

Denticle morphology in the Pacific Spiny Dogfish, *Squalus suckleyi*

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Abstract

To learn more about the denticle morphology and potential for replacement in the denticles of *Squalus suckleyi*, the Pacific spiny dogfish, a survey of denticle shapes using scanning electron microscopy (SEM) was conducted. This provides the knowledge of what is expected in denticle shape and serves as a baseline for future comparisons. Denticle shapes were seen to change over the regions of the body as well as over ontogeny. Denticle shapes were also linked to the function at the different regions of the body. Since there is no dental lamina in denticles, denticles were not seen to replace like oral teeth which went against the predicted expectations. The emergence pattern of the denticles in embryos also went against the predictions as it was different from the previously recorded two dorsolateral rows. These two unexpected findings raise questions and provide a basis for future research. In addition, histology and micro-CT scan data provided information on how the denticles emerge in the embryos and provide a comparison of how similar replacement in adults and this first emergence in embryos look.

Introduction

Denticles (dermal teeth) are teeth like oral teeth, and as such, share similarities. Both denticles and oral teeth are made up of dentine and enamel (Gabler-Smith et al., 2021). However, denticles lack the dental lamina (Berio et al., 2021). This distinction leads to a difference in how the two replace. Oral teeth are replaced continuously throughout the shark's lifetime (Berio et al., 2021). The difference in expression of

SOX2, the marker responsible for dental growth, between oral teeth and dermal teeth may explain the difference in replacement possibilities between oral teeth and denticles (Cooper et al., 2022). Knowledge about denticle replacement is limited and virtually unknown which is why this study and future research into this is needed.

Evolutionarily, denticles are ancient vertebral structures that predate oral teeth and have been around for over 450 million years (Cooper et al., 2018). Denticles are one of the earliest vertebrate traits and are great to study because they are made of enamel, one of the hardest biological materials, and fossilize well which leads to an excellent fossil record to compare against. Their prolonged existence also means there is a plethora of resources available to study denticles as so many organisms have had denticles. The long amount of time also means that denticles have had ample time to become specialized and form different shapes based on their function.

Modern understanding and study of denticles revolves around function. For example, flat leading edge denticles on the caudal tail of the common thresher shark (*Alopias vulpinus*) likely provide protection and limit denticle damage (Popp et al., 2020). Denticles reduce drag based on their streamlined shape, protect from parasites with anti-fouling properties, and aid in mating by their density to allow males to bite the females for better grip (Cooper et al., 2018; Feld et al., 2019; Crooks et al., 2013). Flat and rounded denticles are seen around the nose and leading edges of fins to reduce hydrodynamic drag (Ankhelyi et al., 2018). Denticles can also be cuspid and have a more prominent ridge as seen in the middle parts of fins and trailing edges (Ankhelyi et al., 2018). Because denticles are extremely specialized to serve a specific function of the shark, it is especially important for the shark to replace the denticles as they are damaged.

Teeth have specific and controlled patterns of replacement. This pattern stays the same over ontogeny. Does the replacement pattern in denticles stay the same over ontogeny? Replacement in denticles is not well studied, and this study is looking at the effect of denticle replacement on denticle shape in *Squalus suckleyi*, the Pacific spiny dogfish. Thresher shark tail denticles replace crown first with all surface ridges fully formed (Popp et al., 2020). Do spiny dogfish replace denticles in a similar manner? If denticles are evolutionarily the same as teeth, will they have the same mechanism of replacement?

When looking at the replacement of the denticles, you must also consider the emergence of the denticles for the first time in the embryos. Is there a difference between emergence and replacement or are they the same? If replacement is driven by damage and emergence by growth, we would expect the two to be different. In addition, when looking at the emergence of the denticles, it is worth noting the pattern they follow. Catshark embryos have a development pattern of caudal tail denticles forming first and then in two dorsolateral rows (Cooper et al., 2018), but spiny dogfish embryos do not follow the same pattern (p.r.).

Taking a morphological approach will clarify normal findings in these denticles and their mechanism of replacement. The goals of this study are to find evidence of replacement and find out if replacement in denticles is the same as in oral teeth. Using dissection, scanning electron microscopy (SEM), clear and stain, micro-CT scanning, immunohistochemistry, and geomorph techniques will allow us to complete the goals of this study with a comprehensive survey of the denticles of *S. suckleyi*. We predict that we

will find evidence of replacement of the denticles and that replacement follows a similar pattern in denticles as it does in teeth.

