APPLICATION OF RAMAN SPECTROSCOPY TO BIOMINERALIZATION PROBLEMS IN MARINE CHITONS

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Abstract: This contribution discusses the distribution of iron oxide biominerals in marine chiton teeth.

Chiton teeth, like many biological structures, are sophisticated organic/inorganic composites, the structure and function of which have been refined over millions of years. The result is optimised tools possessing many of the desirable features we seek in biomaterials. Importantly, the extremely hard iron and calcium biomaterials deposited in these teeth are formed at physiological pH and ambient temperatures; conditions not conducive to their formation *in vitro*. Understanding the processes of mineral deposition in these teeth is fundamental to our wider understanding of the biomineralization process and highly relevant to Australia's modern materials focus.

Much of our current understanding of these biomineralized structures has derived from electron microscopy, X-ray and electron diffraction and elemental mapping via X-ray emission spectroscopy. We have initiated extensive studies of biomineralization using a suite of vibrational spectroscopies. Here we apply Raman spectroscopy and microscopy to studies, *in situ*, of the biomineralized structures that comprise chiton teeth. Laser Raman microscopy is a particularly powerful technique for the analysis of iron compounds. The majority of iron oxide and hydroxide vibrational bands occur below 600 cm^{-1}, which renders them inaccessible to routine infrared microscopy. The laser Raman technique also has the advantages of greater spatial resolution, down to ~1.5 μm in contrast to ~20 μm for infrared, and minimal interference from any adsorbed or included water. Therefore, it is possible to focus on very small regions of individual chiton teeth and characterise spectroscopically the chemical species present. Such knowledge is essential if the details of the biomineralization processes in the chitons are to be elucidated and used in laboratory preparations of novel nanocomposite materials.

Results we have recently obtained using the Raman microscopy technique challenge the assertion that all Chiton species can been separated into two general groups based upon the biominerals deposited in their teeth. Two biomineralization strategies are thought to be representative across all species, in one a magnetite surface cap overlays a discrete layer of lepidocrocite and a central tooth core composed of apatite (1-5), in the second strategy a magnetite cap is thought to overlay a ferric phosphate biomineral (6). We show for the first time that a new biomineral, the hydrated iron (III) oxide limonite can be found where ferric phosphate or lepidocrocite are expected (7). We show that deposition of lepidocrocite is limited to a very few species, those most easily collected and therefore the first studied and that in contrast the pseudomineral limonite is the most common FeOOH mineral deposited (7). Where species have been assumed to deposit only a hydrated ferric phosphate we find that despite EDS evidence for large quantities of phosphorus only limonite is observed by Raman spectroscopy(6,7). In fact we have not found evidence for a discrete ferric phosphate biomineral in any chiton species.

Our results suggest that chiton tooth biomineralization can not be divided into two discrete groups, rather, that there is a continuum of biomineralization strategies driven by diet and environmental factors.

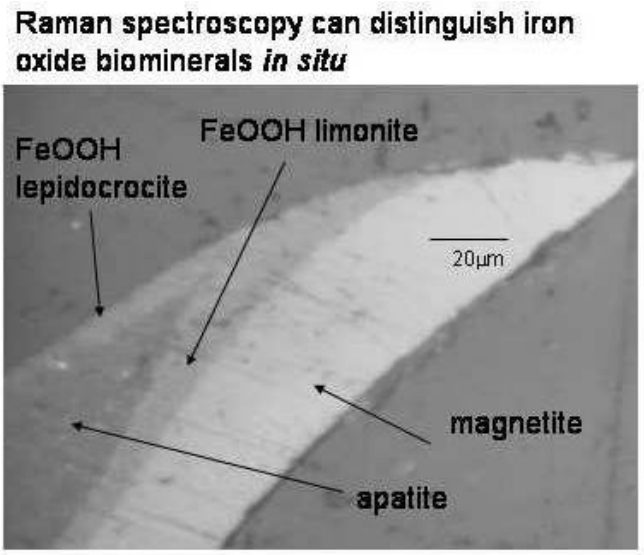

Fig. 1. A single chiton tooth photographed through the microscope of a Dilor Labram instrument showing the deposition of iron oxide biominerals in discrete micro regions.

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