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The Role of Surface Chemistry in Adhesion and Wetting of Gecko Toe Pads

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An array of micron-sized setal hairs offers geckos a unique ability to walk on vertical surfaces using van der Waals interactions. Although many studies have focused on the role of surface morphology of the hairs, very little is known about the role of surface chemistry on wetting and adhesion. We expect that both surface chemistry and morphology are important, not only to achieve optimum dry adhesion but also for increased efficiency in self-cleaning of water and adhesion under wet conditions. Here, we used a plasma-based vapor deposition process to coat the hairy patterns on gecko toe pad sheds with polar and non-polar coatings without significantly perturbing the setal morphology. By a comparison of wetting across treatments, we show that the intrinsic surface of gecko setae has a water contact angle between 70–90°. As expected, under wet conditions, adhesion on a hydrophilic surface (glass) was lower than that on a hydrophobic surface (alkyl-silane monolayer on glass). Surprisingly under wet and dry conditions the adhesion was comparable on the hydrophobic surface, independent of the surface chemistry of the setal hairs. This work highlights the need to utilize morphology and surface chemistry in developing successful synthetic adhesives with desirable adhesion and self-cleaning properties.

Mechanisms underlying the performance of fibrillar adhesives in geckos have been the subject of especially intensive study ever since Autumn and colleagues demonstrated the central importance of van der Waals forces¹. A vast collection of empirical studies, theoretical models and synthetic mimics has demonstrated a deep understanding of the way in which size, shape and scale contribute to a gecko's capacity to climb or adhere to vertical and inverted surfaces². However, despite substantial progress in the translation of the principles of function of the gecko system, gecko-inspired fibrillar adhesives still do not simultaneously capture all of the performance characteristics (e.g., self-cleaning, non-matting, and resilience) observed in free-ranging geckos^{3–5}.

Compared to study of the form of the gecko adhesive system, very little work has actually focused on its material composition. Indeed, most studies have assumed that the gecko setal contact surface is made of β -keratin^{6–9}. Recently however lipids were found in the setae and in footprints left behind as a gecko walks, suggesting that the setal surface chemistry could be hydrophobic^{10,11}. For a typical superhydrophobic surface, like the gecko toe pad, we know that both surface chemistry and surface roughness are accounted for in the Wenzel¹² (homogeneous solid-liquid interface) and the Cassie-Baxter¹³ (heterogeneous solid-liquid and air-liquid interface) wetting theories. Interestingly a water droplet in contact with a gecko toe pad forms a Cassie-Baxter state, with a contact angle of about 150° and a very low contact angle hysteresis (about 2°–3°)^{14,15}. On the other hand, a Wenzel state, corresponding to complete wetting of the toe, is also possible after prolonged or repeated exposure to water^{16,17}. The transition between these states in the gecko toe is non-spontaneous and expected to take place gradually over a period of time (of the order of a few minutes)¹⁷. When tested, we find that this transition is potentially detrimental to geckos, causing them to lose adhesion and slip from surfaces^{16,18}. Thus the metastability of the superhydrophobic state and the estimation of the transition barrier between the superhydrophobic state and the wetted state are important for determining wettability of the gecko toe pad. These factors have a direct relationship in the intrinsic surface chemistry of the setae and the gecko's ability to retain function of their adhesive system.

While much work has been done on the dry adhesion mechanics of the gecko adhesive system, only recently have we begun to focus on more ecologically relevant contexts; specifically, adhesion to wet surfaces (e.g., rain, high humidity, condensation)^{16,17,19–22}. When adhering in dry conditions adhesion is surface-insensitive¹ but



when water is present the substrate wettability is relevant. For example, when tested underwater we find that geckos can expel a thin layer of water on a hydrophilic surface¹⁰, but when this water layer becomes thicker (~ 0.5 cm) it is not sufficiently expelled and adhesion is greatly reduced^{16,22}. This reduction is likely due to water being held between the superhydrophobic toe and hydrophilic surface, a function of the interplay between the wetting behavior of the toe and the wettability of the substrate. When testing gecko adhesion on hydrophobic surfaces submerged underwater however, adhesion was not significantly impacted and in fact, on one surface (polytetrafluoroethylene) adhesion was improved underwater²². Furthermore, thermodynamic models of adhesion reveal the dependence of gecko adhesion on surface chemistry in the presence of water, showing that an oil-like surface (setae), and the wettability of the substrate predict results for whole animal adhesion²². This suggests that the gecko setal surface behaves more oil-like and hydrophobic when contacting surfaces underwater; however the surface chemistry of the adhesive setae has never been directly tested²².

In this study we have developed a strategy to isolate the effect of surface chemistry of the setae from surface morphology (roughness), allowing us to directly investigate the effect of surface chemistry on wettability and adhesion in wet and dry conditions. We present a novel approach to selectively change the surface chemistry of the gecko toe pad by depositing a thin coating of known functionality on the surface of the setae, without significantly affecting setal morphology. We used Plasma Enhanced Chemical Vapor Deposition (PECVD) for this purpose. This dry vapor phase process is particularly advantageous in comparison to a wet process which could result in the collapse or disruption of the setae due to capillary forces. We chose two PECVD precursors with different intrinsic wettabilities (quantified in terms of equilibrium water contact angle on flat surfaces of that particular chemistry, θ_Y); a hydrophilic precursor (maleic anhydride, $\theta_Y \sim 48^\circ$) and a hydrophobic precursor (1H, 1H, 2H-perfluoro-1-dodecene, $\theta_Y \sim 110^\circ$). The deposition using PECVD was carried out on the toe pad skin sheds of naturally molted geckos. We also used a short (1 minute) plasma treatment on the shed surface as a means of altering the inherent surface chemistry of the natural samples, similar to the established technique of lipid stripping wool fibers^{23,24}. Phospholipid, being one of the constituents in the setal surface composition, was thus removed at the surface. The wettability and adhesive performance (in air and water) of PECVD coated sheds with known surface chemistry was compared to the results obtained using non-coated, untreated sheds and plasma treated sheds. Our results have implications for the construction of a gecko-inspired adhesive that can maintain function on various surfaces in dry, humid or wet conditions.

