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ORIGINAL ARTICLE

Enamel Demineralization and Remineralization Detection Using Non-invasive Optical Imaging

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ABSTRACT
Introduction: Optical Coherence Tomography (OCT), is one of the most emerging diagnostic imaging technique. It is capable of producing 3D images using optical scattering media. The fast signal acquisition quality has made it a promising tool to detect early in vivo and in vitro lesions. The aim of this study was to reproduce previous demineralization results and to detect remineralization using OCT.
Methodology: Bovine enamel discs were used thoroughly in this study. The study was done using the flow cell for detecting demineralization and remineralization following 96 hours demineralization and 192 hours remineralization. A time lapse monitoring was done and the lesions were assessed visually. ImageJ software was used to process the images produced through OCT. The lesion depth and intensity was measured across the images produced which helped in assessing the difference between remineralization and demineralization.
Results: OCT B-scan images result in increased backscattering light which is considered the main principle to measure lesion depth and mineral loss. Whereas, in remineralization decreased band of light appeared with reduction in porosity during mineral precipitation. The results for remineralization were diverse and could not be assessed.
Conclusion: OCT is favorable technique to detect demineralization and remineralization but it still needs a lot of improvement especially regarding remineralization there are limitations which need to be improved.

INTRODUCTION

Demineralization is known as the process in which mineral ions are lost from dental enamel(1) whereas remineralization can simply be defined as redepositing of minerals within enamel. Remineralization and demineralization of enamel is a continuous physiochemical process. Mainly the enamel re-deposition can occur by amount of calcium and phosphate ions present in biofilm or can be gained soon after the salivary layer is removed after tooth brushing(2). It is logical to say that the progression of a carious lesion depends on the balance between demineralization and remineralization which may be influenced by three mechanisms - frequency, duration and reaction rates. This remineralization and demineralization is initially visualized in microscopic imaging(3). There have been timely developments in the field of dentistry varying from hard structure imaging to detection of carious lesions and changes in optical properties. OCT can detect changes occurring during demineralization and remineralization(4). OCT is one of the non-invasive imaging technique which provides real-time two- and three-dimensional images of scattering samples in micro-meter resolution(3). It can detect imaging both in vivo and in vitro.

OCT works as light scatters from materials with different densities such as enamel prisms scatter differently from inter-prismatic matrix. So, OCT images shows areas that scatter light differently. The very first vivo and in vitro images were produced by Feldchein et al., 1998(5). The goal was to assess in vivo images of dental microstructures both quantitatively and qualitatively. According to Fried et al., 2002(6) polarization sensitive (PS) OCT can be used to detect surface demineralization by using polarized incident light and backscattering signal which is measured in two axes. Hence, PS-OCT has been successful in imaging the initial lesions to quantify mineral loss and lesion depth(7). Amaechi et al., 2001 carried out an experiment running for three days to assess the demineralization process. He related it to transverse microradiography and proved that OCT can measure mineral loss on a longitudinal basis but on a smaller bovine sample(8).

OCT can detect carious lesions and many researchers have focused on this area. Baumgartner et al., 1999 was the first one to introduce polarized resolved images of dental caries. Than Wang et al., 1999 carried the work forward and introduced that enamel rods acted as wavelengths after being measured(7). OCT has been used to detect enamel cracks as well. The results were compared to the histological slides of individual enamel; a stereomicroscope was used which showed reliable results measuring the crack accurately and size too(9).

In recent years, the leading center for OCT has become the University of California in San Francisco. Several projects have been undertaken both clinically and laboratory based. Different aspects have been covered including remineralization, demineralization and early caries detection. However, the remineralization issue remained unsolved. Thus the aim of the study is:

- To quantify and assess demineralization and remineralization of bovine enamel discs using Optical Coherence Tomography.
- To validate the previous remineralization measurements.

Objectives:

- Visually assess the process of demineralization and remineralization and understand how it applies in OCT images.
- Evaluate and examine the ability of OCT to detect enamel remineralization.
- To compare and assess lesion depth and mean intensity in bovine enamel samples.
- Compare static to flow cell lesion formation.

