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A Comparison of Gecko Adhesion on Soft Substrates

Alexis Schnarrenberger

Introduction:

Gecko adhesion has been studied among various substrates and environments for years. Geckos have highly specialized toe pads that permit adhesion through the use of Van der Waals forces. The gecko toepad is a hierarchal array of beta-keratin setae, that end in nanoscale contact points called spatulae. This allows the gecko to create intimate contact with the surface and adhere to it (Autumn et al., 2014).

Soft substrates have not been heavily investigated in gecko research. In their natural environment, geckos navigate on a variety of substrates, both hard and soft. Therefore, we may expect that geckos have developed a system that allows them to adhere to soft surfaces, as well as hard surfaces. Stark et al. (2015) researched gecko adhesion on a synthetic shark skin called Sharklet ® that was designed to inhibit algae adhesion. As a control, they tested gecko adhesion on a smooth polydimethylsiloxane (PDMS) substrate. Unexpectedly, the geckos could not stick to the smooth PDMS under dry conditions.

Klittich et al. (2017) followed this study to determine if softness was the reason the geckos could not adhere to the PDMS substrate. They investigated gecko adhesion on substrates of PDMS, but with varying degrees of thickness to alter the softness of the material. They found that the geckos were unable to support their own body weight on vertical substrates, except for the PDMS with thickness of 2 nm. This was contrary to what was expected, as they predicted that a softer substrate would increase surface contact for Van der Waals forces to occur. Through additional testing, it was found that the lack of adhesion was not due to a difference in surface chemistry, capillary forces, or coefficient of friction, as these were negligible between the PDMS surface layers. Additionally, they tested adhesion on substrates with higher surface modulus, and adhesion dramatically increased. Thus, it was concluded that surface layer softness was an important factor in gecko adhesion on the PDMS substrates and that geckos cannot stick to soft surfaces. Klittich et al. (2017) came to their conclusion that geckos cannot stick to soft surfaces based on data collected from only one type of soft material. Given that geckos appear to stick to many different kinds of soft surfaces in their natural environments, it begs the question, what kinds of soft surfaces can geckos adhere to? In this study we examined gecko adhesion on various soft substrates and looked for patterns between gecko adhesion and material properties. Based off of previous results, we expected gecko adhesion to be low on all soft surfaces.

Materials and Method:

Whole Animal Experimentation:

Experiments used seven adult tokay geckos (*Gekko gecko*) weighing 74.30 ± 10.97 g and followed the University of Akron IACUC protocol 16-08-14-NGC. The geckos were housed in the University of Akron Research Vivarium, where they were kept at a relative humidity of 75-80% and a temperature of 25°C. The geckos were fed three times a week with vitamin and calcium dusted cockroaches and baby food as a fruit supplement. They were also misted with water daily and health checked to assure they were healthy for experiments. Prior to experiments, the geckos had their toenails clipped as to inhibit clinging with claws on the soft substrates.

Trials were conducted in an environmental chamber with an average temperature of 24.5 ± 0.1°C and relative humidity of 39.8 ± 0.3%. The geckos were placed in the chamber thirty minutes prior to starting trials to allow them to acclimate to the environment. Three substrates were used that have similar relative softness (McMaster Carr), which included neoprene, natural rubber, and nitrile rubber. A gecko was pulled on a substrate three times and the maximum force of the three was recorded for the day, which was then repeated two more times on two separate days for a total of 3 maximum pulls per substrate per gecko (see Niewiarowski et al. 2008). Prior to experimentation, the substrates were cleaned with soap and water and attached using Velcro to a force rig, Substrates were cleaned and dried between each pull for each gecko.

The geckos had two harnesses attached at the pelvis, one dorsally and the other ventrally, which were attached to the force sensor and pulled at a constant speed with a motor. The gecko was placed on the vertical substrate and made to take a step with each foot to allow natural adhesion to the substrate before being pulled. A maximum shear force was recorded when all four feet started to slip on the substrate, with a maximum pull force of 20 N set so as not to hurt the geckos. The geckos had a rest period of at least 24 hours before the next set of pulls. The order of the geckos being pulled, and the substrate being pulled on by each gecko were randomized for each trial day to minimize experimental bias.

