Atomic Force Microscopy Imaging of Enamel versus Dentin Subjected To Critical Ph. Solution

Gokul Vimal Thangaraj[1], R. Gayathri[2*], Jayalakshmi Somasundaram[3], V. Vishnu Priya[4], Kavitha. S[5]

[1]Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[2*]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[3]White Lab - Material Research Centre Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[4]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[5]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Abstract
Introduction:
Enamel is the hardest substance in the human body and contains a high percentage of minerals (96%) primary minerals being hydroxyapatite which consist of crystalline calcium and phosphate. Dentin is a specialized form of tissue that forms the bulk of teeth. AFM (atomic force microscopy) is a scanning probe microscopy.
Aim and Objective:
The aim of the study is to analyze the enamel and dentin subjected to critical pH solution by Atomic force microscopy imaging (AFM).
Materials and Methods:
Patients' posterior teeth were sampled and demineralized in a solution with a pH of 4.8, which is the crucial pH, for 48 hours. The demineralizing solution was changed out for a new one every 8 to 9 hours. Three more recently extracted posterior teeth underwent AFM, and the image was used as a reference. The teeth that had been in contact with the demineralisation solution were imaged using AFM.
Results and Conclusion:
AFM image of pre treated posterior teeth and post treated posterior teeth is compared. AFM images of pre and post treated dentin were compared. A graph was made. The surface roughness of dentin was found out to be greater than that of enamel.

Keywords: Enamel, dentin, AFM, posterior teeth, novel method, surface roughness, innovative technique.

1. INTRODUCTION

The human body's hardest and most robust tissue is enamel. Nanocrystals that are 50 nm broad, microns long, and morphologically aligned are found in enamel. These nanocrystals are either packed into 5-m-wide rods or their interrod-filling interrods. However, little is known about how enamel crystals are oriented. Here, we demonstrate that over the majority of the enamel layer thickness, the crystalline c-axes are homogeneously orientated in interrod crystals. As opposed to what was previously thought, crystals inside each rod are not co-oriented with one another or with the long axis of the rod. Instead, the c-axes of nearby nanocrystals are most typically misoriented by 1° to 30°, and this orientation gradually changes within each rod (1) Tooth enamel is acellular, has a higher mineral content, and is made up of larger, better-oriented crystallites than other mineralized tissues, it is a special type of mineralized tissue. By means of matrix-mediated biomineralization, dental enamel is created. A supersaturated fluid within a clearly defined biological compartment causes enamel crystallites to form. Carbonated calcium hydroxyapatite is not stoichiometrically distributed in mature enamel crystallites.

At the dentino-enamel junction (DEJ), the initial crystallites arise suddenly as thin ribbons that are developing quickly. These crystallites' development patterns and structure provide evidence of an octacalcium phosphate precursor phase (OCP) (2). The difficulty of explaining how enamel developed in an extracellular site was raised by the finding of enamel. For example, interprismatic substance from terminal bars and prism substance from the conversion of ameloblast cytoplasm were suggested as possible explanations (3). The enamel surface becomes rough and rugged upon acid encounter. The enamel demineralised at the oral pH of 5.5. This demineralisation is usually countered by human saliva which acts as a natural buffer and increases the pH to normal pH of the oral cavity.

In a typical tooth, dentin is shielded from view by enamel in the tooth crown and by a thin layer of cementum in the majority of tooth root locations. It has tens of thousands of tiny tube structures that extend from the pulp. The pulp is connected to these dentinal tubules, which are typically 0.5 to 2 microns in diameter and contain biological fluid that resembles plasma. Each tubule has an odontoblast that communicates with the pulp as well as a cytoplasmic cell process known as a Tomes fibre. There are two types of nerve fibers — myelinated (A-fibers) and unmyelinated — within the dentinal tubules (C-fibers). Dentin hypersensitivity, which is felt as pain in response to all stimuli, is caused by the A- fibres.Depending on the depth, about 30,000 tubules can be found in 1 mm2 in a cross- section of dentin(4). The various analytical data on the content of Ca, P, Na, Mg and CO3 as the main components were analyzed for their consistency with a model of variable phase composition of the mineral. This analysis revealed that the probable phase composition of the mineral in dentin and bone is the same, but different from that in tooth enamel. Studies on the solubility behavior indicate that near the physiological pH (7.4) all phases are solubility controlling simultaneously.