Results and Discussion

To investigate the effect of surface chemistry on wetting and adhesion we first discuss the characterization of coated sheds using x-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) measurements. We then discuss differences in wetting as a function of surface chemistry. Finally, we discuss how shear adhesion in air and water varies as a function of setal surface chemistry and substrate wettability.

Surface morphology and chemical analysis. Figure 1 shows SEM images of an untreated shed (B-S, Figures 1a and 1b) and the PECVD coated sheds, using two precursors, maleic anhydride (M-S, Figures 1c and 1d) and 1H, 1H, 2H-perfluoro-1-dodecene (F-S, Figures 1e and 1f) along with an oxygen plasma treated sample (P-S, Figures 1g and 1h). The micrometer-scale tetrad pattern common to Tokay geckos (*Gekko gekko*) can be clearly seen, as can the hierarchal setal branches, terminating into hundreds of finer flattened pads (spatulae) on the untreated and PECVD coated sheds (Figure 1). Visual similarity in these SEMs suggest that PECVD did not result

in changes in morphology. Interestingly however, in the P-S samples we see a unique clumping behavior at the terminal branches of the setae (Figure 1h). We believe this could be due to removal of the lipids and an increase in adhesion energy between setae leading to bunching. The role of adhesion in stabilizing the clumping of fibrillar structure has been studied before and supports our observations of the P-S samples^{25,26}.

To investigate the effect of PECVD and plasma treatment on the samples we used surface sensitive XPS to measure the elemental composition of the toe pad shed surface (the probe depth of XPS is around 10 nm). The survey spectrum of the B-S sample (untreated shed) shows the presence of C1s, N1s, O1s and S2p peaks, representing the primary elements: carbon, nitrogen, oxygen and sulfur (Figure 2). While this is not surprising given the proposed constituents of the setae (β -keratin and lipid)^{8,10}, this scan is the first report of the surface elements involved in the adhesion of the gecko adhesive system. Conversely, when compared to the B-S scan, the survey scan of the P-S sample (oxygen plasma-treated shed) shows an increased relative atomic composition of N1s, implying a change has occurred in the atomic composition of the surface after plasma treatment. The increased atomic composition of N1s in the plasma treatment (P-S) suggests that a lipid layer was removed, similar to the removal of lipids on the surface of wool^{23,24}, except in this case the increased presence of β -keratin at the surface is likely causing the elevated nitrogen signal. It is also interesting to note that the peaks in the C1s spectrum of B-S and P-S samples are at the same positions, however, their relative percentages are significantly different. This provides further evidence that surface lipids are removed upon plasma treatment, at least in fractions if not completely, since we would expect different amounts of carbon in the lipid and keratin components of the setae. It is also important to note that the surfaces of the setae could be partially covered with keratin and the surface structure of keratin may also be affected by oxygen plasma.

In the PECVD coated samples, the absence of N1s or S2p (nitrogen and sulfur) peaks found in the B-S sample implies that the coating was successfully deposited on the sample and allows us to assume that the coating thickness is around 10–15 nm, comparable to the analysis depth of XPS (Figure S1). The M-S sample shows the presence of C1s and O1s peaks (carbon and oxygen) whereas, the F-S sample shows C1s and F1s peaks (carbon and fluorine). These elements are consistent with the chemical structure of the PECVD coatings and the atomic compositions of control measurements. The relative atomic compositions calculated using survey scans and also the results of the high resolution XPS scans are provided in the supporting information (SI Text, section S1, Table S1, Figure S2 and Table S2).

In summary, using XPS and SEM we confirmed that either a thin layer of PECVD coating was deposited on the structured surface of the gecko toe sheds or the surface was at least partially stripped of lipids, such that all four treatments have distinctly different surface chemistry yet similar morphology.

Surface wetting and intrinsic surface chemistry modeling. To study the wettability and thus the ability of gecko toes to self-clean water from their adhesive toe pads, we first measured the water contact angles of the B-S, P-S, M-S and F-S shed surfaces (Table 1). Both the B-S and F-S surfaces show characteristics of a typical superhydrophobic surface, such as a contact angle of 150° (Figure 3) and a very low contact angle hysteresis. The water droplet in contact with both of these surfaces corresponds to a Cassie-Baxter state. In contrast, the water droplet on the P-S and M-S surfaces almost instantaneously spreads on the surface, resulting in complete wetting (Figure 3). The color of the setae on these surfaces also changes from a shiny white to grey once penetrated by water, which is a common indication of surface wetting in gecko toes¹⁶. Because these samples have similar morphology, the differences in surface wettability can be attributed to surface

