollen grains 1 through 5 were determined separately to be 0.007, 0.009, 0.576, 0.29, and 0.65 N/m, respectively, using the methods of Burnham (16) and Hutter et al. (17). A series of 25 force–distance curves were measured for each combination of pollen tip–polymer surface, taken on three separate Si wafers within three randomly chosen 10 $\mu\text{m} \times 10 \mu\text{m}$ areas on each polymer substrate. The typical applied load during force measurements was 2.4 ± 0.3 nN, with the exception of high-loading compliance tests that were performed with a Nanoscope IIIa (Veeco, Santa Barbara, CA) scanning probe microscope under loadings up to 500 nN.

Control and Variation of Relative Humidity. Unless specified otherwise, relative humidity in the laboratory under ambient conditions for the Pico Plus AFM force measurements was 40%. The Nanoscope IIIa AFM was enclosed in a humidity-controlled glass chamber, allowing investigation of the effect of humidity on the AFM force measurements. Relative humidities of 20% and 60% were achieved by adjusting the flow rate ratio of dry N_2 gas and N_2 gas bubbled through water.

Surface Roughness. We used a Thermomicroscopes Explorer AFM (Veeco Metrology Inc., Santa Barbara, CA) with V-shaped silicon nitride cantilevers of nominal spring constant 0.10 N/m to measure the mean (R_a) and root-mean-square (rms) roughness of each surface-coating. For each of three random 10 $\mu\text{m} \times 10 \mu\text{m}$ scans of the substrate surfaces, the image was split into 4 sectors for a total of 12 roughness measurements.

Scanning Electron Microscopy (SEM). The modified “colloidal” AFM probes were characterized by scanning electron microscopy (SEM) (LEO 1530 FEG). This was done after all force measurements were finished on N6, N66, PA12, and PS. Only pollen grains 1–3 were sputtered with gold. All 5 probes were mounted on metal stubs using carbon tape and an accelerating potential of 10.0 kV was applied before imaging.

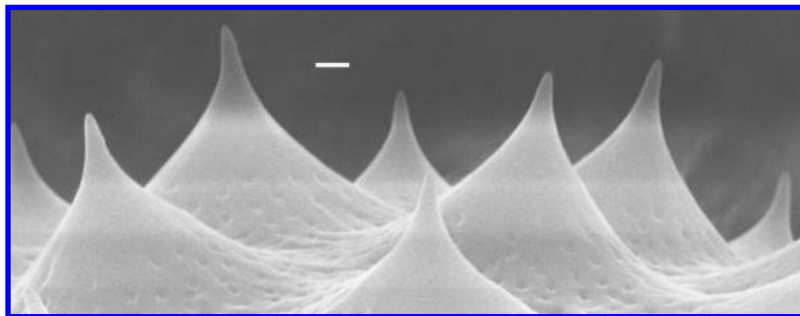


FIGURE 2. Electron micrograph of a section of the ragweed pollen grain exine surface after repeated adhesion force measurements with multiple polymer substrates. The white scale bar represents 200 nm.

Results and Discussion

Adhesive interactions and surface deformation are related, especially in the case of compliant sample surfaces such as many biological and soft polymer surfaces (18). Deformation of either the pollen or the polymer could introduce artifacts by changing the contact area during measurement or, worse, by permanently damaging the pollen through plastic yielding or fracture. From our previous AFM study of the self-interactions of polyamides and polystyrene (19), the elastic deformation of the polymer surfaces under the loadings applied here makes no significant contribution to the adhesion force. To determine whether pollen grains were deformed during subsequent force measurements, the pollen grain-cantilevers were subjected to varying applied force loadings. No apparent permanent damage to pollen grains was observed under typical applied loads (2.4 ± 0.3 nN). This is indicated in Figure 1, which shows SEM images of all 5 probe tips taken after all force measurements were completed. Further, the higher-magnification SEM micrograph in Figure 2 shows no noticeable wearing or chipping of the pollen spikes after repeated adhesion force measurements.

