Suction as a Mechanism of Attachment in Chitons

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Abstract

Chitons, like other mollusks, rely upon a variety of adhesive forces to combat factors such as wave action and predation in the intertidal. In this study, we explore what effect suction has upon a chiton's total force of attachment and tenacity, as well the impact of substrate roughness. In this experiment, we found that suction plays a significant role in chiton attachment over a range of roughness. We also found that a chiton's adhesive tenacity decreases significantly upon rougher textures.

Introduction

In a marine environment, attachment to substrate is vital to the success of many invertebrates, particularly to those in the intertidal. Wave action, tidal flow, and predation are problems that these organisms combat through a variety of mechanisms. Among these attachment mechanisms are friction, suction, glue adhesives, hooks, and clamps, which help the animals stay in place. For many sessile aquatic animals, glue is a dominant source of attachment force (Ditsche and Summers 2014). For many organisms, suction plays a large role in attachment as well. Some organisms, such as many species of mollusk, use a combination of these two mechanisms to stick to a substrate. Cephalopod suckers depend almost solely upon suction (Vogel, 2013). Smith (1991) found that in the case of limpets temporary attachment occurs. Suction is used mostly during locomotion, which depends upon a less stable, more plastic state of attachment. Glue is predominantly used for a stronger, longer-term attachment, suggesting that glue is perhaps a stronger and more reliable mechanism. Transitory attachment involves attachment during locomotion and has been observed in most mollusks. In this case, a viscous film is secreted and an organism will slide over it in order to maintain a constant seal between its foot and a substrate (Ditsche and Summers 2014).

Like limpets, chitons belong to the Phylum Mollusca, which often use a mixture of suction and glue to attach to a substrate. In 1911 and 1916, G.H Parker laid the groundwork for the study of chiton attachment forces with his observation of its attachment and locomotive physiology. These observations were fairly basic and did not involve formal testing, which left the questions of exactly how and with how much force chitons attach to a substrate virtually unexplored. Parker made an important assumption, which will be posed as the hypothesis driving this experiment. Parker asserts that chiton attachment "depends almost exclusively on suction"(Parker, 1916). This suggests that chitons may be very successful with attachment upon solid surfaces, but may be significantly less successful upon surfaces with which it cannot form a watertight seal.

Suction force is based upon a difference in pressure between the air or fluid inside the "suction cup" concavity and its ambient pressure. After an air- or watertight seal is established between the edge of the suction cup and the surface to which it clings, air or fluid is forced out of the concavity in the middle and the suction cup pulls away from the surface to naturally restore its original shape, which expands its inside volume. Because of the seal, no air or fluid is allowed in as the inner cavity expands. This results in a lower pressure inside the cavity compared to ambient pressure outside the suction cup. The higher ambient pressure pushes the suction cup against the surface, allowing it to stick with considerable force.

In the case of chitons, no distinct "suction cup" structure exists. Instead, the ventral area of the chiton consists of a muscular foot surrounded by a cavity and a concentric section of mantle tissue called the girdle (*fig.1*)

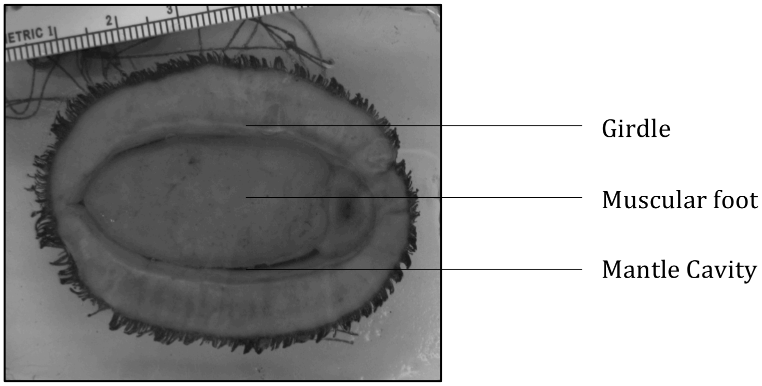

fig. 1: M. muscosa (ventral view)

Despite the relative complexity of the attachment surface of a chiton, attachment forces for the entire area will be investigated rather than each area's contribution to overall force. It is clear, though, that to utilize suction force, a chiton (like a suction cup) must maintain a seal around the edge of its ventral area. Therefore, we should see some decrease in attachment force upon rougher substrates, as it is more difficult for a suction-cup-like structure to maintain a seal on a rough surface.

