TO STUDY THE EFFECT OF DEMINERALISATION OF ENAMEL ON DEPTH OF LIGHT PENETRATION USING OPTICAL COHERENCE TOMOGRAPHY

[1]NAUMAN BARI KHAN, [2]SIMON RAWLINSON, [3]PETE TOMMLINS, [4]PAUL ANDERSON

ABSTRACT

The aim of this research was to study the application of Optical coherence tomography (OCT) which could provide the early, chair-side detection of caries and therefore initiate the prompt treatment strategies required.

Six extracted teeth were selected with no visible carious lesion. All teeth were scanned using X-ray Microtomography (XMT). Short term demineralization was performed in acetic acid (pH) to determine whether Optical Coherence Tomography (OCT) could differentiate between light penetration in mineralized and non-mineralized enamel. Scattering Attenuation Microscopy (SAM) software was used to analyze the depth of penetration.

OCT was helpful in detecting demineralised areas in enamel. Average depth before demineralisation = 10.65±1.55 μm, After demineralisation: 23.29±3.65 μm, p=0.03 Student's paired t-test.

OCT can detect carious lesions that are invisible to the eye, assess mineral density of enamel. Development of the technique to allow chairside diagnosis will permit early intervention to maintain oral and preserve whole body health.

Keywords: *Optical Coherence Tomography, Demineralisation, Early Caries Detection*

This article may be cited as: Khan NB, Rwalinson S, Tommlins P, Anderson P. To study the effect of demineralisation of enamel on depth of light penetration using optical coherence tomography . Pak Oral Dent J 2021; 41(1):18-21.

INTRODUCTION

According to the World Health Organization (WHO), dental caries although largely preventable, is prevalent among 60–90% of school-age children and is virtually universal among adults worldwide.[1]

It necessitates the need for continuous innovation at the level of research, development and implementation of effective diagnostics and preventive strategies. The health gains of prevention are intuitive – it is wiser to prevent a disease than to face its harmful manifestations once it reaches severity.

Dental caries is a chronic microbial disease that results in localized damage of hard tooth structure. The main etiologic agents are acid-producing bacteria, predominantly Streptococcus mutans (in caries initiation) and Lactobacilli species (in caries progression).[2]

Following the production of acid, as the pH drops, the level of calcium and phosphate ions also drops. The risk of demineralization increases as pH lowers and the tooth releases subsurface calcium and phosphate ions into the plaque in an attempt to neutralize the acid. The critical pH at which a tooth starts to dissolve varies among individuals. It may range from 6.5 in individuals with low levels of calcium and phosphate in saliva to 5.5 in those with higher salivary concentrations of calcium and phosphate.[3] It may be as low as 5.1 for dental plaque. When demineralization exceeds the natural re-mineralization capacity of saliva, it will eventually lead to a carious lesion.[4]

Demineralization is the process of removing minerals, in the form of mineral ions, from dental enamel. Demineralization is another term for "dissolving the enamel". Dental enamel is a crystalline latticework composed of various minerals, the principle component

[1] **Corresponding author:** Dr Nauman Bari Khan, BDS, MSc, Assistant Professor, Department of Oral Biology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan. E-mail: nauman@gmail.com Cell: 0300-9562903

[2] Dr Simon Rawlinson, PhD, Senior Lecturer, Institute of Dentistry, Barts and The London, Queen Mary, University of London.

[3] Dr Pete Tomlins, PhD, Senior Lecturer, Institute of Dentistry, Barts and The London, Queen Mary, University of London.

[4] Dr Paul Anderson, PhD, Professor, Institute of Dentistry, Barts and The London, Queen Mary, University of London.

Received for Publication: Jan 17, 2020
Revised: May 15, 2020
Approved: June 16, 2020

of which is a complex calcium phosphate mineral called hydroxyapatite.[5] A substantial number of mineral ions can be removed from hydroxyapatite latticework without destroying its structural integrity; however, such demineralized enamel transmits hot, cold, pressure and pain much more readily than normal enamel.