Methods

Dissection/Sample collection

Took one specimen of *Squalus suckleyi* (TL= 45.2 cm; snout to the last vertebra) out of the freezer and placed it into a tank to thaw (~1.5 hours). Once thawed, photos (taken on a smartphone camera) of the entire specimen and areas of interest (where samples were obtained) were taken. We looked at 16 areas hypothesized to be under different hydrodynamic stressors, these included: top of the nose, nose tip, bottom of the nose, leading and trailing edge of the pectoral fin (left), leading and trailing edge of the dorsal fin, and leading and trailing edge of the caudal fin, trunk, lateral line, spiracle (left), bottom (~50% TL), tongue, gill flap, and upper branchial arch. A scalpel was used to cut out skin samples (~1 cm² of tissue). Samples were then placed into 95% ethanol (190 proof) for 2 days.

Samples from 13 of the areas hypothesized to be under different hydrodynamic stressors: top of the nose, nose tip, bottom of the nose, leading and trailing edge of the pectoral fin (left), leading and trailing edge of the dorsal fin, and leading and trailing edge of the caudal fin, trunk, lateral line, spiracle (left), bottom (~50% TL) were also taken from a specimen of *S. suckleyi* (TL=58.6 cm) freshly euthanized with MS-222. Photos were taken (on a smartphone camera) and a scalpel was used to cut out skin samples (~2

cm² of tissue). Samples were placed in a 4% formalin solution. The samples were then washed with (PBS) for 2 hours then transferred to 50% EtOH for another 2 hours, After, the samples were transferred to 100% EtOH.

Samples from an *S. suckleyi* embryo (TL=24.1 cm) were taken from the same 13 regions under hydrodynamic stressors. Photos were taken (on a smartphone camera) and a scalpel was used to cut out skin samples (~1 cm² of tissue). Samples were placed in 95% ethanol for 6 hours.

Scanning electron microscopy (SEM) and Alizarin red stain

Samples were sonicated using an SPT UC-0609 Ultrasonic Cleaner for four minutes in water and soap to remove any surface debris. Once cleaned, samples were placed back in 95% EtOH for ten minutes and then moved and stored in 100% EtOH until chemical drying. Cleaned samples were chemically dried with hexamethyldisilazane (HMDS) following a modified protocol. Individual samples were placed in glass petri dishes and covered with just enough HMDS to fully cover each specimen. The sample stayed uncovered in the fume hood for an hour before the remaining liquid was removed with a pipette. Samples remained uncovered until all liquid evaporated and the specimens were fully dried. Dry samples were adhered to an SEM stub using a carbon-adhesive sticker, sputter coated (using a Cressington 108 Sputter Coater) for 60 seconds, and covered with a thin film of an electrically conductive material (gold-palladium). Samples were imaged using a NeoScope JCM-5000. Samples from the freshly euthanized shark and embryo were treated with the same protocol stated above.

Adjacent skin samples to those used in SEM were processed with Alizarin red S stain for mineral visualization. Skin segments, after fixation (in neutral buffered formalin (10%) or formaldehyde (37%)), specimens were washed in phosphate-buffered saline, then KOH (0.3%), we stained in a deep red solution of Alizarin Red S (130-22-3) for 24 hours. Once fully stained, samples were transferred into several fresh vials of 95% ethanol to remove excess dye. Denticles were imaged with a Nikon compound microscope (ECLIPSE E600W) and AxioVision Camera (Q-Imaging MicroPublisher 5.0 RTV Microscope Camera) using the QCapture program.

Histology

Samples were fixed in formalin (4% solution) for several hours. Then samples were treated with EDTA to soften mineralized tissues. The samples were embedded with paraffin using xylene and paraffin step sequence (ethanol dehydration sequence, then 50%/50% xylene and paraffin, then 100% paraffin two times). Samples were then made into blocks and sectioned using a Spencer “820” microtome (thickness of cuts being 10 microns). The sections were placed in the water bath, then transferred onto glass slides, and left to dry overnight on the heated plate. The samples were then put through a modified staining protocol (from Ella Nicklin) starting with a 10-minute xylene wash, then an EtOH step sequence of 100%, 96%, and 70% for 8 minutes each. Next, the slides were washed with moving water from the sink for 8 minutes. After that, the histological samples were stained using hematoxylin (2.5 mins), bluing agent (30 s), and eosin (2 mins). Between each stain, the samples were washed running tap water for 1 minute. After the eosin stain, the samples went through the same EtOH step sequence as the

beginning and then a light layer of mounting media was added to the cover slide and the cover slide was placed over the glass slide at a 45-degree angle to ensure no air bubbles. Sections were then observed and photographed under a Nikon compound microscope (ECLIPSE E600W) and AxioVision camera (Q-Imaging MicroPublisher 5.0 RTV Microscope Camera).