In this study, we measure the impact of suction upon the overall attachment force of a chiton. To do so, we measure total force of attachment upon solid substrates and upon those with holes.

Methods

Animals

Mopalia muscosa (Gould, 1846) specimens were collected on-site in the intertidal at the University of Washington Friday Harbor Laboratories on San Jan Island, WA. They were kept in a seawater tank with a flow-through system for the duration of a month until the force measurements were performed.

To account for size variation between chiton specimens, a measurement of each attached chiton's foot surface area was taken. A photograph of each attached chiton specimen was taken with a ruler for size reference before testing began. A computer program (image J) was used to determine the surface area of each attached foot. Total attachment forces were measured in N, and tenacity in N/m^2.

Force Measurements

The contribution made by suction to a chiton's overall adhesive force was determined by measuring the total attachment force and tenacity of chiton specimens. To do this, we used an MTS Synergie 100 (Cary, NC, USA) materials testing system using a 500 N load cell to measure the force necessary to detach chiton from a solid substrate using upward force. 14 total specimens were tested over the course of the experiment. Each specimen was allowed 4 hours to attach to a solid substrate plate before testing. They were then attached to the crosshead of the MTS by an apparatus made of SplashZone epoxy, small Plexiglas plates, and heavy-duty fishing line, which was affixed to their dorsal plates. The specimen, once fully attached to the substrate, was placed in a special aquarium filled with seawater fastened to a base under the crosshead of the MTS. The fishing line attached to the specimen's dorsal surface was looped over the crosshead of the MTS. (*fig. 2).* The MTS pulled upwards at a speed of 1m/min until the specimen was detached completely from the substrate plate. The maximum amount of force necessary to detach the specimen (also known as the force at failure or pull-off force) was recorded. The same tests were done using perforated substrates. The perforations removed the chiton's ability to use suction as a mechanism of attachment. These forces were compared to determine whether suction contributes significantly to the specimen's attachment on a solid surface. To extend the reach of the experiment, we performed the tests described above with a series of substrates of varying roughness. Three levels of roughness were tested, with and without perforation. Using these tests, we investigated the effect of substrate roughness upon *M. muscosa*'s use of overall force of attachment and use of suction.

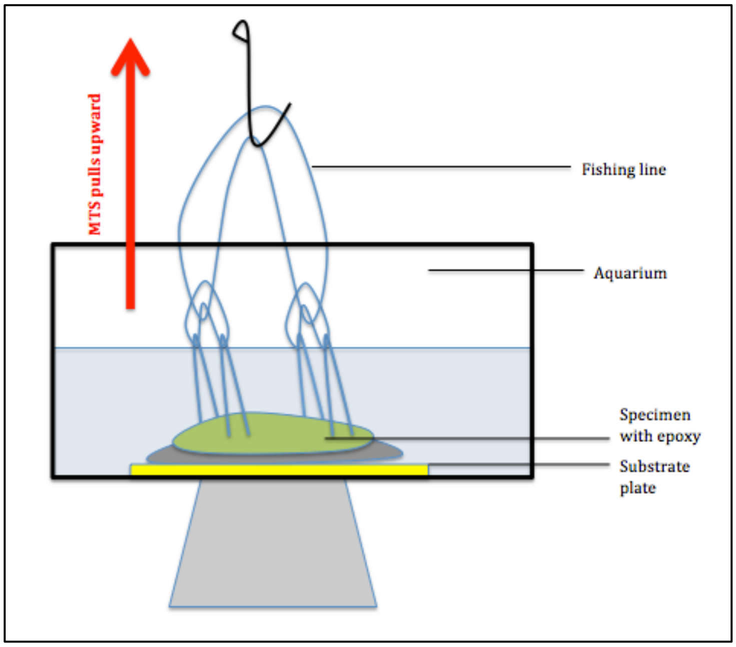

Fig.2: Experimental setup of force measurements

Substrates

Artificial substrates were used in order to provide identical surfaces of varying roughness for each trial, which allowed for consistency between trials and comparability of data. Substrate plates were prepared using a negative molding technique. Each plate was approximately 3in x 3in and about 2mm thick, and was made of an epoxy resin (Low Viscosity Spurr Kit, SPI Supplies®,West Chester, USA). Negative molds were made by casting a smooth glass surface, sandpaper (P60; Buehler® 273 Carbimet, Lake Bluff, Illinois, USA, matching grain size 269 µm), and a coarse texture handmade by Petra