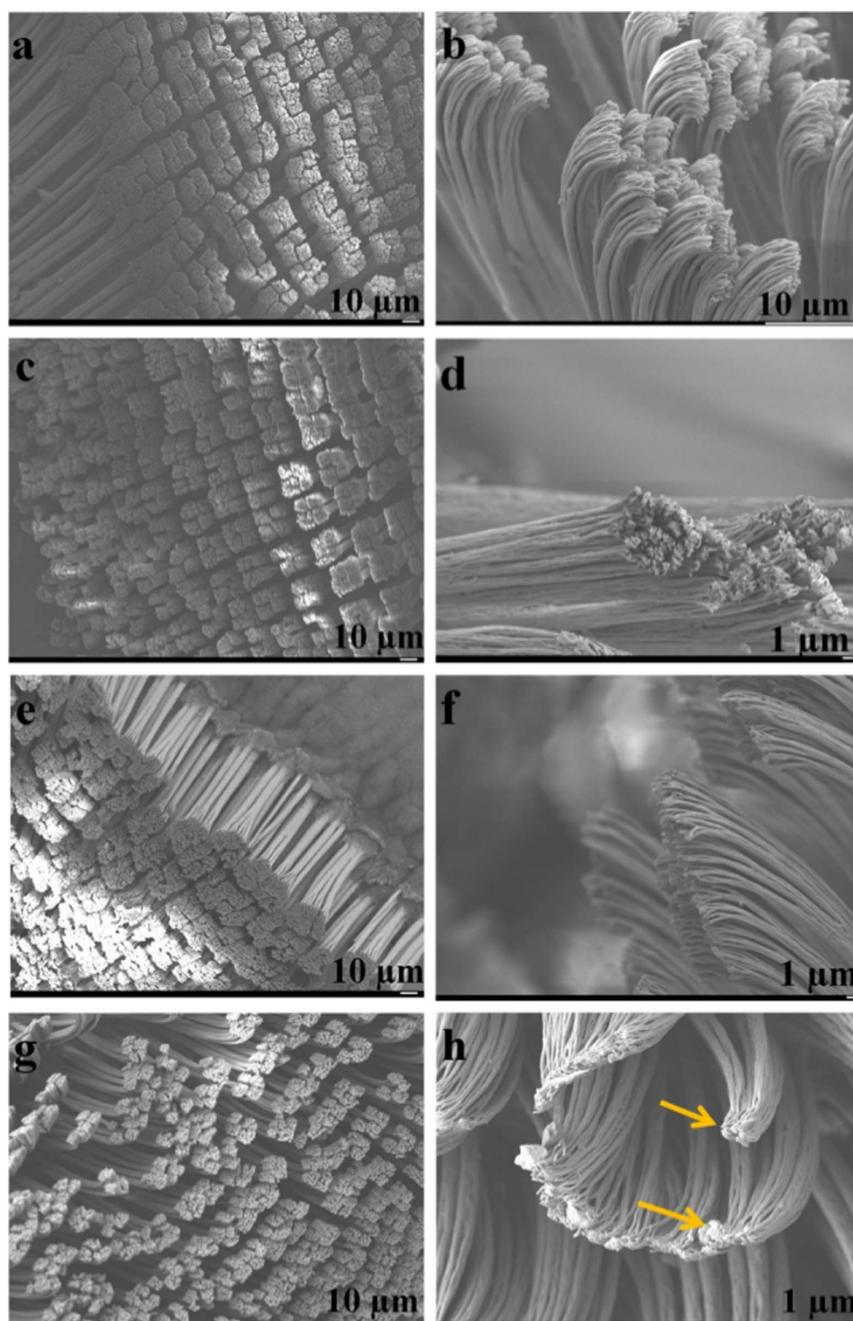


Figure 1 | SEM images of untreated and coated sheds. SEM images of B-S (a,b), M-S (c,d), F-S (e,f) and P-S (g,h) samples. Comparison of lower magnification images (a,c,e and g) indicate that the tetrad pattern of the sheds was retained after PECVD layer deposition. The higher magnification images (b,d,f and h) show the finer structures present on the sheds (spatulae). The arrows in panel h point to the setae that are bunched together.

chemistry. Due to the limitations of SEM however, it is possible that there are morphological differences across samples at the nanometer-level scale which cannot be observed visually. We do not believe this has a significant effect on wetting behavior however because the two PECVD coated samples (M-S and F-S) have such extreme differences in wetting behavior despite likely having similar roughness changes related to the coating process.

To test if the superhydrophobicity of the B-S surface is metastable we measured the B-S surface along with the M-S, F-S and P-S surfaces under water condensation, which is expected to induce a wetting transition^{27,28}. The samples were kept under 100% humidity for 3–4 days, which was then followed by the measurement of water droplet contact angle. We found, as expected, that both the M-S and P-S samples wetted completely under condensation. Similarly, as expected, the F-S surface retained its superhydrophobic character-

istics and continued to remain dry, even after prolonged exposure to high humidity. Conversely, we found that in the B-S surface water began to penetrate inside the surface roughness and resulted in the wetting of the surface, unlike water droplet contact angle measurements made in ambient conditions. Thus the B-S surface changed from a Cassie-Baxter wetting state to a Wenzel wetting state. When comparing the B-S surface (147°) to the known inherent chemistries of the M-S (complete wetting) and F-S (149°) surfaces we find that the B-S surface is inherently hydrophobic, enough to form a stable superhydrophobic Cassie-Baxter state, like the F-S surface. However it appears that this state is only a metastable state and a transition to the Wenzel state, similar to the M-S or P-S surface, occurs after water condensation on the surface. It is important to note here that the wetting transition is completely reversible and the B-S samples regain their superhydrophobicity after drying. Interestingly the B-S sample

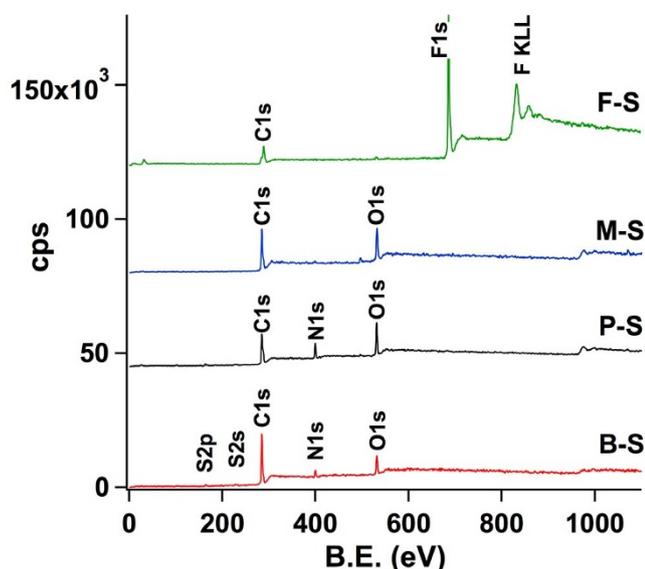


Figure 2 | Surface characterization of untreated and coated sheds.