Optical technology has a distinguished and very long history in the field of medicine. After the invention lasers in the field of surgery, there has been a rapid development in the field of optical fibers which led to the invention of endoscopes which were capable of viewing or imaging internal organs inside the body.[6]

Optical Coherence Tomography (OCT) is a novel Three-Dimensional (3-D) imaging technology which produces cross-sectional high-resolution images of internal microstructures of living tissue (16) Tomographic techniques provide non-invasive diagnostic images by generating slice images of 3-D objects.[7] It should be noted that there is a very fundamental and basic difference between optical tomography and other imaging techniques such as magnetic resonance imaging and X-rays. Optical tomographic (OT) techniques are heavily influenced by diffraction, which suggest that "Fourier slice theorem" cannot be implied in the image construction and analysis.[8]

Optical Coherence Tomography primarily deals with ballistic and near ballistic photons which are basically the photons which travel in a straight line in turbid medium for a specific distance.[9] In order to map the two dimensional (2-D) lateral reflectance of the sample, depth scans are performed at successive depths. (10)

Micro CT shares its basic function with computerised axial tomography (CAT or CT) scans, this equipment is used in the field of medicine for the last thirty to forty years.[11] These techniques which were an extension of conventional projection radiography produce a 2-D image of internal structure when they were given a sufficient powerful source.[12] Because of the variation in x-ray absorption broken bones, decayed teeth can be easily identified when compared with the surrounding bone or tissue. It can scan specimens between one millimeter and one centimeter in size and it can also create images in cross sections which approach around 1um in spatial resolution.[13]

It is used for visualizing tomography, electron-microscopy data and so forth. It aims to ease understanding of the data set and to assist with conveying that understanding to the research community or a lay person.

Scattering Attenuation Microscopy (SAM) software shows demineralized surface area of enamel. It shows Ztop and Zbottom backscattering lines in yellow colour. Ztop showing when light hits the enamel surface and Bottom showing lower end when light it is backscattered. (Figure 3). The green and red lines on the side show the area of enamel taken for scanning.[14]

MATERIALS AND METHODS

Making of the Demineralizing Solution

The demineralizing solution used in this study was 0.1 mol l-1 acetic acid and was prepared using de-ionized water and buffered to pH 4 by adding 1 mol l-1 NaOH. A pH meter (Orion-pH/ISE meter model 710) was used to measure the pH of the solution and then the solution was kept in a bottle. The bottle was sterilized in an autoclave at a temperature of 121°C and pressure 15 p.s.i for about 15-30 minutes.

Method

Six non carious extracted teeth were taken for this part of the project. Samples which had already been scanned using X-ray Microtomography (XMT) and Optical Coherence Tomography (OCT) and determined to have no pathology or abnormality were used for this section. They were covered with nail varnish and a window was left on the enamel surface. As the non-demineralized reading was acquired, the samples were then put in small glass containers and 30ml of demineralizing solution was added to each container. The containers were covered and were kept in a moving incubator for 24 hours. The solution was changed after 24 hours; pH was again taken to confirm the acidity of the solution before changing it. After 48 hours the samples were taken out and were washed with normal running water. After washing the samples, they were scanned with OCT microscope. Images after scanning with OCT were scanned with X-Ray Microtomography (XMT) to confirm the areas of demineralization within the desired window.

Plotting the images for depth of penetration

An excel map was created using the SAM software for measuring the depth of light penetration in the working area. OCT image volume for number of B-scan was set at 400, B-scan width was set 250, A-scan was set 90000 and the scan width was set at 1. In the Excel files, each cell represents a single pixel in the corresponding SAM image. The coordinates are noted for the region of interest in the excel file. Same area was taken for both samples and a histogram were plotted using the selected data. The histogram created was frequency of reading of depth of light penetration plotted against depth of light penetration (frequency distribution histogram). Histogram was helpful in comparing if there is any shift in the graph due to different depth of light penetration between the two samples. A 100x100 pixel area was taken for the plotting of histograms where number of observations (n) was 10,000.

