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# Assessment Of Microbial Modulation Of Chemical Constituents In Enamel

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## ABSTRACT

**Introduction:** Dental enamel is a principal component of the tooth. It has evolved to bear large chewing forces and it can withstand over decades. Functional loss of enamel caused by tooth decay affects the quality of life. Enamel caries are characterized by the demineralization of inorganic and the destruction of organic substances.

**Materials and methods:** FESEM was used to visualize the tooth sections on the glass coverslip. Sections will be dehydrated and nitrogen gas was applied for drying. Further, energy-dispersive X-ray spectroscopy was used for elemental analyses and chemical characterization.

**Results:** In enamel calcium and phosphate were more but the other ions were also present and it got increased more than calcium and phosphate in areas where demineralization happened. Enamel restoration is more challenging than any other part of the tooth.

**Conclusion:** Demineralisation means loss of calcium and phosphate and increase in organic content like carbon and oxygen when this happens the hardness of the tissue will decrease.

**Keywords:** *Dental Enamel, Caries, Hydroxyapatite, scanning electron microscope, chemical changing, electron mapping*

## INTRODUCTION

The chemical changes which occur in the process of carious destruction in enamel are complex due to several factors. The main component of enamel, hydroxyapatite can behave in a complex manner during dissolution. The changes will occur throughout the enamel in the direction of carious attack i.e., from surface to interior. Hydroxyapatite has the ability to accept the substituent ions. There will be a wide range of calcium and phosphate species that can form following dissolution (Robinson et al., 1995).

The physicochemical properties of the mineral comprising the teeth surface modulate the development and remineralization of dental caries. The chemical reactions that occur during acidic conditions when tooth mineral dissolves are determined by the supersaturation of calcium and phosphate ions (Hicks et al., 2005). The association with fluoride has demonstrated the best results in the inhibition of caries development. Hypomaturation amelogenesis imperfecta is a hereditary disorder of the enamel that affects the function and aesthetics of patients. The retention of an organic matrix will influence the quantity and quality of mineral crystals which will demolish the animal enamel and affect the mechanical property (Sa et al., 2014). Dental caries may be defined as a bacterial disease of calcified tissues of teeth and it is characterized by demineralization of the inorganic and destruction of the organic substance of the tooth (Selwitz et al., 2007). Zones of enamel caries are the translucent zone which has an increased concentration of fluoride ions; dark zone; the body of lesion- which has prominent striae of retzius; and the surface zone where calcium and phosphate remineralise r (Nóbrega et al., 2020). Surface enamel is the site of the initial carious attack. Factors that may determine the highly selective dissolution of enamel in initial carious lesions will be assessed both from morphological and chemical points of view since the structures which endure the longest exhibit the characteristics of caries resistance (Brudevold et al., 2009). The fundamental building block of enamel comprises two nanometric layers enriched in magnesium flanking a core rich in sodium, fluoride, and carbonate ions (DeRocher et al., 2020). The aim of this study is to analyze the cariogenic modulation of chemical constituents in enamel. The objective of this study is to assess the ionic modulation due to the caries process in dental enamel.

## MATERIALS AND METHODS

In field scanning electron microscopy we can go two lakh times and see but what you see in the light microscope is thousand times. We will find newer things as we go deeper. Here, we not only see the electron microscopy image, we also access the ions in that particular region specific to the areas of damage so that makes the study interesting and different. A Field emission scanning electron microscope (FESEM -Jeol JSM - IT 800, Tokyo, Japan) was used to visualize the tooth sections on the glass coverslip at a magnification of 5μm. The Section will be dehydrated with 70% ethyl alcohol for 10 seconds and nitrogen gas was applied for drying. After critical point drying, the sections were sputter-coated with platinum for 30 seconds (30mA) to induce conductivity for FESEM analysis. Finally, images were captured at the acceleration voltage of 3.00 kV and projected. Further energy dispersive X-ray spectroscopy was used for elemental analysis and chemical characterization. Further, we also did Electron mapping with energy dispersal spectroscopy and we found that ionic difference was very significant in the demineralized area. An elemental mapping is an image showing the spatial distribution of elements in a tooth. Element maps are extremely useful for displaying element distribution, particularly for showing compositional zonation. The analysis was performed to observe composite material remains on the enamel surface and to evaluate other elements present on its surface. The microscope has a resolving power at a large depth of field, so it is possible to map the surface details of the teeth greatly enlarged.

## RESULTS

The Electron dispersal spectroscopy image showed peaks of ions that are predominantly present. The individual composition of ions distributed in their lesions in the deep enamel caries is observed. The areas of demineralisation exhibited changes in weight percentage significantly differ in terms of oxygen, calcium, Carbon, phosphate, sodium, and magnesium when compared to areas by weight percentage in the adjacent regions.