Surface Characterization:

Contact angle was used to characterize surface energy, where contact angle is the angle between the solid-liquid interface and liquid-air interface. Contact angle was found using a Krüss DSA100 with a separate syringe attached to the instrument to make drops manually. Three squares around 2 square inches each were cut from three separate places on long rubber sheets. They were then cleaned with soap and water as in the gecko adhesion trials. The contact angle syringe was cleaned before each session of contact angle to ensure no contaminants from the glass affected the results. The syringe was cleaned by sonicating in toluene, acetone, ethanol, and ultra pure water (milipore) for one hour each. The syringe was dried in the oven at 125°C and plasma cleaned with a Harrick PDC-32G plasma cleaner for 5 minutes. The syringe was then filled with liquid and placed on the instrument to perform pendant drop and contact angle. A pendant drop was first performed three times to calculate the surface tension of the liquid to make sure the liquid was clear of dirt and abnormalities. Once the pendant drops matched literature values, contact angle was found on each of the three substrates. Each square had three drops placed with a total of nine drops per substrate type, with a picture of the drop taken with Krüss Drop Shape Analysis software. The angle of the drop was analyzed with ImageJ using the contact angle plug-in (Marco Brugnara, NIH). Contact angle was performed with both ultra pure water and with diiodomethane (Sigma-Aldrich) to look at both polar and nonpolar elements of the surface energies for these substrates as suggested in Krüss (1999). The contact angles were used in three equations from Krüss (1999) to find surface energy of the substrates. The first equation, $\sigma_S^D = \sigma_L (\cos\theta + 1)^2 / 4$, where σ_S^D is the dispersive component of surface energy for the solid, σ_L is the surface energy of the liquid, and θ is the contact angle. This equation used the diiodomethane data to determine the dispersive component of surface energy for the substrate. The next equation, $(\sigma_L^D)^{1/2} (\sigma_S^D)^{1/2} + (\sigma_L^P)^{1/2} (\sigma_S^P)^{1/2} = \sigma_L (\cos\theta + 1) / 2$, where σ_L^D is the dispersive component of surface energy for the liquid, σ_L^P is the polar component of surface energy for the liquid, and σ_S^P is the polar component of surface chemistry for the solid. This used the values from the previous equation as well as the water contact angle data to find the polar component of surface energy for the substrates. The final equation, $\sigma_S^P + \sigma_S^D = \sigma_S$, where σ_S is the surface energy of the solid. This equation sums the values from the previous two equations to give total surface energy for the substrates. Two surface modulus measurements were taken from each substrate using a TI Premier nanoindenter to determine stiffness of the rubbers. Procedure was done as explained in Klittich et al. (2017), where the slope of the force displacement curve was used to calculate surface modulus.

Statistical Analysis:

A mixed model analysis of variance (ANOVA) was used to analyze the maximum shear adhesion force across treatments. The overall maximum force per treatment per gecko was used for the analysis, with the independent variable being the substrate and the dependent variable being the maximum shear force. This statistical analysis treated individual geckos as a random effect. A Tukey Honest Significant Difference test was then used to determine significant differences between the specific treatments. All statistical analyses were done using JMP Pro 13.

Results:

Whole Animal Experimentation:

Gecko adhesion was tested by measuring maximum shear force when pulled on three soft substrates: natural rubber, nitrile rubber, and neoprene rubber. There was a significant difference between treatments for maximum shear force ($F_{2,12} = 13.55$, $P = 0.0008$). As shown in **Figure 1**, the natural rubber supported significantly higher maximum shear force than the nitrile rubber ($P = 0.0276$) and the neoprene rubber supported a significantly higher maximum shear force than the nitrile rubber ($P = 0.0006$). The average maximum shear force between the natural rubber and the neoprene rubber were not significantly different ($P = 0.1148$).

