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ABSTRACT

Objectives: This randomized controlled *in vitro* 4-arm trial study aimed to evaluate the efficacy of SoproLife® in detecting and quantifying *in vitro* remineralization with early caries lesions.
Material and Methods: Sixty human teeth were randomly assigned into four equal groups. Groups 1 and 2 were prophylactically cleaned; groups 3 and 4 were not. Group 1 received treatment with MI Varnish® and Recaldent™ for 30 days. Group 2 was treated similarly, but without MI Varnish®. Group 3 was treated as 1 and Group 4 as 2. Mineral composition was obtained using scanning electron microscopy with energy dispersive X-ray analysis SoproLife® camera images on the occlusal surfaces were analysed for grey value distribution and difference in mean intensity values (DI). Paired t-test and Mann-Whitney-U test were used for intragroup comparison between baseline and T1. Kruskal-Wallis followed by Mann-Whitney-U tests were used for inter-group comparisons at T1.
Results: All groups exhibited a significant increase in calcium content and calcium-to-phosphorus ratio ($P < 0.05$), except Group 4 (Group 1 showed the greatest increase, then Groups 3 and 2). Grey intensity values decreased in all groups ($P < 0.05$). Group 1 showed the greatest change in DI (16.82 [SD 12.07]), followed by Group 3 (12.46 [SD 9.41]), 2 (10.45 [SD 7.76]), and 4 (6.46 [SD 6.21]). The difference in DI was different between the compared groups ($P = 0.038$); Groups 1 and 3 exhibited a greater DI compared with 4 ($P < 0.01$).
Conclusions: Within the limitations of this study, SoproLife® is effective for early detection and for longitudinally monitoring the remineralization after Recaldent™ therapy.

Keywords: calcium phosphates; dental caries; dental enamel; tooth demineralization; tooth remineralization.

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INTRODUCTION

Dental caries is highly prevalent chronic disease. It is the result of an imbalance between the processes of demineralization and remineralization of enamel and dentin [1]. It can be stopped and even reversed if diagnosed and controlled at an early stage. This can be achieved by controlling the changes that occur on the early enamel surface lesions; specifically, by ensuring sufficient mineral quantities around the crystals to maintain mineral balance between the tooth enamel and oral fluid [2].

Proper treatment of dental caries requires detection of carious lesions at an early stage, where they still have the potential to be remineralized, presenting the highest tendency for arrest and even reversal, avoiding the need for dental intervention [3,4]. Visual methods and radiography approaches are well known to detect occlusal caries [5]. Nevertheless, monitoring the remineralization of lesions, i.e. changes in the mineral content of early caries lesions, remains a diagnostic problem [6].

The Light Induced Fluorescence Evaluator for Diagnosis and Treatment (LIFE-D.T.) concept which uses the SoproLife® imaging device (SOPRO, ACTEON Group; La Ciotat, France) [7] is a suggested technology in this regard. The SoproLife® camera selectively amplifies fluorescence signals to detect any carious lesion or diseased tissue based on the variation of its autofluorescence compared with the healthy area of the same tooth [7-9].

Numerous studies showed that, compared with other approaches for detecting occlusal carious lesions such as the International Caries Detection and Assessment System (ICDAS), SoproLife® shows high intra- and inter-examiner repeatability and reliability, best caries lesion discrimination, highest sensitivity values and lowest over diagnosis rate *in vitro* and *in vivo* [9-14]. These studies suggested SoproLife® as a useful and superior method in monitoring caries lesions [6,8,11,13-15].

Nowadays, we still lack high-quality studies assessing the efficacy of SoproLife® in monitoring remineralization and changes in the mineral content of caries lesions. The aim of this study is to evaluate the efficacy of SoproLife® in detecting and quantifying *in vitro* remineralization using the ImageJ® software.

MATERIAL AND METHODS

The study design and protocol were reviewed and approved by the Institutional Review Board of the Lebanese University (approval number: CUMEB/D166/42019). The study extended over 2 months, as follows: teeth collection (1 month) and intervention (1 month).