Ditsche (Ditsche, et al., in press) with grain size of 1000-2000 µm. These substrate levels are referred to as "smooth", "0.269mm", and "1-2mm", respectively, in this study. Four substrates of each roughness level were used, two with holes and two without (*fig.3*).

To encourage specimens to attach squarely on the substrate plates, they were placed upon the plate, after which we placed plastic and wire mesh cages over them to inhibit locomotion during the 4-hour attachment process (*fig.4*).

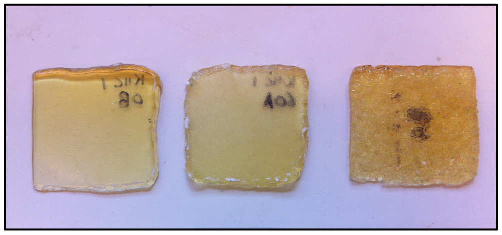

fig. 3: Substrate plates, right to left: smooth, 0.269mm, 1-2mm

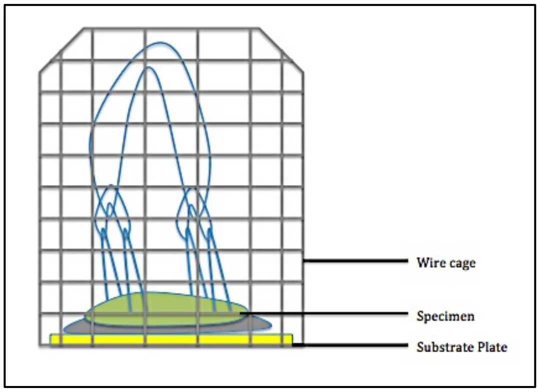

fig. 4: Specimen under cage during

Results

When analyzing the data collected in this study, adhesive forces could be measured as total force at failure measured per specimen (N), or, to account for a possible relation between specimen size and overall adhesive force, as tenacity (kPa). Both data were collected and we determined that there is only a slight trend between specimen attachment surface area (surface area of the ventral side attached to a smooth substrate) and its total adhesive force (linear regression, $R^2 = 0.24$) (*fig.5*). Therefore, either unit of measure may be used.

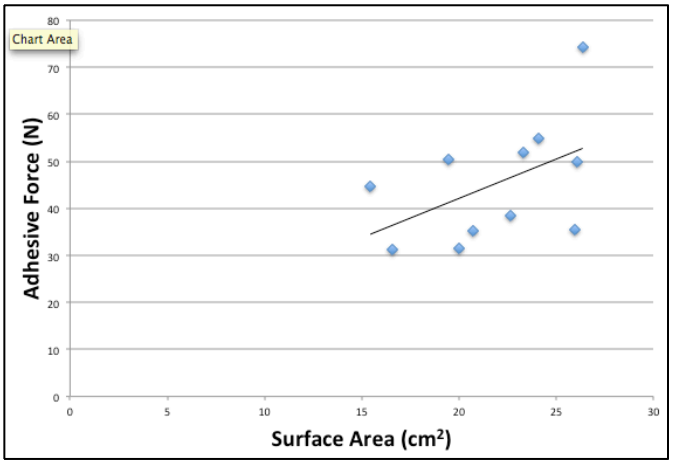

fig. 5: Relationship between surface area and adhesive force of specimen on smooth, solid substrate. $R^2=0.24$

To determine whether holes in a substrate had an impact upon attachment force and tenacity in the case of each roughness, paired and unpaired t-Tests were run comparing the tenacities measured on substrates with and without holes for each roughness. Both kinds of t-Tests were done to account for the fact that in every case there were specimens who had not been tested upon the same roughness with and without holes. This was a result of specimens dying during the testing process. Therefore, sample sizes were smaller for paired t-Tests. A significant difference was found in both in attachment force and tenacity between trials with solid substrates and those of the same roughness with holes. These differences were observed significant for each of the three levels of roughness (t-test, $p < 0.05$, Appendix A.). For the 1-2mm substrate roughness, the paired t-test yielded a p-value >0.05. This may be attributed to its small sample size, as the unpaired t-Test, containing 5 more specimens, yielded a p-value of 0.02.

