3211 The Inhibition of Artificial Lesions by STP: An in vitro Study by SEM

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Introduction

The objective of this study is two fold:
(1) To demonstrate the application of scanning electron microscopy (SEM) in back scattering detection mode, coupled with elemental analysis by x-ray for the qualitative characterisation of artificial caries lesions in enamel, formed by cariogenic acid challenge [1].
(2) To show the efficacy of sodium tripoly phosphate (STP) for preventing artificial lesions in enamel.

Energy dispersive X-ray spectroscopy (EDS) is used for elemental analysis. A sample that is subjected to electromagnetic radiation emits x-rays that are characteristic of the element's atomic structure. A high energy beam of electrons are focused into a sample in the SEM and the resulting x-rays mapped.

Electron backscatter detection (BSE) can be used to differentiate material density, morphology , and crystal orientation. Electrons are focused onto a specimen in the SEM and the resulting backscattered electrons used to construct an image of the specimen. Contrast between areas with different chemical or physical compositions is obtained when there is a difference in the atomic number or density.

Methods

SEM studies

SEM studies were performed using a Zeiss Evo50 environmental microscope. Typical conditions for imaging in BSE mode are: Magnification = 1500, Working distance = 5mm, EHT = 20kV. EDS conditions are: Working distance = 10mm, EHT = 20kV.

Sample preparation

Enamel specimens were prepared from 10mm square sections, 3mm thick, enamel taken from the buccal side of bovine teeth. The enamel sections were embedded in resin and exposed so that the surface of the enamel could be polished and treated. Polishing involved a dedicated machine in combination with an increasingly fine cut SiC abrasive paper with a final five minute polish using 3.0 μm diamond paste.

Post treatment, the specimens were prepared for SEM analysis by cross sectioning through the treated face using a diamond wafering saw. The sectioned face was then polished using 3.0 μm diamond paste to remove the smear layer. The specimens were then mounted, so that the cross section could be imaged. The resulting section is shown in figure1.

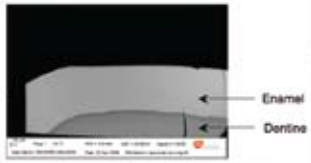

Figure 1 shows the cross section of an enamel specimen in electron backscattering detection mode. Lighter structure indicate a higher molecular density.

[1] Cariogenic buffer comprises of a partially saturated solution with 0.1M lactic acid and 1% carboxymethylcellulose adjusted to pH4.5.

Experimental design

Prepared enamel specimens n=5 were assigned to various treatments:
- Two minute incubation in a 10% w/w solution of sodium tripolyphosphate, then incubation in lactic acid buffer.
- Two minute incubation in a 5% w/w solution of sodium tripolyphosphate, then incubation in lactic acid buffer.
- Incubated in lactic acid solution for 1, 3, 5, 6 and 12 days.
- No treatment.

Sodium tripolyphosphate treatments were incubated in a non stirred solution. Lactic acid buffer treatments were placed onto an orbital agitator set at 50 rpm.

Results

The lesion depth time course study was evaluated by measuring the depth of the lesion across the enamel specimen and averaging over 5 specimens for each treatment leg.

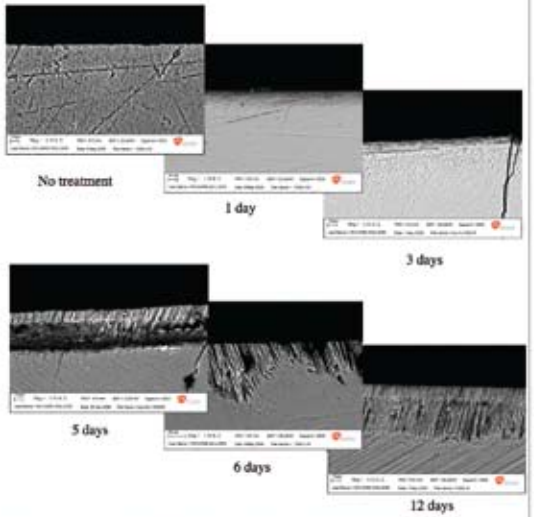

Figure 2 shows a representative BSE image of enamel incubated in lactic acid buffer. The image is of the cross-section with the treated face uppermost.

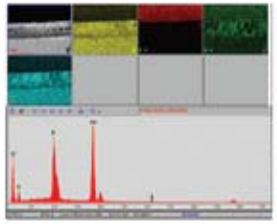

Figure 3 shows a representative example of EDS analysis of enamel after treatment in lactic acid buffer for 5 days. The colour maps are for the elements Ca yellow, C red, O green, P blue. Approximately 40 μm from the treated surface Ca and P show a dark band that can be ascribed to demineralisation of the enamel.

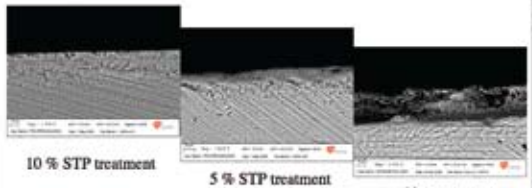

Figure 4 shows representative BSE images of enamel treated with STP solutions prior to incubation in lactic acid buffer for 5 days. The image is of the cross-section with the treated face uppermost.

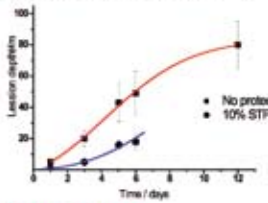

Figure 5 shows the progression of the lesion as a function of incubation time. The limited time course study for STP treated enamel indicates that the formation of lesion is retarded.

Conclusions

- SEM lends itself well to the study of artificial caries lesions, in particular, BSE is rapid and has good resolution. EDS shows promise in the study of de-mineralisation, in practice is highly laborious taking many hours to collect x-ray spectra to give sufficient detail.
- The time course study for the progression of the caries lesion indicates that the conversion of surface erosion to sub-surface lesion takes longer than a day.
- Enamel treatment with a solution of STP significantly inhibits the formation and progression of the caries lesion. Future work is progressing to understand the mechanism of STP interaction with enamel.