(a) XPS survey spectra of B-S, P-S, M-S and F-S. The y-axis is represented as counts per second (cps). The peaks assignable to constituent elements are labeled. The survey spectra were used for calculating surface atomic composition. The high resolution C1s XPS spectra are shown as Figure S2 in the supporting information.

is different than the P-S sample, which does not have a layer of coating deposited but rather has been stripped of surface layers (most likely hydrophobic lipids). We hypothesize that after oxygen plasma treatment a hydrophilic surface has been exposed (likely β -keratin) which results in the complete wetting of the surface, similar to the hydrophilic M-S surfaces.

Since superhydrophobicity is a result of both surface roughness and intrinsic surface wettability, we carried out apparent contact angle calculations using a model unit cell shown as an inset in Figure 4b (SI Text, section 2). The hierarchical unit cell consists of four setae (tetrad pattern), each with a square cross section. The square pillar is 60 μm tall and 4 μm wide. The top face of each pillar consists of a number of cubes, 0.2 μm in size. The roughness parameters were calculated for the unit cell and incorporated into the Cassie-Baxter and Wenzel equations to predict the apparent contact angles (θ_{CB} and θ_W) and to calculate G^*_{CB} and G^*_W , the thermodynamic free energy corresponding to Cassie-Baxter and Wenzel states, respectively^{29–31}. The results show that the range of $70^\circ \leq \theta_Y \leq 120^\circ$ resulted in θ_{CB} of about 145° – 160° (Table S3), matching the range of values measured experimentally on the superhydrophobic B-S and F-S samples. This suggests that the intrinsic wettability of gecko setae can fall somewhere within this range. Figure 4a shows ΔG^* calculated as a function of roughness, R, for different intrinsic wettability θ_Y . Figure 4b shows the expanded region near $\Delta G^* = 0$. Although the range of R where contact angles could be predicted is very small compared to the actual roughness of the gecko setal surface, it qualitatively predicts wettability as a function of θ_Y . For $\theta_Y \leq 90^\circ$, ΔG^* is an increasing function of R, implying that a Wenzel state is thermodynamically more favorable. On the other hand, for $\theta_Y \geq 90^\circ$, ΔG^* is a decreasing function of R, becoming negative at a critical R, implying

that the Cassie-Baxter state is thermodynamically more favorable for a roughness value more than the critical R. We find that the model predictions are consistent with our experimental observations, where M-S surfaces ($\theta_Y \approx 48^\circ$) completely wet with water (Wenzel wetting state), whereas the F-S surface ($\theta_Y \approx 110^\circ$) is superhydrophobic (Cassie-Baxter state). Although the θ_Y for the B-S surface is not known, our model predictions and experimental observations allow us to narrow down a range for θ_Y for the B-S surface to be between 70° – 90° . In this range a stable Cassie-Baxter wetting is possible but also is not the most favorable state thermodynamically, which is in agreement with our observations of B-S surface wetting after exposure to water vapor condensation. It is interesting that this range encompasses the $\theta_Y \approx 90^\circ$ value which was previously suggested to be the intrinsic surface chemistry of the gecko setae based on the contact angle measurement of the smooth gecko eye scale¹⁴ and that it is neither strongly hydrophobic nor is it strongly hydrophilic. Despite this consistency, it is important to be cautious of our predictions, as this predicted range (70° – 90°) does not take into account any changes in surface chemistry which may cause the structure to behave more hydrophobic or hydrophilic when in contact or exposed to water, a behavior mounting evidence suggests may occur (Pesika et al¹⁷ and Hsu et al¹⁰).

To quantify the transition barrier from the Cassie-Baxter to Wenzel wetting states we used a water column to measure the hydrostatic pressure necessary to induce a wetting transition in all four surface treatments. We found that the P-S and M-S sample wetted as soon as they were introduced into the water column, as expected by static water contact angle measurements. Conversely, both the B-S and F-S samples appeared shiny and silvery, implying the presence of an air plastron layer. Both surfaces continued to retain the plastron layer as the immersion depth increased up to a maximum of 4'. The plastron layer did not disappear in either sample, even after 7–8 hours. Thus the transition barrier between the Cassie-Baxter and Wenzel states in both the B-S and F-S samples can be estimated to be greater than 11.95 kPa, almost three orders of magnitude higher than the M-S and P-S samples. Although we anticipate the barrier for the F-S surface to be higher than the B-S surface based on model predictions and the condensation results, it is surprising how resilient the native sample (B-S) is when protected by an air plastron. This has clear implications for the whole animal, as we found previously that the maintenance of an air plastron likely allows geckos to remain adhesive on hydrophobic surfaces^{22,32}. As such we can better appreciate the various scenarios that must occur for natural toe pads to become wet, which seem to be when applied repeatedly on wet surfaces or when agitated on rough, wet surfaces¹⁶. During these instances it is likely that the setal mat first must be penetrated by water and the plastron broken in order to become wet. Similarly in humidity it is likely small droplets of water on the setae cause the plastron to fail to form and allows water to be pulled into the setal mat and wet. Regardless, it is clear that the different transition barriers and wetting behaviors in each of the surface treatments can be attributed to differences in inherent surface chemistry and thus their ability to self-clean water from the adhesive toe pad.