A significant difference was observed between tenacities measured on different solid substrate roughness levels as well. This difference existed between the smooth solid and the 1-2mm solid (t-test, $p < 0.001$) and between the 0.269mm solid and the 1-2mm solid (unpaired t-test, $p < 0.001$, paired t-Test, $p = 0.001$). There was no significant difference between tenacities measured on the smooth solid substrate and the 0.269mm solid substrate (t-test, $p > 0.05$) (Appendix A.)

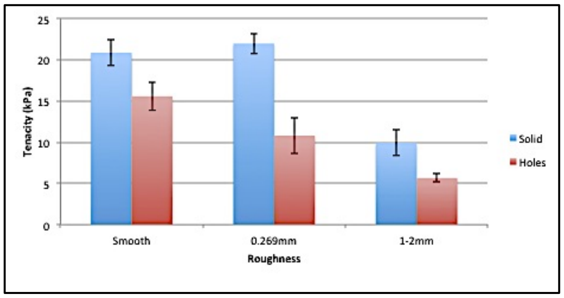

fig. 6: Effect of substrate roughness and holes on tenacity. For each roughness, unpaired t-Test, p-value < 0.05; mean, +/- standard error

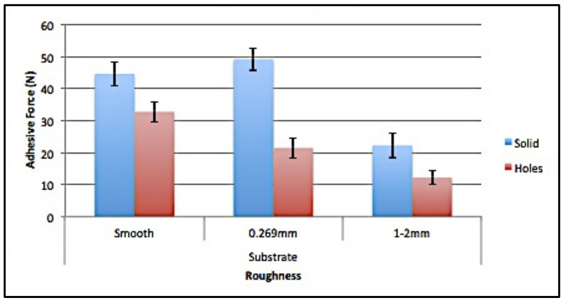

fig. 7: Effect of substrate roughness and holes on adhesive force

Discussion

This study indicates that suction plays a large role in chiton adhesion to substrates of varying roughness. Because chitons displayed a significantly lower tenacity upon substrates with holes than upon solid substrates regardless of texture, we can conclude that this decrease in adhesive strength was due to chitons' inability to utilize suction as an attachment mechanism. When considering this result, it must be noted that some data was missing due to the complications associated with using live animals as specimens. Throughout the course of the experiment, specimens should have been tested upon each combination of roughness and solid/holes twice. By the 7th out of 12 trials, up to 7 specimens were unable to attach to substrates within the 4-hour attachment time given between each trial. Therefore, these specimens were not tested on many of the substrates. Had all of the specimens attached successfully throughout the entire experiment, sample sizes for substrates of each roughness with and without holes would have been larger. The results of this study showed definite trends, but for further experiments, either fewer trials per specimen or more time for attachment between trials would be suggested.

Much like chitons, other mollusks are known to use suction as a force of attachment as well. In a 1991 study, Smith found that limpets use suction exclusively for up to an hour after having been moved, after which they form a stronger bond with the addition of adhesive glue. A film of semi-solid material was left behind on substrates after chiton detachment during this study, which suggests that chitons use glue to account for the remaining adhesive force measured during trials in which the chiton's use of suction was inhibited. A limpet's mean tenacity when using both suction and glue on a smooth substrate is 130 kPa (Smith, 1991), which is much higher than the chitons in this study, whose mean tenacity on a solid substrate was 20.9 kPa. Both of these tenacities could be considered quite low next to that of a mussel or barnacle, which have average tenacities of 105 kPa and 320 – 750 kPa, respectively. This extreme difference in adhesive strength can be explained by a fitness trade-off occurring between attachment force and mobility. Organisms that need move around their environment generally attach with lower tenacity than their completely sessile counterparts (Ditsche and Summers, in press).

