

### **ORIGINAL ARTICLE**

# Preliminary study assessing remineralisation of early caries on posterior teeth using SoproLife®

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#### **KEYWORDS**

CAMBRA; Caries: Caries risk assessment: Fluorescence; LIFEDT concept

Abstract *Purpose:* The aim of this study was to assess the effectiveness of minimal intervention via fluoride therapy in management of early caries lesion with the aid of SoproLife®.

*Materials and methods:* A total of 40 patients were recruited, (mean age = 23 years) and were assigned to low and moderate caries risk groups (n = 20). Eighty occlusal surfaces of posterior teeth were examined for early caries lesion visually and using SoproLife® at baseline and at a recall visit six months later. At baseline visit, patients were given oral hygiene education, fluoridated toothpaste for homecare and topical fluoride application. SoproLife® images acquired were analysed using Image J software version 1.50. Difference in the mean value of intensity of the red wavelength spectrum between baseline and recall visits, ( $\Delta I$ ), were analysed for both risk groups.  $\Delta I$  for upper and lower first molar teeth were also analysed.

*Results:* Results show no statistical difference for  $\Delta I$  between low and moderate risk groups (p = 0.13). There is no statistical difference in  $\Delta I$  within the low caries risk group (p = 0.42) but there is significant difference in the moderate risk group (p = 0.02). No statistically significant difference in  $\Delta I$  value between upper first molars (UFM) (p = 0.80) and lower first molars (LFM) (p = 0.07) were detected. There was also no statistically significant difference in  $\Delta I$  value within the upper and lower first molars (UFM: p = 0.31, LFM: p = 0.27).

Conclusion: SoproLife® generated images did not show significant differences in remineralisation of early caries between low and moderate caries risk patients and between the upper first and lower first permanent molars in these patients.

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#### 1. Introduction

Early detection of caries is important especially when the lesions have the highest tendency for reversal or arrest with the implementation of preventive measures such as fluoride therapies (home and professional). Entering the era of Minimally Invasive Dentistry (MID), the current evidence based on the understanding of caries process, has led to new

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1013-9052 © 2019 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). approaches in caries detection, assessment and management that also include non-cavitated or early caries lesions (Kutsch et al., 2007).

Since early 1900, the importance of detecting early caries lesions has been recognized. Unfortunately, dental caries is usually detected at the cavitation stage and not at the white spot lesion stage (Gomez, 2015). Over the years, methods in detecting early caries lesions have received significant research attention. The latest technology that has been introduced is the LIFEDT (Light Induced Fluorescence Evaluator for Diagnosis and Treatment) concept which uses the SoproLife® imaging (Acteon, La Ciotat, France) device (Terrer et al., 2009). It is a recent technology developed based on imaging using auto fluorescence of dental tissues (Terrer et al., 2009). Sopro-Life<sup>®</sup> camera uses the principle of observation of variations in carious enamel-dentinal tissues' auto fluorescence compared to other healthy areas of tooth (Terrer et al., 2009). The term 'auto fluorescence' refers to the natural fluorescence of the dental hard tissue in the absence of other luminescent substance (Karlsson, 2010). Healthy enamel and dentin have particular fluorescence properties, compared to demineralised dental tissues, which absorb light and thereby have a lower level of fluorescence properties (Panavotov et al., 2013). The spectrum of the fluorescence light is green when the dentine is healthy and dark red when in infected dentine (Terrer et al., 2009). The fluorescence work in a manner in which the wavelength irradiated must cross the enamel in order to excite the dentine (Terrer et al., 2009). Following the excitation of the dentine molecules, the fluorescence signal is sent back, and it must then cross the enamel to be interpreted (Terrer et al., 2009). The thicker the enamel is, the weaker the fluorescence reflected will be (Terrer et al., 2009). Therefore, with demineralisation, the thickness of enamel is reduced and this causes the green light intensity to be weaker and in return the reflection of red wavelength can be observed (Terrer et al., 2009). Fig. 1 shows demineralisation within the occlusal primary fissure of a posterior tooth. SoproLife® can provide overall image and any variations in the optical properties of tooth surface by enabling more than 50X magnifications to aid in caries diagnosis (Terrer et al., 2009). The magnification allows a practitioner to gather additional information regarding caries potential of a tooth surface especially early caries lesions involving the enamel as these lesions have the potential to remineralise (Terrer et al., 2009).