This supports a physiological model according to which the body fluids are close to physicochemical equilibrium with bone mineral. Hence, changes in the chemical and phase composition of the mineral which occur under pathological conditions are related to changes in the activities of the relevant components in the surrounding body fluid. The latter depend mainly on cellular activity and organic transport activity(5).In teeth that had been submerged in the acidic solutions for 60 s, mineral loss could be seen to be progressing at the junction of peritubular and intertubular dentin. Longer immersion times led to total loss of the peritubular dentin, which hollowed out the tubular apertures. There was also surface abrasion and porosity in the intertubular zones.

AFM or atomic force microscopy is a scanning force microscopy with very high and detailed resolution type with upto 2 lakhs magnifying power. The two types are contact and non contact AFM(6). A non contact atomic force microscopy is used for the study. This method is essentially applied to investigate the characteristics of inorganic composites (7). The conta

mode is the simplest and most basic Imaging available. The probe tip comes in constant contact with the sample surface and thus operates in repulsive regimes(8).

Demineralisation solution produced along with saliva during acidic or low pH food intakes, which will demineralise the enamel and dentin(9). Critical pH is the pH at which the minerals are saturated. pH below that causes demineralisation and pH above critical pH causes remineralisation. Our team has extensive knowledge and research experience that has translate into high quality publications (10–19))((20–29). This study focuses on the surface difference between enamel and dentin due to demineralisation solution.

2. MATERIALS AND METHODS

SAMPLE: A total of 5 posterior tooth samples were freshly extracted from the patients visiting the Saveetha dental college and hospital. The patient's teeth which were extracted were severely affected by dental caries. The patients were informed about the study and an informed consent was gathered. The teeth are then subjected to AFM.

3. RESULTS AND DISCUSSION

Afm Analysis:

The atomic force microscope (AFM) system has developed into a useful tool for atomic-resolution characterisation and direct measurements of microstructural parameters and intermolecular forces at the nanoscale. As long as the cantilever tip is in contact with the target sample, the contact mode monitors interaction forces to gather sample properties.(30).5 posterior teeth were freshly extracted from the patients. The teeth were then stored in distilled water from 8 to 9 hours. 2 out of 5 teeth were taken and were subjected to AFM. The image was observed using AFM (FIGURE 1). Then all the 5 posterior teeth were subjected to a demineralisation solution containing a critical pH of 4.8. The critical pH of 4.8 was taken because the enamel demineralised at pH of 4.8 and the dentin demineralized at pH of 5. So an average value was taken as critical pH for the study. The teeth were then placed on wax sheets submerged to the demineralisation solution. The teeth were kept in static condition for a period of 48 hours. The demineralisation solution is changed periodically with an artificially made demineralisation solution. The AFM was used to image the teeth exposed to the demineralisation solution (FIGURE 2) and graph was made comparing enamel and dentin (FIGURE 3)