This study also indicates that a chiton's strength of attachment depends upon the roughness of its substrate as well. While chitons readily attach to many textures of substrate, their ability to cling to these substrates seems to wane as its texture becomes very rough. The specimens in this study attached with similar force to the smooth and the 0.269mm substrates, which were both of relatively similar texture when compared to the more extreme texture of the 1-2mm grit substrates. When attached to the 1-2mm substrates, their strength of attachment decreased dramatically. In a recent study, Ditsche et al. found that Northern Clingfish (*Gobiesox meandricus,* Girard, 1858), which use a specialized suction disc on their ventral surface, have a threshold of substrate roughness to which they can cling as well. In their case, this threshold corresponds to body size (Ditsche, et al., in press), whereas in this study, we found that chiton size does not have a clear effect on the total attachment force.

Our findings suggest that chitons, in order to maximize adhesive force, might prefer to settle upon smoother, solid surfaces in the intertidal rather than coarser or less solid substrate, such as sediment. Chitons are found most frequently in the Lower and Middle Littoral zones of the intertidal (Misra and Kundu, 2005, Veras et al., 2013), which are more exposed to wave action than the Upper Littoral. Species richness is also highest in this area of the intertidal (Veras, et al., 2013). A chiton's need to attach with great force to a substrate is most likely an adaptation to resist detachment due to wave action, as well as a form of defense against predators, which allows it to compete with the abundance of other species in the area.

As a follow-up to this experiment, it would be worthwhile to test Parker's claim in his 1916 review that the chiton foot "sucks locally", meaning that portions of the foot use suction independently of the rest. If this were proven true, then it may be possible to refute the findings of this study, seeing as holes in a substrate would amount simply to a slight decrease in attachment area rather than an inhibition of suction.

The results of this study conclude that suction has a significant impact upon a chiton's overall attachment force; a trait shared by other mollusks as well as benthic invertebrates. We have also found that *M. muscosa*'s adhesive tenacity upon extremely rough substrates is significantly lower than upon smooth or slightly textured surfaces, explaining why chitons may choose certain substrates over others in the intertidal.

Acknowledgements

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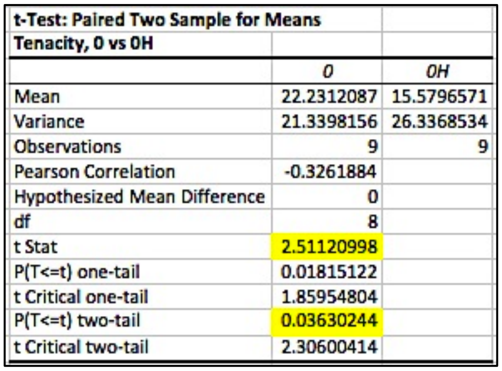
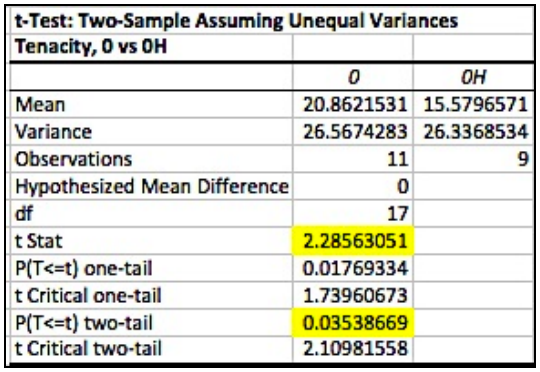
Appendix A. Summary of t-test Data

