Effect of Tooth wear in Lemon Sharks (*Negaprion brevirostris*)

By

Evin M. Fitzgerald

April 1, 2019



Allegheny College

Department of Biology

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**A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College**

**I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code
and to maintain the integrity of both myself and the College community as a whole**.

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Pledge

Approval by the Senior Thesis Committee:

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Dr. Lisa Whitenack

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Dr. Matthew Venesky

Abstract

The majority of studies on shark teeth look at various types of teeth morphologies and observe the purpose for all the different types of shark teeth. In this study I examined the effects of wear on six non-functional teeth of the lemon shark (*Negaprion brevirostris*), and how this can affect to proficiency of the lemon sharks' teeth, potentially affecting the tooth replacement rate. Three trials of 20 punctures were performed using a PVC lever apparatus. This PVC lever apparatus was used to simulate the shark capturing a prey item for ingestion and used to record the puncture force (N) with a fish scale. Before and after the trials the Apices and two identified serrations of the teeth were measured for wear using diameter and perpendicular measurements. Evidence of wear was found at the apices of the six teeth, but no indications of wear were seen at the measured serrations. This study tests the hypothesis that tooth wear will be present from the feeding events, and has an impact on the cutting and penetration ability of the tooth

Introduction

In vertebrates, teeth are a vital component of an organisms feeding apparatus**.** They aid in prey manipulation that allows the prey item to be ingested, allow the capture of desired prey, and aid in the removal of indigestible structures of the prey (Whitenack et al. 2010). When teeth of a shark are observed or studied, often times the only aspects of the teeth taken into account is the relationship between morphology and function (Whitenack et al. 2010, Corn et al. 2016, Bergman et al. 2017). When this is done unworn or barely worn teeth are used to test performance, completely neglecting the change in morphology teeth can have due to repeated use which is experienced because of tooth wear (Ungar & Williamson 2000, Corn et al. 2016).

Tooth wear is a phenomenon that has been observed in current and prehistoric mammals around the world and its severity is driven by the animal's dietary preferences, eating habits, habitat, etc. (Ungar & Williamson 2000, Corn et al. 2016). In mammals, dietary preference of an organism can vary in the same species if different habitats are utilized, given that these habitats differ in resources. This can be seen in northeastern pacific killer whales (*Orcinus orca*), which show three different communities, which are resident, transient, and offshore. The resident *Orinus orcas'* prey on fish populations and inhabit areas around their food source, while the transient communities' prey on mammals such as seals. Lastly the offshore community's prey on fish and sharks and live off the continental shelf. Offshore *Orcinus orca* have been shown to experience more apical tooth wear, than the other two communities based on their dietary preference for sharks. This wear is caused by the placoid scales present on sharks causing offshore *Orcinus orca* to have teeth that are worn down to the gum

line. The observed tooth wear causes large pulp cavities to appear on the anterior teeth and smaller pulp cavities on the posterior teeth. The effect of tooth wear on the offshores fitness level is currently unknown, but if it does affect food sharing that is seen in other whales (Ford et al. 2010), it may potentially hinder members of the offshore community. This correlation between dietary preference and tooth wear is yet to be observed in sharks but, a correlation was shown between wear and tooth morphology. Corn et al. (2016) observed that the various tooth morphologies in sharks appear to play a role in how they are affected by tooth wear. The dynamic testing showed that there appeared to be a tradeoff between cutting ability and the rate at which the tooth is worn. The teeth of the silky shark (*Carcharhinus falciformis*) demonstrated large net wear, similarly the teeth of the tiger shark (*Galeocerdo cuvier*) showed wear and decreased cutting ability after the first five cuts. The teeth of *Carcharhinus falciformis* and *Galeocerdo cuvier* are both advantageous for cutting and therefore are subject to tooth wear faster than the saw-like teeth of the bluntnose sixgill shark (*Hexanchus griseus*), which did not experience significant wear but showed a lack of cutting ability (Corn et al. 2016).

Shark teeth can be divided into six major tooth morphologies and are categorized by their proposed function in prey procurement and processing. The tooth morphologies that are associated with species that feed on soft prey items such as fish and mammals are the clutching, tearing, and cutting type teeth. The tooth morphologies that are associated with species that feed on hard prey items are the crushing, grinding, and clutching grinding type teeth (Ramsay & Wilga 2007). However, the relationship between tooth morphology and feeding ecology is not as straight forward in

elasmobranchs as it is in mammals (Ungar & Williamson 2000, Corn et al. 2016). Common studies observing shark feeding mechanisms have focused on the kinematics and motor patterns in the sharks biting activity (Motta et al. 1997). While this a crucial piece of a shark's ability to survive in the wild, it is just one part of the whole, given that the role of shark teeth in prey capture is ignored (Whitenack & Motta 2010). Even though studies such as Whitenack and Motta, which looks at the performance of shark teeth and the relation between function and morphology are being done, there are still important aspects of shark teeth that have not been fully studied. One of the areas of research on shark teeth that this study observed is the effects of wear and its potential effect on the replacement of shark teeth. Replacement rates of shark teeth have been studied before, but the root cause of replacement has not been given a concrete answer. Since shark teeth are replaced so regularly, one idea has been proposed that the frequency of this replacement may be driven by tooth wear not failure (Whitenack et al. 2011). The teeth of a shark are arranged into files and rows, files are the vertical column of functional and replacement teeth(non-functional) perpendicular to the jaw. Rows are the horizontal group of teeth that consist of teeth from different files and move in a conveyor belt like fashion to replace the worn teeth (Fig.1) (Botella et al. 2008).

The specimen chosen in this study was the lemon shark (*Negaprion brevirostris*), which is a member of the family Carcharhinidae and can grow grow to approximately 2.5 to 3.6 meters. *Negaprion brevirostris*, which ranges from New Jersey to Brazil,and is abundant in the subtropical waters of the Bahamas and Florida. Occupying the areas coral reefs, mangroves, enclosed bays, and river mouths. In these ecosystems *Negaprion brevirostris* feeds primarily on teleosts which may be the reason that

Negaprion brevirostris possesses a cutting-clutching type tooth (Wetherbee et al. 1990, Whitenack & Motta 2010). This is drawn from Wetherbee et al. (1990) where 88% of the stomach content of *Negaprion brevirostris*, the remaining prey items that were ingested consist of marine mammals and other sharks. *Negaprion brevirostris* is estimated to actively partake in in feeding for a duration of 10-11 hours, followed by a 32-hour fasting period. As a result of their abundance and their ability to be observed in captivity, *Negaprion brevirostris* is a well-documented species, making it an adequate specimen for study (Motta et al. 1997). Because of this, their tooth replacement rate has been studied, in Moss (1967) where he references the replacement rate of *Negaprion brevirostris* being 8-10 days/row in the summer time (warm waters), but is susceptible to change depending on certain factors such as starvation. This tooth replacement rate saw a significant decrease when the shark was starved, the starved individual in the study replaced 22 teeth compared to the fed individuals 55. This result indicates that this decrease in replacement rate can be due to the teeth not being used or a slower metabolic rate similar to what was observed in cold waters (Moss 1967, Leur et al. 1990). In this study I aim to present the effects of wear on shark teeth, and how wear impacts the shark's ability to penetrate their prey. I tested the hypothesis that tooth wear will be present from the feeding events and has an impact on the cutting and penetration ability of the tooth. I examined teeth from *Negaprion brevirostris* (cutting-clutching type teeth) and documented the wear on the teeth after feeding sessions. Penetration force was also observed to determine if wear affects the cutting ability of the teeth, potentially indicating a time at which the tooth would be replaced.

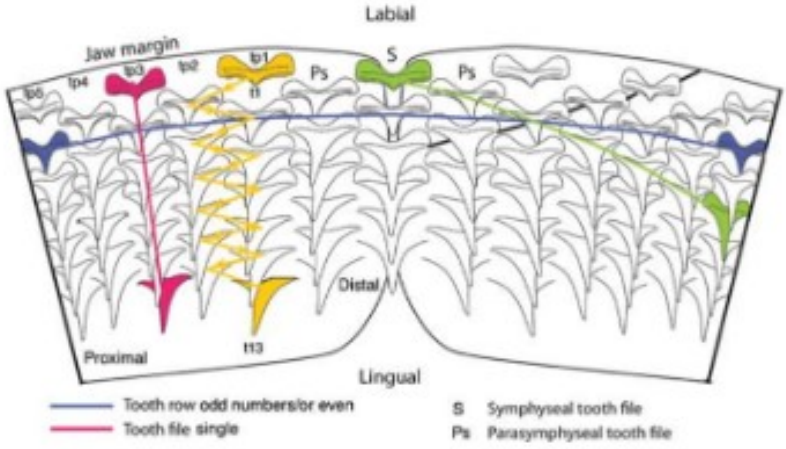


Fig 1. Tooth file indicated by pink vertical column. Tooth row indicated green horizontal line.
Underwood, Charlie & Johanson, Smith (2016)

Methods

Specimen

Teeth from a preserved jaw of *Negaprion brevirostris* from Dr. Whitenack's laboratory at Allegheny College in Meadville, Pennsylvania, were observed in this study (Fig.3). After being soaked in a warm water bath for one hour, six non-functional teeth (not damaged or worn) were manually removed from the second replacement row of the upper jaw of *Negaprion brevirostris*. Six teeth were removed from the upper jaw, three teeth from the right side of the symphysis of the upper jaw and three from the left side of the symphyseal of the upper jaw (Fig 2). These six teeth were then identified based on their position from the upper symphysis position, indicated in Table 1 and Fig. 2.

Table 1. *Negaprion brevirostris* tooth positions and ID's

Tooth ID	Position
L4	4th tooth position on the left of the upper symphyseal
L5	5th tooth position on the left of the upper symphyseal
L6	6th tooth position on the left of the upper symphyseal
R5	5th tooth position on the right of the upper symphyseal
R6	6th tooth position on the right of the upper symphyseal
R7	7th tooth position on the right of the upper symphyseal