An unpaired t-test and paired t-test were used to compare two sample means of pre-demineralization and demineralized samples. The data selected for this is the same which was taken for histograms. Un-paired t-test was used to compare the differences in mean penetration, before and after demineralization

OCT Images were reconstructed using the 3D software 'Drishti' and the effect of demineralization was observed on the surface of the tooth.[15]

RESULTS

There is a significant difference between the mineralized and demineralized enamel surface. Graph showing the average depth of light penetration before and after demineralization. (Fig-1) Scattering Attenuation Microscopy revealing that the back scattering of mineralized area is less and the distance between Z_{top} and Z_{bottom} is very narrow whereas is seen wide in the area which was demineralised as shown in the figure 2. The average depth of penetration in the mineralized zone was found to be 10.65 ± 1.55 µm and when it was compared after the demineralization of the same site it was observed to be: 23.29 ± 3.65 µm and student paired t-test value were 0.03 suggesting a statistically significant result. Thus, suggesting a deeper penetration of light in the demineralized samples. The back-scattering image of mineralized sample showing a blue image and the demineralized area showing a mix of green and yellow scattered all over. This difference in depth in penetration when shown in a histogram shows a different curve as shown in figure 3. There is a shift towards the right side showing greater penetration

SAM processing image showing the small separation between Z_{top} and Z_{bottom} (Fig 2A) A large separation is seen between Z_{top} and Z_{bottom} in demineralized enamel surface (Fig 2B) There is least penetration of light into the mineralized enamel surface (Fig 2C) There is greater penetration of light in the demineralized surface of enamel. (Fig 2D) The histogram is showing the separation between the two means and the data is normally distributed. (Fig 3) There is a shift towards right after demineralization suggesting deeper penetration of light. The mean difference for mineralized sample and demineralized sample is 13.43 µm and 22.33 µm.

DISCUSSION

The penetration of light in demineralized enamel surface is greater than the mineralized surface. The reason for greater penetration is due to loss of dense enamel tissue allowing light to pass through. It can be observed that light has penetrated more in some samples while in some sample the average depth of penetration is less. The degree of demineralization can depend on factors like age suggesting that younger enamel can demineralize much faster than the aged one.[17] It can also depend on the site of enamel as cuspal areas in posterior teeth and cingulum in incisors are more densely mineralized.

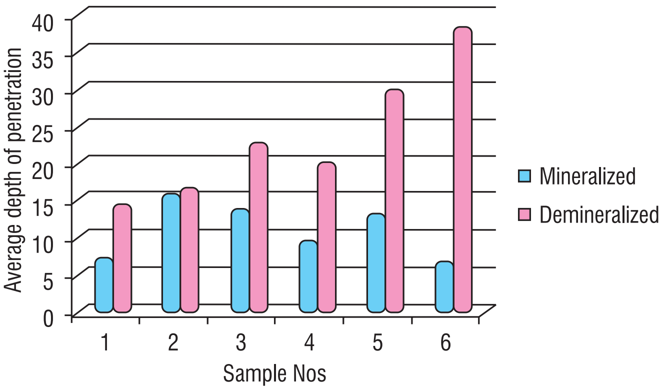

Fig 1: Graph showing six samples before and after demineralization difference

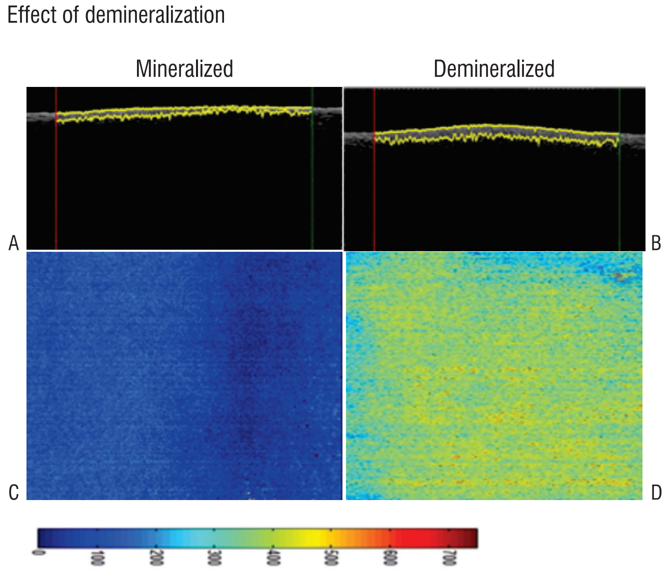

Fig 2: Figure showing depth of light penetration before and after demineralization

