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Enhancing the detection of proximal cavities on near infrared transillumination images with Indocyanine Green (ICG) as a contrast medium: In vitro proof of concept studies

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Abstract

Objectives: The use of near infrared transillumination for caries detection is gaining recognition in daily practice. Differentiation between cavitated and non cavitated proximal lesions is recognized as a threshold for restorative treatment. This investigation focused on the use of a near infrared absorbent dye which may enhance the detection of cavitation on near infrared transillumination images.

Materials and methods: 1: Natural teeth with artificial proximal cavitation were images with 3 different dyes to establish that near infrared absorbent dye can act like a contrast medium. 2: Natural teeth with natural cavitated lesions were used to investigate the contrast enhancing effect of indocyanine green (ICG) on near infrared transillumination images. 3: Artificial teeth with artificial cavitations were used to determine the best consistency of ICG as a contrast medium. 4: natural teeth with proximal lesions were used to confirm that ICG can differentiate between cavitated and non cavitated proximal lesions.

Results: 1: ICG enhanced the contrast of cavitations compared to other dyes (ANOVA; $p < 0.05$). 2: ICG enhanced the contrast of the cavitated area on natural lesions but not significantly (t-test; $p>0.05$). 3: ICG in a gel form enhanced the detection of cavitated lesions when compared to liquid ICG (t-test; $p<0.05$). 4: ICG gel was able to differentiate between cavitated and non cavitated proximal lesions (ANOVA; $p < 0.05$).

Conclusion: ICG can potentially be used as a contrast medium to enhance the detection of cavitated proximal lesions in vitro on near infrared transillumination images. A clinical study is required to validate these results in vivo.

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Conflict of interests:

The authors declare that they have no conflict of interests.

Keywords

cavitated proximal lesions; proximal caries; NIR; Indocyanin green; ICG; contrast medium; Transillumination; Diagnocam; near infrared transillumination

1. Introduction

1.1. Background:

Detection of initial proximal caries in posterior teeth is a difficult task for dentists in general. It often relies on clinician's judgment and experience[1]. Until today, the determination of the presence of cavitation on proximal surfaces is still a challenge, clinically and radiographically. Many clinicians associate the presence of cavitation to radiolucency depth on bitewing radiographs[1]. The statistical probability of cavitation is higher for lesions with radiographic extension into dentin than into enamel only. Lesions at the dentin-enamel junction and lesions with intermediate extension, i.e., those extending from the dentin-enamel junction to half dentin, are considered controversial in treatment decision because the presence of cavitation can range from 30% to 95% [1–4].

In the last few years differentiation between cavitated and non-cavitated proximal lesions has become even more critical, it is now well accepted that as long as enamel is only demineralized but not cavitated, non-operative options should be preferred. Such options may consist of remineralization treatments with fluorides or other prophylactic agents that are capable of restoring the minerals' loss during lesion formation and/or making the tooth resistant to further caries development [5–7]. More recently, the tendency has increased for implementing interceptive but non-invasive techniques such as infiltration, sealing of demineralized surfaces or non-invasive adhesive restoration slowing down or even stopping the progression of the lesion [8–13].

Although bitewing radiography has a higher sensitivity than visual examination for diagnosing proximal caries [14, 15], differentiating enamel demineralization from cavitation is impossible on bitewings. Several authors [14, 16, 17] demonstrated that a great amount of proximal radiolucency confined to enamel or the outer half of dentin in bitewing radiographs corresponded to non-cavitated lesions.

Up to now, the most reliable way to confirm cavitation on a proximal surface is direct clinical examination after orthodontic elastic separation. Studies have shown that tooth separation detected more non-cavitated enamel lesions than visual-tactile examination without separation or bitewing examination [2, 14]. An in vivo pilot study used radiopaque paste pressed in the proximal area to help detecting cavitation of the proximal surface on a bitewing radiograph.[18] However, applying frequent ionized radiation for monitoring might compromise the individual's health.

1.2. Near infrared transillumination:

In the near infrared (NIR) range of light (700 nm to 1500 nm), wavelengths are significantly longer than visible light. Longer wavelengths scatter less and therefore can penetrate objects

more deeply [19, 20]. Several studies have demonstrated that enamel appears to be transparent when NIR light is used to transilluminate the tooth, while dentin scatters more strongly[19, 20]. Furthermore, multiple studies have shown that NIR light transillumination can visualize early caries demineralisation on occlusal and proximal surfaces [21–23].