To further investigate potential artifacts due to pollen grain compliance or humidity, we measured force–distance curves for pollen grains 4 and 5 on a silicon surface at 20% and 60% relative humidity under the maximum applied load of ≈ 500 nN, shown in Figure 3. The plateaus in Figure 3a, b, and d indicate that the limit for the photo detector was reached. Even under such large loadings, approximately 0.5 GPa (assuming only one spike is in contact with the surface), these control experiments indicated no compliance of the grains. In addition the adhesive magnitudes under the highest loadings (pull-off forces of 5–13 nN) were in the same range as under the typically applied loading of 2.4 nN (pull-off forces of 7–13 nN). In addition, there was no significant change in adhesion force even after many repeated measurements, which concurs with the SEM micrographs (Figures 1 and 2) that show no evidence of damage after measurement. It is possible that humidity could affect the adhesion measurement through the formation of capillary bridges via condensed water on the pollen, or by softening the pollen via sorbed water. However, comparison of the retraction force magnitudes indicates no significant changes in the compliance or attraction (pull-off force) as humidity is varied from 20% to 60%.

Figure 4 shows the adhesion forces of pollen grains 1–3 with different polymer substrate surfaces, taken under a typical loading of 2.4 nN. They range between 7 and 13 nN, and adhesive forces for all 3 different pollen grains for the same surface overlap, with one exception: grains 2 and 3 on all 4 polymer surfaces are different by ≈ 3 nN. There is no significant difference in adhesion force for the same pollen grain with different polymer substrates. The dependence of the adhesion force on the specific pollen grain used can be explained by the fact that the spikes of the different grains

have nonuniform radii, and that adhesion force is directly correlated to the contact area between the spike and polymer surface. If we assume that the Hamaker model is valid, eq 1 (explanation in detail given below) show that a change in the spike radius by 1 nm will lead to a change in adhesion force by 0.9 nN, if all other factors are held constant. Hence the variation of the adhesion forces between the 3 different pollen grains from 7 to 13 nN can mean the spike radii differed in size by ± 5 nm.

Surface roughness, as an indicator of morphology, can have an effect on adhesion. Table 1 shows the roughness parameters R_a and rms of the polymer surfaces. If roughness is an important factor, then rougher surfaces (with higher surface area), such as N6 and N66, would be expected to exhibit stronger adhesion forces. But as shown in Figure 4, the forces do not differ significantly as a function of polymer surface when compared within the same class of polymer. For example, PA12 has a smoother surface than N66, yet the interaction force of pollen with PA12 is statistically indistinguishable from pollen–N66. However, because the chemical structure and roughness vary simultaneously it is difficult to make a definitive statement about the roughness effect.

Given the unique spiky surface morphology of the ragweed pollen grain, as shown in Figure 2, it is possible that multiple spikes adhere simultaneously to the polymer substrate surface. Based on these SEM images, the number of possible contacts can be calculated by considering their geometrical distribution on the pollen exine surface. It is estimated that 16 spikes appear around any arbitrarily chosen circumference on a pollen grain. The spikes are arranged in a roughly hexagonal pattern on the spherical surface, with an angle of 24° between the radii drawn from each spike tip to the center of the pollen sphere. Up to three spikes may occupy the same linear plane simultaneously. Thus, it is possible for one, two, or three spikes to contact a flat substrate simultaneously. In addition, simple geometrical calculations show that under these circumstances all noncontacting neighboring spikes will be much too far away ($>1 \mu\text{m}$) to experience VDW attraction with the surface. In about 10% of the measurements we performed, we observed the attachment and detachment of 2 pollen spikes to the polymer surface, as shown in the force–distance curve in Figure 5. These multiple-attachment profiles were omitted from the data in Figure 4, so that Figure 4 most likely represents single-spike adhesion. We did not observe simultaneous attachment of three spikes, and this is probably due to the limited orientations that could be sampled by using a pollen grain glued in a fixed position on the cantilever. From the displacement in Figure 5, the second spike adheres with a force of about twice that of the first spike.