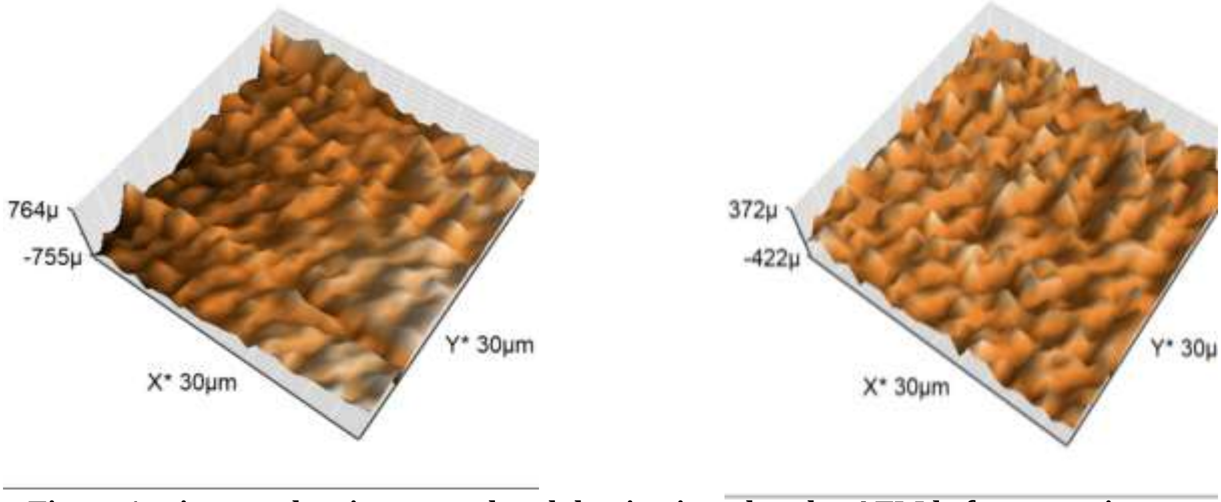


Figure 1: pictures showing enamel and dentin viewed under AFM before exposing to demin solution. Enamel pre demin and dentin pre denim exposure respectively

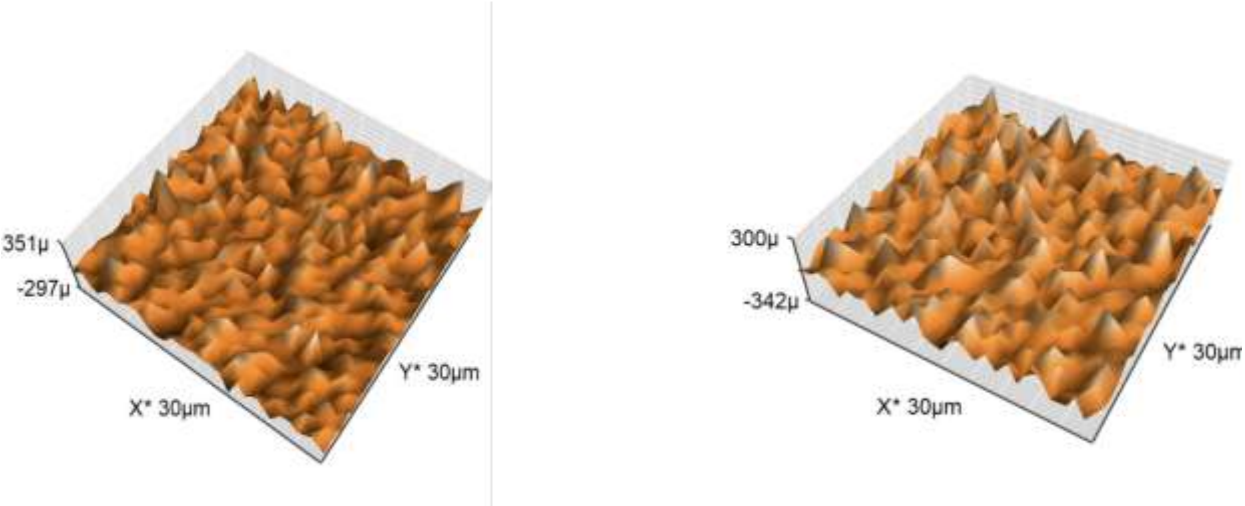
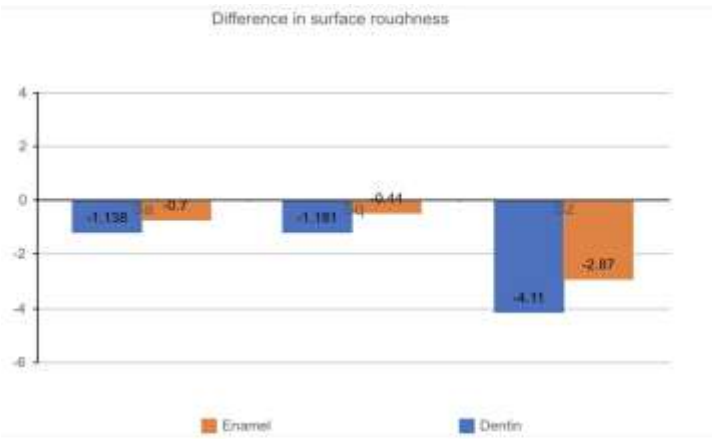