Recent clinical publications [23, 24] and previous in vivo and in vitro studies [25, 26], showed that transillumination with near infrared (NIR) light is a reliable method to detect and monitor carious lesions on proximal and occlusal surfaces. One of the available tools for the detection of proximal enamel caries is the DIAGNOcam (KaVo, Biberach, Germany) [23, 27]. It is a simple, non-invasive, and painless procedure that can be applied repeatedly for monitoring with no radiation risk to the patient. Just like for x-rays, the detection of cavitation on proximal lesions is still not possible by using this tool alone. Differentiation between cavitated and non cavitated proximal lesions is recognized as a threshold for restorative treatments. Increasing the contrast of cavitated lesions can be a game changer for non-invasive dentistry. Therefore, this investigation focuses on the use of a near infrared absorbent dye to enhance the detection of cavitation on near infrared transillumination images.

The material used in this study, Periogreen, is water soluble, greenish in color, semitransparent depending on the concentration and washes away easily after applications. Since Periogreen is aimed and approved for use in the oral cavity it was the product of choice for this study.

Indocyanine green is a water-soluble, fluorescent tricarbocyanine dye with peak spectral absorption between 600–900 nm. Its pH is of approximately 6.5 when reconstituted and it has been used in medicine since 1956[28]. When ingested, ICG is metabolized in the liver and only excreted via the liver and bile ducts; since it is not absorbed by the intestinal mucous membrane. The toxicity is classified as low [29]. ICG has been approved by the United States Food and Drug Administration and the sterile lyophilisate of a water-ICG solution is approved in many European countries and the United States under the names ICG-Pulsion, IC-Green, Cardiogreen and Foxgreen [29, 30]. Since 1956 ICG has been widely used in medical diagnosis as an indicator substance. Its applications include to determine cardiac output, hepatic function, liver blood flow, and ophthalmic angiography [31–33].

Near-infrared (NIR) fluorescence imaging clinical studies have been reported in the literature with six different devices that employed various concentrations of Indocyanine green (ICG) as a non-specific contrast agent. To date, clinical applications range from angiography, intraoperative assessment of vessel patency, tumour/metastasis delineation following intravenous administration of ICG, as well as imaging lymphatic architecture and function following subcutaneous and intradermal ICG administration [34].

ICG has multiple applications in dentistry as well such as photodynamic/photothermal application in periodontology, bacterial and fungal inhibition [35, 36], enhancement of caries removal [37] and caries prevention [38].

1.3. Aim and hypothesis:

In this paper, the aim is to enhance the detection of cavitated lesions on near infrared transillumination images. A series of in vitro studies were designed to test the hypotheses that a near infrared absorbent dye (Indocyanine green (ICG)) can be used to increase the contrast of cavitated lesions on NIR transillumination images. To investigate that we hypothesized the following null hypothesis: 1: ICG does not increase the image contrast of the proximal cavitated area on near infrared transillumination images on natural or artificial cavitations. 2: There is no difference in the image contrast when using liquid or gel ICG. 3: There is no difference in the contrast depending on the lesion's location. 4: There is no difference between the contrast of active or arrested non cavitated lesions and cavitated lesions.

2. Materials and methods

2.1. ICG as a contrast medium:

To test the first null hypothesis, we compared the contrast of the cavitated area with different dyes applied on 10 artificial proximal cavities on natural teeth. To demonstrate that visible colored dyes may not always be observed under NIR imaging at 780 nm we used a near infrared absorbent dye (NIAD), Indocyanine green (ICG) and two non-NIAD dyes used in conservative dentistry for caries detection: Caries detector (Kuraray, Japan) and Sable Seek caries indicator (Ultradent, USA) with bright red and green color respectively.