The pollen exine, which is the outer layer of the grain, is composed of sporopollenin: a complex polymer consisting of carboxylic acids cross-linked with saturated and unsaturated aliphatic chains with varying amounts of aromatics

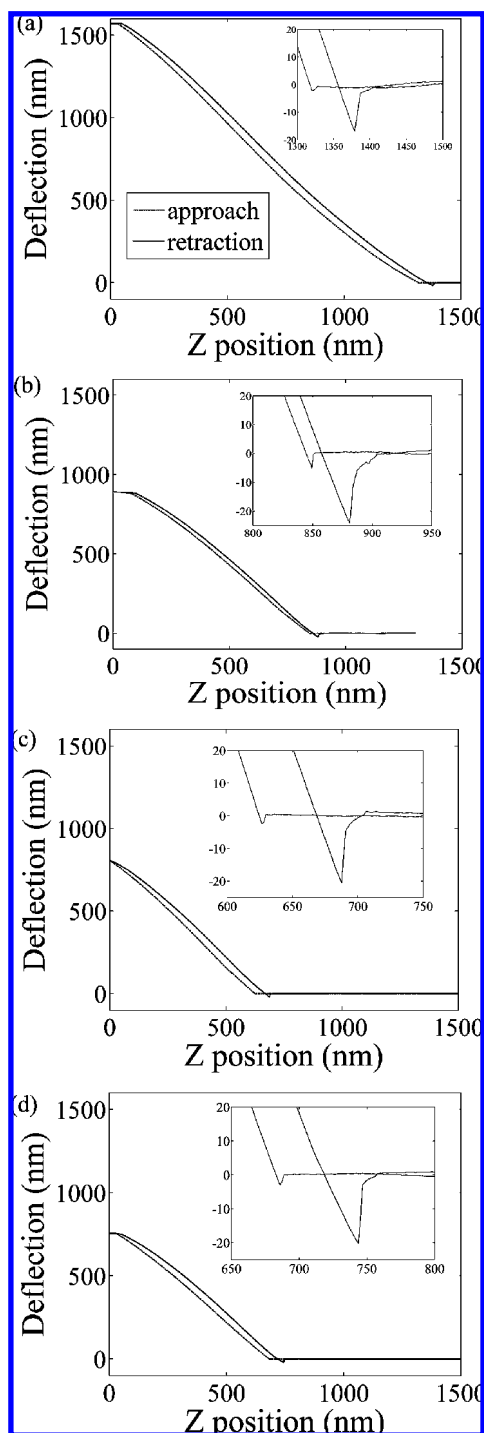


FIGURE 3. Typically observed deflection–distance curves for pollen grains 4 and 5 on Si: (a) pollen grain 4 at 20% relative humidity; (b) pollen grain 4 at 60% relative humidity; (c) pollen grain 5 at 20% relative humidity; and (d) pollen grain 5 at 60% relative humidity. The plateaus at 0 nm Z-position indicate the limit of the Nanoscope IIIa photodetector for our applied loads, and the grains were subjected to a maximum pressure of about 0.5 GPa for all 4 cases (assuming only one pollen spike is in contact with the surface).

(15, 20, 21). We expect that dispersion (London) forces will be the primary interaction between the pollen grain with the various surfaces. However, the possibility of hydrogen bonding also exists. In the case of intact ragweed pollen, protein is believed to be transported from the protoplasm to the external environment via pores on the grain surface (Figure 2) (22–24). In these cases, proteins might contribute

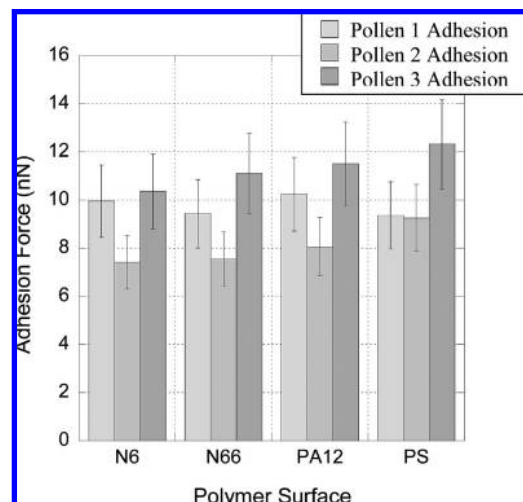


FIGURE 4. Bar graphs of the adhesion forces between the pollen attached AFM tips with various polymer surfaces under typical applied loading of 2.4 nN. Error bars are 95% confidence intervals.

TABLE 1. Surface Roughness of the Various Substrate Surfaces^a

surface	PA6	PA66	PA 12	PS	Si
Ra (nm)	97.7 ± 15.0	79.7 ± 4.2	5.4 ± 0.9	4.9 ± 0.5	0.2 ± 0.2
rms (nm)	111.7 ± 18.4	100.3 ± 5.2	7.0 ± 1.3	6.1 ± 1.1	0.3 ± 0.2

^a Uncertainty is ±95% confidence interval.