METHODOLOGY

Materials

- Bovine Enamel Discs: provided by Modus Laboratories, Reading, and West Berkshire
- Demineralization solution: adding 0.05M acetic acid in deionized water. Than 2.2mM of acetic CaCl2, 2.2mM of KH2PO4 were added in it. To adjust the pH NaOH pellets were added to adjust the pH of the solution to 4.4. The pH was monitored using Orion pH meter.
- Remineralization solution: by 2mM CaCl2, 1.2mmol KH2PO4, 150mM NaCl & 2 ppm NaF in deionized water. The pH was adjusted to 4.9 using Orion pH meter.
- ImageJ software: java based-image processing software which is used to analyze images
- Pump and tubes
- Sample holder: in which the bovine disks could be placed.
- Temperature Probe
- Optical Coherence Tomography

Methods

The study was conducted with five bovine enamel disc samples being embedded in the holder which was then placed on the OCT stage. The sample holder was placed on the stage and the system was set to carry out the different hourly scans. Firstly, we passed ionized water through the tubes connected to the holder. After that demineralization solution was passed through them for 96hours, the channel can bring in the solution to the cell and flushing it out itself because of the pump connected. The OCT software (octView) offers a well-established system, which gives a choice to the run the system at desired positions and intervals. Hence, the intervals were set to different hours, baseline being set at 0 hours. The whole bottle had approximately 100ml of demineralized solution which was enough to carry out the experiment as it was set to flush in and out of the cells to the bottle and back automatically and did not have the need to be changed. After four days of demineralization cycle, the samples were flushed out with ionized water again and then replaced with remineralization solution. This cycle ran for 8 days (192 hours) without the solution being changed.

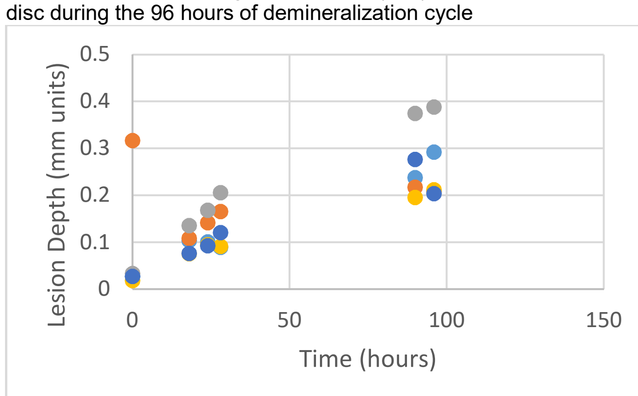
The data analysis was done using ImageJ software in which the images were stacked according to the area of interest and quantitative analysis was done. ImageJ is capable of producing surface images as well in which we can see what changes appeared. The lesion depth and intensities were measured as well according to time lapse.

RESULTS

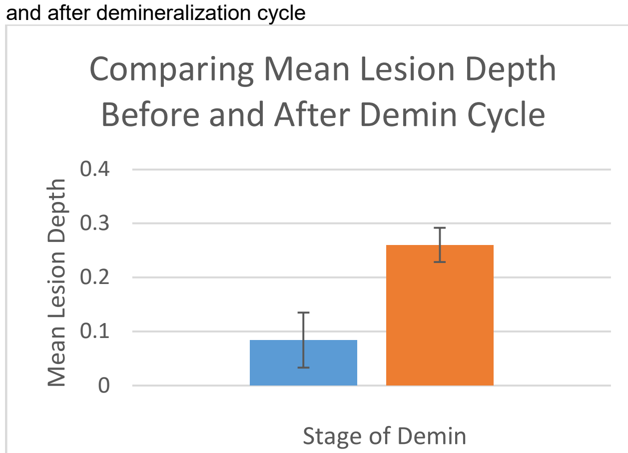
To detect remineralization with OCT, the study was done following the protocol of embedding specimens in the flow cell and running demineralization/remineralization solution through them in at different hours. In this study, all images were produced using OCT and mean lesion depth and intensity were taken using the tool box in OCT by taking 10 measurements throughout, of five specimens and then comparing their mean intensity and depth.

Graph 1 and 3 showed depth of bovine enamel disc during 96 hours of demineralization and 192 hours of remineralization respectively. The specimen are labelled with different color spots: specimen 1 = dark blue, specimen 2= orange, specimen 3=gray, specimen 4=yellow, specimen 5 = light blue. In demineralization cycle at 96 hours, the highest depth is of specimen 3 (0.388mm) and lowest depth is of specimen 5 (0.203mm) whereas in remineralization cycle at 192 hours, the highest depth is of specimen 2 (0.263mm) and lowest depth is of specimen 1 (0.091mm). While comparing lesion depth of bovine enamel before and after demineralization, an increased in depth is seen after demineralization whereas while in remineralization the depth is decreased after remineralization cycle. (Graph 2 and 4 respectively)

