Comparative evaluation of the spectral-domain optical coherence tomography and microhardness for remineralization of enamel caries lesions

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This study aimed to evaluate the Cirrus high-definition (HD) spectral-domain optical coherence tomography (SD-OCT) for the remineralization of artificial enamel caries and to compare it with the comparison surface microhardness (SMH) analysis. Artificial caries lesions were produced on forty human enamel samples. Then, three different remineralization agents containing casein phosphopeptide amorphous calcium phosphate; casein phosphopeptide amorphous calcium fluoride phosphate; calcium glycerophosphate, magnesium chloride, and xylitol; and remineralization solution (control) were applied with pH cycling for six days. The optical depth of backscattered light and microhardness of enamel were measured using SD-OCT and SMH. All remineralization agents were significantly efficient in reducing optical lesion depth on enamels (p_1=0.001, p_2=0.002, p_3=0.006, p_4=0.025), and in increasing the SMH of enamels (p_{1-3}=0.005, p_4=0.017). However, the optical lesion depths of the enamel showed no correlation with the SMH in the groups. In conclusion, demineralization and remineralization of artificial lesions can be assessed with both SD-OCT and SMH.

Keywords: Artificial caries lesion, Demineralization, Optical coherence tomography, Remineralization, Surface microhardness

INTRODUCTION

Continuous demineralization and remineralization of the tooth structure are common in the oral environment. When this balance is disrupted, demineralization may progress and lead to changes in the tooth structure. Preventive and interceptive strategies emphasize the non-invasive management of caries by remineralization of lesions with agents containing minerals to arrest decay and preserve the enamel integrity[1]. If enamel integrity is preserved, it can be reversed by non-invasive means through bioavailable phosphate, calcium ions, fluoride, and xylitol in the environment[2,3]. Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) nanocomplexes exhibit the synergistic effect with fluoride on the remineralization of subsurface enamel lesions by forming casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP)[3]. In the presence of calcium glycerophosphate (CaGP), it provides remineralization to the enamel with the transfer of calcium, phosphate ions, and a certain amount of energy[2].

The objective and quantitative methods for early detection and monitoring of carious tissue are essential in modern caries management strategies[4]. In the carious lesion, the exact diagnosis of the demineralization can be difficult due to the hypermineralization of the outer enamel surface that hides the subsurface lesion[5]. In this regard, apart from the conventional visual, tactile exams and X-ray-based methods, there is an increasing interest in new modalities to detect incipient caries. Conventional methods for caries assessment have some disadvantages; superposition of the surrounding structures, limited expose surface, and potential damage to the enamel surface due to probing[1,6]. Optical coherence tomography (OCT) has considerable potential as a non-invasive imaging technique for carious lesions as it eliminates the need for the removal of the superficial layer and eliminates radiation[7].

OCT is a non-contact and non-invasive technique providing cross-sectional images of microstructure in biological tissues at the submicron scale[8,9]. OCT is an interferometric technique that uses near-infrared (NIR) light that reflects off the internal microstructure by quantitative measurements backscattered light intensity of the enamel layer similar to an ultrasonic pulse echo. It allows the visualization of differences in tissue optical properties with real-time, with three-dimension reconstruction[1,8]. OCT has recently evolved from a time domain to a spectral-domain (SD) system, with increased image acquisition speed and improved image resolution, thereby increasing image quality[10].

The number of several OCT studies already demonstrated in dentistry that OCT can be used to nondestructively measure the severity of the lesion in enamel with cross-polarization optical coherence tomography (CP-OCT), swept-source optical coherence tomography (SS-OCT) images[6,11-13]. The principal purpose of this study was to demonstrate the efficacy of different remineralization agents on artificial lesions using the high-definition (HD) spectral-domain optical coherence tomography (SD-OCT) and to compare it with the surface microhardness (SMH) analysis. The null hypothesis tested was that there were no differences

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between the samples treated with the remineralization agents and the control group.

MATERIALS AND METHODS

Ethical approval was received for the study from the Clinical Ethics Committee of the Marmara University (reference no: 2017-68).