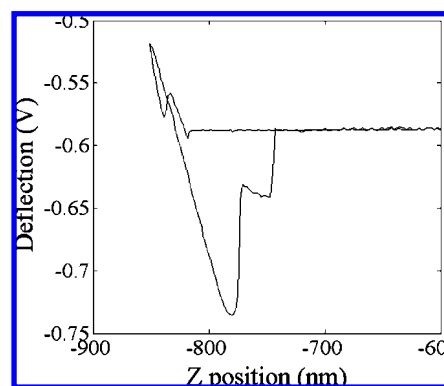


FIGURE 5. Deflection–distance curve indicating sequential attachment and detachment of two pollen spikes to a polymer surface.

functional groups that could form hydrogen bonds with polyamides. However, if present these would have likely been removed during the washing steps. Nevertheless, to test for the presence of specific chemical interactions (hydrogen bonding) in the adhesion, we used one of the pollen-attached AFM tips (pollen grain 1) to measure adhesion before and after coating the grain with a thin (<2 nm) gold layer via sputtering. The thin gold layer prevents short-range contact with the substrate surface necessary for specific interactions, but will still allow VDW interactions to occur (25). The force was measured with polystyrene of various molecular weights and with a piranha-etched (hence, oxide-coated) Si wafer. Table 2 shows the static water contact angle of the various substrate surfaces while Figure 6 presents the adhesion force data of a gold-coated pollen AFM tip with PS of varying molecular weights and silicon.

The comparison of the gold-coated (Figure 6) versus non-gold-coated (Figure 4) pollen force measurements suggests that ragweed pollen adheres to the polymer and silica surfaces

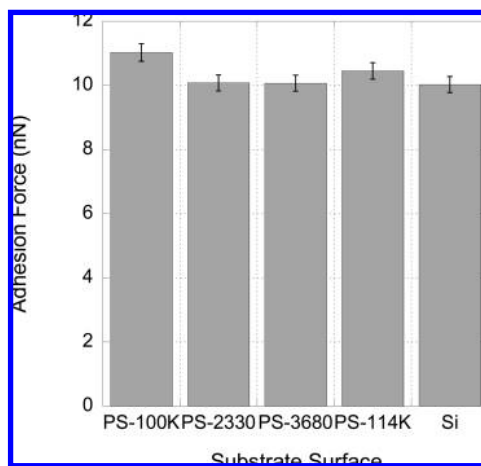
TABLE 2. Static Water Contact Angle of the Substrate Surfaces^a

surface	PA6	PA66	PA 12	PS	Si
	75.2 ± 1.0	67.1 ± 0.6	72.8 ± 1.8	90.6 ± 0.7	13.3 ± 0.1

^a Uncertainty is ±95% confidence interval.

mainly via nonspecific VDW forces. If specific interactions were important, the adhesion forces after gold sputtering would be significantly lower than those prior to the gold coating. However, there was no significant change in the magnitude of adhesion comparing the gold- and non-gold-coated pollen force data, as well as for polystyrene (hydrophobic) and Piranha-etched silicon (hydrophilic) surfaces. There are three important artifacts that must be considered before drawing a conclusion. First, if an oxide layer formed on the gold, then this layer might participate in hydrogen bonding either directly or via an adsorbed water layer (26). We can estimate the number of possible hydrogen bonds to be about 7800 by using the unit cell dimensions of AuO (27), and a pollen spike contact radius of 17 nm. By applying the relation $F_{HB} = (E_{HB}/N_A r)$ (28), where E_{HB} is the hydrogen bonding energy taken to be 30 kJ/mol (29), N_A is Avogadro's number, and r is the distance (0.165 nm) between the two surfaces, we estimate the force of one hydrogen bond to be 0.3 nN. The total adhesion force would be 2340 nN, much larger than any force observed in our data. Second, we did not observe any evidence of electrostatic (Coulombic) interactions that would obscure this conclusion. The force-distance approach and retraction curves did not overlap entirely and there was a distinct jump of the tip from the surface upon retraction at the pull-off distance (where there is no net attractive or repulsive force). Third, it is important to consider that the gold layer might also obscure interpretation by increasing the magnitude of VDW forces. Gold has a higher polarizability, and hence a larger Hamaker constant than PS (45×10^{-20} J for gold versus 6×10^{-20} J for PS). But, the effect of the gold coating on the composite Hamaker constant is scaled by the (gold thickness/total thickness) (3) for nonretarded VDW interactions (25). We estimate that a 2 nm gold coating on a 1000 nm PS film (the minimum PS thickness in our study) raises the effective Hamaker constant by 10% at most. So, it is unlikely that the addition VDW interactions caused by the gold coating could mask the effect of hydrogen bonding.