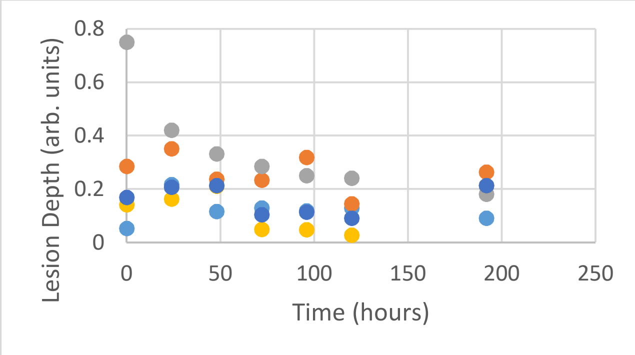
Graph 1: Graph showing lesion depth (mm) of the bovine enamel disc during the 96 hours of demineralization cycle



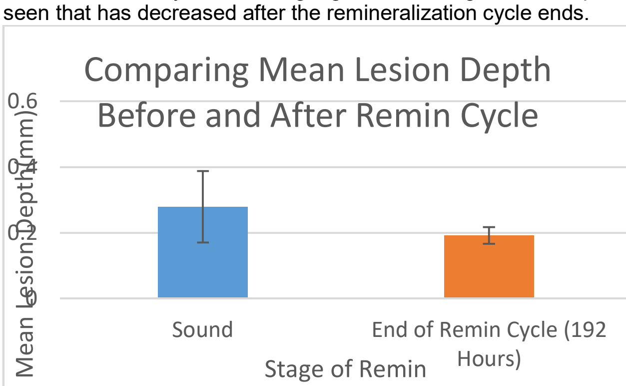
Graph 2: Comparing lesion depth of bovine enamel disc before and after demineralization cycle



Graph 3: Graph showing lesion depth of bovine enamel disc during remineralization cycle from 0-192 hours.



Graph 4: Bar graph comparing mean lesion depth before and after remineralization cycle. Showing significant change in the depth is seen that has decreased after the remineralization cycle ends.



DISCUSSION

Optical coherence tomography has been accepted in dentistry to detect earliest lesions non-invasively producing cross-sectional imaging which makes the visualization of internal structures convenient. From previous studies, it has been cleared that OCT can detect demineralization and remineralization in enamel by measuring changes with respect to backscattering of light(6). Mainly the OCT studies have been based upon the parameters like depth and mineral loss in demineralization. For remineralization, different theories have come forward but nothing has yet been set as consistent. However, in remineralization decrease in reflectivity due to the increased mineral formation that maybe the result of new mineral deposition(10). As seen in previous studies(11), also in this study it was found that previous results with respect to demineralization were produced. OCT can detect remineralization as well but the results vary till today and it is hard to say much because still consistent results have not been found which hold strong conclusion. This dynamic study was conducted with 5 specimens being embedded in a flow-cell. These ran through 4 days (96hr demineralization cycle) and 8 days (192hrs remineralization cycle). The results we got from demineralization cycle clearly indicate that OCT can quantify demineralization as a function of time which was also seen in previous studies(12). In comparison of mean intensity before and after demineralization cycle intensity has changed but the results vary across the specimens so it is not statistically significant which is similar in this study(13).

Optical Coherence Tomography successfully has showed promising results in many fields. Gradually it is opening doors to be practiced in dentistry. Static demineralization did not produce lesions whereas past studies produced consistent lesions using the flow cell dynamic study. Static remineralization produced changes occurring but this questioned all previous assumptions about morphology(14). Basically, increased backscattering light is considered as the main principle to measure lesion depth and mineral loss. With the results, it can be suggested that the backscatter signal intensity increases in demineralization due to the scattering of numerous micro interfaces created in hard tissue caused by dissolution process of the minerals(15). It is reasonable to correlate scattering effects with the structure of enamel. Prismatic crystallites scatter light due to their refractive index which is slightly higher than that of the surrounding medium but the inter-prismatic crystallites do the same but their index is slightly lower. Hence this arrangement of prisms and inter-prismatic material causes diffraction of light waves(16).

Other studies have confirmed this that demineralization process leads to formation of sub-surface band of intense optical backscatter(6). Demineralization changes the subsurface morphology. Remineralization necessarily doesn't return the lesion to original structure. However, it might fill some of the voids (porosity) with new, disordered (or short ordered) mineral crystals. This might reduce backscattering overall from the lesion, but the lesion shape itself is visible though lighter as it can be seen in remineralization images produced through OCT. It is possible that during early stages of remineralization, backscattering intensity might increase and then decrease as porosity is decreased.