Dry and wet adhesion. To study the effect that surface chemistry has on adhesion we tested the shear adhesion of natural, uncoated toe sheds (B-S) and modified sheds (P-S, M-S, and F-S) on a hydrophilic glass surface and a hydrophobic OTS-SAM coated glass surface in air

Table 1 | Summary of water contact angles. The intrinsic contact angles (θ_Y) are measured on flat, control samples and apparent contact angles (θ_{app}) are measured on untreated, plasma treated and PECVD coated shed samples

Sample ID	B-S	P-S	M-S	F-S
θ_Y ($^\circ$)			48 ± 3	110 ± 2
θ_{app} ($^\circ$)	147 ± 5	Complete surface wetting	Complete surface wetting	149 ± 3

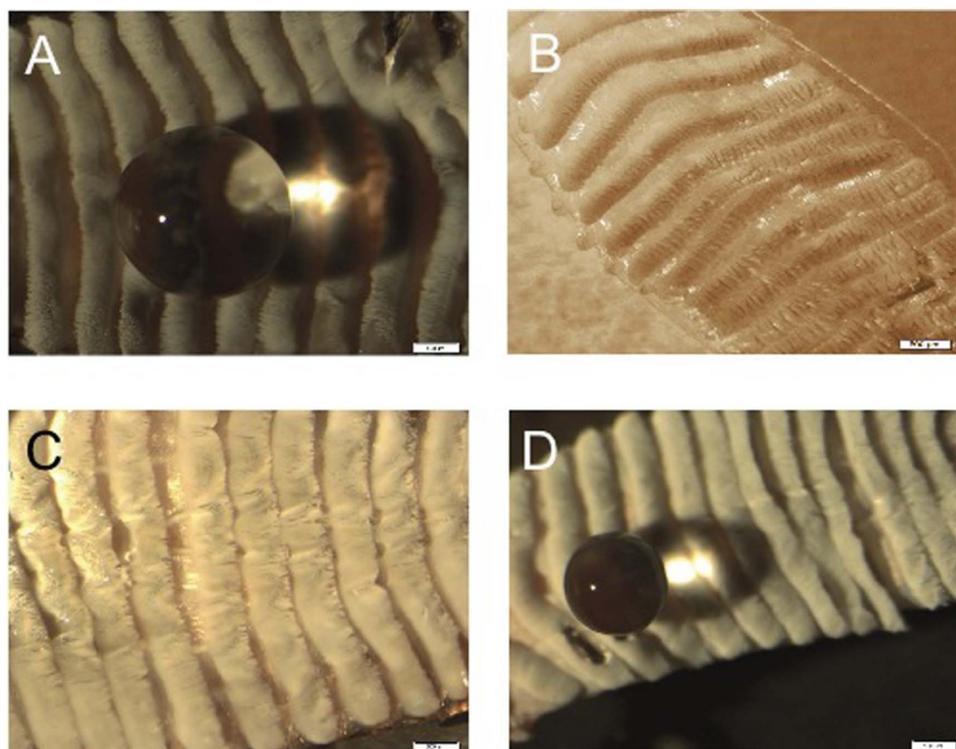


Figure 3 | Optical images of water droplets on the shed surfaces. Droplets of water on B-S (A), P-S (B), M-S (C), and F-S (D) (scale bar for images: 500 μm). The water wets both P-S and M-S samples.

(W_{dry}) and water (W_{wet}). We have measured the forces parallel to the surface, similar to friction measurements, and we refer to these forces as shear adhesion because we expect the origin of this force to be due to adhesion rather than friction. The effect of coating (B-S, M-S, F-S, P-S), test substrate (glass or OTS-SAM coated glass) and treatment conditions (air or water) was highly significant ($df = 16$, $F = 22.7982$, $p \leq 0.0001^*$) as were many of the interactions, including the three-way interaction of coating*surface*treatment (SI Text, section 3, Table S4). Subsequent two-way ANCOVAs, one for each surface demonstrates that the three-way interaction is driven by a strong negative effect of water on adhesion for all four coatings on glass, but the absence of such an effect when adhesion is tested on OTS-SAM coated glass. Specifically, there was a significant drop in force when samples were tested underwater on the hydrophilic glass substrate compared to tests performed in air (Figure 5a). However, this did not occur on the hydrophobic OTS-SAM coated glass

(Figure 5b). Overall force values from samples tested on the OTS-SAM substrate in air were lower than those tested on the glass substrate in air. The ratio of Hamaker constants between OTS-SAM coated glass and glass is 0.87 (SI Text, section 3, Table S5)³³, and we expect the shear adhesion to be lower for OTS-coated glass. However, the measured ratio is much smaller (around 0.14). This ratio is also lower than that measured for adhesion of Tokay geckos on glass and OTS-SAM coated glass¹⁶. The differences could be due to much cleaner glass used in the shed experiments or due to the differences between the animal and shed experiments. In summary, adhesion appears to be highly sensitive to substrate wettability, either reducing adhesion on the hydrophilic glass substrate or maintaining adhesion on the hydrophobic OTS-SAM coated glass. Surprisingly, the surface chemistry of the setae does not have an affect on adhesion across all substrates and treatments, suggesting that setal surface chemistry is not critical to successful adhesion in air or water.

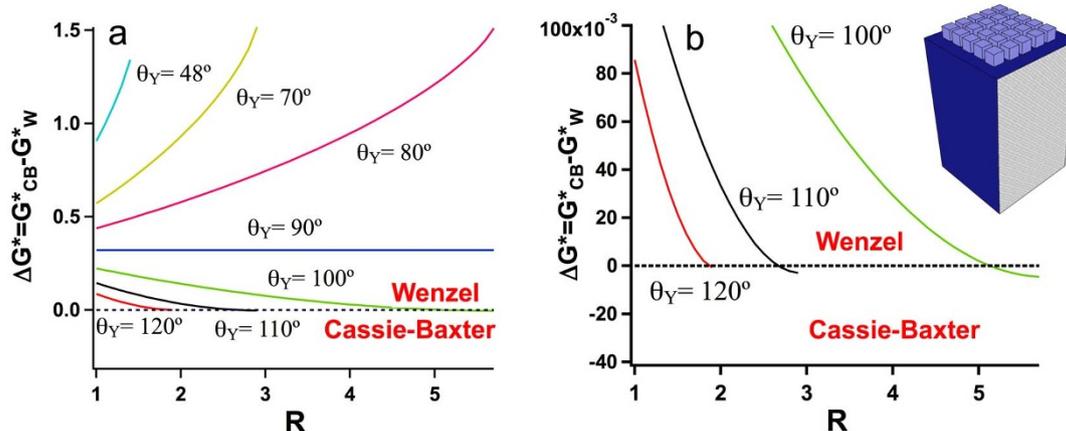


Figure 4 | Predictions of wetting state for untreated sheds as a function of intrinsic contact angles. (a) ΔG^* plotted as a function of R for different θ_Y . (b) The figure shows magnified plots for θ_Y of 100°, 110° and 120°. The inset shows a schematic representation of a section of the unit cell corresponding to gecko toe morphology is shown. A pillar shaped seta has a tier of smaller pillars on top of it. The complete unit cell consists of four such setae.