Micro-CT Scanning

Embryos of *S. suckleyi* and *S. acanthias* (n=4, TL= 19.5 cm, 20.0 cm, 24.1 cm, and 27.5 cm) were scanned using Bruker SKYSCAN 1173. The embryos were placed in a heat-sealed plastic bag with a small amount of 70% EtOH to maintain moisture in the preserved museum specimens and then packed tightly into a tube with packing peanuts and other styrofoam materials for increased stability and wrapped in cling wrap and then placed into the CT scanner. Four full-body scans and two high-resolution scans of areas of interest (head and dorsal spine) were obtained. 3D Slicer and the SlicerMorph extension were then used to visualize 3D images of the CT scans.

Results

Denticle emergence pattern

The embryos of *S. suckleyi* did not have the two dorsolateral rows seen in other shark species, such as *Scyliorhinus canicula* (Copper et al., 2018). The emergence of a new denticle was found to be the same in adults and embryos (Figure 2).

Observations of different denticle shapes

Several different denticle shapes were observed (varying in cusp number, and ridge height) and recorded along the body regions of all three sharks sampled (Figures 3-5). Denticle shapes were not consistent throughout the body, and even varied within the same skin sample (~1-2 cm² of tissue).

Change in denticle shape

A gradient in denticles becoming smaller and more overlapped from the leading edge to the trailing edge on the fins were seen as previously noted (Ankhelyi et al., 2018). A dorsoventral gradient in denticle shape and roughness was also observed as the denticles became less rough and had less prominent ridges as consistent with previous observations (Ankhelyi et al., 2018). Denticle shape was also observed to change over ontogeny (Figure 6).

Replacement

Potential replacement of denticles was observed in several samples in the two adult sharks (Figure 7). Skin samples from the nose tip, bottom of the nose (ventral), pectoral fin, and caudal fin had potential sites of replacement.

Discussion

Denticle emergence pattern

The differing emergence pattern of the denticles in the *S. suckleyi* embryos was unexpected to find. This goes against the previous thought that the emergence pattern of denticles in sharks follows a shared pattern (Copper et al., 2018). Since this pattern is no longer a shared characteristic amongst all sharks, it can be used as a trait in phylogenetic trees to determine how closely or distantly related different species of sharks are.

Denticles typically emerge first as two dorsolateral rows in eggcase-developing sharks, however in *S. suckleyi*, a viviparous shark, we saw no apparent dorsolateral row. This could indicate a difference between internal, uterine, and external, eggcase-developing sharks.

Different denticle shapes

Differing denticle shapes are linked to differing functions at the different regions of the body. The function of the denticles is crucial to the overall functioning of the shark. The differing shapes within small areas of skin mean that function can change quickly throughout the body. The associated function with the corresponding shape could also be used to predict the function of denticles in other shark species based on their shape. The different shapes could also be used to group the different families or to group different shark species based on ecological role or water column depth. For example, if two shark species are both benthic and slow-moving, it could be predicted they could have similar denticle shapes and functions based on their similar lifestyles. If the denticle shape is used to group species based on phylogenetic closeness, the evolution of denticle shape and function over the millions of years sharks have been active could be seen.

Changes in denticle shapes

Changes in the denticle properties (size, roughness, etc.) from the leading edge to the trailing edge and dorsal to ventral suggest that the denticles serve a different function in these regions. As the denticles change in shape, they may no longer serve only hydrodynamic drag reduction purposes (Ankhelyi et al., 2018). Changes over ontogeny are also potentially due to changes in function between adults and embryos. Embryos have not used their denticles but adults have had great use of their denticles. Between adults and juveniles, there is also likely a difference in denticle shapes based on their different lifestyles and usage of the denticles. Differences in denticles and the corresponding function between adults and embryos due to the embryos not using the denticles have been recorded previously in other species (Popp et al., 2020).