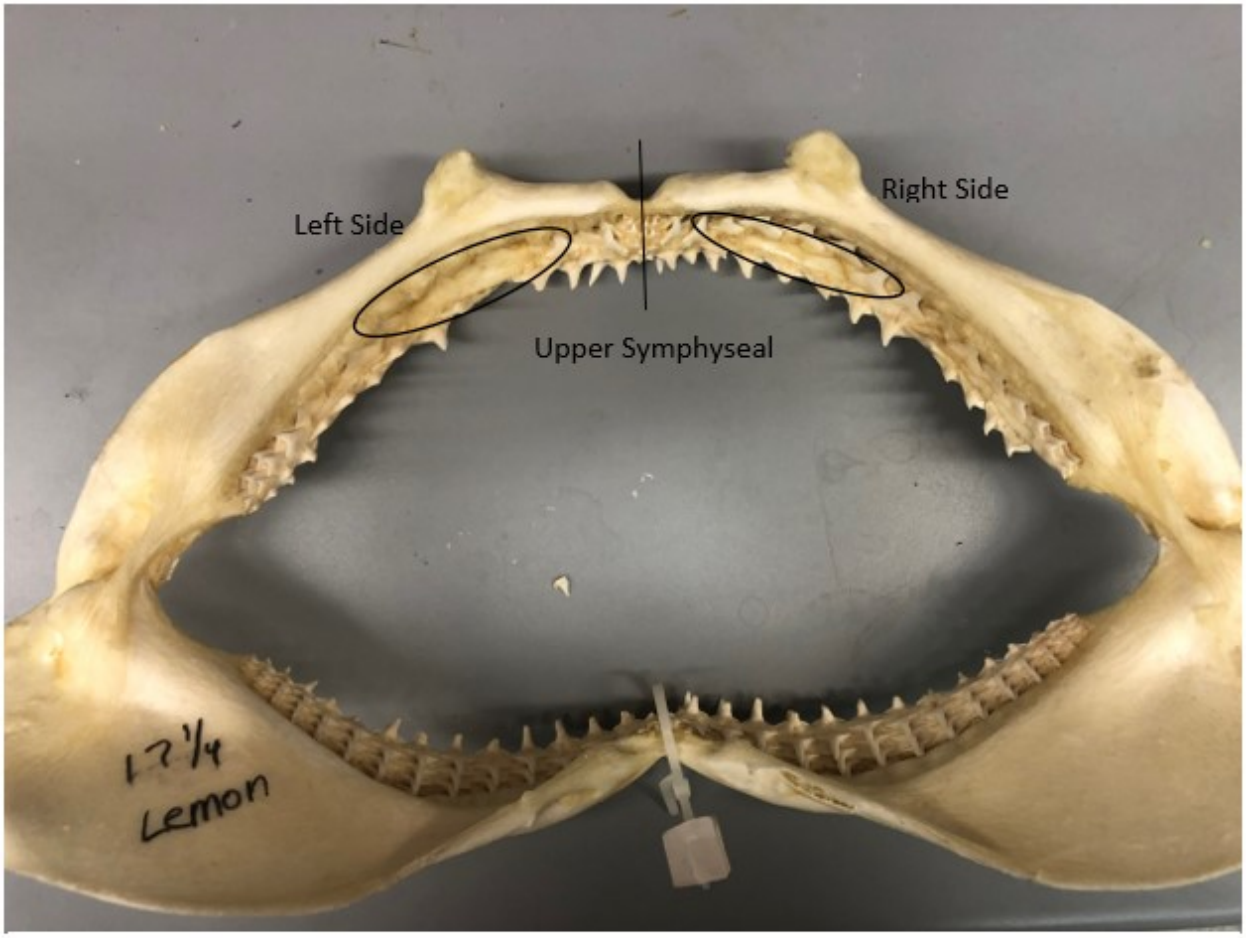


Fig 2. *Negaprion brevirostris* jaw, circled areas indicate the positions where the observed non-functional teeth were removed.



Fig.3 *Negaprion brevirostris* tooth from upper jaw.

Apparatus

The apparatus used in this study (Fig. 4) had a 46cmx23cm baseboard, two post with a height of 30cm and a thickness of 2cm attached to the baseboard. The posts were attached 7cm from the edges at the midline (23cm) of the baseboard. 17.5cm from the base of both posts, a hole was drilled with a 3/32 drill bit allowing the bolt to cross supporting the PVC beam. The PVC beam with a length of 55cm and a diameter of 2cm had a hole drilled 22cm proximal from the anterior end to allow the bolt to pass through. 6.5cm from the posterior end an eye hook was placed to allow the 8kg Rampala Digital Fish Scale to measure the forces of the punctures and depress the anterior end into the prey item.

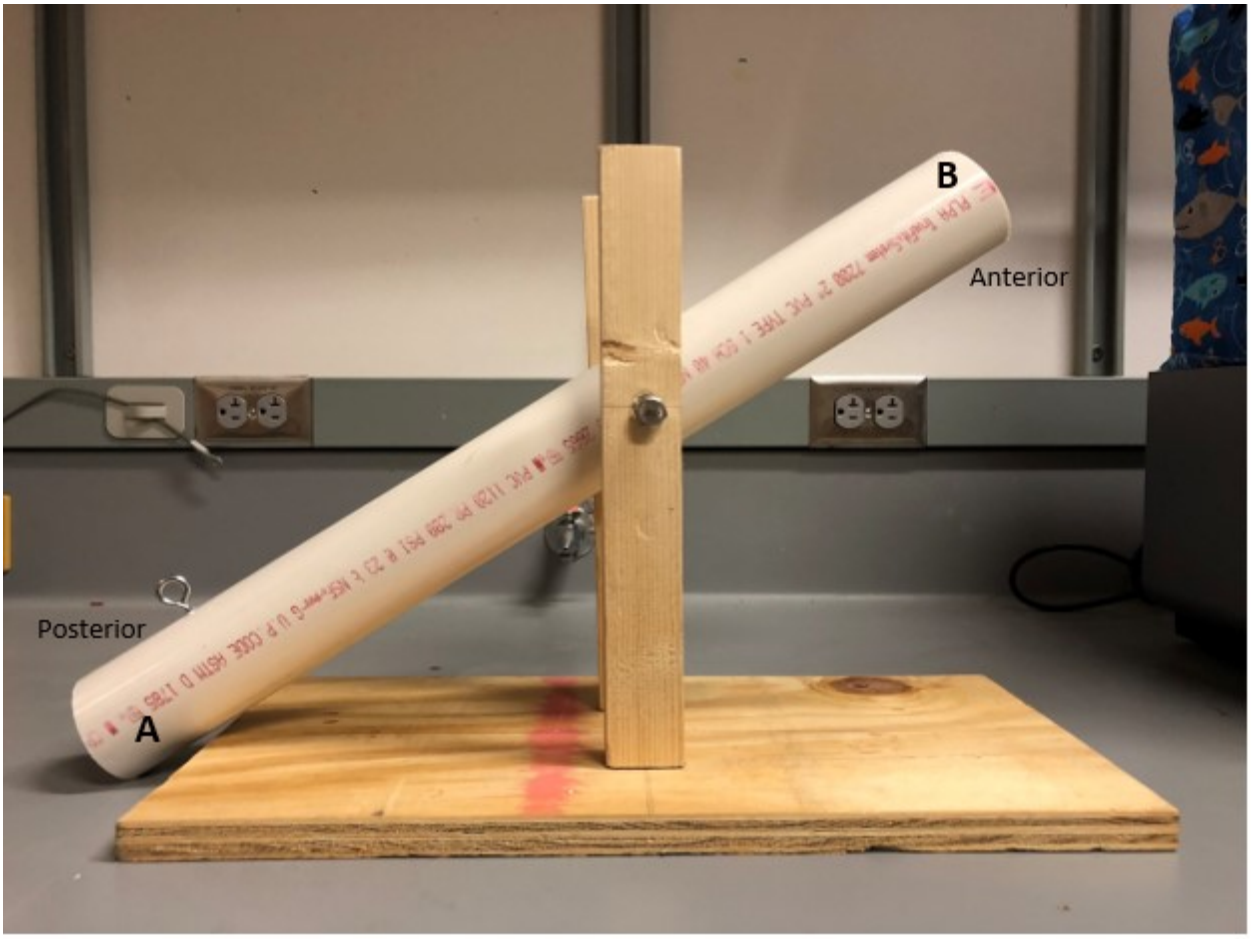


Fig.4 (A) Eye hook allowing the fish hook scale to record puncture force and raise the beam so tooth can penetrate prey item. (B) PVC coupling site

Scanning Electron Microscopy (SEM)

The teeth of *Negaprion brevirostris* were mounted on an SEM stage to be observed under the SEM. Across all observations under the SEM the uniform settings were detector type of SEI, Voltage of 5kV, and a spot size of 50. Under the SEM the apices all of the teeth were observed at x50 magnification for measurements, while the serrations were viewed at a magnification of x150 for measurements (Appendix).

Penetration Testing

Teeth were fixed to a 7.3cm diameter PVC coupling with hot melt adhesive using a glue gun, teeth from the left and right groups (Table 1) were attached to different

couplings marked left and right. The teeth were attached to the outer layer of the PVC coupling by the root of the tooth with the apical end of the tooth being distal from the coupling/root. These couplings were attached to the anterior end of the apparatus, allowing a tooth to face the prey item placed below. On the posterior side of the apparatus, a hook provided a junction for a fish scale (Fig 4). This junction allowed the beam to be driven down into the prey item and record the force applied in the feeding event. The posterior end was raised until the anterior end was 4-6 cm above the prey item, prey items were placed under the tooth (Corn et al. 2016). Once at this point the posterior end was then raised until tooth at the anterior end depressed to puncture the prey (Fig 4). The force of each attempt was recorded in newtons(N), giving each tooth 60 recorded puncture forces. Punctures on the prey items Three trial periods of 20 penetrations where the tooth punctured the prey item were conducted, after a trial the teeth were removed from their PVC coupling, using a hairdryer to soften the hot melt adhesive in order to measure the apices and serrations under the SEM.

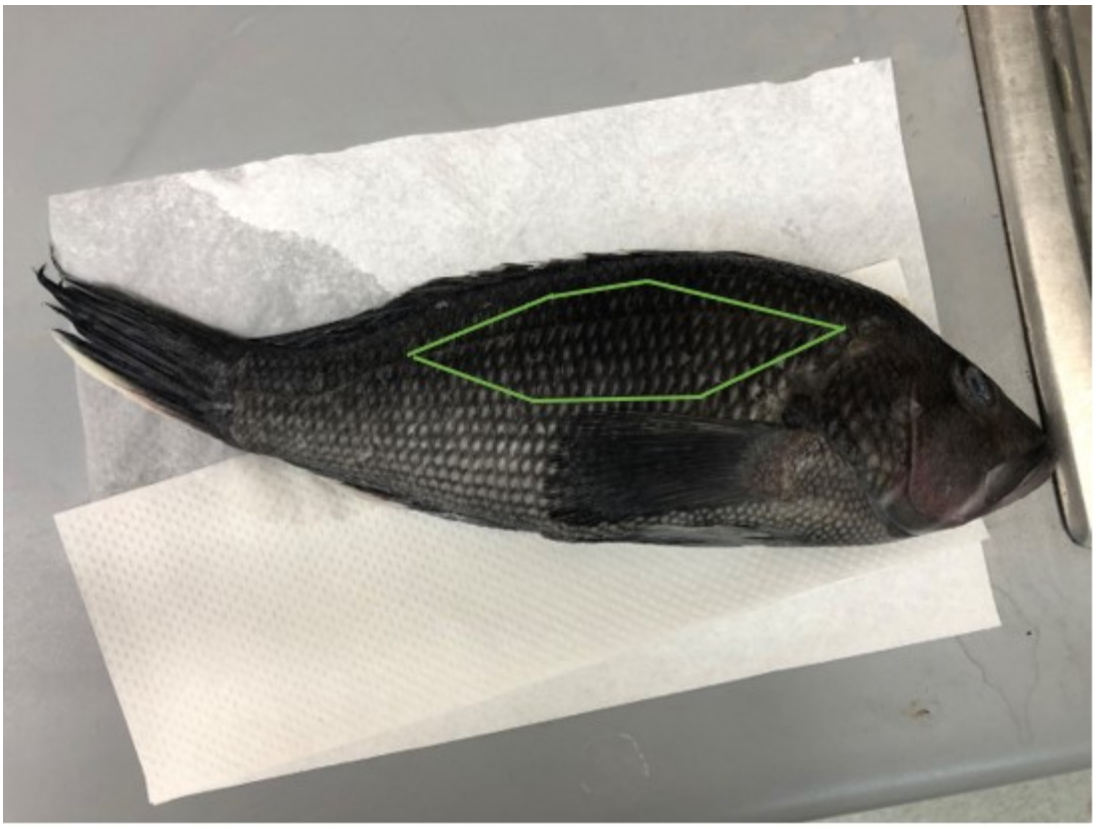


Fig.5 *Centropristis striata* Puncture are during the penetration trials. Penetrations kept above and below the lateral line to avoid contact with bones and to standardize the trials.