Fig. 1 Dark red extended areas indicating demineralisation (black arrow) are confined to the fissures.

In modern cariology, minimum intervention in the management of early caries lesions appears to be the most ethical and widely accepted approach amongst clinicians especially with the introduction of Caries Management by Risk Assessment (CAMBRA). CAMBRA is a system which outlines that treatment for patients is solely based on the risk identified (Kutsch et al., 2007). Clinicians are tasked to identify the cause of disease (caries) by assessing risk factors for each patient. Based on the assessment and evidence gathered, the problems (causative factors) are corrected (risk factor management), using specific recommended treatment of either behavioural, chemical or minimally invasive procedures (Kutsch et al., 2007). The risks assessment in CAMBRA is established using caries risk assessment (CRA) form, which practitioners use to evaluate each patient's disease indicator (Kutsch et al., 2007).

Empowered with the knowledge that caries is a disease continuum, there has been a shift from focusing on restoration of cavities to the investigation of therapeutic approach in arresting or reversing caries progression by remineralising noncavitated early caries lesions. Fluoride dentifrices have been shown to be an effective caries prevention measure available today (Srisilapanan et al., 2013; Yin et al., 2013). Fluoride  $(\bar{F})$  works to promote remineralisation by incorporating fluoride ions into the hydroxyapatite structure forming fluoroapatite and resulting in a stronger crystal lattice (Srisilapanan et al., 2013). Fluoride may be delivered either through home care using fluoridated toothpaste and various mouthwashes or professionally, by an attending clinician in the form of topical fluoride application (Cury and Tenuta, 2009).

With the advent of SoproLife® as a new diagnostic aid, patients are able to view real time images of caries lesions at chair side. This new caries diagnostic tool, combined with other principles of minimal intervention dentistry, perhaps is the way forward in assessment and treatment planning of early caries lesions (Tassery et al., 2013; Walsh and Brostek, 2013).

The aim of this study is to assess the effectiveness of minimal intervention dentistry via fluoride therapy in managing early caries lesions using SoproLife<sup>®</sup>.

The objectives of this study are:

- 1. To assess the remineralisation of early caries lesions after fluoride therapy in low and moderate caries risk patients using SoproLife® generated images.
- 2. To compare the remineralisation of early caries lesions after fluoride therapy between low and moderate caries risk patients using SoproLife® generated images.
- 3. To compare the remineralisation of early caries lesions after fluoride therapy between upper and lower first permanent molars in these two groups of patients using SoproLife® generated images.

#### 2. Methods and materials

#### 2.1. Patient recruitment and inclusion criteria

Study protocol was approved by the Medical Ethics Committee of Faculty of Dentistry [DF RD1606/0020(U)]. During a time period of one month, 46 patients were randomly selected from those who were attending the dental clinic at Faculty of Dentistry, University of Malaya. These patients were screened prior to consenting to this study and were recruited based on the following inclusion criteria: (1) 18–40 years old, (2) patients with low or moderate caries risk, (3) presence of at least 20 teeth intraorally, (4) has at least two early caries lesions on the occlusal surfaces of posterior teeth with ICDAS score 1 and/or 2, (5) no abnormal salivary functions, (6) not on xerostomic drugs or any over-the-counter medications other than analgesics, (7) no advanced periodontal disease (BPE score 4 in at least one sextant), (8) medically fit and able to attend appointments, (9) able to provide written informed consent.

Twenty (n = 20) patients each were selected for the low and moderate caries risk groups respectively. Patients were informed that this is a two-phase clinical study which requires a follow-up appointment after six months.

The American Dental Association's caries risk assessment (CRA) form was used to determine patients' risk of developing caries. Patients were subsequently allocated to low or moderate caries risk groups according to their scores. This was the first-tier of screening. A second-tier screening was done during the clinical examination based on plaque scores.

Clinical examination was performed at a dental unit using an operating light, a 3-in-1 syringe, dental mirror and ballended WHO probe. As a general procedure, oral examinations were performed at baseline and at recall visit based on the sequence below:

- i. Basic Periodontal examination
- ii. Full mouth scaling and polishing
- iii. Caries assessment on occlusal surfaces of posterior teeth.