Preparation of the samples

The number of specimens per group was calculated as 10 samples per group based on published data (α=0.05, 1−β=0.80)[14]. Freshly extracted twenty-five human molars were acquired from consenting patients. The teeth were undergoing extractions for orthodontic and periodontal reasons with no signs of a cavity, caries, crack, restoration, or hypoplasia. Forty enamel blocks 6×3×3 mm^3 (length×width×depth) were prepared using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under running water, and mounted on 3×2 mm^2 acrylic blocks (Imicryl, Konya, Turkey). The enamel surface was sequentially polished using silicon carbide paper from 200 to 1200 grit under running water to create a standard flat smooth surface. Each enamel sample was partitioned into 3 parts using nail varnish (Nail enamel, Flormar, Milan, Italy).

Lesion formation

To produce artificial caries lesions, 40 enamel samples were individually immersed daily in the demineralization solution at room temperature for three days (72 h). The demineralizing solution contained 0.9 mM potassium dihydrogen phosphate, 1.5 mM calcium chloride, and 50 mM acetic acid (pH 4.8)[15].

pH cycling condition

The pH cycling proposed by ten Cate *et al.*[15] was used to simulate oral pH-fluctuation patterns. The enamel samples were immersed in the demineralizing solution for 6 h (30 mL for each sample), then remineralizing agents were applied (after the demineralizing period) for 4 min with micro applicator regular size (2.0 mm). The samples stayed in remineralizing solutions (18 h) without mixing. The remineralizing solution contained 0.9 mM potassium dihydrogen phosphate, 130 mM potassium chloride, 1.5 mM calcium chloride and 20 mM Hepes solution (pH 7.0). Later remineralizing agents were applied (after the remineralizing period) in the same way. Furthermore, the samples were washed with deionized water for 5 s to remove excess solution or agent after each step. This cycle was repeated daily for 6 days. New solutions were used for every cycle. After finishing the pH cycling, all the samples were slightly dried with absorbent paper for the removal of excess liquid and all the samples were subjected to OCT and microhardness measurement after demineralization[15].

Surface treatment and remineralization agents

Forty enamel blocks were randomly allocated into four groups, classified as follows:

Group 1: 10% CPP-ACP (Tooth Mousse®, GC America, Chicago, IL, USA)
Group 2: 10% CPP-ACP+900 ppm fluoride (MI Paste Plus®, GC)
Group 3: CaGP+magnesium chloride ($MgCl_2$)+10% xylitol (R.O.C.S. Medical Minerals Gel®, DRC Group, Moscow, Russia)
Group 4: Remineralizing solution (Positive control)

OCT image analysis

In this study, the surface reflectivity was recorded with a Zeiss Cirrus HD SD-OCT 5000 (Carl Zeiss Meditec, Dublin, CA, USA) with a wavelength of 840 nm. Cirrus HD OCT (model 5000; Carl Zeiss Meditec) is based on SD-OCT technology that uses a superluminescent diode laser wavelength of 840-nm[10]. All samples were scanned using the Anterior Segment Five-Line Raster protocol at the baseline, after the demineralization procedure and after the pH cycling period. For each sample, images were monitored in 3 areas in the same part of the enamel sample (3 per part). The mean of 3 measurements was calculated for each sample. To replicate the imaging position each time, one side of the sample was marked with nail polish and placed in the same direction as previous scans. All the image and microhardness analysis were performed by the same operator. HD-OCT software (ver. 4.0.) was used to import and measure the lesion depth the raw data. The appropriate values that correspond to the visual boundary of enamel lesions were measured. The raw data was analyzed using a computer program written in LabView™ software (National Instruments Austin, TX, USA). It was measured from the deepest part of the boundary representing the optic lesion depth.

The Anterior Segment Five-Line Raster mode scans through 5 parallel lines that can be used to view high-resolution images of tissues. The scan size is 3×1 mm. Cirrus HD SD-OCT acquires HD scans with 4096 A-scans, and the lines are horizontal and separated by 250 µm so that the 5 lines together cover 1mm width. The axial resolution of Cirrus HD SD-OCT in the tissue is 5 µm[16].