Samples

A power analysis was conducted using the G*Power Software, Version 3.1.9.2 (Heinrich-Heine-Universität; Düsseldorf, Germany). Taking into account the repeated measures design of the trial, and accounting for an effect size of 0.2 (small effect size), a 5% level of significance and an 80% power, the required sample size was around 60 teeth.

Sixty human teeth (molar: n = 35; premolar: n = 25) extracted for orthodontic and periodontal reasons and having ICDAS II score of 0 to 3 were analysed. To avoid bacterial contamination, the teeth were rinsed with water and stored in a 0.1% thymol solution at a pH of 7, for 1 hour, and then were rinsed with water and examined for mineral composition targeting their occlusal surface.

Procedures

Details of the procedures are published elsewhere [16]. The teeth were randomly assigned to 4 equal groups.

Group 1 teeth (n = 15) were prophylactically cleaned for 20 seconds, using AIR-N-GO® (Acteon; Bordeaux, France) combining water, air and pearl powder (natural calcium-carbonate-based, diameter: 55 µm), at 1 mm distance from the surface. The cleaned 15 teeth were then rinsed with water, had MI Varnish® (GC Corporation; Tokyo, Japan) applied according to manufacturer instructions for 4 minutes, and soaked with artificial saliva overnight. In the morning, the teeth were then brushed with Oral B brush (Oral B Laboratory; Kildare, Ireland), rinsed with water, and soaked again in artificial saliva until night. Afterward, the teeth were rinsed with water, treated with GC Tooth Mousse Plus® (GC America Inc; Illinois, USA) - which contains casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Recaldent™), a special milk-derived protein - for 4 minutes, brushed with a micro-brush on the surface, and then soaked again in artificial saliva. The teeth underwent these same procedures for 30 days (T1). T1 is defined as the period where the concerned teeth received different interventions post-baseline.

- Group 2 teeth (n = 15) received the same interventions as Group 1 teeth, but were not treated with MI Varnish®.

- Group 3 teeth (n = 15) underwent the same procedure as Group 1 teeth, except for initial prophylaxis cleaning.
- Group 4 teeth (n = 15) received the same treatment as Group 2 teeth, except for initial prophylaxis cleaning.

Analysis of mineral composition

The specimens were evaluated for mineral composition (% weight) of calcium (Ca) and phosphorus (P), as well as for calcium-to-phosphorus ratio (Ca/P) at baseline and after 30 days of intervention (T1) using scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX) analysis. SEM, complemented by X-ray analysis, is a relatively inexpensive, rapid, and non-destructive approach to surface analysis. It is often used to examine surface analytical problems before proceeding to advanced surface-sensitive and specialized techniques.

SoproLife® imaging

SoproLife® images were taken on the occlusal surfaces of selected posterior teeth using the diagnostic aid mode with a visible blue light frequency (wavelength 450 nm) and saved in JPEG file format. Only 1 examiner traced all images for baseline and at T1 to allow standardization. The one-month period was chosen to detect any remineralization events.

The SoproLife® images were compared and analysed to quantify the remineralized areas using the open source software - ImageJ® version 1.52p (Fiji distribution; National Institute of Health, Bethesda, Meryland, USA). The mean and standard deviation of the grey value distribution from each group was computed. Then, a grey scale histogram displaying the distribution of the grey values in the image, i.e. the number of pixels detected for each grey value, was obtained. The histogram was generated by converting each pixel of the RGB image into grayscale using the equation: grey = (red + blue + green)/3, taking into consideration that for a 16-bit image, the grey values range between 0 and 255 bins. The outcome measure for the mean intensity values was expressed as the difference in the mean intensity (DI) at baseline and after undergoing various interventions.

Statistical analysis

The normality of the data was checked using Shapiro-Wilk test. Paired t-test and Mann-Whitney-U test were used for the intragroup comparison of the difference in grey intensity values, and mineral composition, respectively, between baseline and T1. Kruskal-Wallis followed by Mann-Whitney-U tests were used for inter-group comparisons of the DI at T1. $P < 0.05$ was considered significant except for the results of the inter-group comparisons where $P < 0.01$ was considered significant (Bonferroni correction for the 5 comparisons done between the 4 groups). The Statistical Package for Social Sciences (SPSS), version 21.0 (IBM SPSS Statistics, IBM Corporation; Armonk, New York, USA) was used for statistical analysis. Parametric data were expressed as mean and standard deviation (M [SD]).

