FOLLOWING BIOMINERAL DEPOSITION FRONTS IN MARINE CHITON TEETH BY RAMAN SPECTROSCOPY

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Abstract: *In situ* Raman microscopy has been used to follow biomineral deposition wavefronts in single chiton teeth. These results demonstrate that the elements detected by energy dispersive spectroscopy (EDS) may not always represent the presence of a deposited mineral.

The structure and composition of the major lateral teeth of chitons (Mollusca: Polyplacophora) is of considerable interest due to their use of biomineralized forms of iron and calcium(1-4). In most chitons, the major lateral teeth comprise an iron-oxide cap that extends down the posterior (or cutting) surface which overlies a softer central tooth core composed of either calcium phosphate or phosphate containing oxyhydroxide of iron (5).

Prior to the availability of Raman spectroscopy through microscope optics the mineral components of the core region and deposition processes involved in their formation had not been investigated extensively in any chiton species owing to difficulties in sample preparation. Whilst Heinz Lowenstam first observed that the mineral in the core region of teeth of three chiton species, *Acanthopleura echinata*, *A. spiniger,* and *Chiton tuberculatis* was an apatitic calcium phosphate (5) his investigation used an *ex situ* analysis protocol. The tooth cusps from many teeth were disected out of subject animals, the black magnetite capping material carefully separated from the softer lighter core material before the latter was crushed and examined by infrared spectroscopy to identify the mineral component. Further *ex situ* investigations later concluded that an initial deposit of an amorphous calcium phosphate was formed in the tooth core and that this mineral matured into an apatitic calcium phosphate (dahllite) over the course of a number of rows (6). However, the processing method employed in both these studies precluded a detailed study of the progress of mineral deposition within microregions of individual teeth.

Laser Raman microscopy, analysing as it does small areas, offers the opportunity to study micro domains of individual teeth *in situ* and identify the mineral components present.. To demonstrate the power of the *in situ* Raman microscopy technique for the analysis of very small biomineral structures we show here the results of a complementary study using energy dispersive and Raman spectroscopies to characterise the progress of biomineralization in the the cores of teeth from the species *Acanthopleura echinata*. This species is idea for study since it possesses a radula with teeth much larger than most other chitons, effectively increasing the resolution of our study (7).

Our studies show that mineralization in chiton teeth proceed as an organised process from the tooth tip down and from the posterior to anterior surfaces. We also show that regions of high Fe, Ca, and P, ion density, as measured by energy dispersive spectroscopy, are not necessarily associated with any mineral deposit (7). Our results challenge previous *ex situ* investigations that concluded that the biominerals in chiton teeth are deposited first as amorphous materials that undergo a maturation process.

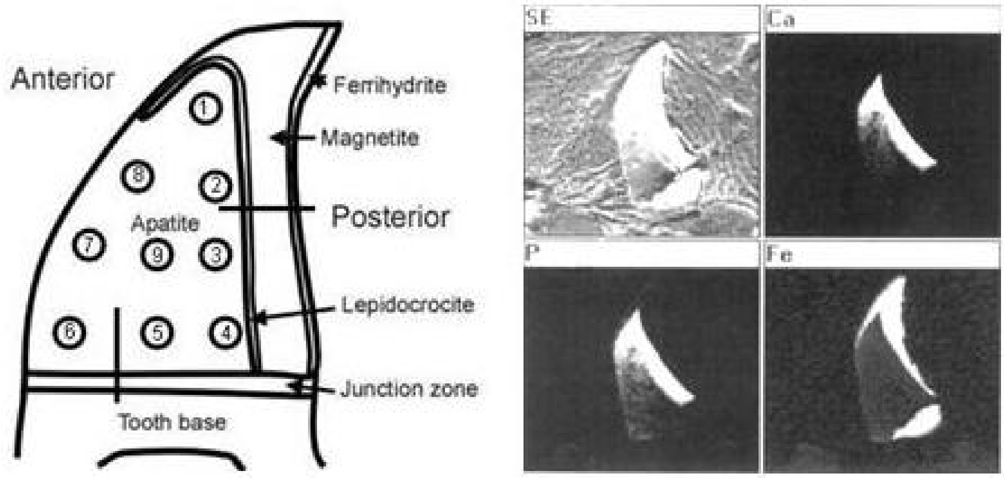

Fig. 1. A schematic of a chiton tooth and EDS element maps showing individual micro-regions examined by Raman spectroscopy and the element distribution of a semi mature tooth.

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