Microhardness analysis

The SMH was measured at the baseline, after the demineralization and the pH cycling period defined for each group using a Vickers microhardness tester (Wolpert Wilson Instruments Model 402 MVD, Norwood, MA, USA). A load of 300 g was applied for 15 s in 3 areas (3 per part). Three indentations spaced 100 µm away from each other. All the length measurements were performed manually. From those data, the microhardness analyzer automatically provides the Vickers hardness number. The mean of 3 measurements was calculated for each sample. The percentage recovery of the surface microhardness (%SMHR) was then calculated.

Statistical analysis

All statistical analyses were performed with Statistical software package (SPSS for Windows 22.0, Chicago, IL, USA). Evaluation of normality distribution of the data was confirmed using the Shapiro Wilk test. One-way ANOVA test was used for normal data, and Kruskal Wallis test was used for non-normal data for compare four groups. Wilcoxen signed rank test was used for non-normal data to compare two dependent groups. Spearman's rank correlation test was used to examine the relationship between non-normal numerical variables. All statistical analyses were performed with a 95% level of confidence.

RESULTS

OCT images

An example of the OCT image of an enamel sample is shown in Fig. 1. An area of weak signal and backscattering intensity were observed in the baseline in OCT images. In the artificial caries samples, the backscattered signal from the enamel surface deepened. After pH cycling, the signal from the enamel surface appears as a slight backscattered light.

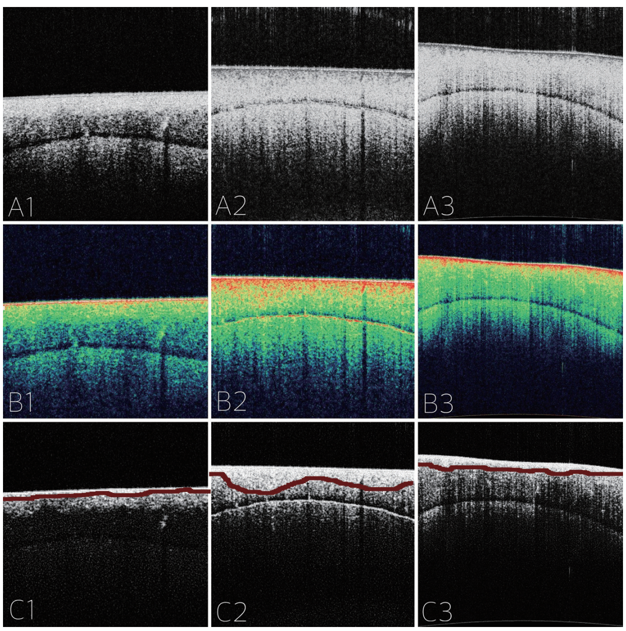

Fig. 1 SD-OCT images obtained at baseline (Column 1: A1, B1, C1), after the demineralization (Column 2: A2, B2, C2), and after pH cycling (Column 3: A3, B3, C3).
A: SD-OCT images in dark scale, B: SD-OCT color images, and C: An illustrative representation of what is shown in the images. A. There is minimal reflectivity consistent sound enamel. B. OCT identified the demineralized region from sound tissue through an increase in reflectivity through dentinal tubules. The presence of demineralization was determined as a strong scattering signal from the top of the enamel and drops off with depth in that area. The decreased reflectivity from the lesion reveals a lesion boundary. SD-OCT image of the demineralized enamel appeared representative lesions of layer type. C. There was an apparent decrease in the optical lesion depth and distinct remineralization after exposure to pH cycling.