RESULTS

A total of 60 teeth (molar: n = 35; premolar: n = 25) were included in this study. Their distribution is detailed in Table 1.

The whole sample was examined clinically and imaged with SoproLife® using ICDAS scores 0 to 3 at baseline and T1. An example of figures provided by SoproLife® is shown in Figure 1.

Table 1. Distribution of teeth included in this study (n = 60)

Tooth	Maxilla (n = 36)	Mandible (n = 24)
First premolar	16	2
Second premolar	2	5
First molar	12	13
Second molar	6	4

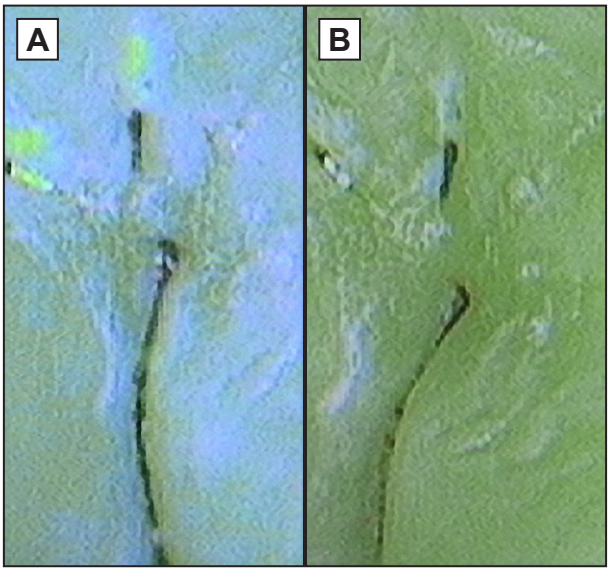

Figure 1. A = primary fissures on occlusal surface of posterior tooth using ImageJ® at baseline. B = primary fissures on occlusal surface of posterior tooth using ImageJ® at T1.

The chemical composition was obtained for all included teeth at the above-mentioned time-points. Table 2 details the mineral content (% weight) of Ca and P, as well as Ca/P at baseline and T1. All groups exhibited a significant increase in their Ca content and Ca/P ($P < 0.05$), except Group 4. Group 1 showed the greatest increase in its Ca/P (mean increase: 0.4), followed by Group 3 (mean increase: 0.31), then by Group 2 (mean increase: 0.14), and finally by Group 4 (mean increase: 0.06).

At baseline, Group 3 exhibited the least mean difference in grey intensity values (136.22 [15.24) followed by Group 4 (139.9 [9.61]), then by Group 2 (145.27 [18.29]), and finally by Group 1 (145.45 [14.95]), as shown in Table 3.

Table 2. Mineral composition evaluated by SEM-EDX at baseline and T1 (n = 60)

	Mineral composition	Baseline	T1	P-value[a]
		Mean (SD)	Mean (SD)	
Group 1	Calcium (% weight)	19.09 (5.57)	31.3 (13.69)	0.001
	Phosphorous (% weight)	13.01 (3.41)	16.2 (2.78)	0.47
	Ca/P	1.45 (0.07)	1.85 (0.47)	0.003
Group 2	Calcium (% weight)	18.05 (5.85)	24.93 (1.55)	0.001
	Phosphorous (% weight)	12.4 (3.64)	15.71 (0.79)	0.001
	Ca/P	1.44 (0.06)	1.58 (0.03)	0.002
Group 3	Calcium (% weight)	18.31 (6.08)	28.48 (8.34)	0.001
	Phosphorous (% weight)	16.01 (1.56)	12.48 (3.71)	0.15
	Ca/P	1.45 (0.07)	1.76 (0.42)	0.001
Group 4	Calcium (% weight)	18.47 (6.12)	21.83 (1.66)	0.17
	Phosphorous (% weight)	12.62 (3.76)	14.53 (0.49)	0.649
	Ca/P	1.44 (0.07)	1.5 (0.06)	0.124

[a]Statistically significant at level $P < 0.05$, Mann-Whitney-U test.
Ca/P = calcium-to-phosphorus ratio; SD = standard deviation.