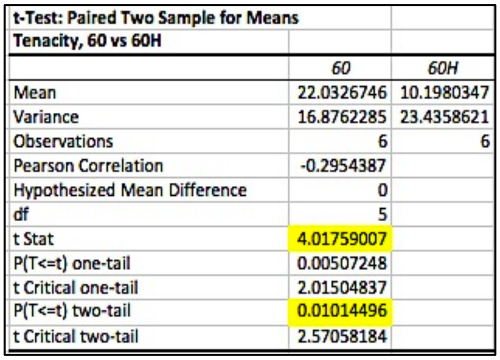
Holes versus no Holes Comparisons



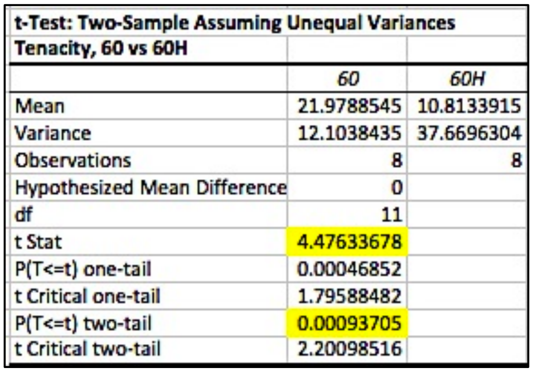
t-Test: Paired Two Sample for Means		
Tenacity, 0 vs 0H		
	0	*0H*
Mean	22.2312087	15.5796571
Variance	21.3398156	26.3368534
Observations	9	9
Pearson Correlation	-0.3261884	
Hypothesized Mean Difference	0	
df	8	
t Stat	2.51120998	
P(T<=t) one-tail	0.01815122	
t Critical one-tail	1.85954804	
P(T<=t) two-tail	0.03630244	
t Critical two-tail	2.30600414	



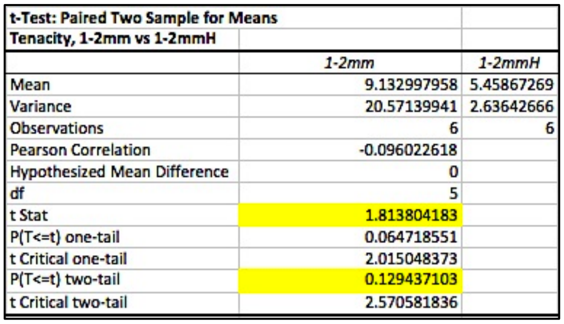
t-Test: Two-Sample Assuming Unequal Variances		
Tenacity, 0 vs 0H		
	0	*0H*
Mean	20.8621531	15.5796571
Variance	26.5674283	26.3368534
Observations	11	9
Hypothesized Mean Difference	0	
df	17	
t Stat	2.28563051	
P(T<=t) one-tail	0.01769334	
t Critical one-tail	1.73960673	
P(T<=t) two-tail	0.03538669	
t Critical two-tail	2.10981558	



t-Test: Paired Two Sample for Means		
Tenacity, 60 vs 60H		
	60	*60H*
Mean	22.0326746	10.1980347
Variance	16.8762285	23.4358621
Observations	6	6
Pearson Correlation	-0.2954387	
Hypothesized Mean Difference	0	
df	5	
t Stat	4.01759007	
P(T<=t) one-tail	0.00507248	
t Critical one-tail	2.01504837	
P(T<=t) two-tail	0.01014496	
t Critical two-tail	2.57058184	



t-Test: Two-Sample Assuming Unequal Variances		
Tenacity, 60 vs 60H		
	60	*60H*
Mean	21.9788545	10.8133915
Variance	12.1038435	37.6696304
Observations	8	8
Hypothesized Mean Difference	0	
df	11	
t Stat	4.47633678	
P(T<=t) one-tail	0.00046852	
t Critical one-tail	1.79588482	
P(T<=t) two-tail	0.00093705	
t Critical two-tail	2.20098516	