Patients' teeth were stained using two-toned dye solution (Mira-2-Tone® by Hager &Werken, GmbH&Co., Germany). The resulting plaque score was used to determine patients' caries risk group (low or moderate). Patients with plaque score of more than 35% were categorized as high caries risk and excluded from this study. As mentioned previously, this procedure was a second-tier screening to categorise patients into low and moderate caries risk groups.

Subsequently, full mouth scaling was performed for all patients. In addition, the occlusal surfaces of posterior teeth (not including third molars) were cleaned using pumice prior to taking SoproLife® (Acteon, La Ciotat, France) images. The occlusal surfaces of posterior teeth were examined for early caries lesions using the International Caries Detection and Assessment System (ICDAS). For patients to be included in this study, at least 2 early caries lesions with ICDAS scores 1 and/or 2 must be present on the occlusal surfaces of posterior teeth. Two examiners performed the clinical examination. Prior to performing the clinical examination, both examiners were calibrated for ICDAS assessment, achieving weighted Kappa scores of 0.8 indicating almost perfect agreement.

#### 2.2. SoproLife® (Acteon, La Ciotat, France) imaging

SoproLife® images of early caries lesions on the occlusal surfaces of selected posterior teeth were taken using the diagnostic aid mode with a visible blue light frequency (wavelength 450 nm). Examiners used tripodisation of finger rest to standardize the images captured between visits as seen in Fig. 2. Images at baseline and recall visit for each patient were taken by the same examiner, also to allow standardization. The



Fig. 2 Tripodisation of finger rest by the examiner.

images captured were later recorded in Sopro Imaging software and were saved in JPEG format. At the six months recall visit, all the clinical procedures and acquisition of SoproLife® images were repeated.

#### 2.3. Non-surgical intervention for caries management

As a preventive strategy, oral health education and fluoridated toothpaste with 1450 ppm Sodium Monofluorophosphate (NaMFP) (Colgate-Palmolive, Malaysia) were given to all patients regardless of caries risk group, for home oral care regime. The acquired real time SoproLife® images were displayed to each patient and the examiner interpreted these images, highlighting the presence of early caries lesions. Patients were also informed of the remineralising potential of early caries lesions with improved oral hygiene. Also, at baseline visit, fluoride varnish Duraphat® (Colgate-Palmolive, Denmark) was applied on occlusal surfaces of posterior teeth with ICDAS scores 1 and/or 2 as per manufacturer's instructions.

## 2.4. Processing of SoproLife® (Acteon, La Ciotat, France) images

The baseline and recall visit SoproLife® images were compared and analyzed using ImageJ software version 1.50. Only primary fissures of the occlusal surfaces of posterior teeth were included in the analysis. Markers were determined along the primary fissures and the resulting surface was traced for analysis (Fig. 3). Only one examiner traced all images for baseline and recall visit to ensure standardization. A resulting histogram displaying the intensity of the red wavelength was obtained, a sample of which is shown in Fig. 4. The counts for the red wavelength indicate a range of intensity from 0 to 255 and the mean intensity of red wavelength was recorded at 111.07 (SD 12.51).

The outcome measure for the mean intensity values is expressed as the difference in the mean intensity ( $\Delta I$ ) at baseline and recall visit, calculated using the formula below:

$$\label{eq:distance} \begin{split} \Delta I &= Mean \mbox{ Intensity } (I) \mbox{ at } BV - Mean \mbox{ Intensity } (I) \mbox{ at } RV \\ &= I^{Bv} - I^{Rv} \end{split}$$



Fig. 3 Marking and tracing of primary fissures on occlusal surface of posterior tooth using Image J.



**Fig. 4** Histogram displaying the intensity values of red wavelength corresponding to the primary fissures of tooth in Fig. 3.

#### 2.5. Statistical analysis

Data was collected and analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, US), version 12.0.1. Paired *t*-test was performed for low and moderate caries risk groups to determine if there was any statistically significant difference in the outcome measure, ( $\Delta I$ ). Similar test was used to determine statistically significant differences in  $\Delta I$  for upper and lower first molar teeth. For inter-group comparisons, Mann-Whitney U test was used to compare  $\Delta I$  between low and moderate caries risk groups. Independent *t*-test was used to compare  $\Delta I$  between upper and lower first permanent molars. p < 0.05 was regarded as indicating statistical significance.