Prey Item

The item used as prey in this study was the black sea bass (*Centropristis striata*). The prey items were purchased from the Robert Wholey Company in Pittsburgh Pennsylvania and they were stored in a freezer at -16℃ in Dr. Whitenack's laboratory. Before puncture testing, the fish were thawed in a bath of cold water for one hour, the water was running for the full duration of this hour. Punctures on the prey item were all placed on the area indicated in Fig 5.

Tooth Measurements

The following tooth measurements were collected from teeth of *Negaprion brevirostris*: The apex of each tooth and two serrations from the distal edge of each tooth were measured. The apices of each tooth were measured using the diameter tool provided by the SEM InTouch software. This tool created a circle at the apex of each tooth and gave diameter measurement (μm) (Moyer & Bemis 2017). The serrations were measured using the perpendicular line tool provided by the SEM InTouch software. The perpendicular tool drew a line at the base of the serration, and a line perpendicular to this base measured the height of the serration (Fig 6). All measurements were standardized by fitting the previous measurement to the desired portion of the tooth. Side by side comparisons of the measurement images were also used to determine the best placement of the software's measurement tools.

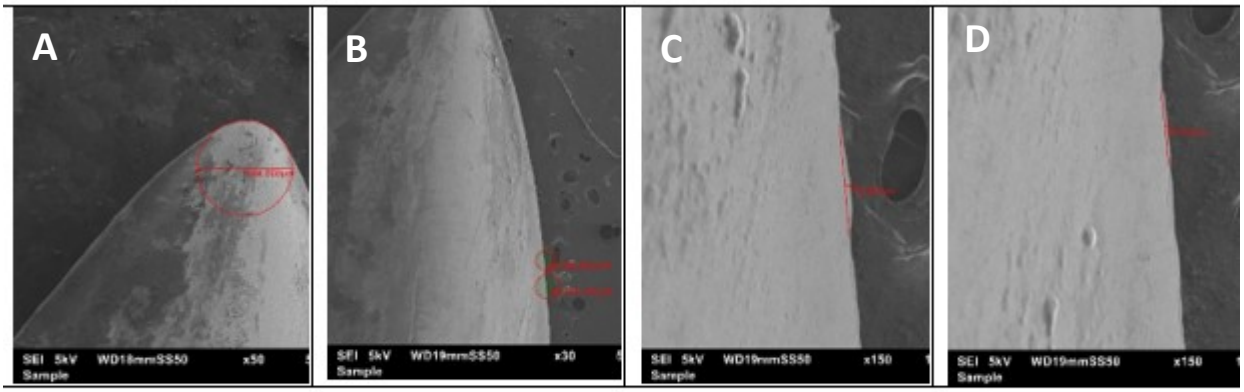


Fig.6 (A) Apex measurement using diameter tool under SEM. (B) Selection of the two serrations to be measured under the SEM. (C&D) Serration perpendicular line measurements under SEM

Statistical Analysis

Differences among the measurements of the apices and serrations of the teeth of *Negaprion brevirostris* were evaluated using random effects model in repeated

measures analysis of variance (ANOVA) using JMP Pro 12. Three different repeated measure ANOVAs were produced for three different variables, the apices and the two serrations. The random variable that was set in all three repeated measure ANOVAs was the teeth and the fixed variable in all three repeated measure ANOVAs was the trials. Post hoc analysis was run with a Tukey honestly significant difference (HSD) test on results that were statistically significant. A linear regression was run with the mean force of each puncture on the Y axis and the puncture number on the X axis, to show the effect of the puncture number of the force required to puncture the prey. The linear regression was run using the Fit Y by X function through JMP Pro 12.

Results

A repeated measures ANOVA was conducted to compare the effect of tooth wear on the apices and serrations of nonfunctional teeth of *Negaprion brevirostris,* before, during, and after the feeding trials. Overall, the observed teeth of *Negaprion brevirostris* expressed signs of wear at their apices that were statistically significant (Fig. 7, Table 2, Table 3). Post hoc analysis with a Tukey HSD test on the diameters of the apices showed that there was an increase in the mean diameter of the teeth from before the start of the trials (M=576.67) to the end of the trials(M=593.33). The wear on the serrations was not statistically significant (Table 2), so the Tukey HSD could not be used (Fig. 8, Table 2, Table 3).

Table 2. Results of a repeated measures ANOVAs on Apices, Serration 1 and 2

Random Effect: Tooth

Fixed Effect: Trials

Variables: Apex, Serration 1, and serration 2 measurements

Source	df	F Ratio	P-value
Trial			
Apex	3	13.801	0.001
Serration 1	3	0.4621	0.7129
Serration 2	3	0.1185	0.9479

Table 3. Tooth position, penetrations, and measurements for teeth used in the analysis

Tooth ID	Penetration #	Tip Diameter(μm)	Serration 1 (μm)	Serration 2 (μm)
L4	0	664	46.476	34.975
L4	20	664	46.671	34.986
L4	40	668	20.276	17.751
L4	60	676	21.685	20.87
L5	0	628	17.951	18.884
L5	20	640	17.951	19.195
L5	40	656	25.024	36.326
L5	60	656	24.676	34.493
L6	0	596	11.831	16.368
L6	20	600	11.926	16.826
L6	40	600	13.92	19.437

L6	60	600	13.728	13.283
R5	0	552	14.634	13.735
R5	20	556	14.636	13.744
R5	40	564	14.636	23.324
R5	60	576	13.877	23.113
R6	0	554	13.67	11.727
R6	20	556	13.679	11.87
R6	40	560	12.806	11.353
R6	60	568	10.934	8.537
R7	0	466	11.353	7.22
R7	20	472	11.353	7.364
R7	40	476	12.074	6.7
R7	60	484	10.687	6.037

Penetration # indicates the number of times the tooth penetrated the prey item and indicate when the tooth was measured.

Throughout each of the trials the force required to penetrate the prey item started to form a consistent pattern. A linear regression was used ($F(_{1, 58})=62.09$, $p<.001$) to determine the relationship between the trial number and the force required to puncture the prey item. The linear regression did show that there was a statistically significant relationship, while also producing the equation $y=-.0.0124x + 3.7342$ and an $R^2=0.519$. The slope of -0.0124 indicates a slight decreasing trendline and the R^2 shows that 51.9% of the variance in the “bite” force can be explained by the trial number. The negative trending relationship depicts that as the number of trials increases the amount of force required to penetrate the prey item decreases, therefore showing a negative relationship (Fig 9).

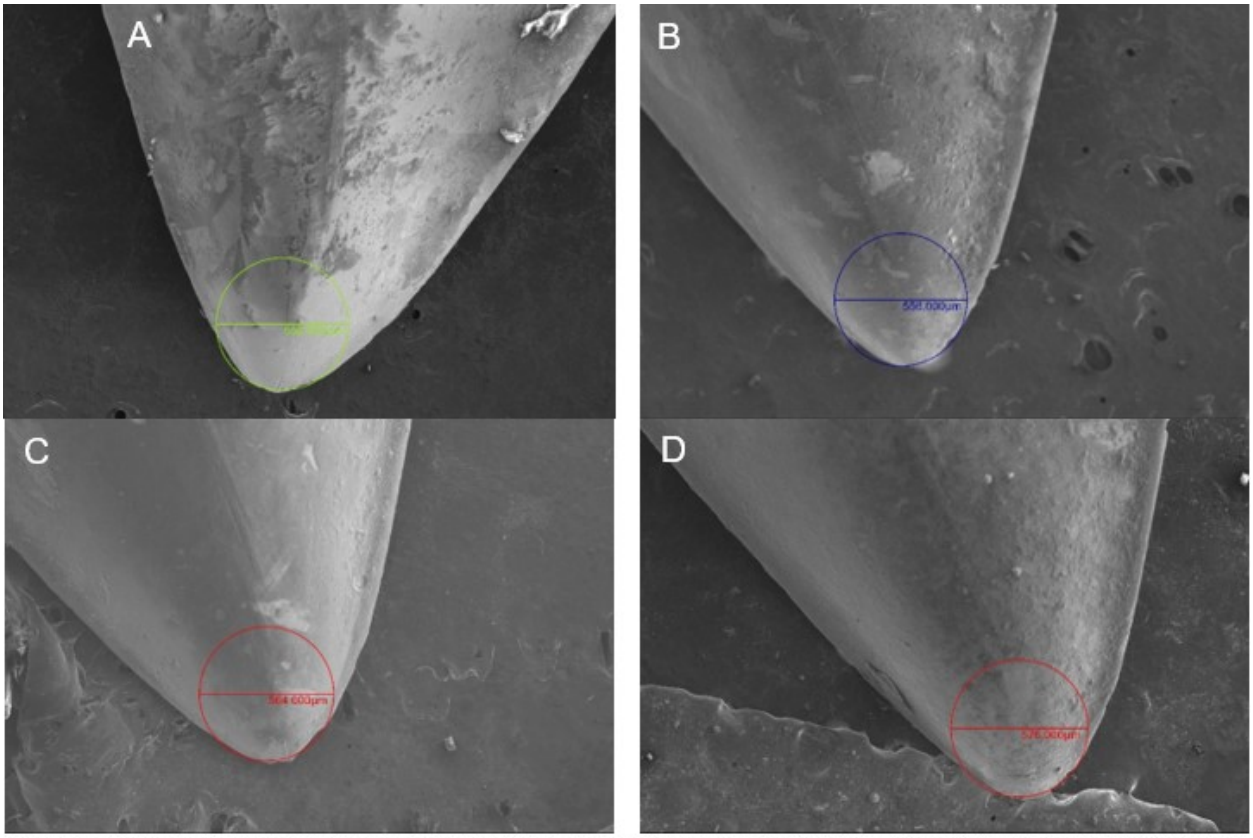


Fig.7 SEM micrographs of the R5 tooth apex of *Negarprion brevirostris*. (A) Shows the apex diameter before the trials is 552.00µm. (B) Shows the apex diameter after trial 1 is 556.00µm. (C) Shows the apex diameter after trial 2 is 564.00 µm. (D) Shows the apex diameter after trial 3 is 576.00 µm