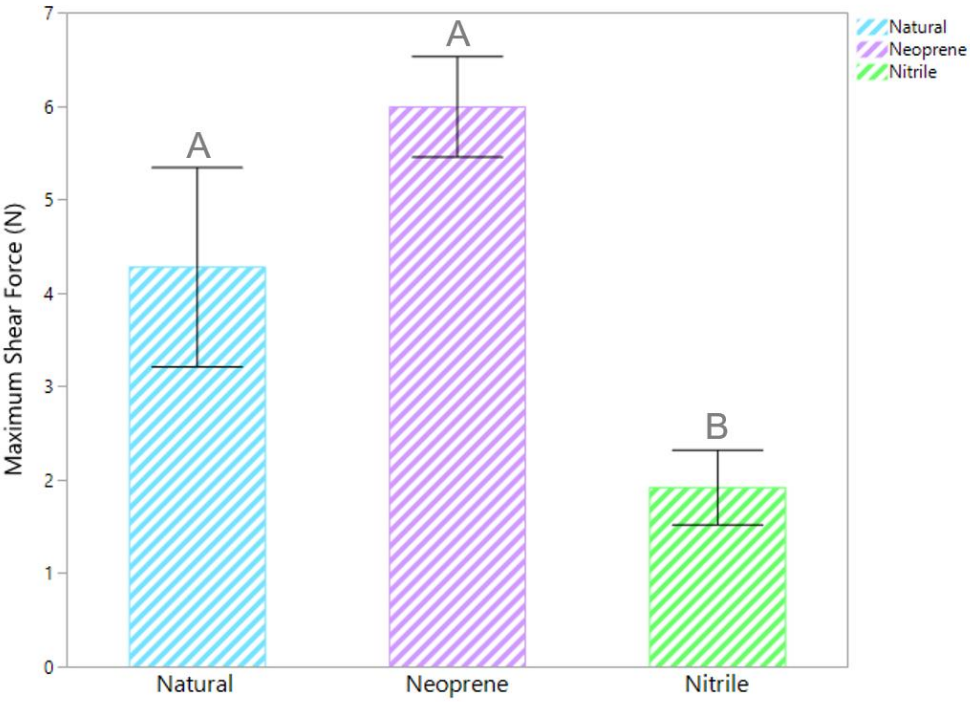

Figure 1. Average maximum shear force for each of the three substrates. There was a significant difference in maximum shear force between natural rubber and nitrile rubber (P = 0.0276) and a significant difference in maximum shear force between neoprene rubber and nitrile rubber (P = 0.0006). Different letters indicate significant differences between treatments. Error bars represent ± 1 SEM.

Surface Characterization:

Water contact angle was measured for the three substrates of nitrile rubber, natural rubber, and neoprene rubber. The pendant drop to determine surface tension of the water was found to be 73.01±0.63 mN/m, which matches literature value for water (Krüss, 1999). The natural rubber had an average water contact angle of 117.04 ± 1.81° which was the largest of the three rubbers as shown in **Figure 2**. The next largest water contact angle was the nitrile rubber with an average water contact angle of 103.61 ± 0.91°. The lowest water contact angle was the neoprene rubber with an average water contact angle of 99.37 ± 1.08°. All of the rubbers, however, were hydrophobic and the water contact angles did not appear to follow any trends in relation to whole animal adhesion.