t-Test: Paired Two Sample for Means		
Tenacity, 1-2mm vs 1-2mmH		
	1-2mm	*1-2mmH*
Mean	9.132997958	5.45867269
Variance	20.57139941	2.63642666
Observations	6	6
Pearson Correlation	-0.096022618	
Hypothesized Mean Difference	0	
df	5	
t Stat	1.813804183	
P(T<=t) one-tail	0.064718551	
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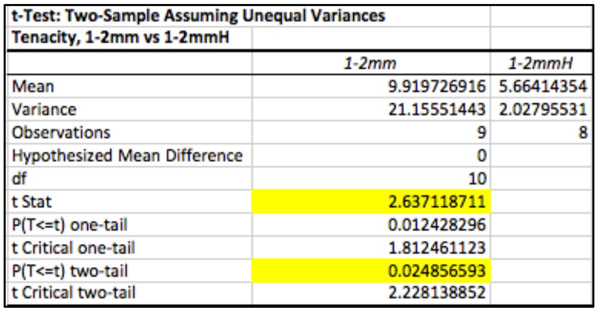
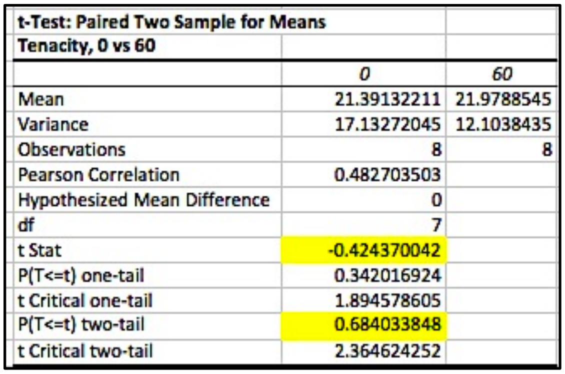
P(T<=t) two-tail	0.129437103	
t Critical two-tail	2.570581836	

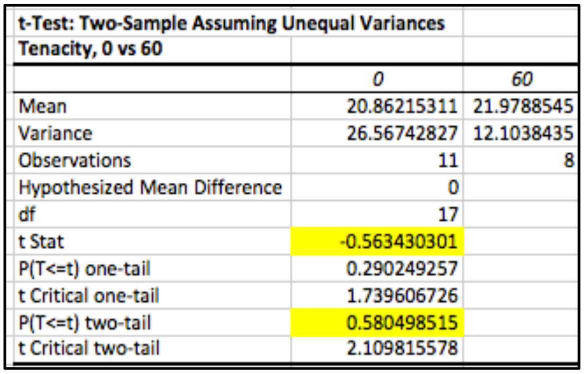


t-Test: Two-Sample Assuming Unequal Variances		
Tenacity, 1-2mm vs 1-2mmH		
	1-2mm	*1-2mmH*
Mean	9.919726916	5.66414354
Variance	21.15551443	2.02795531
Observations	9	8
Hypothesized Mean Difference	0	
df	10	
t Stat	2.637118711	
P(T<=t) one-tail	0.012428296	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.024856593	
t Critical two-tail	2.228138852	

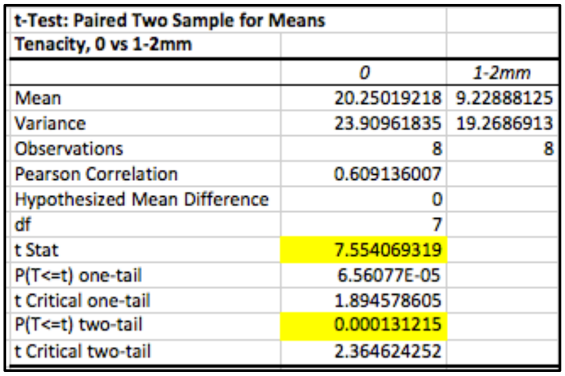
Substrate Roughness Comparison



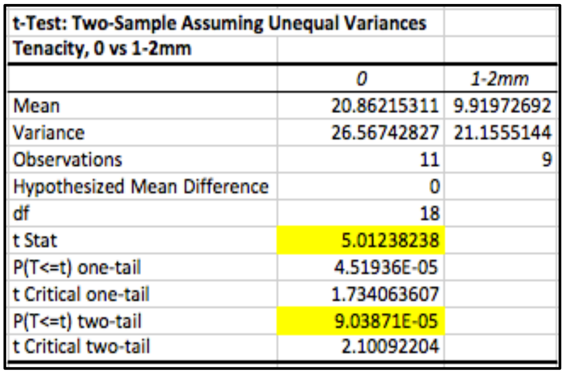
t-Test: Paired Two Sample for Means		
Tenacity, 0 vs 60		
	0	*60*
Mean	21.39132211	21.9788545
Variance	17.13272045	12.1038435
Observations	8	8
Pearson Correlation	0.482703503	
Hypothesized Mean Difference	0	
df	7	
t Stat	-0.424370042	
P(T<=t) one-tail	0.342016924	
t Critical one-tail	1.894578605	
P(T<=t) two-tail	0.684033848	
t Critical two-tail	2.364624252	