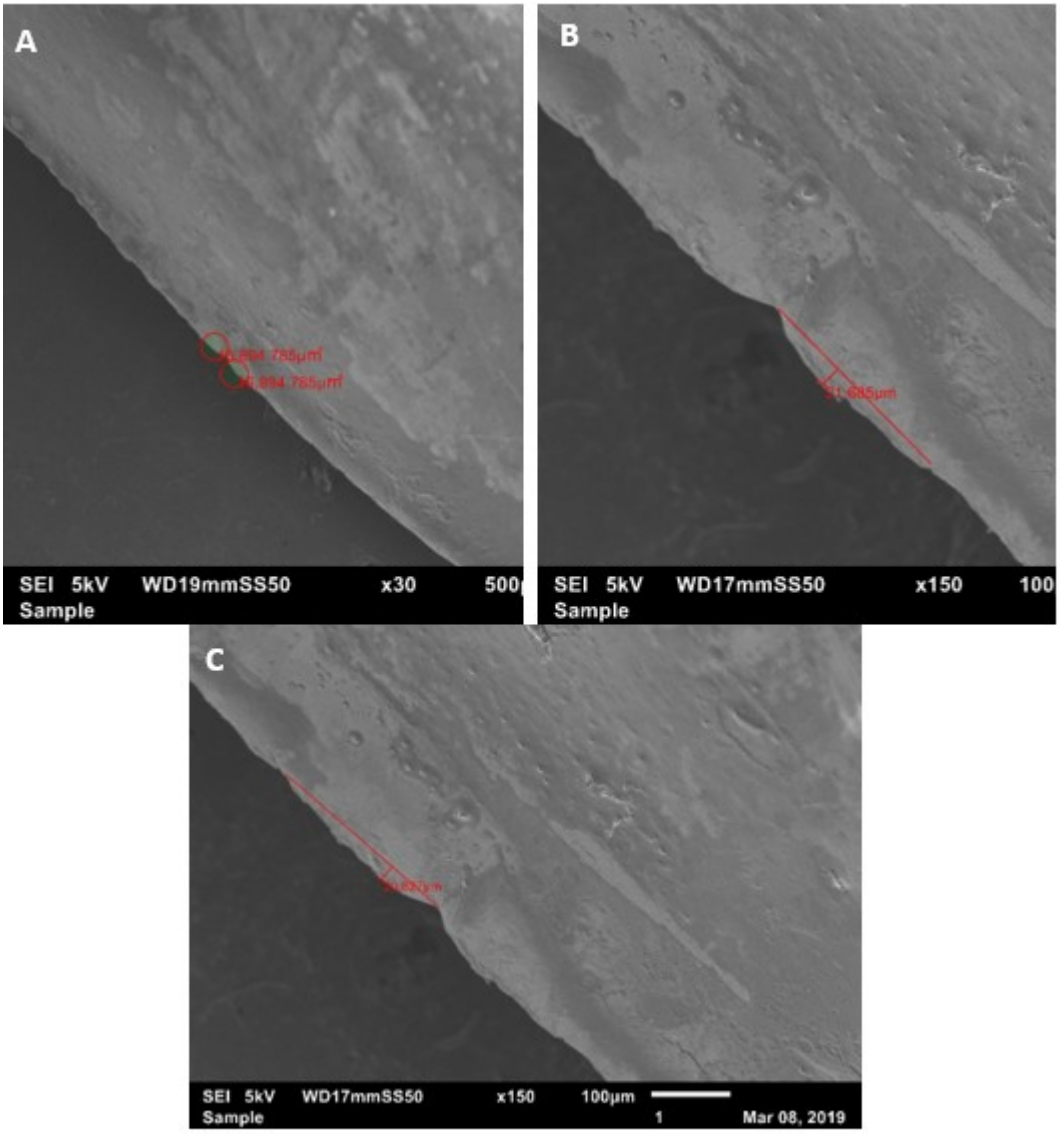


Fig. 8 (A) Indicates the selected serrations that were measured. (B) The perpendicular measurement of serration 1 on L4 after trial 3, 21.685µm. (C) The perpendicular measurement of serration 2 on L4 after trial 3, 20.827µm.

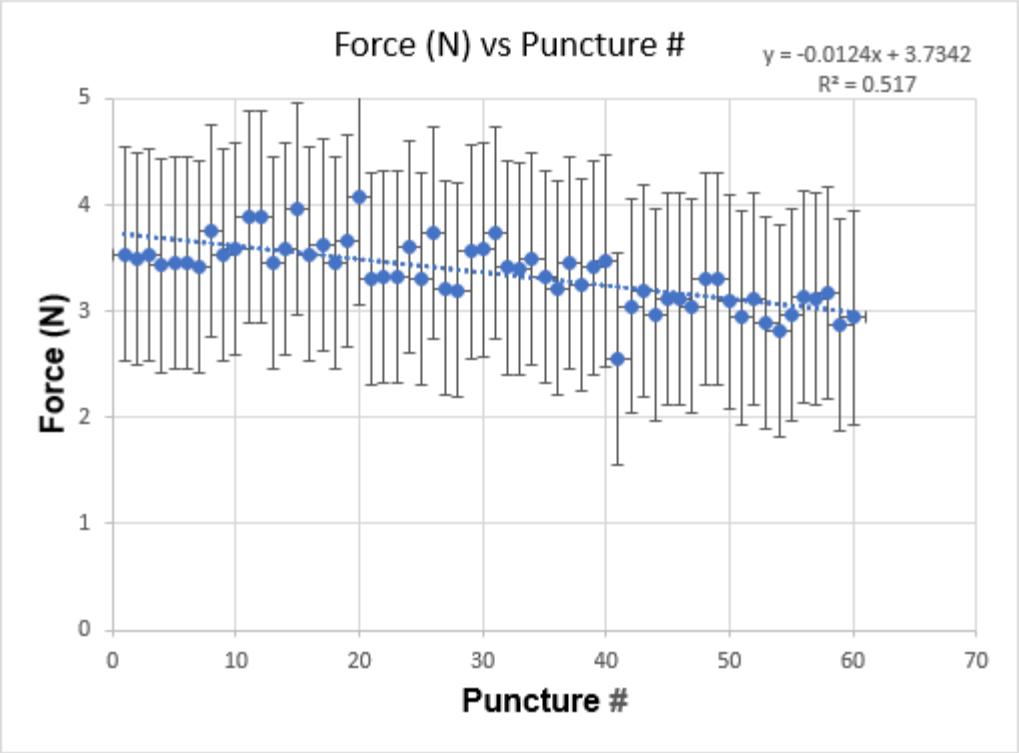


Fig. 9 Linear regression of the relationship between the Force and the Puncture number. Each point is the mean force of the six teeth at each trial. Error bars are standard error.

Discussion

Multiple studies on the feeding apparatus of sharks have been conducted, mainly focusing on tooth morphology, kinematics, and prey interactions (Motta et al. 1997, Whitenack & Motta 2010, Whitenack et al. 2010, Moyer & Bemis 2016, Corn et al. 2016, Bergman et al. 2017), while largely ignoring the role or implications of tooth wear. In this research, evidence of wear occurred on the apices of the six observed teeth of *Negaprion brevirostris* after the testing sessions (Table 2). This result was expected due to the results of Corn et al.2016, and it occurred in the form of an increase of the diameter in the apices of the observed teeth. This increase of apical diameter shows

that wear was a present, this is because, an increase of the edge diameter indicates that the apex has dulled over the course of the trials (Botella et al. 2008, Whitenack et al. 2010, Moyer & Bemis 2016). The implications of this finding provide another step in finding the proposed relationship between tooth wear and tooth replacement. That relationship being that tooth replacement in elasmobranchs is driven by tooth wear. The results regarding the apices support part of the hypothesis that tooth wear will be present over the span of the experiment, but the force to penetrate the prey item did not increase (Bergman et al. 2017). Even though this decreasing trend of puncture force was experienced and was statistically significant, it is not biologically significant. Huber et al. (2005) observed the bite force of the blacktip shark *Carcharhinus limbatus* and observed that its bite force ranged from 32N to 423N. This indicates the 1N to 2N variance seen in the penetration trials would is not likely affect the performance of *Negaprion brevirostris* during feeding.

While there was a presence of wear at the apices, there was not any expression of wear in the serrations that were measured (Table 2). Since serrations offer the tooth a greater surface area, it is proposed that this would lead to easier penetration into prey due to the increased distribution of forces (Moyer & Bemis 2016). On the other hand, *Negaprion brevirostris'* teeth are categorized as “cutting-clutching” teeth. Resulting in high and narrow cusps, this shape may be responsible for the tooths cutting ability rather than the present serrations (Whitenack & Motta 2010). In this case the hypothesis is not supported by the findings, since there was no wear present on any of the serrations and the force to penetrate the force continued to decrease overall from trial 1 to trial 3.

Given that there was no evidence supporting a relationship between the increase in wear would lead to an increase in penetration force like it was seen in Corn et al. (2016), it further begs the question about when the wear on a subject's teeth become a hindrance to the fitness of the animal. Moss (1967) found that tooth replacement rates in *Negaprion brevirostris* were higher when they were fed than when they were starved. While this finding does lean towards the theory of wear causing the tooth replacement, it also shows that replacement still happens if the shark does not eat. Despite the fact that tooth replacement was less frequent in the starving individuals, it provides evidence that there are more factors that affect tooth replacement than wear. One of these factors that affects tooth replace is the growth rate of *Negaprion brevirostris*. Growth curves appear to indicate that as the body length and the size of the teeth of the shark increases, the number of teeth replaced also increases (Moss 1967). Another factor on replacement rates that is highlighted in Luer et al. (1990) is the temperature of the water. In nurse sharks (*Ginglymostoma cirratum*), it was shown that during seasons where the water was warm (26-28°C) there was an increase in the tooth replacement rate, while during the seasons where the water was colder (21-24°C) there was a decrease in the tooth replacement rate in some species. The decrease in the replacement rates in the colder water could potentially be attributed to the metabolism of the shark slowing down. This could cause a decrease in the shark's food intake, while also causing a decrease in teeth usage. The decrease of teeth usage would then mean that the teeth are not being worn down as quickly as they are in the warmer season, resulting in a slower replacement rate (Luer et al.1990). These are the factors that need to be accounted for

when studying tooth replacement rates, so observations need to be made in various seasons.

Motta et al. (1997) observed the feeding mechanism of *Negaprion brevirostris* and showed that the shark has different biting techniques depending on the prey. In some cases, the shark captured the prey by ram feeding and was either ingested almost immediately or manipulation bites would be used to make the prey easier to swallow. Whenever the prey item was too large however, the shark captured the item and shook its head vigorously to tear off an ingestible piece. These varieties of feeding were not able to be recreated by the apparatus in this study. The apparatus allowed for single punctures at a time and could not accomplish the rapid succession of bites or head shaking maneuvers that are present in *Negaprion brevirostris* and most sharks (SA Moss 1977, Motta et al. 1997, Moyer & Bemis 2016, Corn et al. 2016). Corn et al. (2016) findings showed noticeable decrease in cutting ability in *Carcharhinus falciformis* after 17 cuts with an apparatus that attempted to mimic head shaking. This result show that these factors should be taken into account when wear tests are being studied.

The findings of this study present evidence of tooth wear at the apices but was not able to convey this wear at the serrations or show a relationship of the effect of wear on the force required to penetrate the prey. Even though these results were not anticipated, it shows that there is room for improvement in the research of this area. This study examined teeth from only one species, inclusion of a variety of tooth morphologies as seen many puncture studies (Whitenack & Motta 20010, Moyer & Bemis 2016, Corn et al. 2016, Bergman et al. 2017) may provide further results to understand the effects of tooth wear on performance. Furthermore, a similar application

to this study could have been multiple prey items, in order to test for any variance in wear based on prey. This concept was used in Whitenack & Motta (2010) and Berman et al. (2017) but neither of these studies measured the wear on the tooth after testing, just the penetration force. Another change that should be implemented in the future is that the manner in which serrations are measured should not follow the procedure of this study. In Moyer & Bemis (2016) they found that serrations were difficult to measure with diameter due to the potential of them getting damaged during handling or extraction from the jaw. In this instance the serrations were too elongated in order to fit a diameter, so I used the perpendicular tool provided by the SEM. This tool was useful but not feasible to recreate each measurement session and was reflected in the results. Lastly, the study should have implemented more trials, this study was too short and in order to see more wear or a trend with the penetration force more trials need to be added.