In the first part of the study which evaluated the proof of concept, ten sound posterior extracted human teeth where cleaned and stored in 0.1% thymol solution after extraction. The teeth are chosen from a pool of anonymous extracted teeth at the university dental clinics. The teeth were fixed on holders to help to obtain a sequence of reproducible images with the DIAGNOcam (KaVo, Biberach, Germany). For each tooth, 4 images (Fig. 1a–d) were captured: 1st image (Fig.1a) without any dye, 2nd image (Fig.1b) with the red non-NIAD dye Caries detector, 3rd image (Fig.1c) using the dark green dye Sable Seek caries detector and 4th image (Fig.1d) after application of the NIAD dye ICG-pulsion (Diagnostic Green, Ohio, USA) onto the same sound proximal tooth surface of 6 teeth each. The teeth were thoroughly cleaned and hydrated after each dye application. A small cavity was then prepared into the enamel in the interproximal area with a round diamond-coated bur (Fig. 1e). A DIAGNOcam image was captured of the empty cavity and then with each of the dyes applied separately (Fig. 1.f–h). Analyses of all DIAGNOcam images were carried out by a computer software (Image J, NIH, Bethesda, MA, USA) to reveal changes in the grey level of the pixels in the proximal ROI with and without cavity for each of the four dyes.

Images were manually exported from the DIAGNOcam software to ImageJ (NIH, USA). After selecting the same ROI on each set of images, the average grey values were calculated and compared before/after dye application.

The differences between groups, in terms of changes in mean grey level within the ROI were assessed applying one-way ANOVA and Duncan post-hoc test. An independent sample t-test was used to detect differences between cavities with and without ICG. The level of confidence was set to 95%.

2.2. Liquid ICG on natural lesions:

For the second part of the experiment, two groups of 6 extracted human teeth were used. In the first group (Fig. 2) with natural proximal cavitated lesions DIAGNOcam images were taken. Then ICG (Periogreen, Elexxion, Radolfzell, Germany) was applied to the proximal surfaces and another set of images was taken with the DIAGNOcam. The same procedure was repeated with the second group of six natural non cavitated proximal lesions. ImageJ (NIH, USA) was used to calculate the intensities (I) of the healthy (I_h) and caviated lesion area (I_c). The contrast was calculated using the formula = $(I_h - I_c)/I_h$. Contrast is obtained as a value ranging between 0–1. The higher the value the more visible the lesion is. An independent sample t-test was run to detect differences between cavities with and without ICG. The level of confidence was set to 95%.

2.3. ICG Gel on different lesion location:

To test the second hypothesis, ICG was used in liquid form (ICGLIQ) and in a gel form (ICGGEL) to compare the contrast of artificial cavities in 2 different locations, Gr. 1: under the contact point and Gr. 2: cervical location.

For this part of the study 20 bilayer (enamel/dentin) artificial resin teeth (Ivoclar, Vivadent) were used. These teeth were chosen as they present similar contrast to natural teeth when illuminated with near infrared light. Artificial cavities were prepared on the proximal surfaces of each tooth.

The Indocyanine green powder (Periogreen, Elexxion, Germany) was mixed with either sterile water or glycerine to provide a liquid or a gel contrast medium.

Images of each cavity was recorded before and after the application of ICG, the product was applied in the contact point area and is distributed using a floss to ensure its introduction into the cavity. The tooth was then removed from the model and cleaned then another image was obtained with the liquid form of ICG applied in the contact area using a syringe with a brush tip.

The same protocol was carried on with group 2. In total, 3 images were taken for each tooth in group 1 and group 2 for the final contrast analysis. (Fig.3)

ImageJ (NIH, USA) was used to calculate the contrast of the area of interest. The contrast was calculated using the formula = $(I_h - I_c)/I_h$. Contrast is obtained as a value ranging from 0 to 1. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test was used to analyze the results.

2.4. ICG on natural lesions:

To test the fourth hypothesis, 45 teeth with natural proximal active white spot lesions (WSL), arrested brown spot lesions (BSL) and cavitated proximal lesions (CAV) where imaged using near infrared transillumination after applying Gel ICG (GICG), after washing ICG in water for 24h (24hW), after applying ICG liquid (LICG), and washing in water for 3 weeks (3WEEKS), and finally after applying ICG in a thick gel form (TGICG). ImageJ (NIH, USA) was used to calculate the grey level intensity of the area of interest. The

contrast was calculated using the formula= $(I_h - I_c)/I_h$ Contrast is obtained as a value ranging from 0 to 1. Polarization sensitive optical coherence tomography (PS-OCT) was used to confirm the cavitation and the activity of the lesion by the observation of hypermineralised layer. The difference in the contrast between groups was assessed applying one-way analysis of variance (ANOVA) and Tukey's multiple comparison test, the level of confidence was set to 95%.