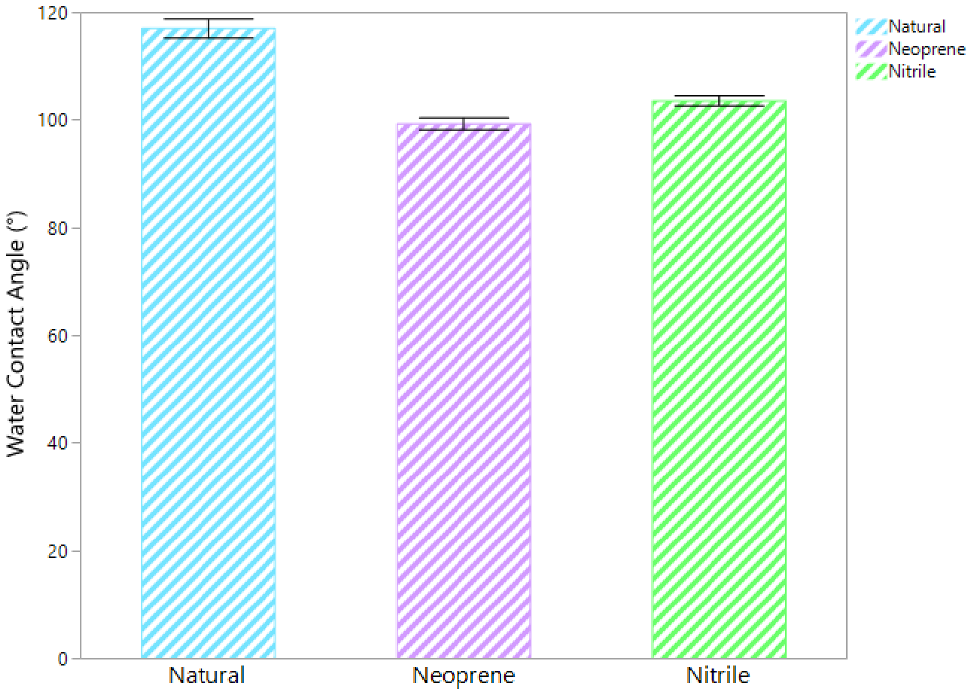

Figure 2. Average water contact angle for each of the three substrates. Error bars represent ± 1 SEM.

Diiodomethane contact angle was also found for the three rubber substrates. The pendant drop to determine surface tension of the diiodomethane was found to be 50.98 ± 0.13 mN/m, which matches literature value for diiodomethane (Krüss, 1999). The natural rubber had an average diiodomethane contact angle of 76.30 ± 0.92° which was the largest of the three rubbers as shown in **Figure 3**. The next largest diiodomethane contact angle was the nitrile rubber with an average diiodomethane contact angle of 70.00 ± 1.67°. The lowest diiodomethane contact angle was the neoprene rubber with an average diiodomethane contact angle of 39.50 ± 1.78°. The neoprene rubber had a much smaller diiodomethane contact angle than the other two rubbers, which were more similar in their contact angles. While the neoprene rubber had what looked like a major difference in contact angle, the diiodomethane contact angles did not seem to follow any trend with differences in gecko adhesion.

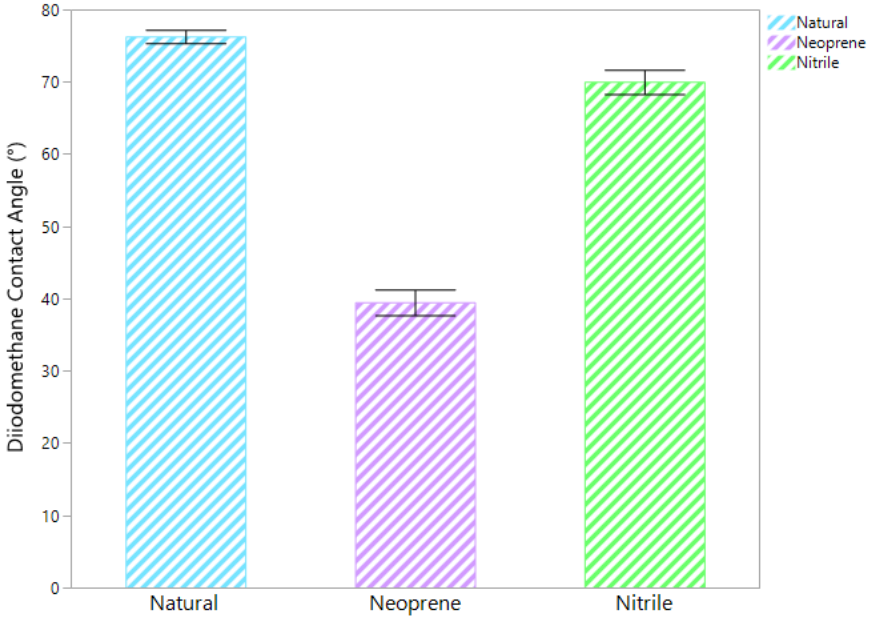

Figure 3. Average diiodomethane contact angle for each of the three substrates. Error bars represent ± 1 SEM.