To investigate the experimentally measured results, we calculated the W_{wet}/W_{dry} ratio for B-S, P-S, M-S and F-S shed samples in contact with glass or OTS-SAM coated glass and modeled the predicted W_{wet}/W_{dry} ratios of shed samples in contact with the coated glass surface (SI Text, section 3.3, Table S6 and Table S7) using models derived elsewhere²². Model calculations for the glass surface were not possible in this context. Experimental ratios on glass clearly show that dry adhesion is favored (the ratio is below 1), whereas on coated glass adhesion in dry and wet conditions are virtually the same (the ratio is near 1) in all sample treatments. In contrast, model calculations predict that only the B-S and the F-S samples should be lower in air compared to water on the coated surface and the hydrophilic samples (P-S and M-S) should have much weaker adhesion in water than air. This is clearly inconsistent with our experimental results. During tests with the B-S and F-S samples in water, we could clearly see that a layer of air was always present at the shed surface, consistent with our hydrostatic pressure experiments. When testing on a hydrophilic glass surface, water formed a lubricating layer between the air plastron and the glass slide, preventing the hydrophobic sheds from contacting the surface and thus resulting in much lower adhesion (ratios are below 1). This is consistent with whole animal adhesion to wet glass^{16,22}. Likewise, when testing on the hydrophobic OTS-SAM coated glass, the air plastron on the B-S and F-S samples allowed for dry contact to occur, similar to whole-animal experiments which show a dry region of the toe is maintained when in contact with a hydrophobic surface²². What was intriguing however was the adhesion results of the hydrophilic P-S and M-S samples on the coated surface. As expected, the M-S and P-S samples wetted immediately underwater. However, we found that wetting only affected adhesion when tested on the hydrophilic glass surface, not the hydrophobic OTS-SAM coated glass. Our results suggest that wetting due to inherent surface chemistry does not affect adhesion in wet conditions, and rather substrate wettability is the driving factor in wet conditions. This is clearly contradictory to what we expect from theoretical models for adhesion in wet environment and observations of geckos sliding down surfaces when their toes become wet¹⁶.

Several factors may explain differences between the experimental and theoretical results. Firstly, small differences in mechanical properties, surface roughness and clumping in the P-S samples may have a significant effect on adhesion between uncoated and coated samples. In wool fibers plasma treatment may increase roughness by about 5 nm³⁴, and in a PECVD coating similar to that used in this study a Wenzel value of 1.19 (ratio of actual area divided by projected area) was found on flat surfaces after PECVD treatment³¹. Secondly, model predictions are based on normal adhesion between the shed surface

and substrate, however experimental measurements use shear geometry. Even though there is a correlation between normal and shear adhesion³⁵, in the case of M-S and P-S which wet almost instantaneously in water, it is possible that water drains out of micro and nano channels of the shed to establish temporary dry contact with the surface as it is sheared over the shed^{36,37}, a behavior which is not accounted for in the model. Additionally, it is possible that coating the setae with either polymer coating or by plasma treating the setae alters the mechanical properties of the setal array. However, it is unlikely that the large difference in shear adhesion force between the glass and the OTS-SAM coated glass is due to changes in the modulus of the setal array alone. Instead we expect changes in modulus to affect the shear adhesion in wet and dry conditions, where plasma treated setae or setae that are coated with a hydrophilic polymer may become softer in water, similar to results found when measuring setal modulus in highly humid conditions (>80% RH)³⁸. Finally, measurements of shear adhesion in shed samples suggest that maximum force (F_{max}) is reached by significant differences in the force profiles. F_{max} was reached either just before the sliding started, followed by a decrease in the force as the sliding continued, a case we refer to as stiction; or F_{max} was reached after sliding started, in which case the force either plateaued at the maximum value or continued to increase until it reached F_{max} , a case we refer to as friction (Figure 6). When we compared stiction and friction responses in air and water we found that P-S and M-S tested on OTS-SAM coated glass were significantly different, and the B-S samples tested on OTS-SAM coated glass were nearly significantly different. In fact, when comparing instances of friction to stiction it is very clear that friction dominates in all surface chemistries tested on OTS-SAM coated glass in air (Table S8). When tested in water however, there is no clear sample behavior on OTS-SAM coated glass (Table S8). Conversely on glass we only found that adhesion behavior in air and water was significantly different for B-S samples where stiction was the dominant behavior in air. Interestingly it appears friction may be more dominant in water for the B-S samples tested on glass. The association of either stiction or friction with specific treatment groups was very intriguing and clearly suggests an influence of shed surface chemistry on adhesion behavior and performance.

