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Connected Microscopy to Characterise the Dermal Denticle of *Raja clavata*, the Thornback Ray

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Thornback ray, *Raja clavata*, have multiple dermal denticles positioned on the dorsal side of their bodies. These dermal denticles are formed through the deposition of minerals by the epidermal and dermal cells, and their structural makeup is a dentine protrusion with vitrodentine on the outside (1,2). The possible function of dermal denticles across different species has led to debate, ranging from protection of predators and mechanical abrasion, to enhanced swimming performance, or deterring attachment of ectoparasites (3). The dermal denticles found on skates are larger, therefore the widely accepted function is defence (3). Biological materials and biomineralisation is dependent on the environment, utilising moisture, temperature fluctuations, loading, and chemistry to create complex chemical and biological structural relationships with often enhanced structural integrity (4). Analysing and understanding the complex ways the microstructure is arranged to achieve stiffness, strength and toughness can be used for bioinspiration (5).

This work demonstrates the potential of X-ray microCT/X-ray microscopy for 'bioprospecting' functional layers in biological materials. The initial characterisation step for the thornback ray denticle was X-ray microscopy on a lab-based system (Zeiss Xradia Versa 520) capable of spatial resolution down to 700 nm. An initial speculative scan of a sample retrieved from the campus beach at Swansea University, UK, revealed a clear and distinct micro-layering and interface, through a difference in X-ray greyscale. This indicates a contrast in adjacent materials with possible chemical variation, and potentially mechanical differences too, as seen in similar work on cuttlebone (6,7). Subsequently, a fresh sample was acquired from the food industry for this study. Targeted imaging was applied to study these layers and their interface further and understand if there is a difference in mechanical and chemical properties across the two regions, as seen in similar layered shark teeth samples (8). Secondary electron microscopy (SEM) and energy dispersive spectroscopy (EDS) was applied to a sample targeting the interface region, including the outer and inner layers. SEM and EDS characterisation was carried out using a Zeiss Evo LS25 SEM. The sample was prepared by cold mounting in CHEM 1000 Blue resin and ground down to reveal the interface between the two layers, with polishing down to a 0.04 µm surface finish. Nanoindentation was carried out on a Bruker Hysitron Ti950 using a Berkovich tip geometry, 500 µN load at 2 µm spacing generating XPM (accelerated property mapping) hardness and reduced Modulus maps of the dentine and vitrodentine. EDS and SEM captured the microstructural and chemical differences between these layers.

In this study multimodal microscopy was applied to multiple denticles for different aspects of characterisation. High resolution XRM, SEM imaging of microstructures, EDS imaging to understand the chemical composition, and nanoindentation to quantify the micromechanical properties. This develops understanding of the structure-property relationship in thornback ray denticles, potentially informing understanding of their function. Also, lessons learned from nature in terms of layering of

materials with Modulus mismatch can inspire materials design for components requiring similar function. The XRM highlighted a potential discrete difference in microstructure and chemistry across the interface from the differences in attenuation greyscale. This was observed across multiple denticle samples where the interface could be observed. Damage to the vitrodentine layer was observed in some denticles, with the outer layer fractured and delaminated from the dentin layer, potentially from an impact in the wild. Beneath regions of damage the dentin layer is seemingly intact, and it's possible the vitrodentine is acting as a sacrificial layer from observations with XRM and SEM. EDS tests reveal a higher calcium (Ca) and phosphorus (P) content in the outer layer – the vitrodentine region. Nanoindentation revealed a doubling of hardness and reduced Modulus from the inner dentin to the outer vitrodentine layer. The nanoindentation coupled with the chemical analysis reveals a large mechanical difference which arises from a comparatively low difference in counts per second of Ca and P. A 25-percentage increase in Ca and 11 percentage increase in P in the vitrodentine region imparting a 2 times higher reduced Modulus and hardness of this layer.

A multi-modal connected approach to characterising biological materials enables greater insight into the structure-property relationships fundamental to material behaviour, but also aids understanding of form and function. This approach can be used to identify features of interest in nature, to understand them chemically, mechanically, microstructurally, and morphologically, and to inspire new manufactured material developments based on these insights [9].

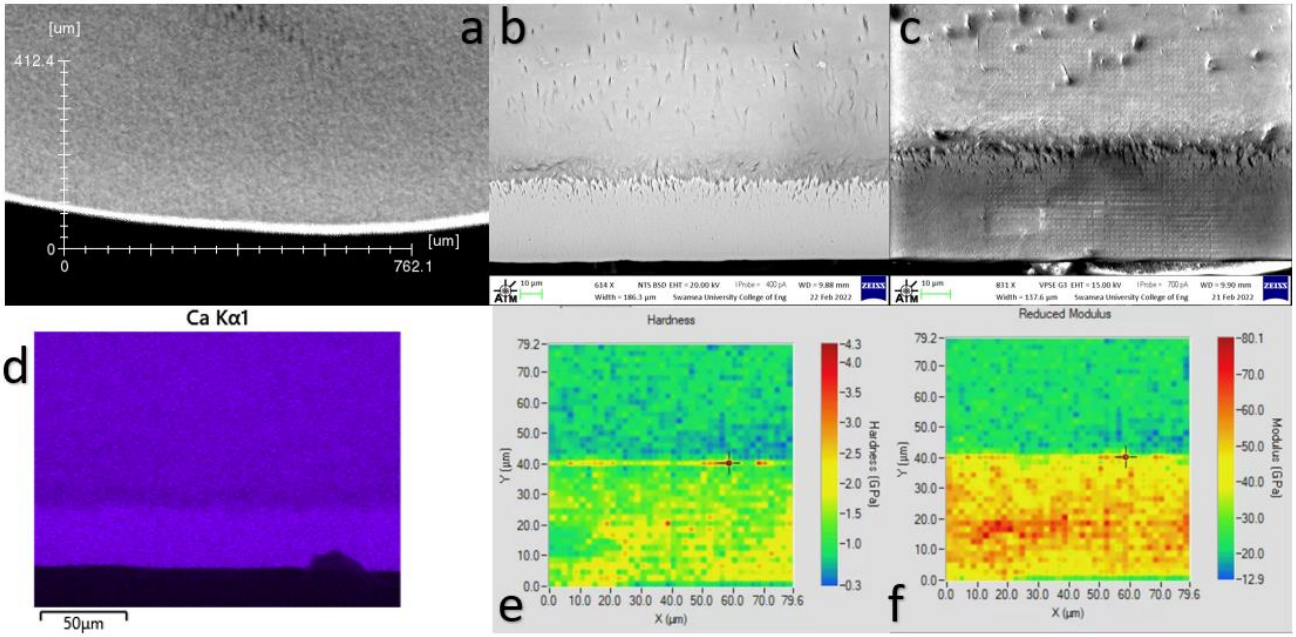

Figure 1. Each pane (a-f) represents the outer region of the thornback ray dermal denticle in cross section imaged/characterised with different modalities. In each pane, the outer vitrodentine is at the bottom and the inner dentine is towards the top. (a) XRM characterisation showing the bright region (higher density) of a potential functional layer. (b) Backscatter electron microscopy of interface between the two layers of vitrodentine and dentin. (c) Secondary electron microscopy of interface between two layers, with indents from XPM visible. (d) Energy dispersive spectroscopy of calcium across vitrodentine and dentin, with higher Ca content present in outer vitrodentine. (e) Nanomechanical XPM hardness map. Difference in hardness across the two layers. (f) XPM reduced Modulus map. Distinct difference in indentation reduced modulus E' between the layers.

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