The measurement of the optical lesion depth (OLD) in OCT

The OCT images of 40 enamel samples were obtained before starting the demineralization procedure and after the demineralization, and the pH cycling. Then the backscattered light was measured for each image and recorded as OLD by SD-OCT (Fig. 2). At the baseline, there was no statistically significant difference in the OLD of the sound enamel in the four groups (p=0.551). The OLD values after demineralization and pH cycling were compared with the Wilcoxen signed rank test. A significant increase in the light scattering on the enamel surfaces in all groups was observed following the demineralization (p_1=0.000, $p_{2,3}$=0.002, p_4=0.014). After six days, remineralization of the lesion surface using CPP-ACP; CPP-ACFP; CaGP, magnesium chloride, and xylitol with pH cycling was significantly efficient in reducing OLD on enamels (p_1=0.014, p_2=0.004, $p_{3,4}$=0.025). One-way ANOVA results indicated the presence of significant influence of all treatment procedures in the OLD at the baseline, after demineralization and remineralization (p_1=0.001, p_2=0.002, p_3=0.006, p_4=0.025). CPP-ACFP showed the highest rate of remineralization effect, among the agents tested. But there was no significant difference between the groups in terms of the amount of reduction in the depth of the lesion (p=0.683, Table 1).

Microhardness analysis

The SMH values were obtained at the baseline and after the demineralization and after the pH cycling period (Fig. 3). The percentage recovery of the SMH was then calculated. The baseline SMH values were not significantly different between the four groups (p=0.164). The treatment of the immersion in the demineralizing solution for 72 h significantly reduced SMH in each

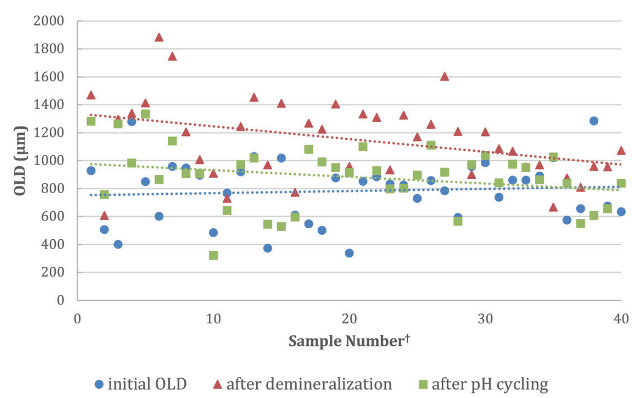

Fig. 2 The optical lesion depths (OLDs) of all groups at baseline, after demineralization, and the pH cycling period.
†Sample number: 0–10=Group 1, 11–20=Group 2, 21–30=Group 3, 31–40=Group 4 (Control)

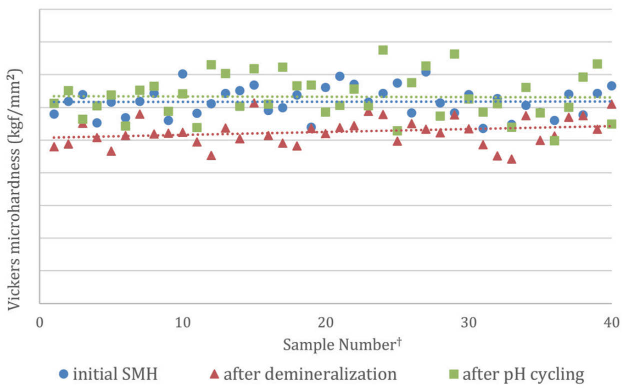

Fig. 3 The Vickers microhardness numbers of all groups at baseline, after demineralization, and the pH cycling period.
†Sample number: 0–10=Group 1, 11–20=Group 2, 21–30=Group 3, 31–40=Group 4 (Control)

group (p_{1-4}=0.001). The post-demineralization SMH was not significantly different between the four groups (p=0.302). The SMH values post-demineralization and post-remineralization were compared with the Wilcoxen signed rank test. All groups were statistically efficient in increasing the SMH of enamels (p_{1-3}=0.005, p_4=0.017). There was a significant difference in SMH in all groups at the baseline, after demineralization and pH cycling (p_{1-3}=0.001, p_4=0.006). Besides, there was no significant difference between the groups in terms of the amount of advancing in the %SMHR (p=0.290) (Table 2). However, the mean microhardness values after six days of pH cycling were not significantly different in groups (p=0.247). No correlation between the OCT measurement and the SMH in the groups (Table 3).