Figure 2: pictures showing enamel and dentin viewed under AFM after exposing to demin solution for 48 hours. Enamel post demineralisation and dentin post denim exposure respectively



	Pre dentin	Post dentin	Pre enamel	Post enamel		Dentin	Enamel
Sa	3.212	4.35	3.83	4.53	Sa	-1.138	-0.7
Sq	3.839	5.02	4.12	4.56	Sq	-1.181	-0.44
Sz	16.45	20.56	16.69	19.56	Sz	-4.11	-2.87

Figure 3: picture showing surface roughness between enamel and dentin before and after exposing to demineralization solution. Blue represents dentin and orange represents enamel.

In the figure 3, the Sa, Sq and Sz are the surfaces of enamel and dentin seen using the atomic force microscopy. On viewing the AFM of the extracted teeth and demineralisation solution incubated teet, it is observed that there exists a surface roughness whose peak and valleys (elevation and depression on the teeth surface) was greater in dentin rather than the enamel. A clear distinction of surface differences can be visualized between the enamel and dentin where dentin is more affected than the enamel. Dentinal loss is increased with increasing acid flow rate, demineralisation Time and was higher for demineralisation with pH of 4.8.(31)

Very few researches were previously done on this topic. A study done on Atomic force microscopy analysis of the surface alterations of enamel, dentin, composite and ceramic materials exposed to low oral pH in GERD supports this study. The study concluded that the findings demonstrated that enamel and dentine exposed to a lower pH level underwent significant changes that started even within a little immersion duration.(32)Another study conducted on the influence of Carisolv on enamel and dentine surface topography supports the study and concluded that the surface roughness of enamel and dentine was increased by phosphoric acid etching, however. Caries removal with Carisolv did make the surface rougher as compared to traditional caries removal methods(33). Another study on The effect of pH on the erosion of dentine and enamel by dietary acids concludes that the results demonstrated that under low pH, citric acid induced significantly more erosion over the pH range tested for both tissue types than phosphoric acid.

When hydrochloric acid and citric acid were compared, the effects of chelation and dissolution were highlighted. When used over pH 3 for enamel and pH 4 for dentine, phosphoric acid barely eroded either. These elements might be taken into account to lessen how erosive acidic soft drinks are. The article also concludes that pH is a significant factor, and at high pH levels, chelation has a notable impact on tooth tissue deterioration. (34)

A study conducted on effect of home bleaching agents on the roughness and surface morphology of human enamel and dentine was in opposition of the study, the

study concluded that There were no statistically significant differences between the surface roughness of untreated control specimens and the specimens treated with the bleaching materials (10% and 15% CP) for both enamel and dentine at any given measurement time.(35) (36), (37), (38), (39), (40,41), (42), (43), (44), (45), (46), (47), (48), (49).
The study is however, in vivo, acid stability will surely contribute and be a significant factor. This has to be investigated further, especially using a model created by this group that can be used in vivo. (50)

4. CONCLUSION

The surface roughness of dentin was found out to be greater than the enamel because of organic composition being greater in content than that of the enamel and it is dentin which is exposed to a demineralisation solution than the enamel.

Future Scope:
Increase in surface roughness can cause increase in dentinal caries and other oral diseases. Creating an awareness on food to be avoided and food intake. An in vivo study can be done regarding the surface roughness and its importance.

Conflict of Interest:
The authors hereby declare that there is no conflict of interest in this study.

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Author Contribution:
A) Gokul Vimal Thangaraj - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
B) Dr. Jayalakshmi - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
C) Dr.V.Vishnu Priya - contributed in study design, guiding the research work, manuscript correction.
D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.
E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

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