Summary. Tokay geckos (*Gekko gekko*) are native to wet tropical regions of South East Asia and because of this we expect their adhesive system to remain functional at high humidity and in the presence of surface water. We find that toe pad sheds coated with a nanometer-thick hydrophilic coating wet immediately, creating a super wetting state. In contrast, sheds with a hydrophobic fluorinated coating are superhydrophobic, even after prolonged

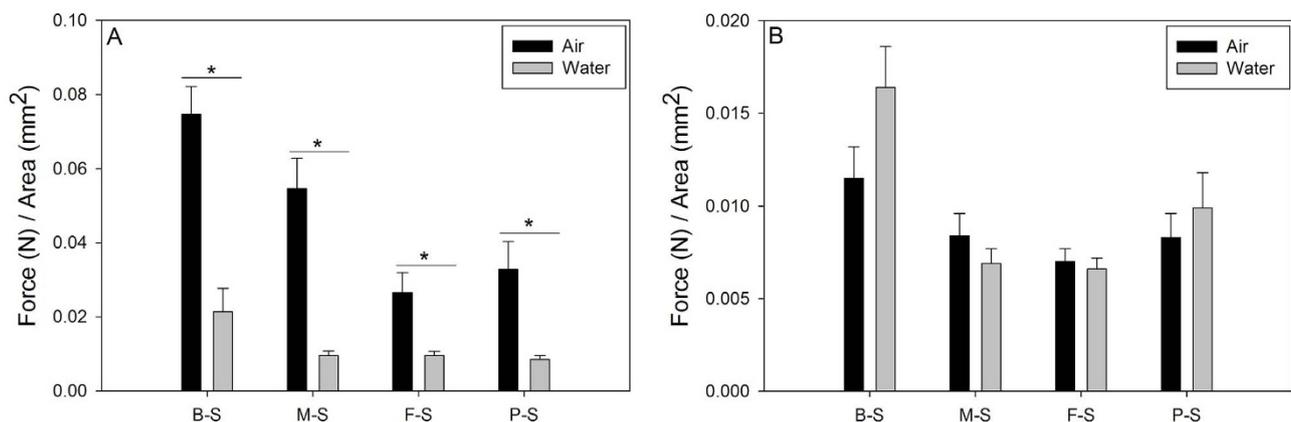


Figure 5 | Shear adhesion measurements as a function of surface treatments. Average force per unit area of Tokay gecko (*Gekko gekko*) skin sheds with either untreated (B-S), M-S, F-S or P-S treatments. Samples were tested on a clean hydrophilic glass slide (A) or on a glass slide coated with hydrophobic OTS-SAM (B). Eight samples in each coating and surface group were tested in air (black bars) and eight were tested in water (grey bars). Error is reported as 1 s.e.m. and significant differences are indicated with a *.



exposure to humidity. From these results we infer that the intrinsic surface chemistry of the gecko setae is more similar to hydrophobic surfaces with high water contact angles (150°). Nevertheless, after prolonged exposure to humidity, untreated samples wet similarly to hydrophilic-coated samples, although they do recover their superhydrophobic state upon drying. This suggests that the water contact angle for intrinsic surface chemistry of gecko setae is between 70 – 90° , resulting in a metastable Cassie-Baxter superhydrophobic state that changed to the thermodynamically more stable Wenzel state upon exposure to humid air. However, we cannot exclude the possibility that the wetting behavior results from an intrinsic surface chemistry of greater than 90° that restructures to a more hydrophilic surface after prolonged exposure to water or humid air. We also found that adhesion is significantly impacted by the surface chemistry of the substrate. As expected, adhesion in wet conditions on hydrophilic glass is much lower than dry adhesion. Surprisingly, adhesion in wet conditions for hydrophilic-coated sheds on hydrophobic OTS-SAM coated glass was almost the same as dry adhesion. This suggests that the hydrophilic structured surfaces (toe pad sheds) do not show the same trends as the hydrophilic flat surfaces (substrates where adhesion is made).

Our results raise several important questions about the characteristics of the gecko adhesive system. Gecko adhesion is very sensitive to the presence of water on hydrophilic surfaces but not hydrophobic surfaces. If the latter are better reflective of the surface chemistry of natural surfaces, this could help explain the abundance and success of geckos in wet tropical habitats. The hydrophobicity of gecko toe pads arises from roughness (fine hair-like structures) as well as surface chemistry (likely lipids), and the non-wetting (Cassie-Baxter) state of the toe pads should enhance the ability of the toe pads to exclude water at the contact interface on hydrophobic surfaces. Interestingly, our F-S coated toe pad sheds remained non-wettable under conditions that led to the wetting of the native toe pad sheds, raising the question of whether the critical pressure to prevent transition from the Cassie to Wenzel state in the natural toe pad is 1) not attainable by the natural system, 2) trades-off with some other performance characteristic (e.g., self cleaning, resiliency, etc.), or 3) does not translate into an adhesion effect that affects organismal fitness. Until the material components of gecko setae are better understood, we will not know the answers to these and other questions relevant to a deeper understanding of the ecology and evolution of gecko adhesion and the current limitations in synthetic mimics. We hope our findings motivate more research into the role of chemistry and the nature of the materials making up adhesive gecko toe pads.

Methods

Sample preparation. Samples were prepared from skin sheds that were collected from eight naturally molting Tokay geckos (*Gekko gecko*). Sheds were stored in a

-20°C freezer until sample preparation. This method preserved both the adhesive setal structure and multiple lamellae of each toe that contact the surface during adhesion, similar to the configuration we expect for whole-animal studies. We do not anticipate any significant structural, mechanical or chemical differences at the scale of the setal mats between the toe pad shed and the natural setae found on the toe pad of the animal. Samples were made by cutting away the setal toe pad region from the rest of the foot shed (non-adhesive region) and mounting the toe shed on adhesive tape (Scotch tape) with the adhesive hairs facing up. Sheds were attached by applying pressure to the inter-lamellae region (hairless region) using forceps. This was done along the toe shed, taking care to not disturb the adhesive hairs. Excess tape was cut away from the sample so that none of the synthetic adhesive could interfere with adhesion trials. A vacuum operated PECVD set up was used for the PECVD coating of the sheds. The PECVD of maleic anhydride was carried out at about 100 mTorr vapor pressure and PECVD of 1H,1H,2H-perfluoro-1-dodecene was carried out at about 200 mTorr. The PECVD process consisted of a standard three steps: precursor vaporization, plasma ignition and precursor vaporization. In synthetic systems the PECVD coating penetrates through carbon nanotube (CNT) forests³⁰ and also into the space between colloidal spheres³¹. In carbon nanotube forests, transmission electron microscopy shows that the coating is about 10 nm thick and even along the length of the nanotubes. We expect similar behavior in the gecko shed samples. All procedures using live animals were approved by the University of Akron IACUC protocol 07-4G and are consistent with guidelines published by the Society for the Study of Amphibians and Reptiles (SSAR 2004).