Overall, the observed teeth from the upper jaw of *Negaprion brevirostris* displayed wear at the apices of the teeth, but not at the serrations. The Forces required to penetrate the prey item however, decreased as the study progressed. This finding was not consistent with the decrease in cutting performance over time that was seen in other research (Corn et al. 2016, Bergman 2017). These findings did not support the hypothesis that tooth wear will be present from the feeding events and has an impact on the cutting and penetration ability of the tooth. Even though wear as detected at the spices, there was no effect observed on the penetration force. The findings of this research do provide a gateway to further observing tooth wear and its effects on teeth, so that we can get closer to understanding its role in tooth replacement. To understand the implications of tooth wear and its effect on replacement, we must be able to

understand the extent to which a tooth is still productive in increasing the shark's fitness.

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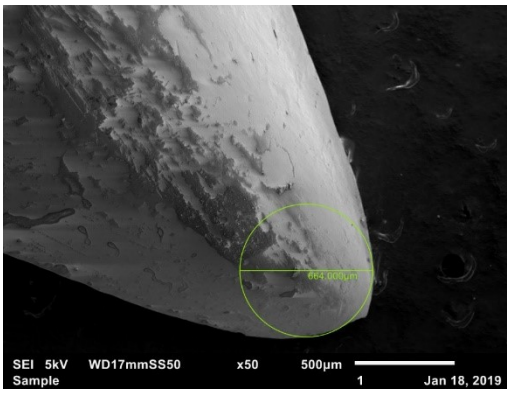
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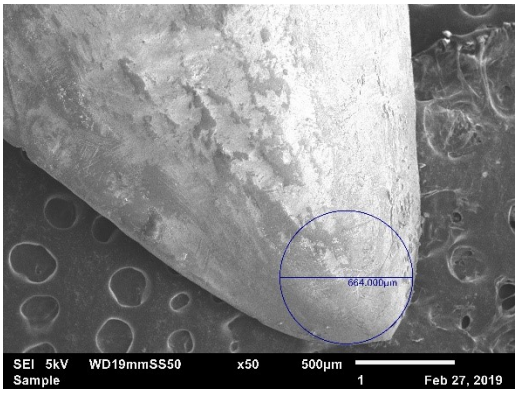
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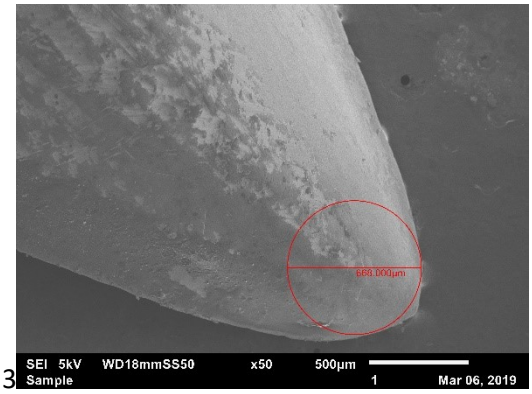
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Appendix

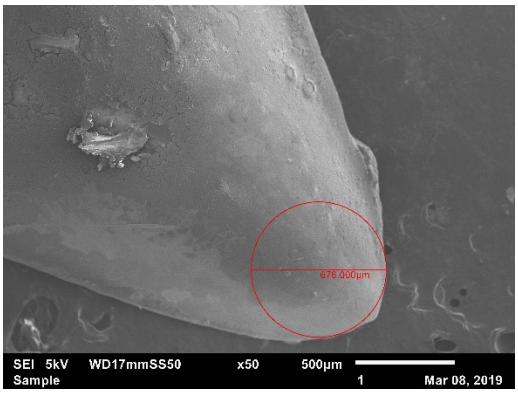
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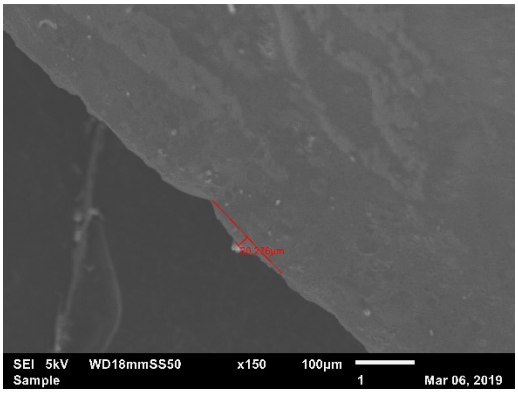


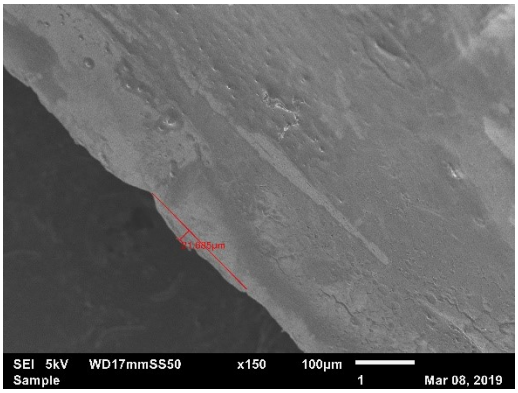


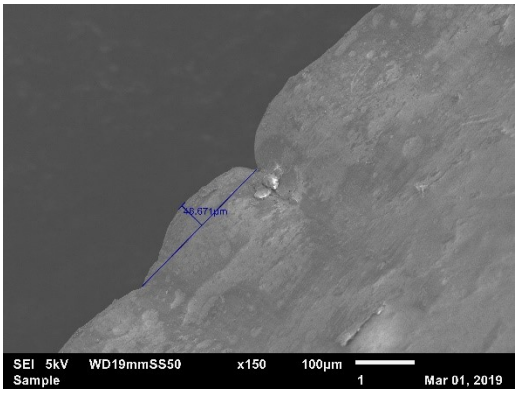
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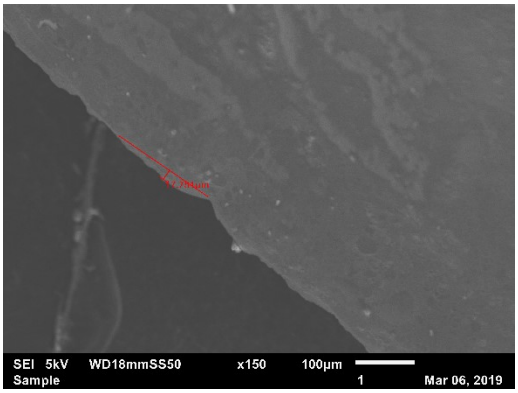
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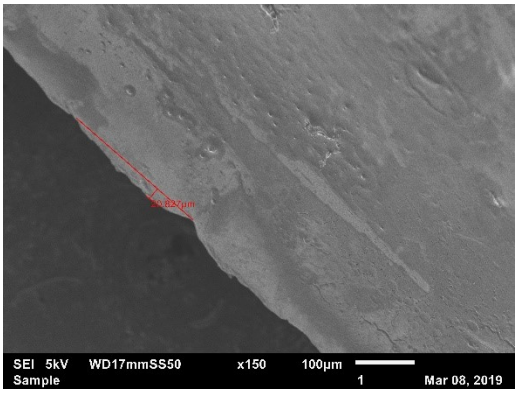


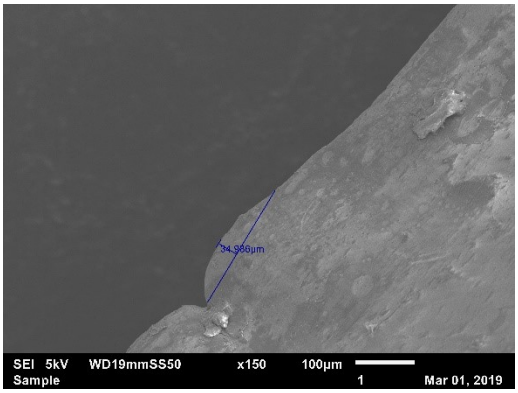




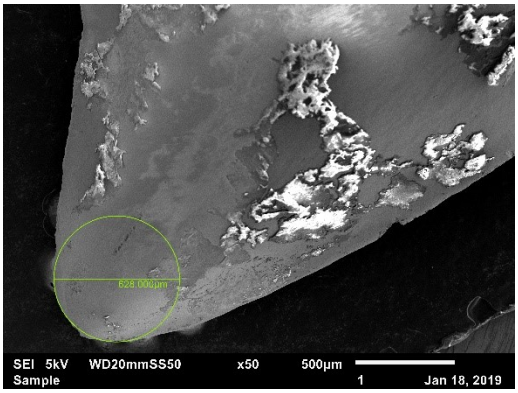
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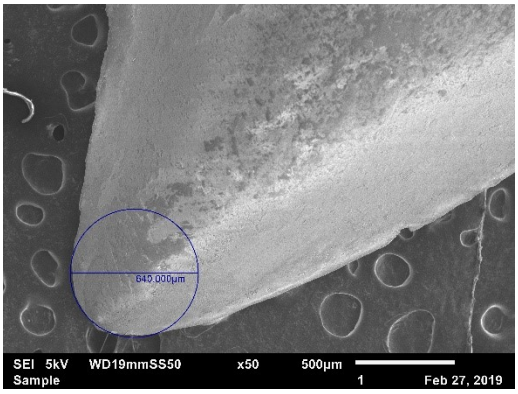


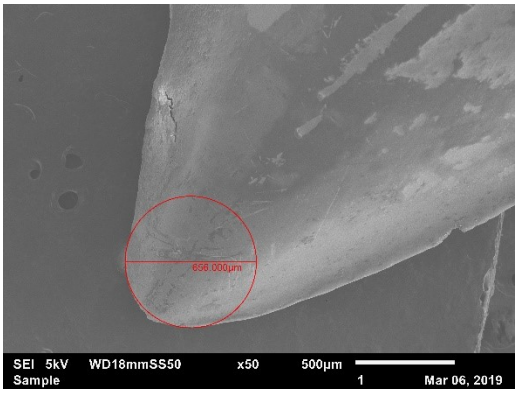


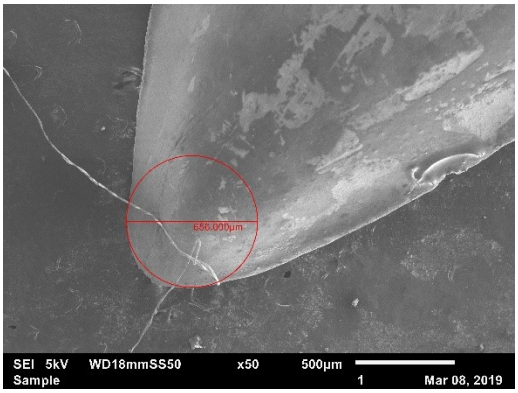


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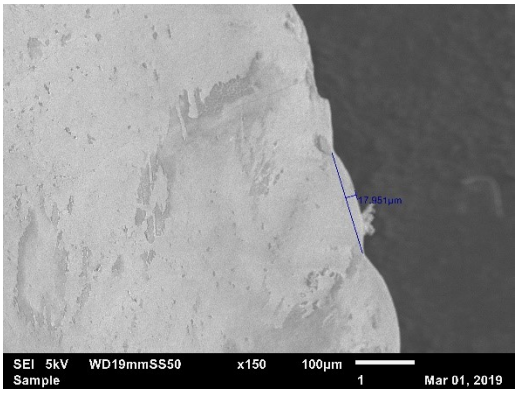