#### 3. Result

#### 3.1. Descriptive analysis

A total of 40 patients were recruited for this study. Among them, 26 were males and 14 females and the mean age was 23 years. 20 patients each (n = 20) were assigned to the low and moderate caries risks

groups respectively. However, 6 patients dropped out due to personal reasons and there were only 17 patients each (n = 17) for both groups at the recall visit.

A total of 63 out of 68 occlusal surfaces of posterior teeth were examined clinically and imaged with SoproLife® for ICDAS score 1 and/or 2 lesions at baseline and recall visit. 5 teeth were excluded at the recall visit as the early caries lesions were restored at private clinics during the six-month interval. Overall for both arches, 43 first molars, 2 s molars, 6 first premolars and 12 s premolars were examined as shown in Table 1.

#### 3.2. Statistical analysis

Paired *t*-test (Table 2) showed no significant difference in  $\Delta I$  value in the low caries risk group (p = 0.42). However, there is a significant difference in the moderate risk group with p = 0.02. Mann-Whitney U test was used to compare  $\Delta I$  between low and moderate caries risk groups. The results showed p = 0.13 indicating that there is no statistically significant difference in  $\Delta I$  between low and moderate risk groups.

 $\Delta I$  for the upper and lower first molars were also tabulated and analysed. Shapiro-Wilk test showed that the data were normally distributed for both groups (p > 0.05) at baseline and recall visit. Subsequent paired *t*-test showed no statistically significant difference in  $\Delta I$  for both groups as shown in Table 3.

A normality test was also done on the data sample for UFM and LFM. The result indicated that the data was normally distributed only for the upper first molars (p = 0.125) but not normally distributed for lower first molars (p = 0.00) group. Subsequently, an independent *t*-test showed there is no statistically significant difference in  $\Delta I$  between upper and lower first molars with p > 0.05 (Table 4).

#### 4. Discussion

Among 40 patients selected at baseline, the mean age calculated for this sample population is 23 years. It has been shown that prevalence of caries increases with age (Panayotov et al., 2013). Therefore, young adults were included in this study to be able to identify and promote remineralisation of early caries lesions. The authors hope this intervention could prevent progression of caries lesions which may result in cavitation. A study done by Basavaraj et al. reported that young adults are susceptible to caries because of erupting third molars and changes to their dietary habit, oral hygiene practice and lifestyle (Tassery et al., 2013).

In this study, SoproLife® was used to aid in detecting early caries lesions as a supplement to visual examination. This device will visualize healthy dentine as acidic green and infected or affected dentine as bright red fluorescence on images captured under diagnostic aid mode (Panayotov et al., 2013). A review by Tassery et al, 2013 compared various caries detection techniques and devices and it showed that SoproLife® has high levels of specificity (0.63) and sensitivity (0.93) (Tassery et al., 2013). When compared to visual inspec-

Table 1	Distribution of posterior teet	h included in this study.
Tooth	Maxilla n = 22	Mandible $n = 41$

Tooth	Maxilla $n = 22$	Mandible $n = 41$
First premolar	3	3
Second premolar	3	9
First molar	15	28
Second premolar	1	1

**Table 2** Paired *t*-test for low and moderate risk groups.

Group	Mean difference in intensity ( $\Delta I$ )	Standard deviation	p value
Low risk Moderate risk	0.72 4.77	4.98 10.88	0.42 0.02*

\* Statistically significant difference.

Table 3 Paired *t*-test for UFM and LFM groups.