Characterization. The untreated samples and those treated with a deposited PECVD layer or by oxygen plasma treatment were characterized using SEM and XPS. A JEOL JSM-7401F field emission scanning electron microscope was used for SEM imaging. The samples were sputter coated with silver prior to imaging. Imaging was carried out in LEI mode with very low accelerating voltage, typically 1–2 kV, and an emission current of $20\ \mu\text{A}$. Chemical composition analysis was carried out using XPS. The spectra were acquired using PHI Versaprobe II that uses Al K α radiation. The data for survey spectra were obtained over 0 eV–1100 eV range of binding energy using a pass energy of 117.5 eV and step size of 0.5 eV. The high resolution spectra were acquired over a narrower range of binding energies (typically about 20 eV) using 11.75 eV pass energy and 0.1 eV step size. The IR spectra for fluoropolymer and maleic anhydride coatings on flat surfaces are provided in Figure S4 (SI) and Siffer et al.³⁹, respectively. The direct IR or Raman analysis of PECVD coatings on the sheds was not possible due to limited surface sensitivity of these two techniques. These measurements probe a depth of microns compared to 10–15 nm using XPS.

Wetting and adhesion tests. Contact angle measurements were carried out using 10–15 μL droplets of de-ionized water. At least two measurements were done for each sample and the average of the measurements was reported as the final value. PECVD coating deposited on a flat Si wafer surface was used for the intrinsic contact angle (θ_y). The water column measurements were done by introducing the sample into a water column contained in a transparent glass tube. We allowed the samples to equilibrate at a given height for about 5 minutes and then observed them for their appearance. Superhydrophobicity of the surface is characterized by shiny silver color of the sample whereas the wetted sample looks grey in color. Adhesion tests were performed at room temperature and 30–40% relative humidity. Samples were attached to a horizontally mounted force apparatus using double sided copper tape²¹. A nylon thread was fitted to the top of a clean glass slide and hooked to a motorized force reader which pulled at a controlled rate (Figure S3). Glass slides were cleaned in a base bath, blow dried with compressed N_2 followed by oven drying at 120°C prior to use. A second glass slide was coated with a self-assembled monolayer of octadecyltrichlorosilane (OTS-SAM), the water contact angle on the surface of OTS-SAM was $95^\circ \pm 2^\circ$ ⁴⁰. The method for forming the OTS-SAM coating is outlined elsewhere²². The glass plates used in these experiments weighed about 46 g and were laid over the sample prior to sliding, thus a uniform pre-load of 46 g was applied to

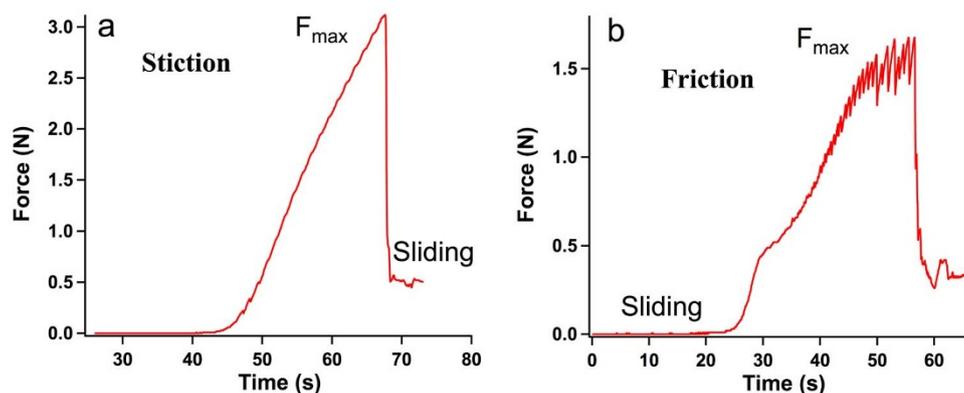


Figure 6 | Sticking and friction force profiles. The force profiles typically observed in the case of stiction (a) and friction (b). Stiction refers to F_{max} attained in the sliding experiments just before the sliding started; whereas, friction refers to reaching F_{max} after sliding has occurred in the shear adhesion measurements.



each sample. The weighted slide was then pulled along the sample in the direction of adhesion. Slides were cleaned with ethanol and water after each sample. We tested each coating group (B-S, P-S, M-S and F-S) on the hydrophilic glass slide and the hydrophobic OTS-SAM coated slide in both air and water. Water trials were done the same as air trials, except that after the sample was positioned inside a small open chamber water was poured in so that the shed was completely submerged and covered with a layer of water. Only the maximum force reading during the sliding was used for analysis. Occasionally we used the maximum force prior to sample failure (ripped or damaged) for analysis. Sample sliding behavior was also noted in each sample to assess whether stiction or friction occurred. Total two-dimensional area of the samples was measured after the experiment using a dissecting microscope and ImageJ software (National Institutes of Health, Bethesda, MD, USA). Area was included as a co-variate in statistical analyses however area was not significant and all interactions with area were subsequently removed from analysis.

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Author contributions

I.B., A.Y.S. and E.L.P. performed the experiments. I.B., A.Y.S., P.H.N. and A.D. analyzed the data. I.B., A.Y.S., P.H.N. and A.D. wrote the paper. I.B., A.Y.S., E.L.P., P.H.N. and A.D. discussed the results and commented on the manuscript.

Additional information

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