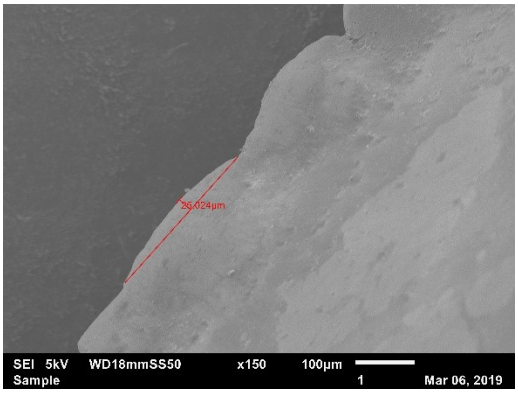


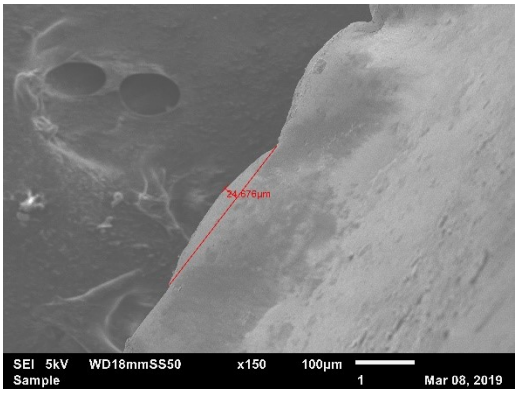




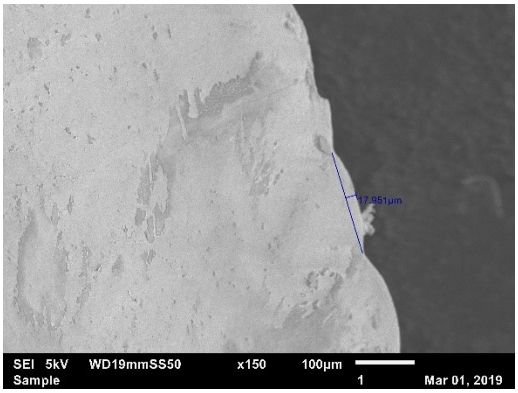
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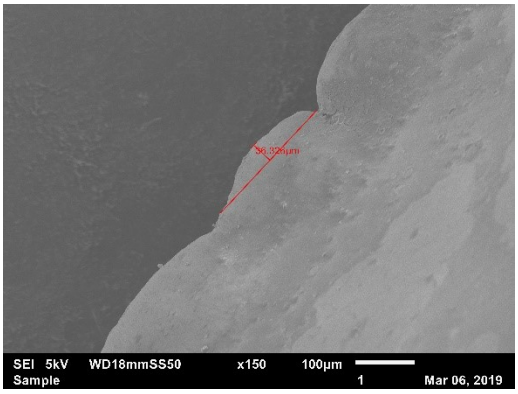


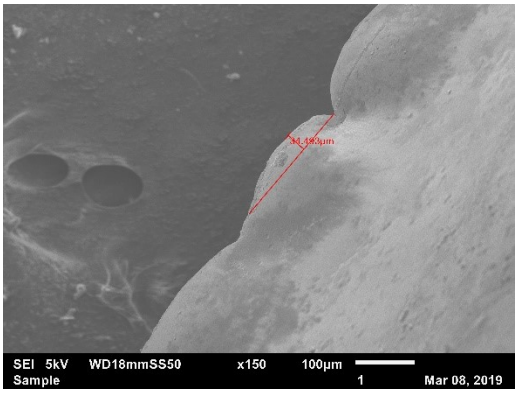




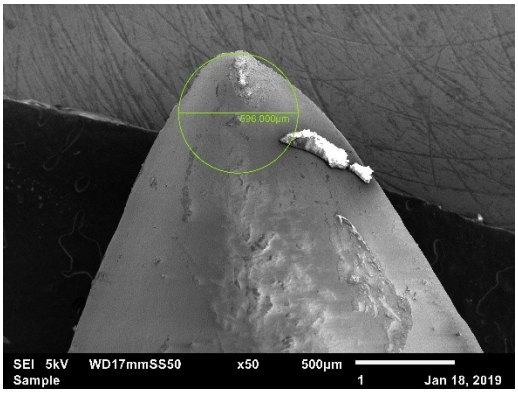
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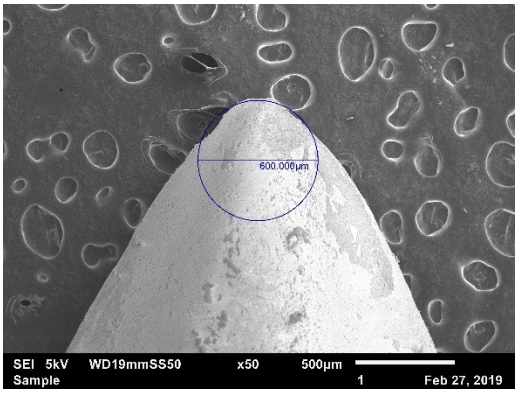


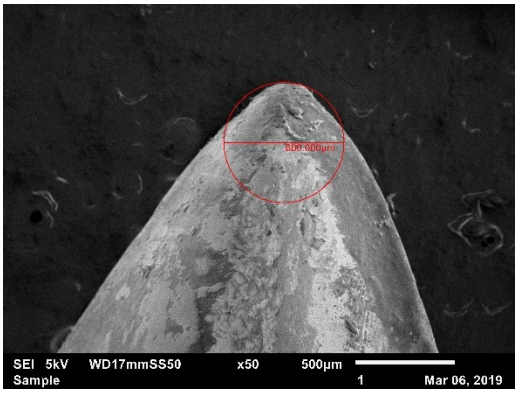


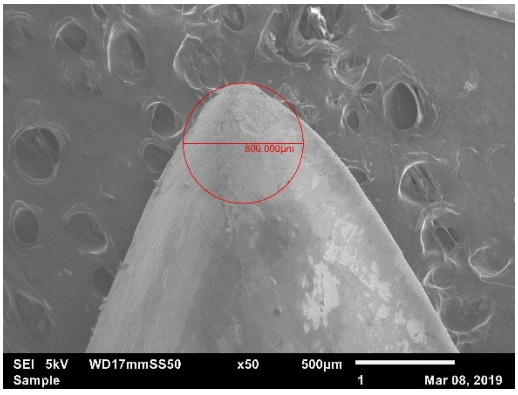


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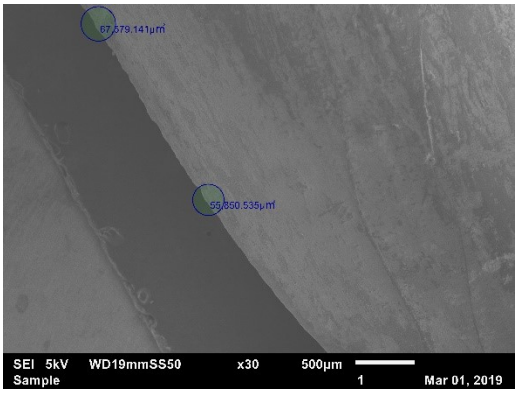


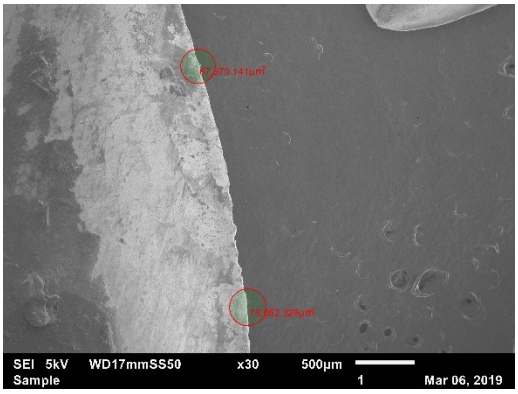


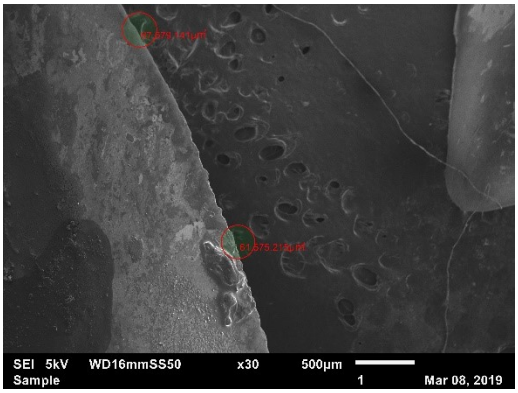




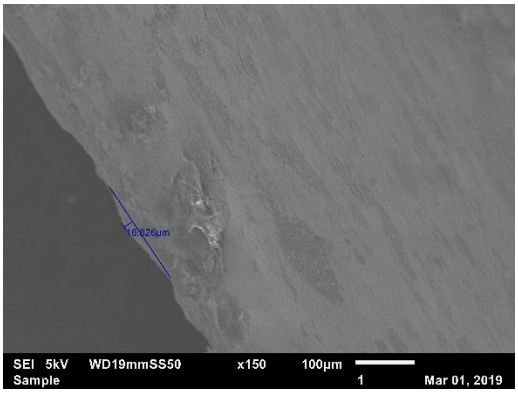
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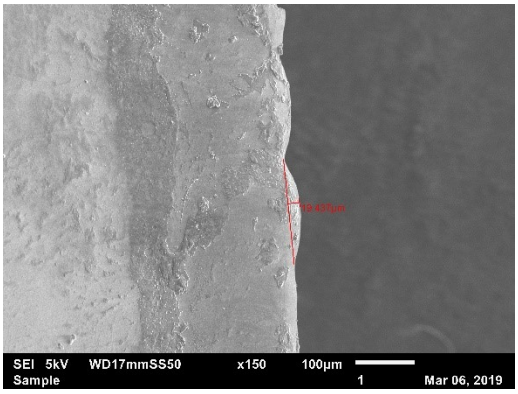