3. Results

3.1. Liquid ICG as a contrast medium:

The first hypothesis was rejected. The application of ICG significantly increases the visibility of the lesion on near infrared transillumination images. In the non-cavitated group, significant differences in grey levels were observed between the tooth without dye (171.8±6.7) and the two groups where non-NIAD dyes were applied (157.6±11 (red), 157.9± 9.6 (green)). Nevertheless, for the ICG group the differences in grey levels (111.4±10.9) were more significant (ANOVA; $p < 0.05$) in comparison to the other two dyes. No significant difference in grey level was observed when a green or red dye were applied into the artificial cavity. However, after ICG application a significantly higher grey level difference was detected (64.2±6.1, ANOVA; $p < 0.05$). This part was merely a proof of concept to establish the visibility of ICG on NIR transillumination images compared to other dyes, only ICG was used for subsequent measurements.

3.2. Liquid ICG on natural lesions:

When liquid ICG applied to natural cavitated (n = 6) and non cavitated (n = 6) lesions the change in contrast was also visible, however not significant (p=0.076). (Fig.4) The wide variation in the contrast of non cavitated lesions with ICG (NCICG) is probably affected by lesion activity.

3.3. ICG gel on different lesion locations:

The second and third hypothesis were also rejected, one-way ANOVA and Tukey's multiple comparison test confirmed significant change in the contrast values when comparing the groups ($p<0.05$). The gel form of ICG seems to significantly enhance the visibility of artificial cavitated lesions and to render the cervical cavitations more visible compared to the liquid ICG. The detailed results are presented in (Fig. 5).

3.4. ICG on natural lesions:

The fourth hypothesis was rejected. ICG in a thick gel form produced the most significant increase in contrast, making the difference between cavitated (n = 15) and non cavitated lesions most visible. Even though without ICG application the contrast of the active (n = 15) and arrested lesion (n = 15) was statistically significant ($p = 0.046$), with liquid ICG the p value was reduced to ($p = 0.036$), meaning it was still able to differentiate between active and arrested lesions. Results are detailed in (Fig. 6). The significance level between groups is presented in (Table 1).

4. Discussion

It has been previously proposed that administrating a topical intraoral radiographic contrast agent can be useful in imaging of dental caries, diagnosing or monitoring periodontal disease or evaluating the three-dimensional shape of root canals before root canal therapy (WO/2012/151464). Based on the same principle, the present study combined the use of ICG and NIR-DIFOTI trying to enhance the detection of proximal cavitated lesions using a safe and non-invasive procedure.

ICG has an absorption peak at 780 nm, and it was demonstrated in the present study that ICG will be visible under the recently introduced NIR caries detection camera (DIAGNOcam) that uses 780 nm diodes for transillumination and that the application of ICG into the interproximal region enhanced the visualization of cavitation in proximal carious lesions.

In the present study the application of ICG on a cavitated proximal surface resulted in a significant reduction in the relative intensity of the cavitated area compared to an intact surface in both natural and artificial cavities. These results indicate that ICG may help differentiate between cavitated and non cavitated proximal lesions. ICG acted here by absorbing the light and preventing it from going through the tissue, thus presented as a dark area on the NIR transillumination image. This method we describe here is different from previous literature [39–41], those studies exploited ICG fluorescence as opposed it's NIR absorption at 780 nm. Moreover, some of those studies used ICG delivered systemically, requiring 24 hours after administration for the product to be detected. In our study the application is topical to the area of interest and the results are obtained immediately. Our approach also provides the possibility to wash off the product directly after obtaining the images in the clinical setting.

To facilitate the use of ICG and increase its retention in cavities during clinical application, we considered using it as a paste. We added glycerin to provide a water-soluble mixture that can be removed easily. We obtained better results with gel and the thick gel form of ICG. When mixed in glycerin, in the thick gel form it will only occupy the cavitated area rendering this zone darker than the rest of the lesion. While when mixed with water the diffusion of the ICG into active non cavitated lesion pores would increase its contrast as well.

In the last part of the study, natural arrested non cavitated lesions showed higher contrast than active lesions without applying ICG. This could be attributed to lesion staining over time, as some stains are still visible with this NIR wavelength. When using gel ICG the difference between active and arrested lesions was not significant, however when using liquid ICG active lesions where significantly more visible. Which as discussed earlier, could be attributed to the diffusion of ICG into the lesion pores. This aspect of differentiating between active and arrested lesions requires further investigation.