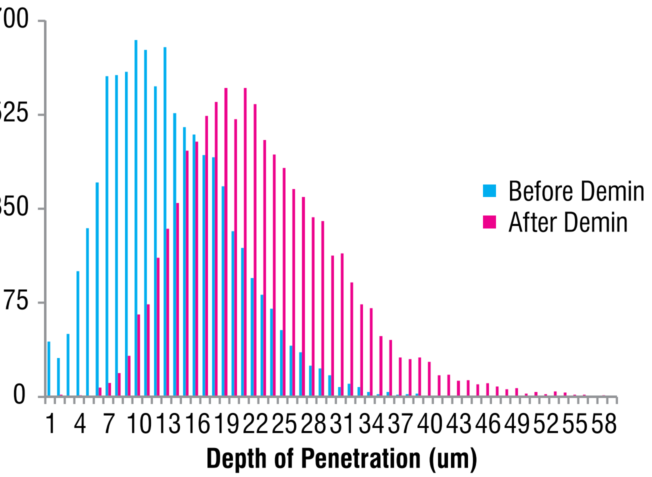

Fig 3: Sample showing before and after demineralization of enamel.

In a study done by *Al Sayed*, it was observed that Optical Coherence Tomography can be used to non-destructively monitor the integrity of such coatings, as well as enamel changes adjacent and beneath to them.[18] In another study done by *Tsai et al* illustrates that OCT was helpful when used as an indicator of early-stage cavity formation and demineralization of the tooth surface can be successfully detected by Optical Coherence Tomography.[19]

Austin Rs et al detected Optical Coherence Tomography (OCT) signal changes in the superficial sub-surface enamel of upper central incisor teeth of healthy individuals after rinsing from orange juice.[20] *Shimada et al* used OCT for diagnosis of cracks, caries and defects in tooth restorations.[21] *Kim JM* used optical coherence tomography for automatic detection of tooth cracks in images. They were able to distinguish split and craze lines, structural cracks, in tooth cracks using OCT images, and automatically detected the position of various cracks in the optical coherence tomography images.[22] In a study done by Chew HP, concluded that Optical Coherence Tomography was able to detect demineralization after 10 minutes of erosive stimuli and could be used to monitor the progression of demineralization of initial enamel erosion in vitro.[23]

CONCLUSION

Keeping in mind the results of this study it can be conducted that OCT is able to detect changes in the degree of mineralization in enamel surface. This suggests that early changes in degree demineralization in a tooth surface can be detected by OCT. This degree of demineralization can be confirmed using the software called'Scattering Attenuation Microscopy" which measures the volumetric data and produces SAM images for colour coding of light penetration.

Though the resolution and the structure cannot be compared to XMT but it is safe and useful in detecting early changes in enamel mineral concentration.

REFERENCES

1 Anil S, Anand PS. Early childhood caries: prevalence, risk factors, and prevention. Front Pediatr 2017;5:157

2 Hujoel PP, Hujoel MLA, Kotsakis GA. Personal oral hygiene and dental caries: a systematic review of randomised controlled trials. Gerodontology. 2018;35(4):282–89.

3 Delgado AJ, Olafsson VG. Acidic oral moisturizers with pH below 6.7 may be harmful to teeth depending on formulation: a short report. Clin Cosmet Investig Dent. 2017; 9:81–83.

4 Carounanidy U, Sathyanarayanan R. Dental caries: a complete changeover, Changeover in the treatment decisions and treatments. J Conserv Dent. 2010;13(4):209–217. doi:10.4103/0972-0707.73383

5 Abou Neel EA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, et al. Demineralization–remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016;19(11):4743–763.