Replacement

If true replacement was seen and not growth, this has larger significance and implications for our current knowledge of shark denticles. Denticle replacement is virtually unknown and possible observations are few and far between. If we are able to record examples of replacement, we can prove that it does actually occur, and this could be the first step towards finding the mechanism that denticles replace since it is different than in oral teeth. It is also interesting or worth noting what regions of the body had potential areas of replacement and whether certain areas had more occurrences. This could tie into function and where the denticles need to replace the most based on damage and need. Replacement denticles were superficial to the dermal region of the skin and

not associated with any identifiable dental lamina-like structure, which answers the question of how denticles are able to replace without a dental lamina.

Biomimicry application

Shark denticles can be used in biomimicry to inspire product designs. Finding the most efficient denticle shape based on what function the product is looking for could be very important for this emerging market. Concerning the antifouling property of shark denticles, finding the best shape for antifouling could have a large impact on the way we prevent germ spread and disease outbreaks through films (based on denticle shapes) on top of high contact areas like doorknobs. The hydrodynamic drag-reducing qualities of shark denticle shape can also be used to design more efficient products that move in the water like swimsuits or hydroelectric turbines.

Further studies and future directions

This study was a larger overview of what the denticles of *S. suckleyi* look like and how they function. The knowledge from this qualitative study can further information on shark denticles specifically concerning replacement. Figuring out the emergence pattern in *S. suckleyi* embryos and figuring out how and where replacement occurs in this species and others are further areas that need to be looked into. Gathering data from juvenile *S. suckleyi* is a potential future study to close the gap between the embryo and adult denticle data we have to see how denticles change shape between juveniles and mature adults.

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References

Ankhelyi, MV, Wainwright, DK, Lauder, GV. (2018) "Diversity of Dermal Denticle Structure in Sharks: Skin Surface Roughness and Three-Dimensional Morphology." *Journal of Morphology*, vol. 279, no. 8, pp. 1132–1154., <https://doi.org/10.1002/jmor.20836>.

Berio, F, Evin A, Goudemand, N, Debiais-Thibaud, M. (2020) "The Intraspecific Diversity of Tooth Morphology in the Large-Spotted Catshark *Scyliorhinus Stellaris*: Insights into the Ontogenetic Cues Driving Sexual Dimorphism." *Journal of Anatomy*, vol. 237, no. 5, pp. 960–978., <https://doi.org/10.1111/joa.13257>.

Cooper, RL, Thiery, AP, Fletcher, AG, Delbarre, DJ, Rasch, LJ, Fraser, GJ. (2018) "An Ancient Turing-like Patterning Mechanism Regulates Skin Denticle Development in Sharks." *Science Advances*, vol. 4, no. 11, <https://doi.org/10.1126/sciadv.aau5484>.

Cooper, RL, Nicklin, EF, Rasch, LJ, Fraser, GJ. (2022) "Teeth Outside the Mouth: The Evolution and Development of Shark Denticles." bioRxiv preprint,

<https://doi.org/10.1101/2022.07.13.499989>.

Crooks N, Babey L, Haddon WJ, Love AC, Waring CP (2013) Sexual Dimorphisms in the Dermal Denticles of the Lesser-Spotted Catshark, *Scyliorhinus canicula* (Linnaeus, 1758). *PLoS ONE* 8(10): e76887.
doi:10.1371/journal.pone.0076887

Feld, K, Kolborg AN, Nyborg, CM, Salewski, M, Steffensen, JF, Berg-Sørensen, K. (2019) “Dermal Denticles of Three Slowly Swimming Shark Species: Microscopy and Flow Visualization.” *Biomimetics*, vol. 4, no. 2, p. 38.,
<https://doi.org/10.3390/biomimetics4020038>.

Gabler-Smith, MK, Wainwright, DK, Wong, GA, Lauder, GV. (2021) “Dermal Denticle Diversity in Sharks: Novel Patterns on the Interbranchial Skin.” *Integrative Organismal Biology*, vol. 3, no. 1, <https://doi.org/10.1093/iob/obab034>.