Table 1 The amount of reduction in the OLDs after remineralization treatment with pH cycling

Groups	The reduction in the OLD (μm) Mean (SD)	p
CPP-ACP	311.80 (344.38)	0.683
CPP-ACFP	320.10 (244.36)	
CaGP+MgCl$_2$+Xylitol	312.70 (203.80)	
Control	242.73 (11.01)	

OLD: optical lesion depth, SD: standard deviation *: p<0.05

Table 2 The SMHR% after remineralization treatment with pH cycling

Groups	SMHR% Mean (SD)	p
CPP-ACP	20.10 (11.71)	0.290
CPP-ACFP	27.94 (16.55)	
CaGP+MgCl$_2$+Xylitol	19.45 (11.85)	
Control	16.05 (15.12)	

SMHR: recovery of the surface microhardness, SD: standard deviation *: p<0.05

Table 3 The correlation between OLD and SMH

Groups			SMH
CPP-ACP	OCT	r	0.018
		p	0.960
CPP-ACFP	OCT	r	0.297
		p	0.405
CaGP+MgCl$_2$+Xylitol	OCT	r	0.261
		p	0.467
Control	OCT	r	0.152
		p	0.676

DISCUSSION

The demineralization starts in the subsurface zone on enamel. There is strong evidence that the incipient lesions can be remineralized with remineralization treatment if the intact surface layer is preserved[17]. Application of high-concentration fluoride provides rapid remineralization in the superficial layer of enamel, but it is not effective in the deeper layers of the lesion. Instead of high-concentration fluoride application, it is recommended to perform low-concentration fluoride applications that allow slower penetration of calcium and fluoride ions from saliva[18]. Therefore, in the present study, different remineralization agents containing bioavailable phosphate, calcium, and fluoride ions for the remineralization of artificial caries lesions were applied with a pH cycling that mimics the 6 days intraoral condition.

OCT has been used since 1998 for diagnosis in dentistry. The Zeiss Cirrus HD SD-OCT device used in the current research received FDA approval in 2015. The capture of an image takes less than 2 s with Cirrus HD SD-OCT, making this suitable for routine clinical use[19]. Therefore, in this study, SD-OCT was used, and its correlation with a traditional method, SMH, was evaluated.

Most dental OCT uses low numerical aperture imaging lenses and light source in 1,300 nm wavelength and provides a 2 mm depth image with 10 micrometers axial resolution. However, the OCT with high numerical aperture and shorter wavelength like 850 nm gives a shallower image, but the axial resolution of the image increases[20]. The probing wavelength of the SD-OCT system is 840 nm. There are studies in the literature where OCT with a wavelength of about 800 nm has been successfully used to investigate biological tissues, including ocular tissues[21], and dental tissues[22,23]. The maximum probing depth is 1–2 mm in dental tissue at a wavelength of 850 nm. However, demineralization appears within the first 300–400 micrometers of the enamel[23,24].

Today, there is a lot of research with some kind of OCT techniques on acquiring images of incipient carious lesions in order to quantify lesion depth, their remineralization, for determining the efficiency by different chemical agents on smooth enamel surfaces in the inhibition of the demineralization in an artificial caries model[25,26]. Jones and Fried stated that the optical reflectivity of the lesion area can be easily determined and quantified with OCT[27]. Ngaotheppitak *et al.*[28] reported that when the lesion occurred, the signal intensity increased in the OCT images, resulting in a marked increase in penetration depth in most lesions. However, this increase was correlated with the mineral loss on microradiography. Matsuyoshi *et al.*[29] observed a decrease in depth of the artificial lesions on OCT images after remineralization treatment. Similarly, in the current study, it was observed that the depth of the lesion increased when artificial caries occurred in the enamel and the depth of the lesion decreased when remineralization treatment was applied. After demineralization, the backscattering was consistent with lesion depth in enamel. A reduction in the reflectivity of light in the enamel was observed after pH cycling in all groups. There was no statistically significant difference between the groups. The minerals in the remineralization agents can affect the porosities on the enamel surface and the optical properties of the enamel and differences in the light backscattering can be observed[30].