Table 3. Within-group differences in grey intensity values (n = 60)

	Baseline		T1		P-value[a]
	Mean	SD	Mean	SD	
Group 1 (n = 15)	145.45	14.95	128.63	13.67	< 0.001
Group 2 (n = 15)	145.27	18.29	134.82	15.98	< 0.001
Group 3 (n = 15)	136.22	15.24	123.75	11.77	< 0.001
Group 4 (n = 15)	139.9	9.61	133.43	9.18	0.001

[a]Statistically significant at level $P < 0.05$, Paired t-test.
SD = standard deviation.

Table 3 also shows the within-group changes in grey intensity values for the 4 groups between baseline and T1. All groups exhibited a statistically significant decrease in grey intensity values ($P < 0.05$).

As shown in Table 4, Group 1 showed the greatest mean change in DI (16.82 [12.07]), followed by Group 3 (mean change: 12.46 [9.41]), then by Group 2 (undergoing initial prophylactic treatment, then treatment with Recaldent™) (mean change: 10.45 [7.76]). Finally, Group 4 (undergoing only treatment with Recaldent™) exhibited the least mean change (6.46 [6.21]). The difference in DI was significantly different between the four compared groups ($P = 0.038$), whereby Groups 1 and 3 exhibited a greater DI compared with Group 4 ($P < 0.01$).

DISCUSSION

Dental caries is a complex disease of the calcified tissues of the teeth, characterized by a demineralization of the inorganic portion and destruction of the organic substances of the tooth, i.e. dissolution of the mineral content of enamel and dentine caused by acids from bacterial metabolism. Despite advances in preventive and curative measures, dental caries remains one of the most common diseases, affecting nearly 95% of the global population [17].

Remineralization consists of regaining calcium, phosphate, and fluoride ions within the tooth structure in the form of fluorapatite crystals. The latter are more resistant to acidic dissolution and considerably larger than the original crystals [18]. As the remineralization of enamel requires the availability of Ca and P in the oral environment, materials providing essential elements for remineralization are needed. Among these, CPP-ACP (Recaldent™) emerged as a novel material that prevented demineralization, and showed enhanced remineralization of initial enamel lesions *in vitro* and *in vivo* studies [18,20-25]. Additionally, the concomitant use of CPP-ACP (Recaldent™)

Table 4. Between-group differences in intensity (n = 60)

	Mean	SD	P-value[c]
Group 1 (n = 15)[a]	16.82	12.07	0.038
Group 2 (n = 15)	10.45	7.76	
Group 3 (n = 15)[b]	12.46	9.41	
Group 4 (n = 15)[ab]	6.46	6.21	

[a]P = 0.007, [b]P = 0.009 statistically significant after applying Bonferroni correction.
[c]Kruskal Wallis test followed by Mann Whitney-U test were used.
SD = standard deviation.

and fluorides (MI Varnish®) showed better remineralization effect in terms of higher mineral content and difference in grey intensity values [26,27], as well as reversal of early enamel lesions [28].

SoproLife® is a camera that illuminates tooth surfaces within an excitation radiation band of light to induce fluorescence and it facilitates a high magnification image. By combining the advantages of laser fluorescence device and intraoral camera, SoproLife® proved to be a non-destructive, clinically applicable, sensitive and specific diagnostic method [8,9,11] in detecting three types of enamel caries [29]:

- Enamel caries on the surface.
- Suspicious grooves with positive red signal.
- Suspicious grooves with neutral dark signal.

The use of SoproLife® in caries detection is especially relevant for early caries lesions, because of their potential to remineralize. Recently, SoproLife® was suggested as a useful device in monitoring caries lesions; specifically, remineralization of these lesions [6,11], and due to the lack of empirical evidence, longitudinal studies to further establish its use for this purpose were needed [30].

The present study assessed the superiority of Recaldent™ and MI Varnish® in enhancing remineralization of early dental caries compared with the use of Recaldent™ alone. Then, it assessed the efficacy of SoproLife® in detecting *in vitro* remineralization of these lesions and compared it with the SEM-EDX approach. The latter quantitatively assesses the changes in mineral content during demineralization and *in vitro* remineralization processes [22], and is considered the gold standard for the evaluation of mineral loss or gain in experimentally induced initial caries lesions [31].