Furthermore, the liquid form of ICG showed some other unexpected advantages such as the detection of fissures and fractures (Fig.7) and enhancing the detection of ill-adapted fillings with open margins (Fig.7). These fortunate findings will be the subject of future research.

A limitation of this study is that it didn't look at sensitivity and specificity of this technique, including healthy surfaces would help see if the contrast medium can differentiate between healthy surfaces and cavitated lesions. However, the idea was that the contrast medium would be used in case of lesion detection to differentiate between cavitated and non cavitated lesions. This is why the authors thought it was justified to keep this experiment for the second phase of the project. Another limitation is that using DIAGNOcam in vitro is not ideal. According to the experience of the authors, the images obtained in vivo are usually of much better quality. This is most probably due to near infrared light absorption/scattering by the periodontal tissues before reaching the tooth and the rapid dehydration of the tooth when manipulated in vitro.

To ensure practical and rapid clinical application, further research should focus on developing software models that can detect lesions on NIR transillumination images and provide instant measurements after the application of ICG. A further development of the recently published caries diagnostic software using artificial intelligence model based on near infrared transillumination images could be interesting in this aspect [42].

Despite the limitations of this study, it demonstrated that ICG can be used as a contrast agent in combination with NIR transillumination imaging technologies. Still, questions about details of this technique, such as the optimal concentration and time needed for penetration, its influence on subsequent adhesion procedures, resin infiltration or sealing is yet to be performed.

Nevertheless, if this simple and non-invasive technique is proved effective in vivo, it might be a revolutionary method in confirming proximal caries lesions with cavitation limiting restorative procedures to indicated cases.

5. Conclusion

The results obtained in this paper support the hypothesis that the dye can help differentiate between cavitated and non cavitated lesions by enhancing the contrast of the cavitated area. ICG in a thick gel form is best suited as a contrast medium for the detection of cavitated proximal lesions using near infrared transillumination. A clinical study should be the next step to confirm these results in vivo.

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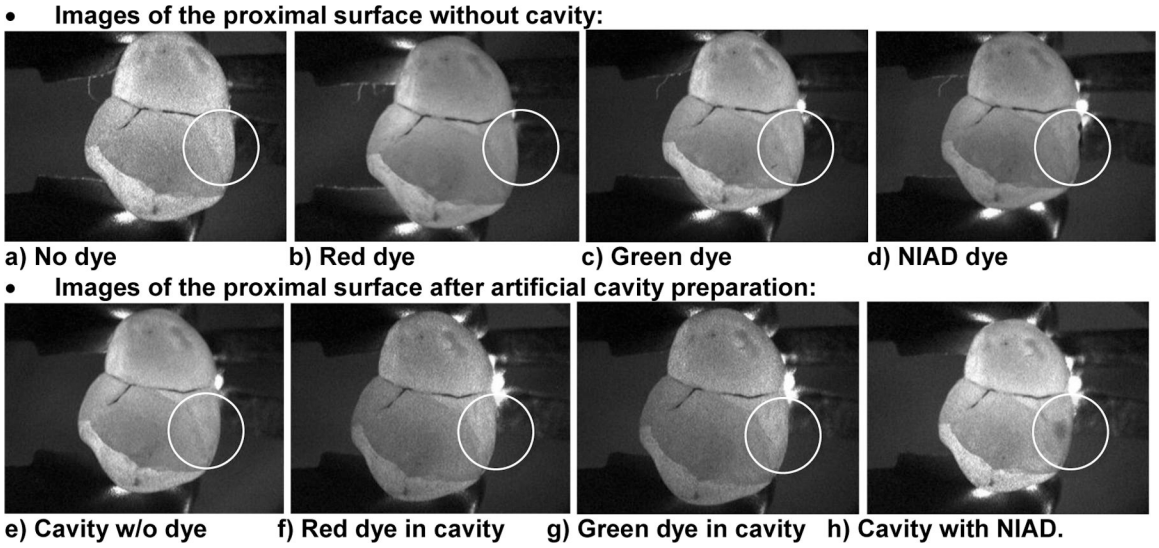

Fig. 1:

Images comparing the effect of application of 3 different dyes on the mesial surface with and without cavitation. Notice that in figures d and h, the dye can easily be observed on the surface. (NIAD: Near Infrared Absorbent Dye (ICG))