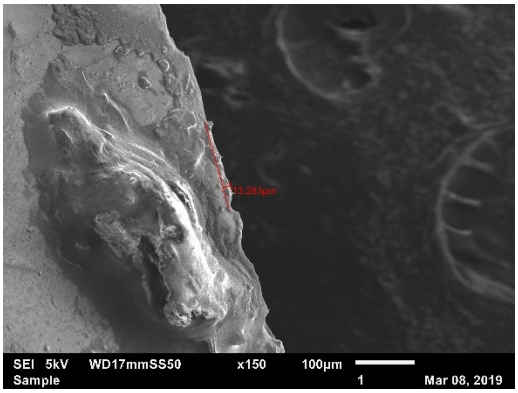




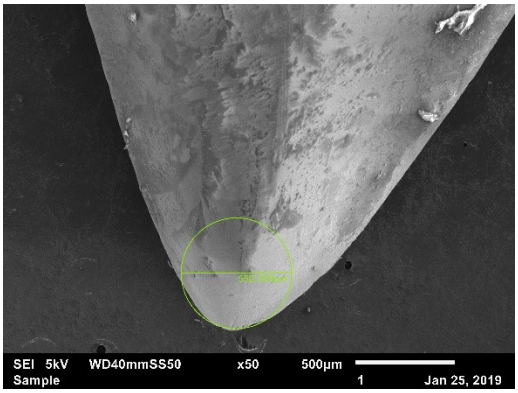
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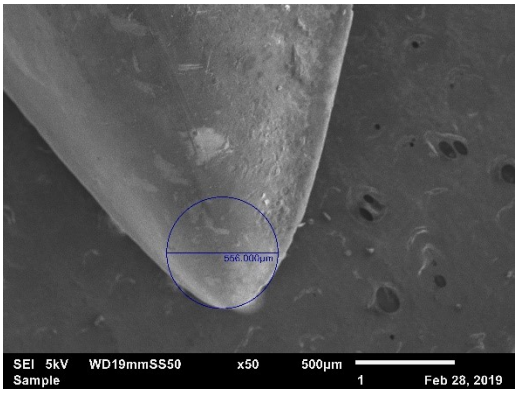


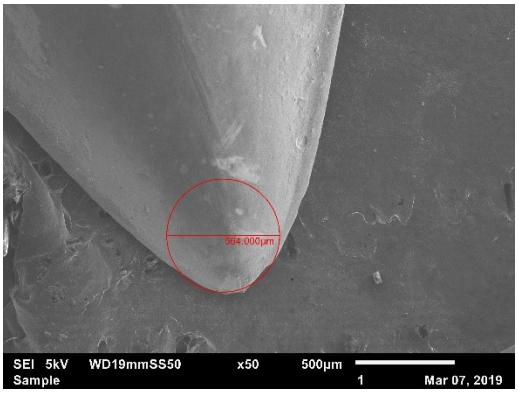


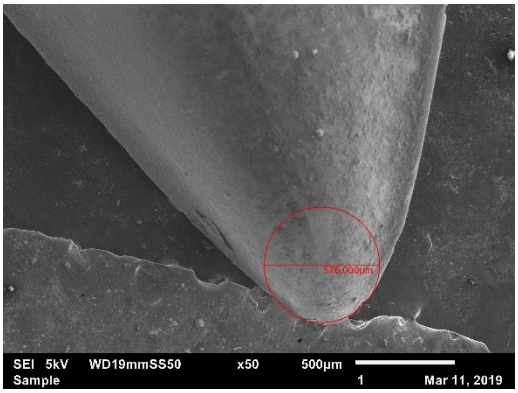


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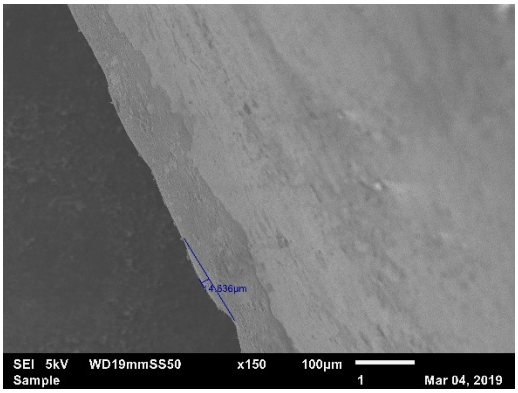


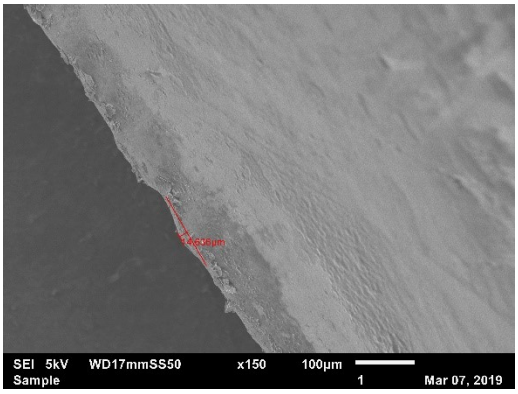


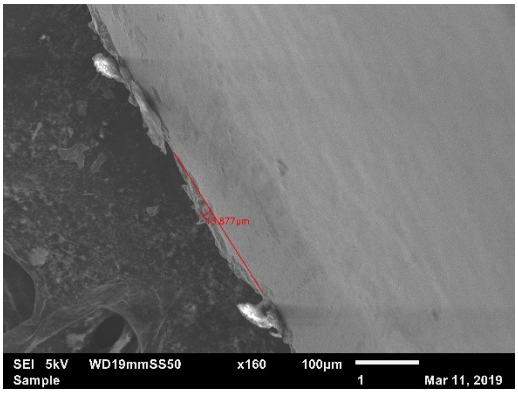




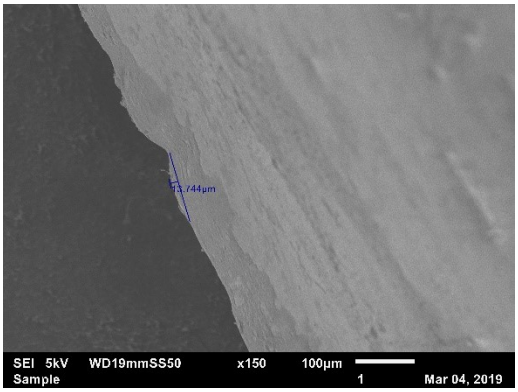
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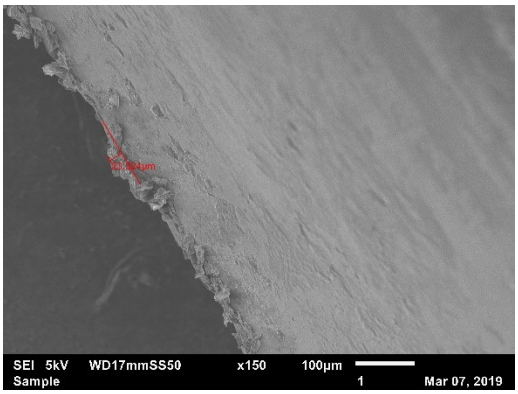


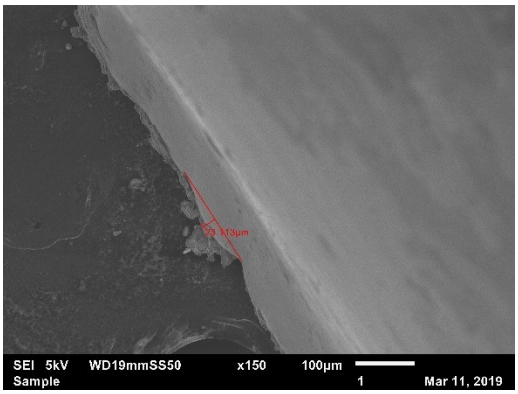




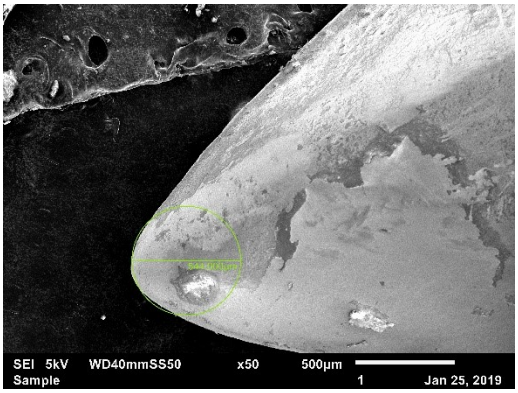
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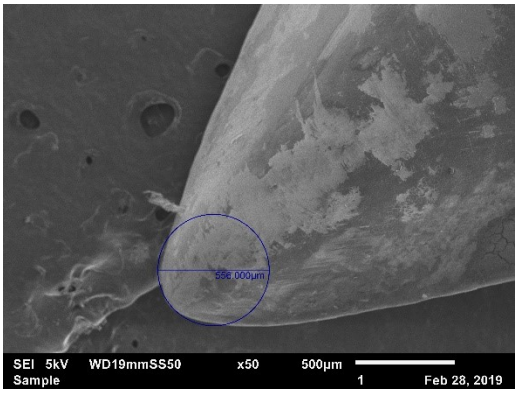


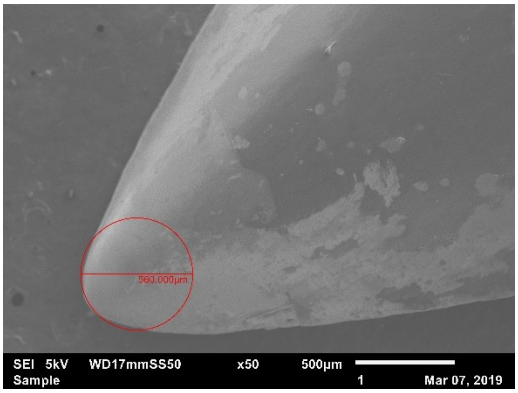


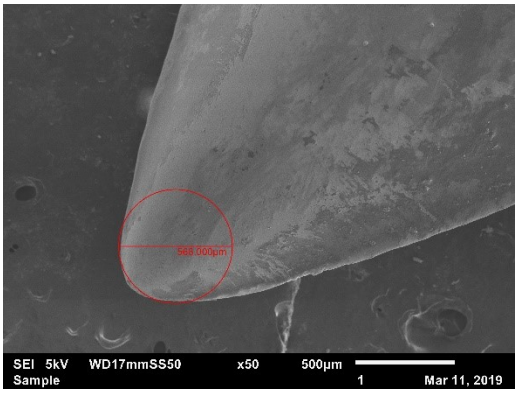


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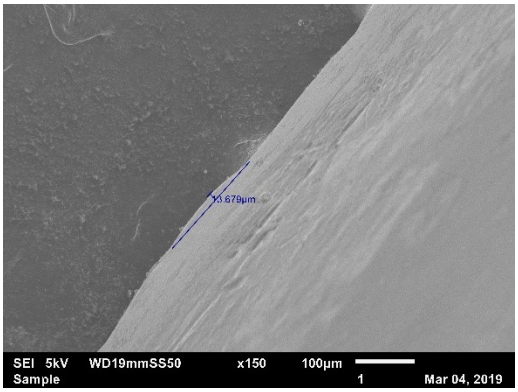