## DISCUSSION

If you see this picture there are green and pink colored dots, which shows the difference in ions. So, overall it indicates that the green color is calcium. And then, they are the elemental mapping of the other ions which are present. The orange color is chloride, the turquoise blue is phosphate, the green is magnesium and the red color is carbon. Calcium and phosphate are more, whereas other ions are also present and it will increase more than calcium and phosphate in the region where demineralization happened. This indicates that the caries process replaces the calcium and phosphate ions and then increases the organic content. This intrinsic molecular level nanoscale characterization helps to understand the enamel caries better. Aluminum interacts with most physical and cellular processes in humans. Metal ions including aluminum are present on the enamel surface after the completion of orthodontic treatment. The presence of aluminumwas detected after cleaning the enamel using a polisher with aluminum oxides (Machoy et al., 2016). Enamel lesions formed in-vivo do not have a surface layer initially but develop this mineral rich layer later and the fact that the fluoride level in the solid enamel is not determining the subsurface lesion formation. The observations that in-vitro fluoride ions in the liquid at a very low level (approximately 0.02 ppm) determine surface layer formation are difficult to explain. A new kinetic model for subsurface lesion formation is described, in which inhibitors such as fluoride play an important role(Arends & Christoffersen, 1986). Fluorine content showed no significant statistical trend although the center traverse showed a slightly higher fluorine content at the enamel surface. The SEM shows the surface of white spot lesions to consist of an increased number of eroded focal holes and numerous other irregular holes (Mann & Dickinson, 2006)**.** The chemistry of enamel apatite is probably the best understood of all the biological apatites. It is an imperfect apatite, low in calcium and hydroxide ions, but rich in substitutional impurities. A few impurities, like fluoride, are beneficial but appear to disrupt the lattice structure (Eanes, 1979). The chlorine was determined by the silver nitrate thiocyanate procedure in an aqueous extract and on NO2 solution. The CO2 determinations were also carried out on samples after they had been heated in a furnace to a temperature and for a time previously determined as sufficient to drive off the CO2 from CaCO3 and MgCO3. Distillation of the F as H2SiF6 by treatment of the material with HCIO4 and then applying the Zr-alizarin colorimetric determination (Bowes & Murray, 1935)**.** Dental enamel crystallites consist of highly crystalline biological apatite, which includes a large number of carbonate ions in the apatite crystal lattice. The chemical composition of dental enamel is a major factor in determining the physical-chemical properties, using non-destructive high-accuracy analytical instruments, such as FT-IR spectrometry, FT-Raman spectrometry, and X-ray diffraction. Variations in the enamel structure observed by instruments other than electron microscope were not reported in relation to tooth type differences. These variations are referred to as variations in enamel microstructure, such as micropores (Sakae, 2006)**.** The chemical potentials of both the organic and phosphoric acids are higher in the surface zone than in the inner enamel. Diffusion of acid constituents occurs from the surface zone into the inner enamel. The reverse is true for the chemical potential of calcium hydroxide. Furthermore, as the acid constituents diffuse deeper into the enamel, they are neutralized by the dissolution of the inner enamel mineral. The chemical potential of calcium hydroxide in this neutralized solution is higher than that in the solution of the surface zone (Moreno & Zahradnik, 1974)**.** Raman microspectrometry allows thorough molecular analysis of mineralized dental tissues. Modifications due to the acidic attack essentially concern the phosphate group (PO43), which represents the mineral phase in enamel (hydroxyapatite); on Raman spectra, changes in the intensity of the (PO43–) band are linked to the type of enamel, to its anatomical location (Tramini et al., 2000)**.** The scanning electron micrographs revealed hypoplastic, rough, uneven, cracked and pitted enamel surfaces covered with granular deposits as a result of excessive intake of fluoride. It can be concluded that long-term fluoride administration leads to severe structural alterations on the enamel surface, possibly through defective mineralization (Susheela & Bhatnagar, 1993). The chemical composition of bovine enamel is inhomogeneous at the atomic scale. The Mg impurities were significantly segregated throughout the enamel, such clustering influenced the variation of Ca/P ratios. The increase in Mg concentrations, near the Mg clusters, correlated with increased Ca and decreased P concentrations (Licata et al., 2020). Zinc is the most abundant microelement detected, followed by Pb, Fe, Mg, and Al. Morphological features observed include enamel rods in the rodent teeth, while incremental lines and semi-prismatic enamel were observed in the alligator species. The fossil enamel was in an excellent state for microscopic analyses. Major dental enamel's physical, chemical, and morphological features are present both in extant and extinct fossil tooth enamel (Pessoa-Lima et al., 2022). The use of Biomimetic Hydroxyapatite toothpaste has proven to be a valuable prevention measure against dental caries in primary dentition since it prevents the risk of fluorosis (Bossù et al., 2019). Enamel remineralisation is a challenging aspect of restorative dentistry.Nanoscale characterization and validation will assist in deriving advanced remineralization strategies. Our team has extensive knowledge and research experience that has translate into high quality publications

(Felicita et al., 2012; Lakshmi et al., 2015; Menon & Thenmozhi, 2016; Rajeshkumar et al., 2019; Sahu et al., 2014; Saravanan et al., 2021; Sathivel et al., 2008; Sekar et al., 2019; Thejeswar & Thenmozhi, 2015; Wang et al., 2019)

## CONCLUSIONS

More than any other part of teeth enamel restoration is very challenging, understanding it at a nanoscale characterizationand validation will help in deriving advanced remineralization strategies. Enamel undergoes remineralisation due to the acidic effects of cariogenic bacteria and other demineralization mechanisms. Ionic changes in various planes of enamel have been demonstrated in this study. FESEM images reveal the breakdown of enamel rods which exhibited an increase in carbon and oxygen molecules then calcium and phosphate. Further, elemental mapping with energy dispersive spectroscopy reveals a significant loss of calcium and phosphate when demineralization happens the hardness of the tissue will decrease.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.


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