As we know that remineralization is process of incorporation of ions such as calcium, phosphate and fluoride that reduces the porosity of enamel so following the demineralization of bovine enamel discs which were then exposed to remineralization solution could have caused the lighter appearance.

CONCLUSION

The results of the study conclude that OCT can detect demineralization with lesion depth being the best indicator in assessing it. Whereas, the remineralization data was variable which made it hard to assess visually due to unstable positions thoroughly. The intensity change could be due to light scattering properties. However, overall data represented much uniform platform for demineralization when compared to remineralization.

FUTURE RECOMMENDATION

- To check if dynamic and static study can be compared. Keep a uniform length of bovine disks by polishing and trimming them and to use same bovine discs in demineralization solution and remineralization solution to confirm if the static study show different results in comparison to dynamic study.
- To check if results produced from static remineralization studies were real.

- To compare the results using hardness test.
- To check if any other software than ImageJ can be used.

REFERENCES

1. Yu OY, Zhao IS, Mei ML, Lo EC-M, Chu C-H. A review of the common models used in mechanistic studies on demineralization-remineralization for cariology research. Dentistry journal. 2017;5(2):20.
2. Pini NIP, Theobaldo JD, Lima DANL, Aguiar FHB. Consumption of Different Energy Beverages and Oral Health. Sports and Energy Drinks: Elsevier; 2019. p. 441-81.
3. Ibne Mokbul M. Optical coherence tomography: basic concepts and applications in neuroscience research. Journal of medical engineering. 2017;2017.
4. Korotkova O, Gbur G. Applications of optical coherence theory. Progress in Optics. 2020;65:43-104.
5. Feldchtein FI, Gelikonov G, Gelikonov V, Iksanov R, Kuranov R, Sergeev AM, et al. In vivo OCT imaging of hard and soft tissue of the oral cavity. Opt Express. 1998;3(6):239-50.
6. Fried D, Xie J, Shafi S, Featherstone JD, Breunig T, Le CQ. Imaging caries lesions and lesion progression with polarization sensitive optical coherence tomography. Journal of biomedical optics. 2002;7(4):618-27.
7. Everett MJ, Colston Jr BW, Sathyam US, Da Silva LB, Fried D, Featherstone JD, editors. Noninvasive diagnosis of early caries with polarization-sensitive optical coherence tomography (PS-OCT). Lasers in dentistry V; 1999: International Society for Optics and Photonics.
8. Amaechi B, Higham S, Podoleanu AG, Rogers JA, Jackson DA. Use of optical coherence tomography for assessment of dental caries: quantitative procedure. Journal of oral rehabilitation. 2001;28(12):1092-3.
9. Zhou Y, Shimada Y, Matin K, Sadr A, Sumi Y, Tagami J. Assessment of bacterial demineralization around composite restorations using swept-source optical coherence tomography (SS-OCT). Dental Materials. 2016;32(9):1177-88.
10. Kang H, Jiao JJ, Lee C, Le MH, Darling CL, Fried D. Nondestructive assessment of early tooth demineralization using cross-polarization optical coherence tomography. IEEE journal of selected topics in quantum electronics. 2010;16(4):870-6.
11. Aden A. Developing Optical Coherence Tomography for the Quantitative Study of Erosive and Carious Lesions in Dental Enamel in vitro: Queen Mary University of London; 2017.
12. Ferreira RI, Haiter-Neto F, Tabchoury CPM, Bóscolo FN. In vitro induction of enamel subsurface demineralization for evaluation of diagnostic imaging methods. Journal of Applied Oral Science. 2007;15(5):392-8.
13. Nourolahian H, Parisay I, Mir F. The effect of Remin Pro on the microhardness of initial enamel lesions in primary teeth: An in vitro study. Dental Research Journal. 2021;18.
14. Schneider H, Park K-J, Häfer M, Rüger C, Schmalz G, Krause F, et al. Dental applications of optical coherence tomography (OCT) in cariology. Applied Sciences. 2017;7(5):472.
15. Majkut P, Sadr A, Shimada Y, Sumi Y, Tagami J. Validation of optical coherence tomography against micro–computed tomography for evaluation of remaining coronal dentin thickness. Journal of endodontics. 2015;41(8):1349-52.
16. da Silva CMF, de Menezes Costa AF, Costa AR, Neves JG, de Godói APT, de Góes VFF. Influence of different acid etching times on the shear bond strength of brackets bonded to bovine enamel. The Saudi Dental Journal. 2020.