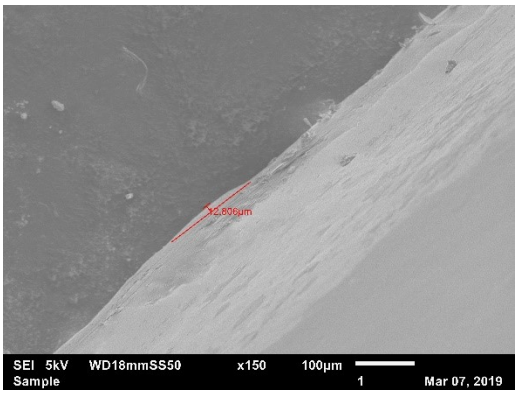


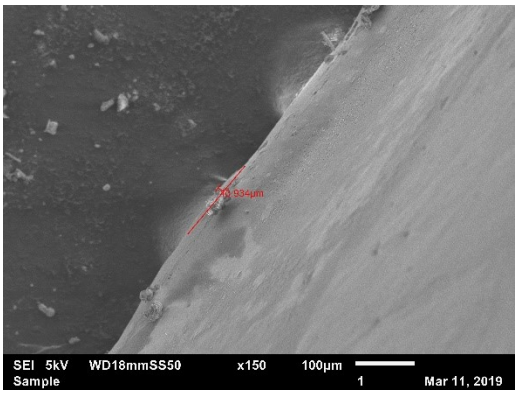




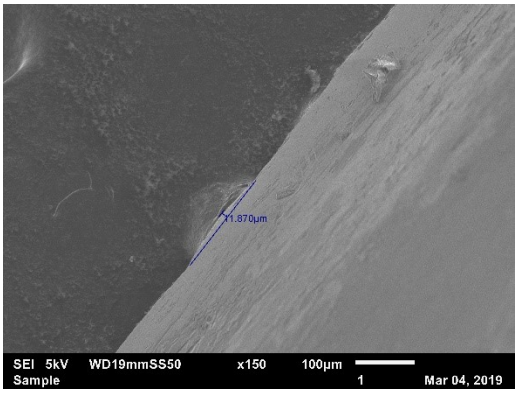
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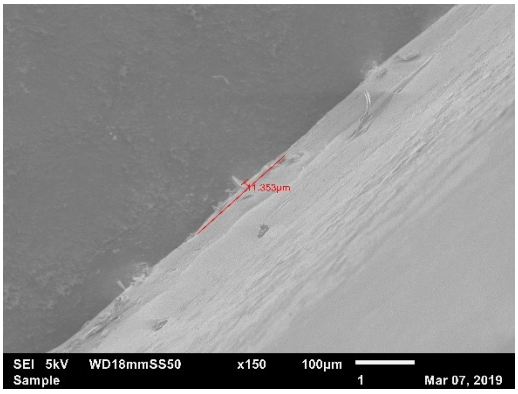

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SEI 5kV WD19mmSS50 x150 100µm
Sample 1 Mar 04, 2019

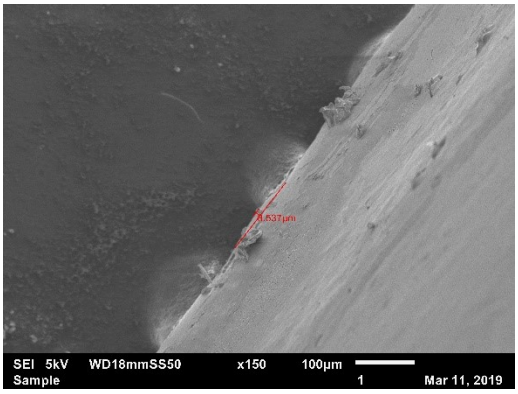

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Sample 1 Mar 07, 2019


SEI 5kV WD18mmSS50 x150 100µm
Sample 1 Mar 11, 2019

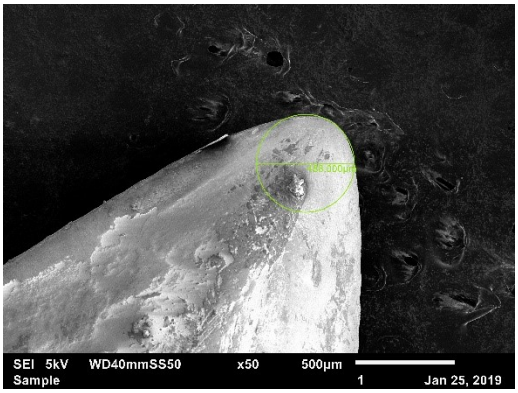
R6 S2

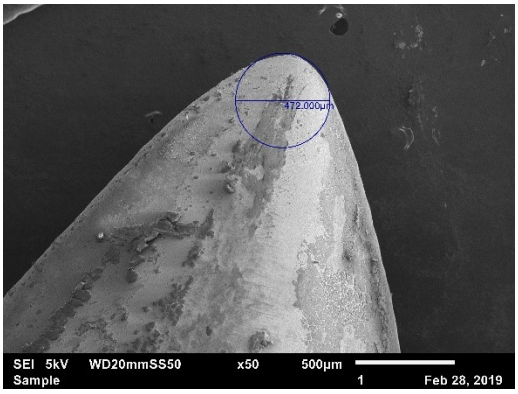

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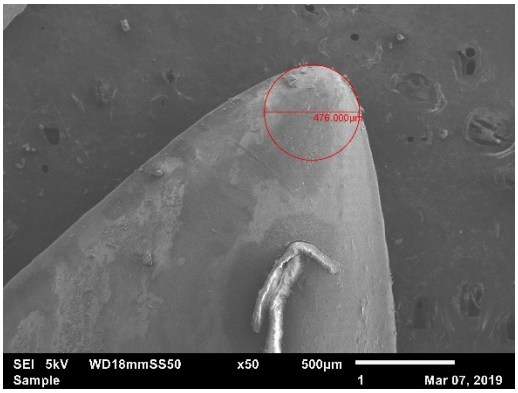

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Sample 1 Mar 07, 2019

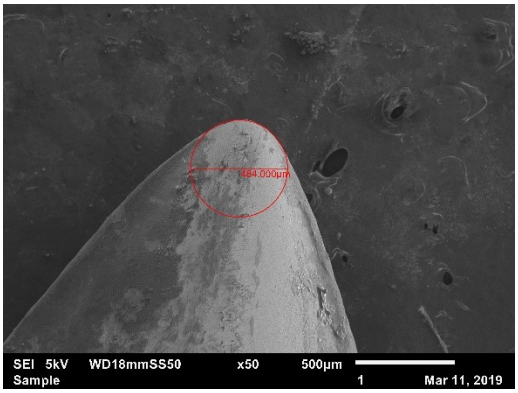

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SEI 5kV WD18mmSS50 x150 100µm
Sample 1 Mar 11, 2019

R7 Apex

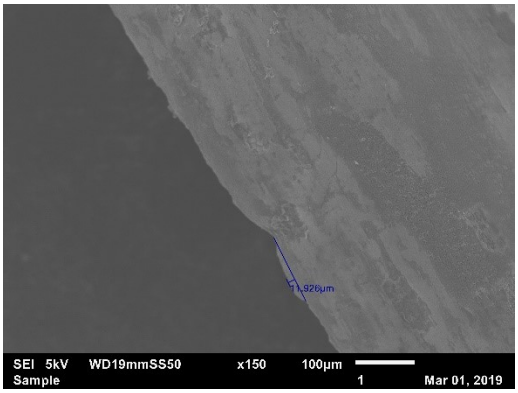

SEI 5kV WD40mmSS50 x50 500µm
Sample 1 Jan 25, 2019

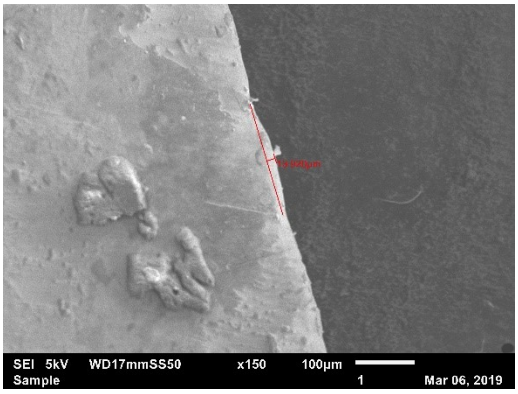

472.000µm
SEI 5kV WD20mmSS50 x50 500µm
Sample 1 Feb 28, 2019

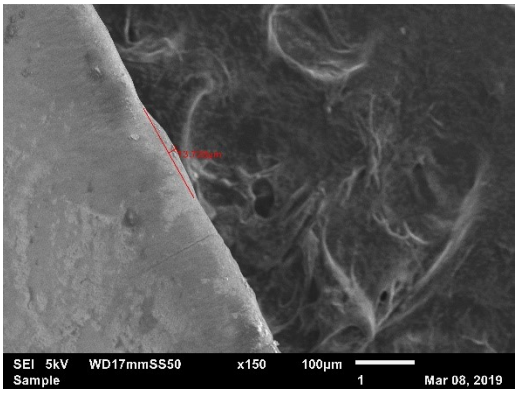

476.000µm
SEI 5kV WD18mmSS50 x50 500µm
Sample 1 Mar 07, 2019


484.000µm
SEI 5kV WD18mmSS50 x50 500µm
Sample 1 Mar 11, 2019

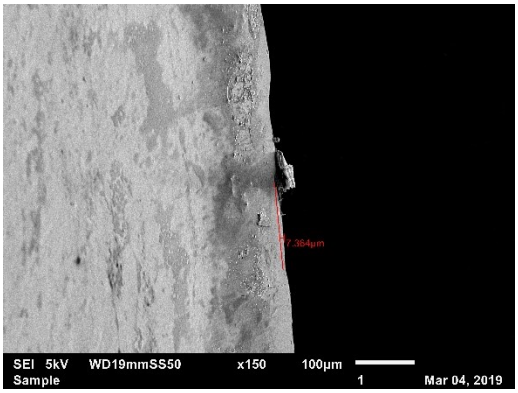
R7 S1

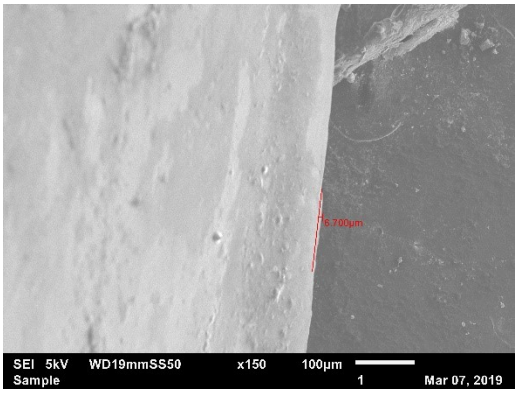

1.926µm
SEI 5kV WD19mmSS50 x150 100µm
Sample 1 Mar 01, 2019

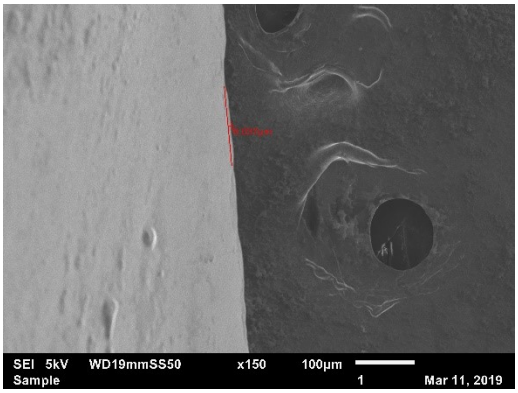

SEI 5kV WD17mmSS50 x150 100µm
Sample 1 Mar 06, 2019


SEI 5kV WD17mmSS50 x150 100µm
Sample 1 Mar 08, 2019

R7 S2







Note: The pre-trial serrations photos were not saved but were recorded in the lab notebook