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TO STUDY THE EFFECT OF REMINERALISATION ON ENAMEL USING OPTICAL COHERENCE TOMOGRAPHY

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ABSTRACT

The aim of this research was to study the application of Optical coherence tomography (OCT) which could provide changes in degree of mineralization in tooth enamel.

In vitro Study was planned at Dental Physical Sciences Unit, Barts & the London Institute of Dentistry. Six extracted teeth were taken for this part of the project which detected demineralize area using X-ray Microtomography (XMT) out of ten samples. These samples were then scanned using OCT to detect changes in remineralisation. For making the remineralising solution, the main ingredients used were calcium and phosphate and a pH of 7 was maintained for the remineralising solution

OCT was helpful in determining the remineralised areas in enamel. The average depth of penetration in the pre-mineralized zone was found to 11.27 +/- 7.5 µm and when it was compared after the remineralized of the same site it was observed to be 6.39 +/-4.52 µm and unpaired t-test value was <0.0001 suggesting a statistically significant result.

OCT can detect and assess changes in degree of mineralization that are invisible to the eye. Development of the technique to allow chair-side diagnosis will permit early intervention to maintain oral and preserve whole body health.

Keywords: *Scattering Attenuation Microscopy, Early Caries Detection, X-Ray Microtomography*

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INTRODUCTION

Dental caries is a sugar-driven, biofilm-mediated, multifactorial disease that leads to the phasic remineralization and demineralization of dental hard tissues.[1] Caries can occur in permanent and deciduous dentitions during anytime of life which can result in damage of tooth surface and then further damage the roots.[2]

Despite the decrease in the prevalence of tooth decay in children of western countries, caries index in preschool children remain a major concern in developed as well as developing states.[3] A review of the literature suggests that in most developed countries the prevalence rate of early childhood caries is between 1% and 12%. The prevalence has been reported to be as high as 70 percent in less developed countries and also in developed countries among minority groups.[4]

Tooth decay in primary teeth was among the 12th most prevalent diseases in all ages together according to the Global Burden of Diseases in 2015. The importance of the dental, social, medical and economic costs of Early Childhood Caries has risen in all parts of the world.[5]

Biomineralization is a dynamic, complex and a continuous mechanism which produces a unique synthesized biological tissue in which inorganic nanocrystals are precipitated by the living organism within the organic matrix.[6]

Saliva, fluoride, probiotic bacteria and diet control are known to act as preventive regimes for tooth demineralization. Saliva protects enamel from demineralization and erosive acidic changes.[7] Calcium phosphates (CaPs) incorporated in dental composites are discussed as potentially curative method for tooth demineralization.[8] The use of nanotechnology in pre-

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ventive dentistry, as biomimetic remineralization and antibacterial nanotherapy for reversing a recurrent decay or an incipient caries has been documented.[9]

The diagnosis of diseases frequently moves more from the WHO traditional DMFT requirements to the use of the International Caries Detection and Assessment Framework (ICDAS), where non-cavitated enamel lesions are also included.[10] This has raised the proportion of people diagnosed with dental caries, offering a substantial opportunity to use regenerative medicine-based dental methods for non-operative treatment and secondary prevention. Such minimally invasive remineralization techniques are clearly needed by modern-day dentistry, not only to increase clinical performance, but also to improve patient well-being and experience.[11]

Te previous reports indicated that the teeth demineralization or remineralization causing the changes in surface morphology and optical properties of enamel which can be identifed by OCT. However, the dynamic progress of remineralization or demineralization is rarely discussed, and it might be a problem for evaluation of remineralization with the previously proposed methods for clinical practice since it is difcult to repeatedly scan the same tooth location. Therefore, a higher axial or transverse resolution would be a better solution for providing more accurate information of optical properties and surface structure of enamel.

Optical coherence tomography (OCT) has been recognized as one of the most effective and vital optical techniques for clinical biomedical imaging and biophotonics, especially in the field of ophthalmology.[12] Optical Coherence Tomography(OCT) has the ability to perform non-invasive, real time and noncontact measurements in reflection, providing three-dimensional (3D) sample visualization.[13]

X-ray microtomography (also known as micro-CT or XMT) is a radiographic imaging technique that can produce 3D images of material internal structure better than 1µm spatial resolution.[14] There is minimum preparation for the specimen and also it is a non-destructive procedure. This allows it to take many scans for the same specimen without destroying it. This approach complements lower resolution 3-Dimensional (3D) ultrasonic imaging higher resolution 2D microscopy.[15] The purpose of this research was to study the application of Optical coherence tomography (OCT) which could provide changes in degree of mineralization in tooth enamel.

MATERIALS AND METHODS

Plotting the images for depth of penetration

An excel map was created using the Scattering Attenuation Microscopy (SAM) software for measuring the depth of light penetration in the working area. OCT image volume for the 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 was 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 the number of observations (n) was 10,000. Remineralising solution was made to remineralise the lesions seen with OCT and XMT to compare the differences.