After applying different remineralization agents to enamel samples with pH cycling, it was observed that OLD decreased in OCT images of all groups. Also, due to the increase in mineral density, the signal intensity within the depth profiles in artificial caries lesions decreased. The amount of reduction in OLD is CPP-ACFP>CPP-ACP>CaGP, magnesium chloride, and xylitol, respectively. Although each agent provided more remineralization than the control group, no statistical difference was observed between the groups (p=0.683). When the effect of the remineralization agents on the SMH of enamel samples was examined, the findings were similar to OCT analysis. All remineralization agents increased SMH more than the control group, but there was no statistical difference between the groups (p=0.247). Unlike OCT findings, the remineralization agent containing calcium glycerophosphate, magnesium chloride, and xylitol provided more remineralization that CPP-ACP. However, due to the difference in the groups are not statistically significant, this result may be inconsiderable.

Jayarajan *et al.*[31] showed by using a scanning electron microscope that CPP-ACFP and CPP-ACP have remineralization capacity more than artificial saliva. Several studies concluded that the combined use of fluoride with CPP-ACP is more efficient in increasing the SMH of enamels[32,33]. Manton *et al.*[34] reported that chewing gum containing xylitol has less remineralization capacity that chewing gum containing CPP-ACP. However, the combined use of calcium glycerophosphate with xylitol remineralize 80% of the white spot lesions in 15 applications[35]. Zhitkov[2] stated that in the presence of calcium glycerophosphate, remineralization increases.

SMH is widely accepted as an effective, relatively simple, and non-destructive method for demineralization and remineralization of the hard dental tissue[36,37]. A current meta-analysis evaluating the methodological quality of SMH in *in vitro* experiments by remineralization of white spot lesions with CPP-ACP showed that the SMH values increased compared to the control group[36]. In the demineralization process, the depth of artificial lesion in the enamel increased in OCT images, while enamel SMH decreased, and vice versa in the remineralization process. Given this knowledge, it was expected that there is a negative linear correlation between the OLD in OCT images and SMH. But the OLD of the enamel showed no correlation with the SMH in the groups. Cara *et al.*[38] implied that there is a correlation between the optical attenuation coefficient and the SMH of the enamel samples, but it was not statistically significant. However, Cara *et* *al*.[14] also founded that the average areas under the curves of OCT are correlated with the areas under the curves of microhardness. Hariri *et al*.[4] investigated the availability of the refractive index, which is the result of the scattering of light, in measuring the mineral content of demineralized enamel and dentin. OCT was used to measure the refractive index and transverse microradiography was used to measure the mineral content. There is a positive correlation between mineral content and SMH of tissue[39]. Unlike our findings, Hariri *et al*.[4] found the linear correlation between the refractive index and the mineral content of tissue. Besides, Ibusuki *et al*.[40] stated that the visible boundary in OCT images from bright to dark areas was consistent with the depth of the lesion. The porosity of enamel increases due to the dissolution of minerals in demineralization. As a result, scattering at many micro interfaces between media with different refractive indices and an increase in lesion depth are observed.

Based on the current study, it was concluded that the intensity of artificial lesions can be determined after demineralization and remineralization with both OCT and SMH. The pH cycling regimen induced a significant reduction in the OLD with the decrease of the backscattering. However, the performance of the investigated remineralizing agents did not appear to be any better than each other. Therefore, the null hypothesis of the study was accepted. One limitation of the study that only the depth of the lesion is measured in OCT analysis. Measuring the reflection density in the lesion could support the findings. Another limitation of studies on OCT is the lack of standardization of methodology. Further *in vivo* studies are needed to better understand the correlation between the backscattered light and SMH of enamel and the clinical practice of SD-OCT.

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CONFLICT OF INTEREST

There are no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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