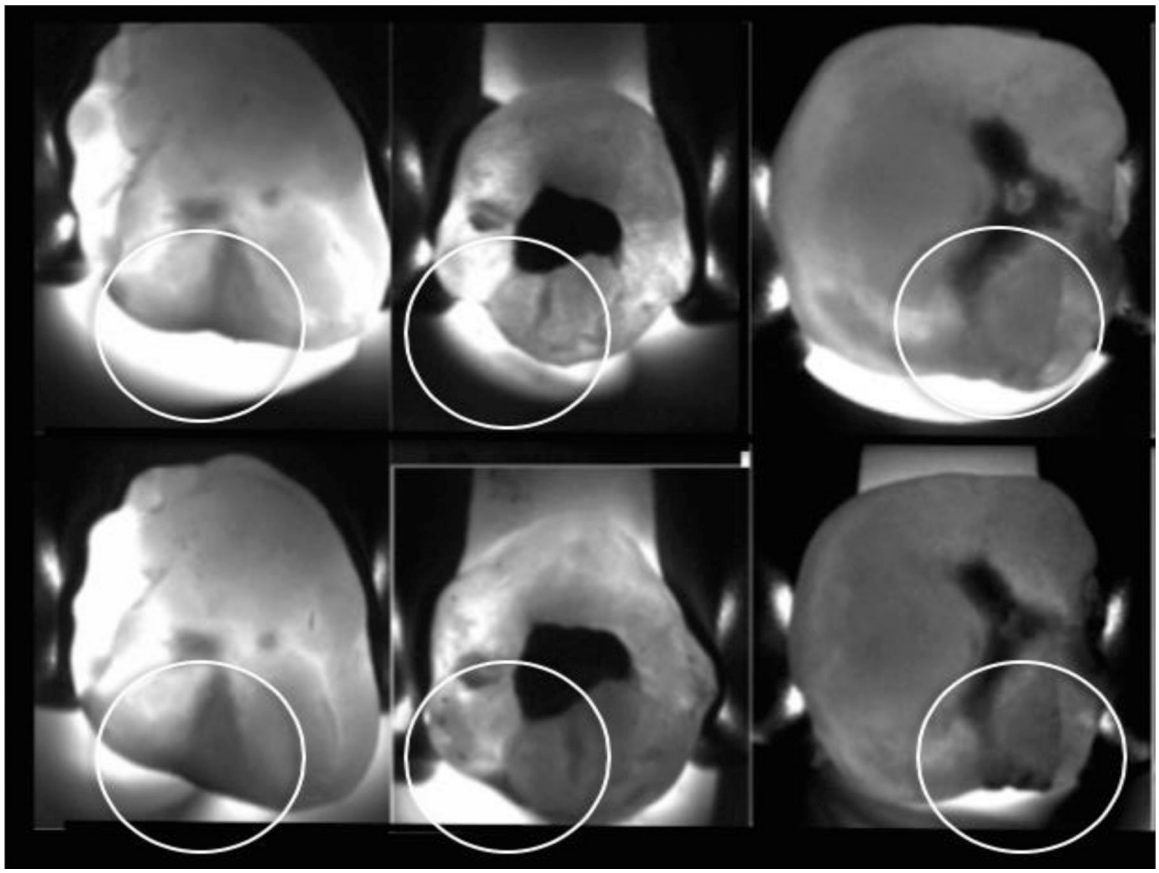

Fig. 2:
The upper row of images represents natural cavities on proximal surfaces imaged by DIAGNOcam, the lower row shows the same lesions after adding the ICG on the surface, the enhanced contrast is noticeable by the naked eye on most images.

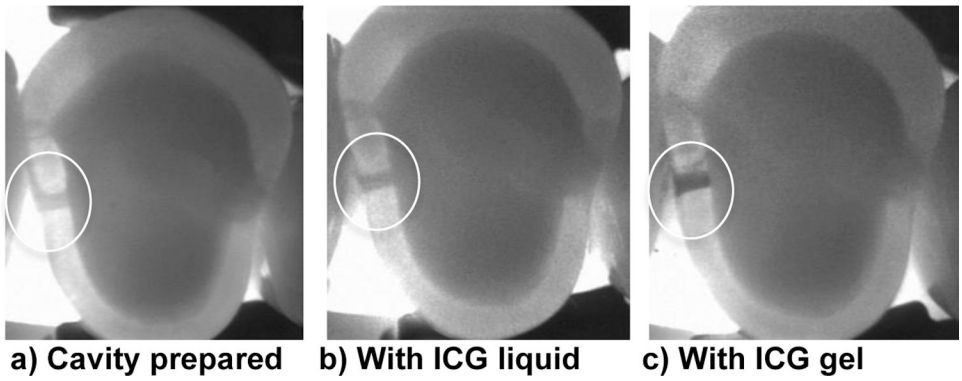

Fig. 3:

Artificial bilayer resin tooth, white circles are enclosing the region of interest after preparing the cavity (a), after applying liquid ICG (b) and after applying gel ICG (c). The increase contrast is clearly visible.