In Figure 6, we also varied the molecular weight of polystyrene used as a substrate as a rough probe of the effects

**FIGURE 6. Bar graphs of the adhesion forces between a gold-coated pollen attached AFM tip (pollen grain 1) with polystyrene surfaces of various molecular weight and Piranha-etched silicon. Error bars are 95% confidence intervals.**

of polymer dynamics on the measured adhesion. The glass transition temperature decreases dramatically when $M_w < 10,000$, from ≈ 100 °C for $M_w = 100,000$ to ≈ 50 °C for $M_w = 1000$. From Figure 6, there is no evidence that the change in M_w , and hence glass transition temperature, is a factor over the measurement time scale.

To provide further rationale supporting the controlling role of VDW forces, we use a simple Hamaker model to predict the adhesive forces expected. If we assume that the tip of the spike of the pollen grain is a hemisphere with radius $R \approx 17$ nm, from Figure 2, that is separated from an infinitely large, flat surface, we can estimate the adhesion force of one exine spike to the surface by (30):

$$F = \frac{A_H R}{6D^2} \quad (1)$$

where A_H is the material-dependent nonretarded Hamaker constant. We take A_H from our previous paper measuring adhesion between *E. coli* and Nylon in air (7), where A_H ranged from 0.5 to 2×10^{-19} J. The value of D is taken as 0.165 nm, the nominal value used for cutoff separation distance. This theoretical estimate gives an adhesion force for an exine spike to be between 5 and 20 nN. From Figure 4, our ragweed pollen grain has an average measured adhesion force ranging from 7 to 13 nN, which is well within the range of the predicted adhesion forces. These values, together with the deflection-distance curve in Figure 5, support the contention that only one or at most two spikes are in contact with a flat surface in our measurements.

How might this dependence on van der Waals forces for adhesion be used to provide useful design principles for indoor carpet and upholstery materials? Ideally, one desires a material that allows moderate adhesion so that routine foot traffic does not dislodge particles (effectively “cleaning” the air); however, the same material should release the particles upon cleaning, e.g., by vacuuming. Ragweed pollen grains, like gecko setae (31–35), appear to adhere to surfaces depending mainly on surface contact geometry rather than on surface chemistry. In our experiments, pollen grains were fixed onto AFM cantilevers and only one orientation was sampled in adhesion measurements. For pollen dispersed in air, the grain can rotate freely and would be expected to easily make multiple contacts with 3 exine spikes. In addition, a single grain might also contact more than one Nylon fiber simultaneously in “real” carpet. Hence, we would expect that in indoor environments the forces experienced will be higher. Since the pollen forces in Figure 4, which average 10 ± 3 nN, are for single spikes contacting with a flat surface, we expect that free pollen adhesion to carpet fiber would be between 2 and 3 times higher, or 20–30 nN. If a single pollen grain were able to contact multiple carpet fibers simultaneously, which is possible given its large diameter of 10–20 μ m, then the grain would adhere by an appropriately scaled total force. Hence, a grain adhering to 2 fibers with 3 contact spikes each could reach an adhesive force of 60 nN.

Following the approach in our previous publication (7), we estimate the drag force induced by foot traffic (0.3 m/s average air velocity) on a 15 μ m smooth sphere to be 3 nN. Vacuuming, which can produce velocities on order of 40 m/s, leads to much larger drag force of 6800 nN. While these are only intended to be rough, order-of-magnitude estimates, one predicts that pollen grains adhering via even a single spike (10 nN adhesion) would not be dislodged by foot traffic (3 nN drag force). However, vacuuming would easily be able to remove grains adhering with many spikes. The dependence of adhesion on the number of spike contacts suggests that opportunities exist for developing fiber morphologies engineered to control multispike or multifiber contacts, e.g., by carefully designing the interfiber spacing or the fiber

curvature based upon the pollen grain's curvature and spike distribution. Dislodging a pollen grain from a fiber involves pulling polymer chains away from a surface. Such pollen–fiber interactions might be modeled also by applying scaling results from studies of grafted polymers interacting with an AFM tip (36). In addition, we point out that the antisoiling additives typically added to commercial carpet will likely change the values measured here. Since the forces are VDW in nature, we would expect that fluorinated treatments commonly used will further decrease the adhesion force. These are important areas for future research.