6 M Andresen ER, Sivankutty S, Tsvirkun V, Bouwmans G, Rigneault H. Ultrathin endoscopes based on multicore fibers and adaptive optics: a status review and perspectives. JBO. 2016;21(12):121506

7 Aden A, Anderson P, Burnett GR, Lynch RJM, Tomlins PH. Longitudinal correlation of 3D OCT to detect early stage erosion in bovine enamel. Biomed Opt Express. 2017;8(2):954–73.

8 Podoleanu AG. Optical coherence tomography. J Microsc. 2012;247(3):209–19.

9 Agrawal A, Pfefer TJ, Woolliams PD, Tomlins PH, Nehmetallah G. Methods to assess sensitivity of optical coherence tomography systems. Biomed Opt Express. 2017;8(2):902–17.

10 Ali ZA, Karimi Galougahi K, Maehara A, Shlofmitz RA, Ben-Yehuda O, Mintz GS, et al. Intracoronary Optical Coherence Tomography:current status and future directions. JACC Cardiovasc Interv. 2017;10(24):2473–87.

11 Park Y-S, Bae K-H, Chang J, Shon W-J. Theory of X-ray microcomputed tomography in dental research. J Korean Conserv Dent.2011;36(2):98-107

12 Davis GR, Evershed ANZ, Mills D. Quantitative high contrast X-ray microtomography for dental research. J Dent. 2013;41(5):475–82

13 du Plessis A, Broeckhoven C, Guelpa A, le Roux SG. Laboratory x-ray micro-computed tomography: a user guideline for biological samples. Gigascience. 2017;6(6):1▯11.

14 Wang RK, Tuchin VV. Optical coherence tomography: light scattering and imaging enhancement. In: Tuchin VV, editor. Handbook of Coherent-Domain Optical Methods: Biomedical Diagnostics, Environmental Monitoring, and Materials Science. Springer New York; 2013 p. 665–742.

15 Semple TL, Peakall R, Tatarnic NJ. A comprehensive and user-friendly framework for 3D-data visualisation in invertebrates and other organisms. Journal of morphology. 2019 1;280(2):223–31.

16 Spaide RF, Fujimoto JG, Waheed NK, Sadda SR, Staurenghi G. Optical coherence tomography angiography. Progress in Retinal and Eye Research. 2018;1(64):1–55.

17 Philip N. state of the art enamel remineralization systems: the next frontier in caries management. Caries Res. 2019;53(3):284–295.

18 Alsayed EZ, Hariri I, Sadr A, Nakashima S, Bakhsh TA, Shimada Y, et al. Optical coherence tomography for evaluation of enamel and protective coatings. Dent Mater J. 2015;34(1):98–107.

19 Tsai M-T, Wang Y-L, Yeh T-W, Lee H-C, Chen W-J, Ke J-L, et al. Early detection of enamel demineralization by optical coherence tomography. Sci Rep. 2019; 20;9(1):1–9.

20 Austin RS, Taha MH, Festy F, Cook R, Andiappan M, Gomez J, et al. Quantitative swept-source optical coherence tomography of early enamel erosion in vivo. CRE. 2017;51(4):410–8.

21 Shimada Y, Sadr A, Sumi Y, Tagami J. Application of optical coherence tomography (oct) for diagnosis of caries, cracks, and defects of restorations. Curr Oral Health Rep. 2015;2(2):73–80.

22 Kim J-M, Kang S-R, Yi W-J. Automatic detection of tooth cracks in optical coherence tomography images. J Periodontal Implant Sci. 2017;47(1):41–50.

23 Chew HP, Zakian CM, Pretty IA, Ellwood RP. Measuring initial enamel erosion with quantitative light-induced fluorescence and optical coherence tomography: an in vitro validation study. Caries Res. 2014;48(3):254–62.

CONTRIBUTIONS BY AUTHORS

1	**Nauman Bari Khan:**	Principal Author, Manuscript Writing
2	**Simon Rawlinson:**	Project Co-Supervisor
3	**Pete Tomlins:**	Project Co-Supervisor, OCT
4	**Paul Anderson:**	Project Supervisor, XMT