Making of remineralising solution

For making a remineralising solution, the main ingredients necessary are calcium and phosphate, and a pH of 7 plays the crucial role in remineralising. For this project 1 liter of remineralising solution was prepared with 0.59 g of calcium nitrate (AnalaR, BDH Chemicals Ltd., Poole, England) which was dissolved in about 700 cm3 of distilled water, 8.77 g of sodium chloride (AnalaR) were added to stabilise the final solution. 198 g of ammonium phosphate (AnalaR) was dissolved in 100cm3, of distilled water. The phosphate solution was slowly added to the calcium solution, continuously stirring to prevent precipitation. NaOH was added to attain a pH of 7. Thymol was added to prevent bacterial growth and the solution was stored in a refrigerator.

METHOD

This In-vitro study was carried out a Dental Physical Sciences Unit, Barts & the London Institute of Dentistry. 10 extracted teeth with no lesions visible to naked eye were included for this experiment. These samples were scanned using X-Ray Microtomography (XMT). Six extracted teeth were taken for this part of the project which detected demineralized area by XMT out of 10 samples. These samples were then covered with nail varnish and a window was left on the enamel surface where the lesion was detected on X-ray micro tomography. The samples were then put in small glass containers where 30ml of remineralising solution was added to each container. The containers were kept in a moving incubator for 24 hours. The solution was changed after every 24 hours for 7 days; pH was maintained at 7 and was rechecked each time on changing the solution. After 7 days, samples were taken out and were washed with normal running water. After washing the samples, they were scanned with an OCT microscope with the exact same direction and dimensions. Images after scanning with OCT were scanned with XMT to confirm the areas of remineralization within the desired window. An unpaired t-test was used to compare two sample means. Un-paired t-test was used to compare the differences in before and after remineralization.

RESULTS

Effect of remineralization

There is a significant difference between the mineralized and remineralized enamel surface. Scattering Attenuation Microscopy reveals that the back scattering of mineralized area is less and the distance between the two lines is very narrow and is seen wide in the area which was demineralized as shown in the figure 1. They average depth of penetration in the pre-mineralized zone was found to 11.27 +/- 7.5 µm and when it was compared after the remineralized of the same site it was observed to be 6.39 +/-4.52 µm and unpaired t-test value was <0.0001 suggesting a statistically significant result. Thus, suggesting a less deep penetration of light in the remineralise samples. The back-scattering image of a mineralized sample showing a blue image and the pre-mineralized 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 1B. There is a shift towards the left side showing lesser penetration in re-mineralized samples.

SAM processing image showing the large separation between Ztop and Zbottom. (fig 1A) A smaller separation is seen between Ztop and Zbottom in remineralized enamel surface There is least penetration of light into the mineralized enamel surface (fig 1A) There is greater penetration of light in the demineral-