Acknowledgments

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Literature Cited

- (1) Weiss, K. B.; Gergen, P. J.; Hodgson, T. A. An Economic Evaluation of Asthma in the U.S. *New Engl. J. Med.* **1992**, 326, 862.
- (2) Weiss, K. B.; Sullivan, S. D. The health economics of asthma and rhinitis. I. Assessing the economic impact. *J. Allergy Clin. Immunol.* **2001**, 107 (1), 3–8.
- (3) Davidson, L. Ventilation by displacement in a three-dimensional room, a numerical study. *Build. Environ.* **1989**, 24 (2), 363–372.
- (4) Chen, F.; Yu, S. C. M.; Lai, A. C. K. Modeling particle distribution and deposition in indoor environments with a new drift-flux model. *Atmos. Environ.* **2006**, 40 (2), 357–367.
- (5) Tian, Z. F.; Tu, J. Y.; Yeoh, G. H.; Yuen, R. K. K. On the numerical study of contaminant particle concentration in indoor airflow. *Build. Environ.* **2006**, 41 (11), 1504–1514.
- (6) *Trends in Asthma Morbidity and Mortality*; American Lung Association Epidemiology and Statistics Unit, Research and Scientific Affairs, Washington, D.C., 2003.
- (7) Thio, B. J.; Meredith, C. J. Quantification of *E. coli* Adhesion to Polyamides and Polystyrene with Atomic Force Microscopy. *Colloids and Surfaces B: Biointerfaces* **2008**, 65 (2), 308–312.
- (8) Behrendt, H.; Becker, W.-M. Localization, release and bioavailability of pollen allergens: the influence of environmental factors. *Curr. Opin. Immunol.* **2001**, 13 (6), 709–715.
- (9) Traidl-Hoffmann, C.; Kasche, A.; Menzel, A.; Jakob, T.; Thiel, M.; Ring, J.; Behrendt, H. Impact of Pollen on Human Health: More than Allergen Carriers. *Int. Arch. Allergy Immunol.* **2003**, 131, 1–13.
- (10) Solomon, W. R. Airborne pollen: A brief life. *J. Allergy Clin. Immunol.* **2002**, 109 (6), 895–900.
- (11) Binnig, G.; Quate, C. F.; Gerber, C. Atomic Force Microscope. *Phys. Rev. Lett.* **1986**, 56, 930–933.
- (12) Dufrene, Y. F. Atomic Force Microscopy, a Powerful Tool in Microbiology. *J. Bacteriol.* **2002**, 184 (19), 5205–5213.
- (13) Dufrene, Y. F. Recent progress in the application of atomic force microscopy imaging and force spectroscopy to microbiology. *Curr. Opin. Microbiol.* **2003**, 6, 317–323.
- (14) Ducker, W. A.; Senden, T. J.; Pashley, R. M. Direct measurement of colloidal forces using an atomic force microscope. *Nature* **1991**, 353 (6341), 239–241.
- (15) Dominguez, E.; Mercado, J. A.; Quesada, M. A.; Heredia, A. Pollen sporopollenin: degradation and structural elucidation. *Sex Plant Reprod.* **1999**, 12, 171–178.