Group	Mean difference in intensity ( $\Delta I$ )	Standard deviation	p value
Upper first permanent molar (UFM)	0.53	7.77	0.80
Lower first permanent molar (LFM)	3.65	10.43	0.07

**Table 4** Independent *t*-test showed there is no significant difference in  $\Delta I$  between UFM and LFM.

Group	Mean difference in intensity ( $\Delta I$ )	Standard deviation	p value
Upper first molars (UFM)	0.53	7.77	0.31
Lower first molars (LFM)	3.65	10.43	0.27

tion of caries, SoproLife® posted higher sensitivity and comparable specificity values (visual inspection: specificity 0.73; sensitivity 0.6). Sensitivity is determined by the proportion of actual caries lesions which are correctly diagnosed by Sopro-Life® whereas specificity is determined by the proportion of non-carious lesions which are correctly diagnosed by Sopro-Life<sup>®</sup> (Zeitouny et al., 2014). The potential for SoproLife<sup>®</sup> to detect a significant difference in  $\Delta I$  for the moderate risk group and between the upper and lower first molars in this study may once again indicate the sensitivity of this device in monitoring caries lesions. There appears to be justification for the use of SoproLife® as a supplementary tool to visual inspection in caries detection especially early caries lesions which have potential to remineralise with the application of minimal intervention dentistry. However, there may are some limitations with the use of SoproLife® mainly related to presence of organic deposits, porosities and crystalline disruption, which are all able to disrupt the auto fluorescence signal, discolouring and modifying the brightness of the hard tooth structures (Gomez et al., 2013). SoproLife® generated images serve as a vital tool for communication between patient and practitioner, which allows patient to view site(s) of early carious lesions at various appointments, at chair side. Also, all SoproLife® generated images can be safely stored using Sopro Imaging<sup>®</sup> software to monitor regression or progression of caries lesions.

From previous studies conducted, it was reported that almost 60% of dental caries occurred on occlusal surfaces of permanent dentition involving both children and adults despite accounting for only 12.5% of the total teeth surface area (Ito et al., 2016; Lussi, 1991). In this study, posterior teeth were

selected as the presence of pits and fissures contribute to a complex occlusal morphology which render these teeth more vulnerable to caries development (Ito et al., 2016). Previous clinical studies had revealed that anatomical fissure morphology such as depth, width and shape have a strong association with caries susceptibility (Ito et al., 2016). Bossert, in his study also observed the relationship between incidences of caries with the steepness of wall of grooves down to the fissures (Bossert, 1933). It reported that most caries lesions occurred in the deepest grooves with steeply sloping sides. Similarly, for this study, authors decided to assess and monitor for early caries lesions only along the primary fissure as these are the primary regions for the initiation of caries lesions. Deep fissures are common obstacles that will hinder plaque removal and penetration of fluoride on teeth surfaces (Ito et al., 2016). The complexity of these pits and fissures may also result in over diagnosing of caries lesions by SoproLife® as the red fluorescence imaged on the fissure system could be either due to demineralisation of enamel in early caries lesion or presence of organic deposits (Terrer et al. 2009). In order to prevent such over diagnosis, cleaning of the fissure system using an ultrasonic tip or pumice with rubber cup is strongly advocated and such practice is in line with the recommendations of the ICDAS system as well (Terrer et al, 2009). Aligning ourselves with these recommendations, we ensured that the occlusal surfaces which were to be imaged were plaque-free in order to reduce any potential confounding factor which could alter the acquired image.

Also, in analysing the SoproLife® images, only the primary fissures of posterior teeth were selected. The slopes of the cusps were not included during tracing of the surface to be analysed, as residual plaque deposits on these surfaces may be viewed as confounding factors which could result in over diagnosing. One of the steps undertaken during tracing of the primary fissures was to identify structures along the fissures which functioned as markers. These markers were reproduced during tracing of SoproLife® images at the recall visit to ensure the surface traced between visits were identical.

The results showed that there was no significant difference between the two risk groups in the remineralisation of early caries lesions after 6 months of recall. A review by Azarpazhooh and Main reported that clinically significant remineralisation can only be observed at six months post-application of topical fluoride varnish (Azarpazhooh and Main, 2008). The review also wrote that evidence was found for slow fluoride release of up to six months post-application for Durafluor and Duraphat products, in which greatest fluoride release is within the first 3 weeks post-application and more gradual release afterwards (Azarpazhooh and Main, 2008). Additionally, it has also been reported that subsurface lesions need minimally a few years for complete remineralisation. The authors postulate that potentially, remineralisation of lesions in the low risk group were incomplete and therefore, a statistically significant difference in  $\Delta I$  could not be established. However, it is well documented that fluoride has a huge contribution in reduction of caries prevalence (Kutsch et al., 2007; Cury and Tenuta, 2009; Bossert, 1933). Fluoride plays a role in the secondary caries prevention approach especially in early and noncavitated caries lesion (Kutsch et al., 2007; Cury and Tenuta, 2009). This preliminary clinical study could pave way for a longitudinal clinical study monitoring remineralisation of early caries lesions using SoproLife® after fluoride intervention