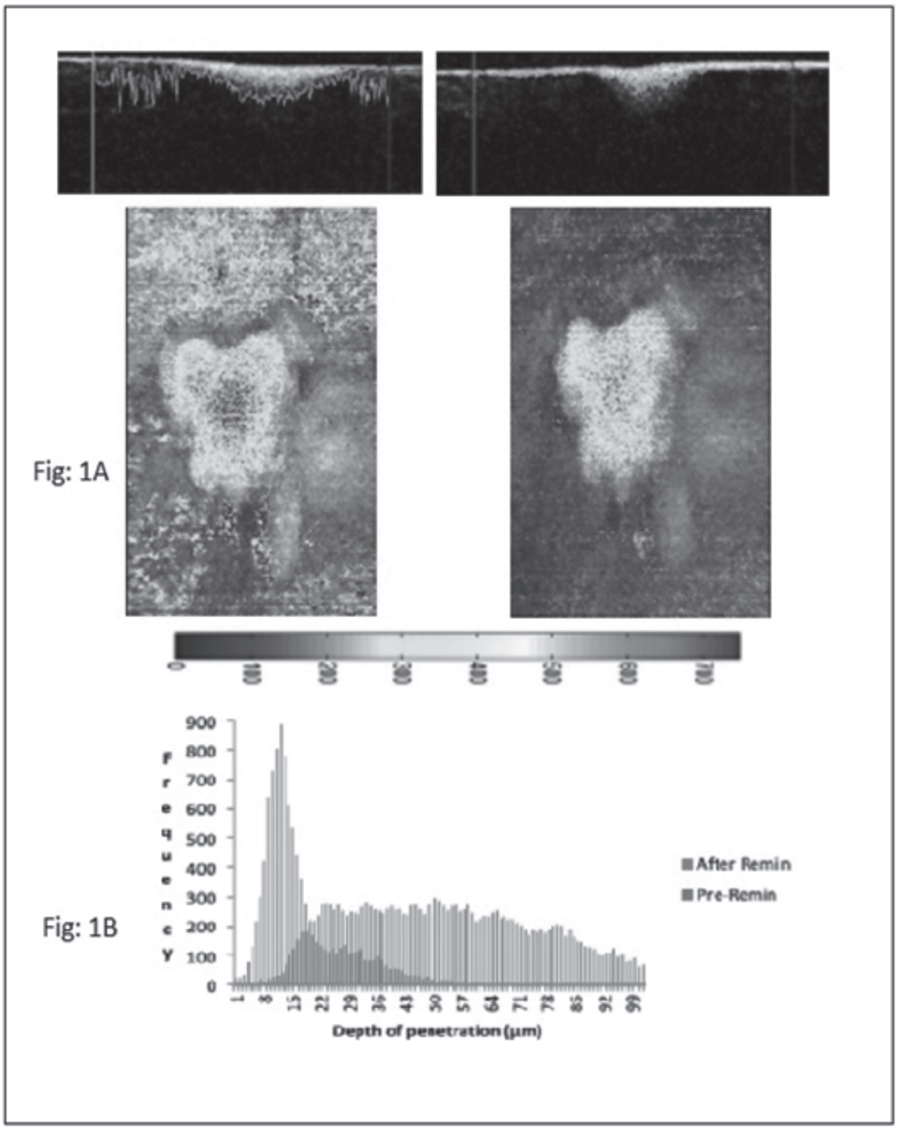

Fig 1 : Scattering Attenuation Microscopy showing back scattering ized surface of enamel. The histogram is showing the separation between the two means. There is a shift towards left after remineralization effect suggesting deeper penetration of light. (Fig 1B)

Comparison of enamel surface before and after remineralization

The depth of penetration of light in enamel was less in the remineralized samples as compared to pre-remineralized ones. Due to remineralizing the enamel, depth of penetration decreased. There is a difference between the process of amelogenesis and remineralization. In amelogenesis the nano-spheres act as spacers between the hydroxyapatite crystals to control the growth, inhibiting crystal fusion and also making space for new crystal deposition. Enamel crystals run parallel to the long axis of the rods at the head of the enamel rod. They elongate with a specific pattern during amelogenesis whereas in remineralization there is no fixed or perfect at which the crystals elongate. The difference in scattering can also be due to the haphazard pattern of crystal growth in remineralized teeth.

DISCUSSION

Early detection of changes in degree of mineralization is one of the most popular research fields.[16] Iatrogenic damages caused to the dental tissue by dentists are of great concerns.[17] The field of minimal invasive dentistry is gaining more popularity among the dentist and the patients.[18] Awareness of oral hygiene and its implications are increasing among the new generation.[19]

OCT can be used to detect early carious lesions and areas of demineralization in the clinic. Dental clinics use normal peri-apical x-rays to determine the loss of tooth structure and also keeping in mind the radiation hazards caused to the patient but according to one of the research it is determined that the carious lesion should be 30-50% demineralized in hard dental tissue to be evident in conventional radiographs.[20] A portable optical probe can be designed for the dental clinical use, for easy scanning inside the oral cavity.

In a study done by *Al Sayed*, it was observed that Optical Coherence Tomography can be used to non-destructively measure the changes in enamel as well as structure beneath them.[21] In another study done by *Tsai et al* illustrates that Optical coherence tomography (OCT) was helpful when used as an indicator for initial cavity formation and detection of surface demineralization.[22]

Austin Rs et al detected changes in superficial enamel after rinsing healthy upper central incisors with orange juice. Changes in the superficial subsurface enamel were deducted by Optical Coherence Tomography (OCT).[23] *Shimada et al* used OCT for diagnosis of fractures, tooth decay and defects in tooth restorations.[24] *Kim JM* used optical coherence tomography for automatic detection of tooth cracks in images. They were able to identify craze lines, splits and structural fractures in tooth using Optical Coherence Tomography images,[25] In a study done by Chew HP, he concluded that after 10 minutes of exposure to erosive stimuli, progression in demineralization of early enamel erosion can be monitored in vitro.[26] Another study shows that changes in degree of demineralization can be deducted using OCT when compare to micro-CT.[27] *Kitasako* observed white spot lesions in enamel in a study done with OCT to see the remineralization ability of enamel. In another study *Matsuyoshi* observed the remineralization of enamel effect of a dentifrice containing calcium sodium phosphosilicate using OCT and found remineralization changes can be deducted.

Future Work

OCT can be used under conditions similar to oral cavity for the determination of changes in the enamel surface. A detailed study to determine why there is different back scattering of hydroxyapatite crystals in remineralized and demineralized enamel can be done using SEM in observing the different orientation of the crystals. This study was done on enamel only, a study to determine the difference in back scattering of enamel and dentine can be performed. Interesting findings can be determined to check if dentinal tubules play a role in giving different back scatter than enamel. Dentinal hypersensitivity can be detected using OCT. OCT equipment can be redesigned to make it more portable for clinical application.

CONCLUSION

Optical Coherence Tomography was able to detect the changes in difference in degree of remineralization.

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