Johanson, Z, Tanaka, M, Chaplin, N, Smith, M. (2007) “Early Palaeozoic Dentine and Patterned Scales in the Embryonic Catshark Tail.” *Biology Letters*, vol. 4, no. 1, pp. 87–90., <https://doi.org/10.1098/rsbl.2007.0502>.

Popp, M, White, CF, Bernal, D, Wainwright, DK, Lauder, GV. (2020) “The Denticle Surface of Thresher Shark Tails: Three-Dimensional Structure and Comparison to Other Pelagic Species.” *Journal of Morphology*, vol. 281, no. 8, pp. 938–955., <https://doi.org/10.1002/jmor.21222>.

Figures



Figure 1 Shark A (TL= 45.2 cm), shark B (TL=58.6 cm), and shark C (TL=24.1 cm).

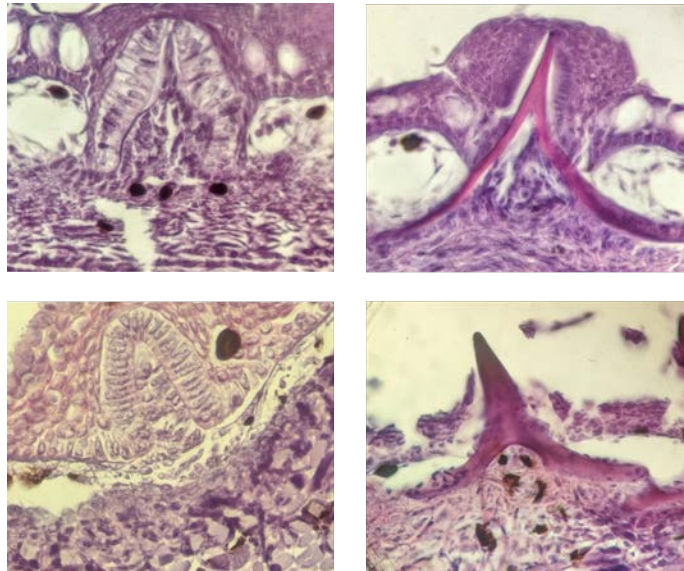


Figure 2 Developing (left) and mineralized tooth (right) in embryos (top) and adults (bottom).

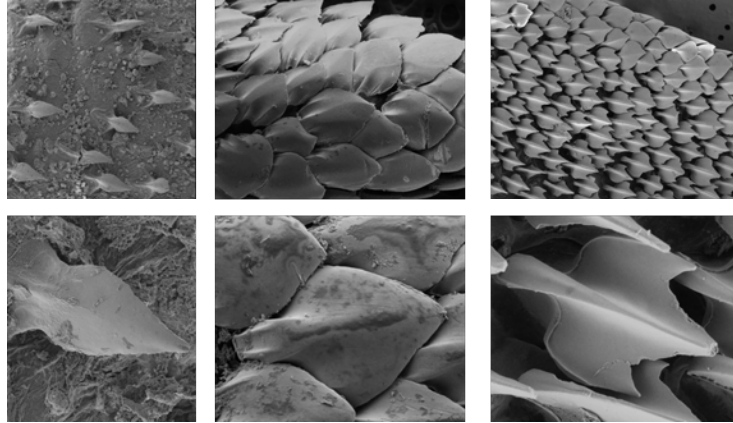


Figure 3 Denticles from three different regions in shark A: tongue caudal leading edge, and dorsal trailing edge.

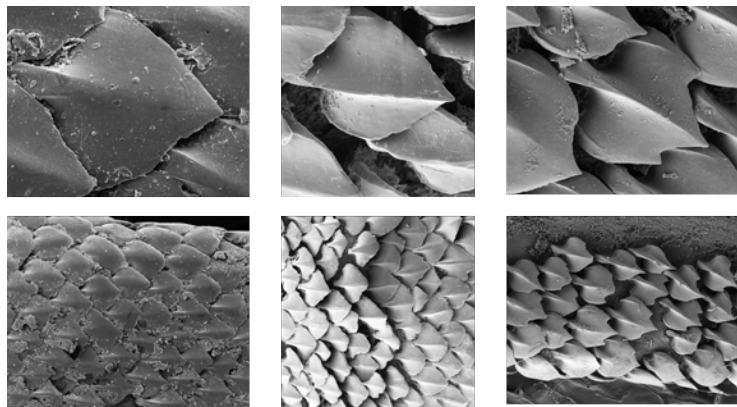


Figure 4 Denticles from three different regions in shark B: dorsal leading edge, nose, and dorsal trailing edge.