and behavioural modification between low, moderate and high caries risk patients. Caries risk assessment can be used to predict caries experience of a patient over time, the likelihood to develop new caries lesions and the progression rate of existing caries lesions, if there were any. Assessment and prediction of future caries activity may facilitate development of an individualised minimal intervention dentistry (MID), including nonsurgical and minimal surgical management and an appropriate recall period for each patient.

At baseline visit, examiners noticed patients in the moderate caries risk group lacked oral health related knowledge and practice. The examiners had used a more open and conversational style when delivering oral hygiene education (OHE) as it has been suggested that acceptance of information with such format is better amongst patients (Salter et al, 2007). At the six-month recall, the results also showed that there was a statistically significant difference in  $\Delta I$  (positive) value for the moderate risk group. This may indicate that remineralisation has occurred in this group of patients. It is highly likely this could be due to better compliance to oral hygiene education amongst patients in this group despite having higher plaque scores at baseline. A multicentre study has shown that reduced compliance with OHE may contribute to the onset of primary caries (Arino et al., 2015). Information related to plaque scores have also proven to motivate and educate patient on oral hygiene as patients are able to view for themselves the stained plaque (Tassery et al., 2013). In addition to plaque disclosure, patients in this study were also able to view real-time images of early caries lesions present in their oral cavity. The real-time images generated by SoproLife® serve as a vital tool in communicating with patient on the risk of caries incidence. Patients are able to view for themselves on the potential sites for caries occurrence as indicated by the red fluorescence and this could possibly lead to keener brushing on these sites and modification in diet, eventually leading to remineralisation of early caries lesions. Subsequent recall visits showing previous early caries lesions with reduced red fluorescence will further motivate patients to continue with their improved oral hygiene behaviour and diet modification.

Numerous studies have shown the importance of saliva as one of the indicators required in caries risk assessment (Fontana and Zero, 2006; Stookey, 2008; Basavaraj et al., 2011). Dawes showed that concentration of bicarbonate is directly proportionate with the salivary flow rate (Dawes, 2008). The study by Dawes also reported that bicarbonate diffused within the plaque is able to neutralise acid produced by bacteria hence enhancing the remineralisation process of the demineralised enamel (Dawes, 2008; Lenander-Lumikari and Loimaranta, 2000). Additionally, studies by Papas and Spak reported that higher caries incidence was found in individuals with salivary hypo function (Papas et al., 1993; Spak et al., 1994). Despite acknowledging the significance of assessing salivary function as one of the components in caries risk assessment (CRA), the authors were not able to perform this test due to cost limitation. However, patients recruited for this study were carefully selected and patients with salivary hypo function due to medication, physiological or pathological reasons were excluded. Selection were done at the initial screening and while assessing patients' caries risk using CRA form.

Although SoproLife® has shown potential to be used as a supplementary tool to visual inspection in detecting early

caries lesions, the authors would like to propose to include an algorithm software within the device. At present, images generated from SoproLife® have to be analysed using separate software which is not part of the SoproLife® system. Installing a software to analyse the images would allow clinicians to quantify caries lesions at chair side based on the real-time images acquired. This could further help in motivating and initiating behaviour modification in patients. Moreover, clinicians would also be able to monitor the progression or regression of caries lesions, all done at chair side. Nevertheless, the authors feel that further studies need to be done to ensure the reliability of using SoproLife® in detecting and managing caries lesions.

#### 5. Conclusion

Within the limitation of this study, the authors conclude that SoproLife® generated images did not show significant differences in remineralisation of early caries lesions after fluoride therapy between low and moderate caries risk patients and between the upper first and lower first permanent molars in these patients. However, it a sensitive enough tool to aid in caries monitoring and more longitudinal studies are needed to further establish its use for this purpose.

#### Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

#### Ethical statement

The study protocol was approved by the Research and Ethics Committee of the Faculty of Dentistry, University of Malaya [DF RD1606/0020(U)].

#### **Declaration of interest**

The authors declare no personal interest in this study.

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