Surface energies were calculated for the three rubber substrates using the water contact angles and the diiodomethane contact angles. The neoprene had the largest surface energy with 40.14 ± 0.94 mJ/m^2, as shown in **Figure 4**. The natural rubber and nitrile rubber had similar surface energies, with nitrile having a surface energy of 23.30 ± 0.88 mJ/m^2 and natural having a surface energy of 19.91 ± 0.62 mJ/m^2. Changes in surface energy do not appear to reflect changes in adhesion.

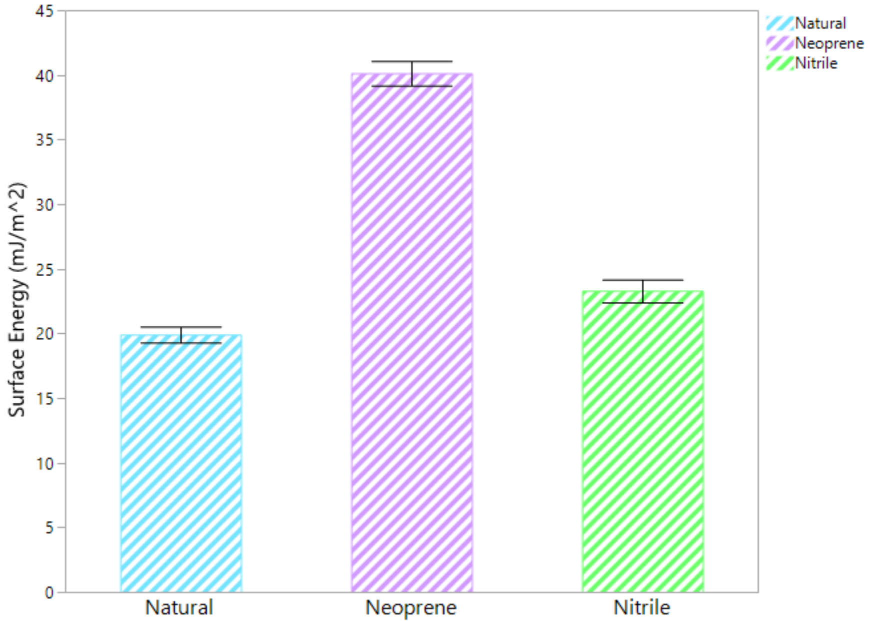

Figure 4. Average surface energies for each of the three substrates. Error bars represent ± 1 SEM.

Nanoindenter was used to determine surface modulus of each of the three rubber substrates: nitrile, natural, and neoprene. Neoprene rubber had an order of magnitude larger surface modulus than the other two rubber substrates, as shown in **Figure 5**, with a modulus of 81.38 ± 23.60 MPa. The other two substrates were an order of magnitude smaller that neoprene rubber, with nitrile rubber slightly higher with a modulus of 11.36 ± 4.42 MPa. The smallest modulus was natural rubber with a modulus of 8.44 ± 0.05 MPa. This shows that the neoprene rubber was much harder than the other two rubber substrates while the natural rubber and nitrile rubber were not that different in terms of softness, but this does not seem to explain any differences in gecko adhesion.