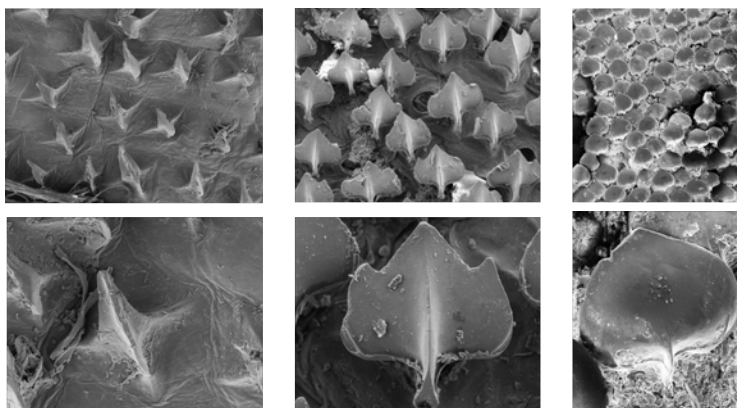


Figure 5 Denticles from three different regions in shark C: lateral line, nose tip, and pectoral trailing edge.

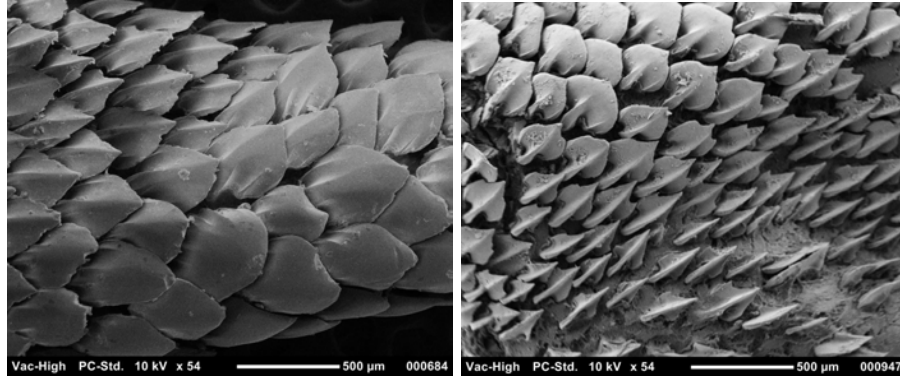


Figure 6 Pictures highlighting gradient in denticle flatness from leading to trailing edges.

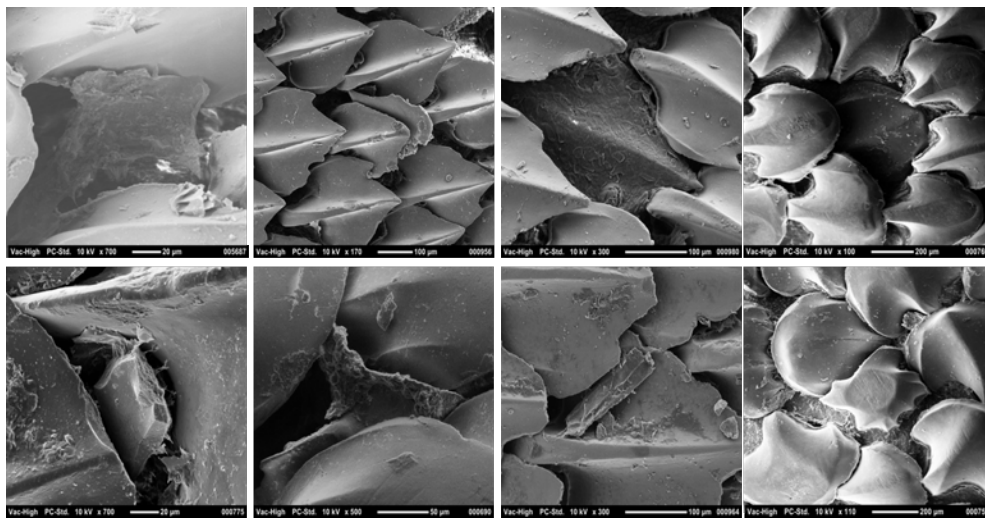


Figure 7 Sites of potential replacement in denticles (from shark A).