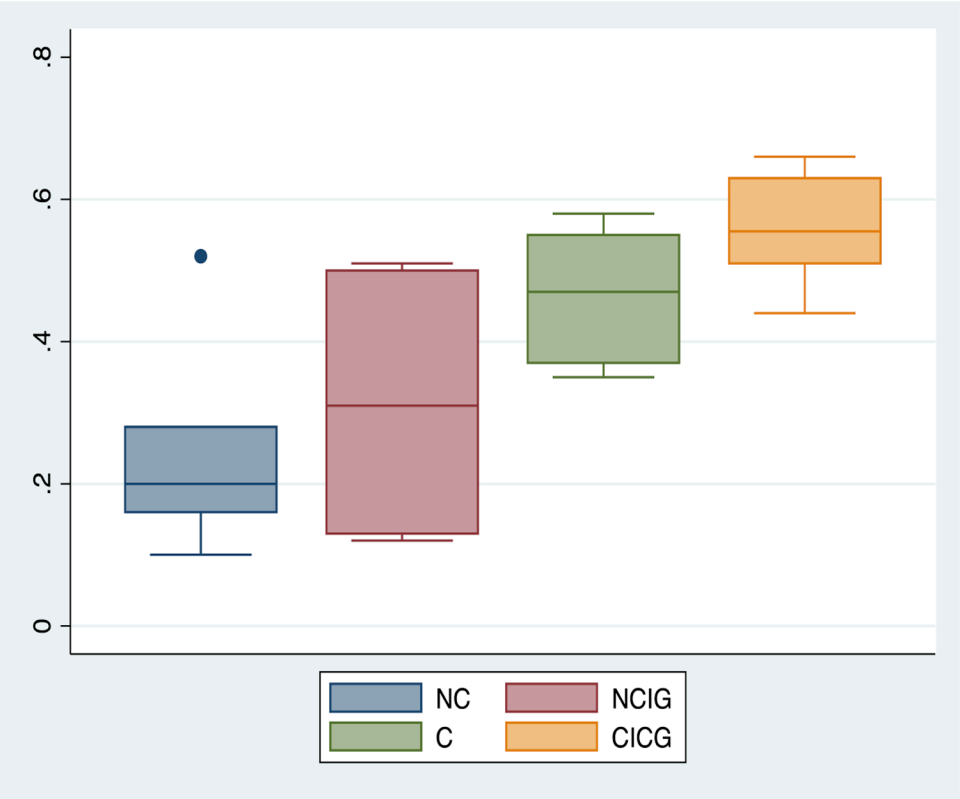

Fig. 4:

The contrast of the lesions before and after the application of ICG on natural non cavitated lesions (NC) and cavitated (C) lesions. The wide variation in the contrast of non cavitated lesions with ICG (NCICG) is probably affected by lesion activity.