First, the results of this study found that initial prophylactic treatment, MI Varnish® and treatment with Recaldent™ has the most remineralizing potential followed by MI Varnish® and treatment with Recaldent™ without initial prophylactic cleaning, then by initial prophylactic cleaning and treatment with Recaldent™ and finally only treatment with Recaldent™. These results are in line with the existing literature.

Most importantly, this study provided empirical longitudinal evidence that SoproLife® is a reliable tool in monitoring the remineralization of early caries lesions in *in vitro* conditions. In addition, the study found a perfect agreement among the two analytical methods used, suggesting that the diagnosis made with SEM-EDX is roughly the same as the one made by SoproLife® The agreement between the two techniques found in our study was previously confirmed [30]. Indeed, our results are in line with the existing literature. Kockanat and Unal [11] showed that SoproLife® gave a high sensitivity *in vivo* and *in vitro* in detecting occlusal caries in 120 primary molar teeth. Similarly, Rechmann et al. [13] showed the highest sensitivity *in vivo* of SoproLife® in detecting caries lesions in 433 posterior permanent teeth in comparison with other diagnostic tools, such as ICDAS-II. Further, the authors found that SoproLife® had the highest ability to discriminate between individuals with dental caries and those without the disease [13]. A review by Tassery et al. [8] showed that SoproLife® has high levels of specificity (0.63) and sensitivity (0.93) in comparison with various caries detection techniques and devices. The same finding was reported more recently by Sukumaran et al. [30] in 63 premolars and molars.

This result might be explained by the SoproLife® characteristics themselves. The device visualizes healthy dentine as acidic green and infected or affected dentine as bright red fluorescence on images captured under diagnostic aid mode [32].

Further, the device combines a high-magnification intraoral camera and a laser fluorescence device allowing the lesion and its real topography to be seen in a magnified enlarged view [13], facilitating a better visibility [33,34], as well as a high discrimination, more particularly in anfractuous permanent premolars and molars [35]

With SoproLife®, clinicians may visualize and record the lesion, obtain information about the success of long-term protective applications and increase patient motivation by enabling re-evaluation of treatment. Thus, SoproLife® may support the patient-practitioner communication; and since it is free of ionizing radiation, it could be useful in the carious lesion detection in children and pregnant women [35].

As SoproLife® has shown potential to be used as a sensitive tool to the visual inspection and monitoring of the remineralization of early caries lesions, it would be useful to include algorithm software within the device to analyse the images. Currently, the storage and analysis of the images provided by SoproLife® use a separate software - Sopro Imaging® (SOPRO, ACTEON Group, La Ciotat, France) which is not part of the SoproLife® system. Installing such software allows the clinicians to quantify caries lesions and monitor their remineralization at chair side based on the real-time images acquired, allowing for a greater motivation and initiation of behaviour modification by the patients.

This study is limited to *in vitro* conditions which may be different than the *in vivo* dynamic complex biological system. Accordingly, further clinical trials

are necessary to assess the potential of SoproLife® in monitoring dental remineralization in the real-world clinical setting. On the other hand, SoproLife® may suffer from interference since it is light-based, and might give false positive results if images are magnified above a certain threshold [36]. These two issues were not addressed within this study. Further, the use of SoproLife® may be limited mainly by the presence of organic deposits, porosities and crystalline disruption, which might disrupt the auto fluorescence signal, discolouring and modifying the brightness of the hard tooth structures [6].

CONCLUSIONS

Within the limitations of this study, we conclude that SoproLife® is effective not only for early detection but also for longitudinally monitoring the remineralization of early caries lesions after Recaldent™ therapy in first and second premolars and molars. These results are equivalent with scanning electron microscopy/energy dispersive X-ray spectroscopy analysis. However, more longitudinal *in vivo* studies are needed to further establish its use for confirming the success of prevention and remineralization efforts.

SoproLife® is easy for clinicians since it is a simple evaluation of images. Further, the better visibility of such images coupled with the potential of SoproLife® in monitoring caries activity could prevent unnecessary operative intervention, which could translate into an individualized minimal intervention dentistry [37] and probably a cost-effective dental care.

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