- (16) Burnham, N. A.; Chen, X.; Hodges, C. S.; Matei, G. A.; Thoreson, E. J.; Roberts, C. J.; Davies, M. C.; Tendler, S. J. B. Comparison of calibration methods for atomic-force microscopy cantilevers. *Nanotechnology* **2003**, 14, 1–6.
- (17) Hutter, J. L.; Bechhoefer, J. Calibration of atomic-force microscope tips. *Rev. Sci. Instrum.* **1993**, 64 (7), 1868–1873.
- (18) Sun, Y.; Akhremitchev, B.; Walker, G. C. Using the Adhesive Interaction between Atomic Force Microscopy Tips and Polymer Surfaces to Measure the Elastic Modulus of Compliant Samples. *Langmuir* **2004**, 20, 5837–5845.
- (19) Thio, B. J.; Meredith, C. J. Measurement of polyamide and polystyrene adhesion with coated-tip atomic force microscopy. *J. Colloid Interface Sci.* **2007**, 314 (1), 52–62.
- (20) Ivleva, N. P.; Niessner, R.; Panne, U. Characterization and discrimination of pollen by Raman microscopy. *Anal. Bioanal. Chem.* **2005**, 381 (1), 261–267.
- (21) Kano, H.; Hamaguchi, H. Vibrational Imaging of a Single Pollen Grain by Ultrabroadband Multiplex Coherent Anti-Stokes Raman Scattering Microspectroscopy. *Chem. Lett.* **2006**, 35 (10), 1124–1125.
- (22) Howlett, B. J.; Knox, R. B.; Heslop-Harrison, J. Pollen-Wall Proteins: Release of the Allergen Antigen E from Intine and Exine Sites in Pollen Grains of Ragweed and Cosmos. *J. Cell Sci.* **1973**, 13, 603–619.
- (23) Piffanelli, P.; Ross, J. H. E.; Murphy, D. J. Biogenesis and function of the lipidic structures of pollen grains. *Sex Plant Reprod.* **1998**, 11, 65–80.
- (24) Knox, R. B. Pollen Wall Proteins: Pollen-Stigma Interactions in Ragweed and Cosmos (Compositae). *J. Cell Sci.* **1973**, 12, 421–443.
- (25) Sharma, A.; Reiter, G. Instability of Thin Polymer Films on Coated Substrates: Rupture, Dewetting, and Drop Formation. *J. Colloid Interface Sci.* **1996**, 178, 383–399.
- (26) Gordon, J. S.; Johnson, D. C. Application of an electrochemical quartz crystal microbalance to a study of water adsorption at gold surfaces in acidic media. *J. Electroanal. Chem.* **1994**, 365, 267–274.
- (27) Chesters, M. A.; Somorjai, G. A. The Chemisorption of Oxygen, Water and selected Hydrocarbons on the (111) and Stepped Gold Surfaces. *Surf. Sci.* **1975**, 52, 21–28.
- (28) Bolland, T.; Ratner, B. D. Direct measurement of hydrogen bonding in DNA nucleotide bases by atomic force microscopy. *Proc. Natl. Acad. Sci. USA* **1995**, 92, 5297–5301.
- (29) Emsley, J. A. Very strong hydrogen bonding. *Chem. Soc. Rev.* **1980**, 9, 91–124.
- (30) Israelachvili, J. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: London, 1992.
- (31) Tian, Y.; Pesika, N.; Zeng, H.; Rosenberg, K.; Zhao, B.; McGuiggan, P.; Autumn, K.; Israelachvili, J. Adhesion and friction in gecko toe attachment and detachment. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, 103 (51), 19320–19325.
- (32) Huber, G.; Gorb, S. N.; Spolenak, R.; Arzt, E. Resolving the nanoscale adhesion of individual gecko spatulae by atomic force microscopy. *Biol. Lett.* **2005**, 1, 2–4.
- (33) Autumn, K.; Peattie, A. M. Mechanisms of Adhesion in Geckos. *Integr. Comp. Biol.* **2002**, 42 (6), 1081–1090.
- (34) Autumn, K.; Liang, Y. A.; Hsieh, S. T.; Zesch, W.; Chan, W. P.; Kenny, T. W.; Fearing, R.; Full, R. J. Adhesive force of a single gecko foot-hair. *Nature* **2000**, 405, 681–685.
- (35) Zhao, B.; Pesika, N.; Rosenberg, K.; Tian, Y.; Zeng, H.; McGuiggan, P.; Autumn, K.; Israelachvili, J. Adhesion and Friction Force Coupling of Gecko Setal Arrays: Implications for Structured Adhesive Surfaces. *Langmuir* **2008**, 24 (4), 1517–1524.
- (36) Jimenez, J.; Rajagopalan, R. Interaction between a Grafted Polymer Chain and an AFM tip: Scaling Laws, Forces, and Evidence of Conformational Transition. *Langmuir* **1998**, 14 (10), 2598–2601.

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