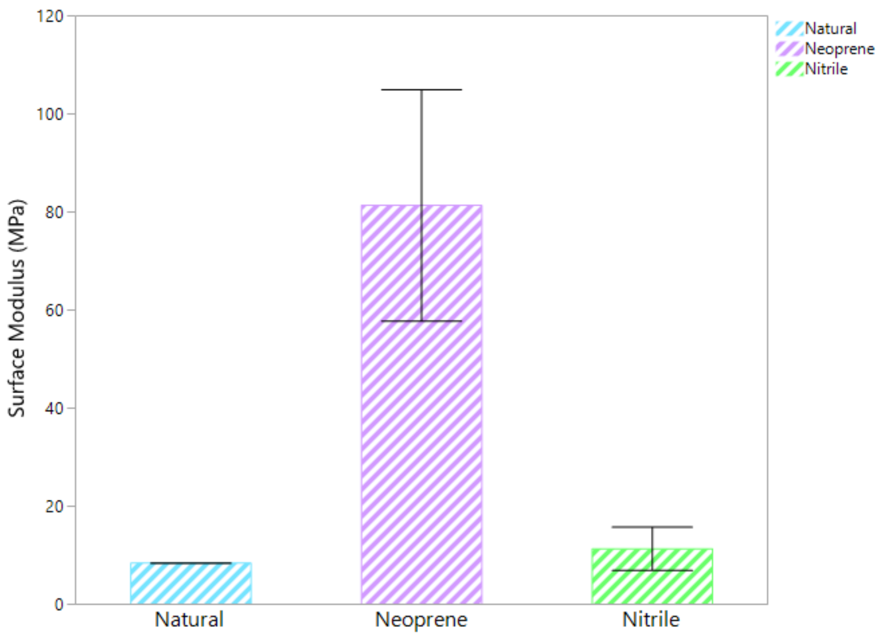

Figure 5. Average surface modulus (MPa) for each of the three treatment groups with error bars representing ± 1 SEM.

Discussion:

This experiment tested gecko adhesion on soft substrates. Due to the intimate contact the gecko spatulae make with the surface, it was thought that soft substrates should allow closer contact with more of the toepad than hard surfaces, and therefore they should be able to stick. However, previous work on this subject concluded that geckos cannot stick to soft surfaces (Klittich et al., 2017).

The results from the current experiment showed that geckos can stick to soft substrates, although to some better than others. When trying to understand why geckos adhered to some of the soft substrates more strongly than others, surface characterization tests were performed. The water contact angles are similar between all of the three substrates, in addition to being similar to 1000 μm PDMS from Klittich et al. (2017), which should then not be expected to cause any dramatic difference in adhesion. The differences between the water contact angles also did not follow any trend to suggest that water contact angle alone would cause any differences in adhesion. The diiodomethane contact angles were very close between the natural rubber and the nitrile rubber while the neoprene rubber had a far lower contact angle. Even though not all of the substrates were close to each other in diiodomethane contact angles and the neoprene was much lower, the data does not follow trends that would explain differences in gecko adhesion; there was no significant difference in maximum shear adhesion between natural rubber and neoprene rubber while the diiodomethane contact angles had a large difference between natural rubber and neoprene rubber. These contact angles measured were then used to calculate the surface energy for the three substrates. The surface energy of nitrile rubber and natural rubber were closer together than the neoprene rubber, which was much larger than the other two. However, this data did not seem to follow any trends that would explain that surface energy alone caused changes in gecko adhesion.

The surface modulus data found from the nanoindenter showed that neoprene was notably harder that the other two substrates. However, the surface modulus data did not follow a trend that would suggest that it caused differences in adhesion. While the neoprene had the largest surface modulus and the natural rubber and nitrile rubber were very similar, the shear adhesion testing showed both the natural rubber and neoprene rubber had significantly higher shear adhesion than the nitrile rubber. Furthermore, surface modulus found in Klittich et al. (2017) for the 1000 μm PDMS substrate, which they did not stick to, was 7 MPa which is similar to the surface modulus of the natural rubber and nitrile rubber. This suggests that while surface modulus may have an effect of gecko adhesion on soft substrates, it is not the sole reason gecko adhesion varies in this experiment.

In contrast to previous work under laboratory conditions, this study demonstrated that geckos can stick to soft substrates. While this study seems to counter the results of previous work suggesting that geckos have trouble sticking to soft substrates, it raises more questions for the future. One such question is that if contact angle and surface modulus do not affect gecko adhesion alone, what causes geckos to adhere to some soft substrates better than others? An idea to be explored in the future is the role of roughness on adhesion, not just in relation to soft surfaces, but all surfaces. Other factors may be important when looking at gecko adhesion, and gecko adhesion on soft surfaces has been shown to be more complex than previously thought.

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