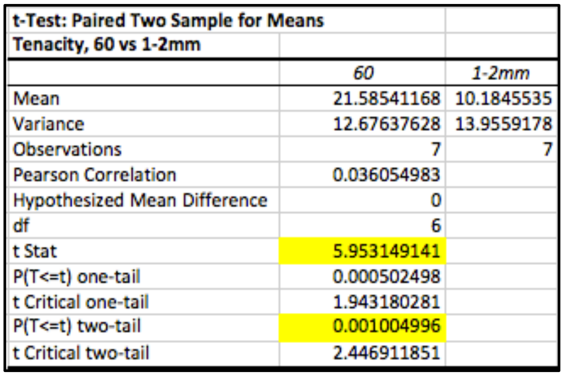
t-Test: Two-Sample Assuming Unequal Variances		
Tenacity, 0 vs 60		
	0	*60*
Mean	20.86215311	21.9788545
Variance	26.56742827	12.1038435
Observations	11	8
Hypothesized Mean Difference	0	
df	17	
t Stat	-0.563430301	
P(T<=t) one-tail	0.290249257	
t Critical one-tail	1.739606726	
P(T<=t) two-tail	0.580498515	
t Critical two-tail	2.109815578	



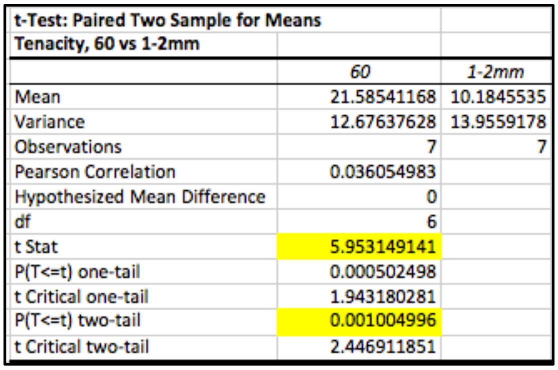
t-Test: Paired Two Sample for Means		
Tenacity, 0 vs 1-2mm		
	0	*1-2mm*
Mean	20.25019218	9.22888125
Variance	23.90961835	19.2686913
Observations	8	8
Pearson Correlation	0.609136007	
Hypothesized Mean Difference	0	
df	7	
t Stat	7.554069319	
P(T<=t) one-tail	6.56077E-05	
t Critical one-tail	1.894578605	
P(T<=t) two-tail	0.000131215	
t Critical two-tail	2.364624252	



t-Test: Two-Sample Assuming Unequal Variances		
Tenacity, 0 vs 1-2mm		
	0	*1-2mm*
Mean	20.86215311	9.91972692
Variance	26.56742827	21.1555144
Observations	11	9
Hypothesized Mean Difference	0	
df	18	
t Stat	5.01238238	
P(T<=t) one-tail	4.51936E-05	
t Critical one-tail	1.734063607	
P(T<=t) two-tail	9.03871E-05	
t Critical two-tail	2.10092204	



t-Test: Paired Two Sample for Means		
Tenacity, 60 vs 1-2mm		
	60	*1-2mm*
Mean	21.58541168	10.1845535
Variance	12.67637628	13.9559178
Observations	7	7
Pearson Correlation	0.036054983	
Hypothesized Mean Difference	0	
df	6	
t Stat	5.953149141	
P(T<=t) one-tail	0.000502498	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.001004996	
t Critical two-tail	2.446911851	



t-Test: Paired Two Sample for Means		
Tenacity, 60 vs 1-2mm		
	60	*1-2mm*
Mean	21.58541168	10.1845535
Variance	12.67637628	13.9559178
Observations	7	7
Pearson Correlation	0.036054983	
Hypothesized Mean Difference	0	
df	6	
t Stat	5.953149141	
P(T<=t) one-tail	0.000502498	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.001004996	
t Critical two-tail	2.446911851	