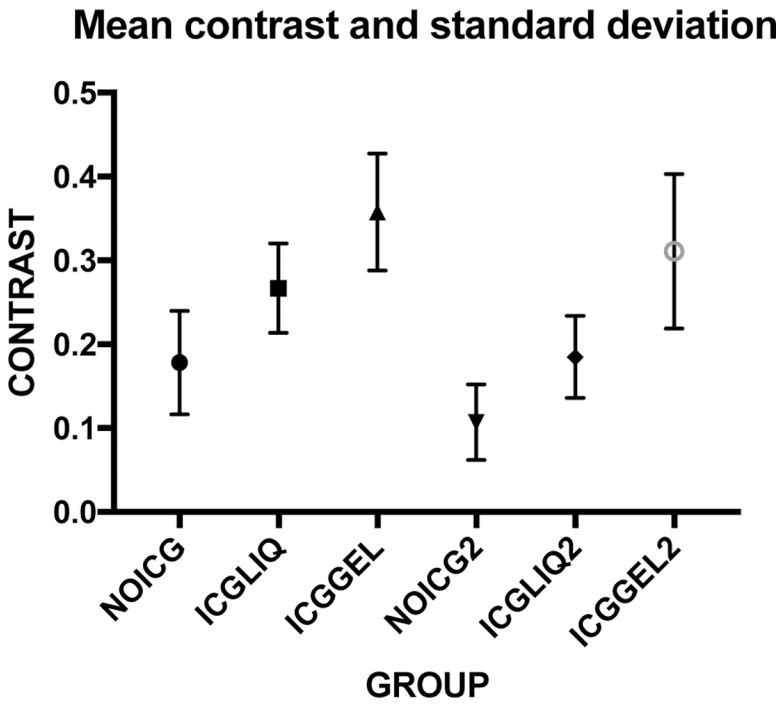

Fig. 5:

The contrast increases significantly with liquid ICG but ICG gel seems to increase it more. In G2 with more cervical cavitations, ICG gel seems to render the cavities as visible as the lesions near the contact point.

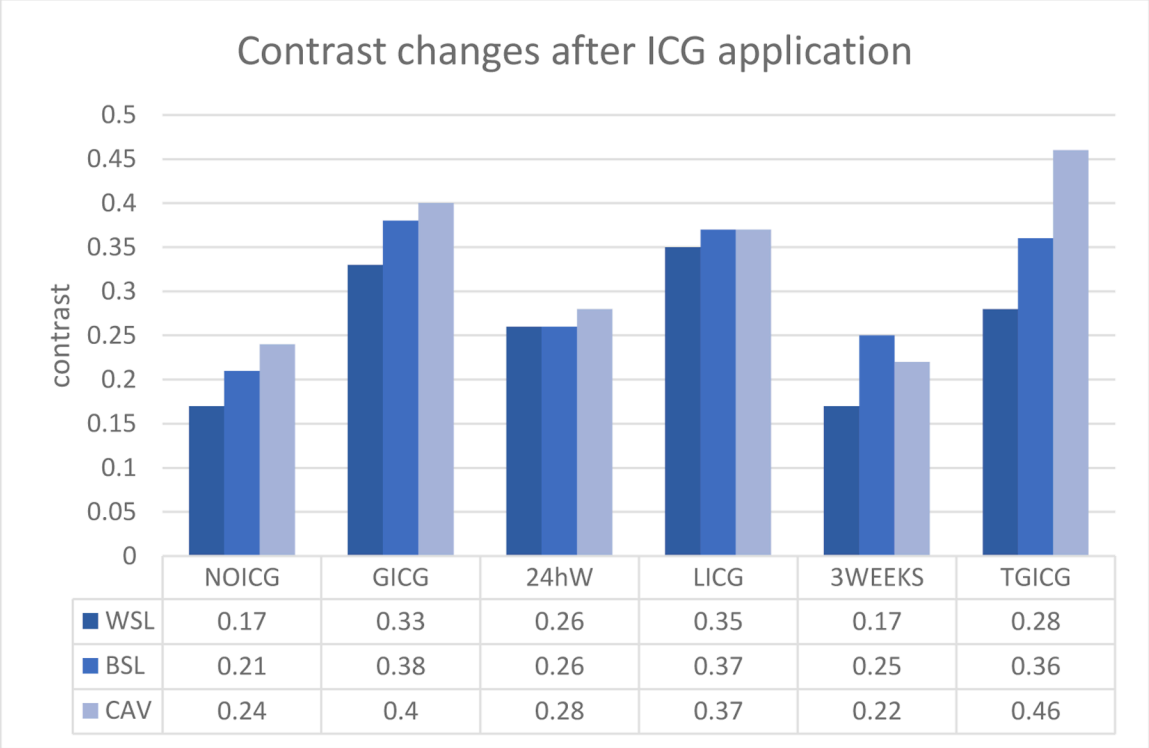

Fig. 6:

The average values of contrast measured on NIR images with no ICG on the lesion (NOICG), applying gel ICG (GICG), after one week in water, liquid ICG (LICG), three weeks in water (3Weeks) and thick ICG gel (TICGG). The three colors represent the natural proximal active white spot lesions (WSL), arrested brown spot lesions (BSL) and cavitated proximal lesions (CAV).

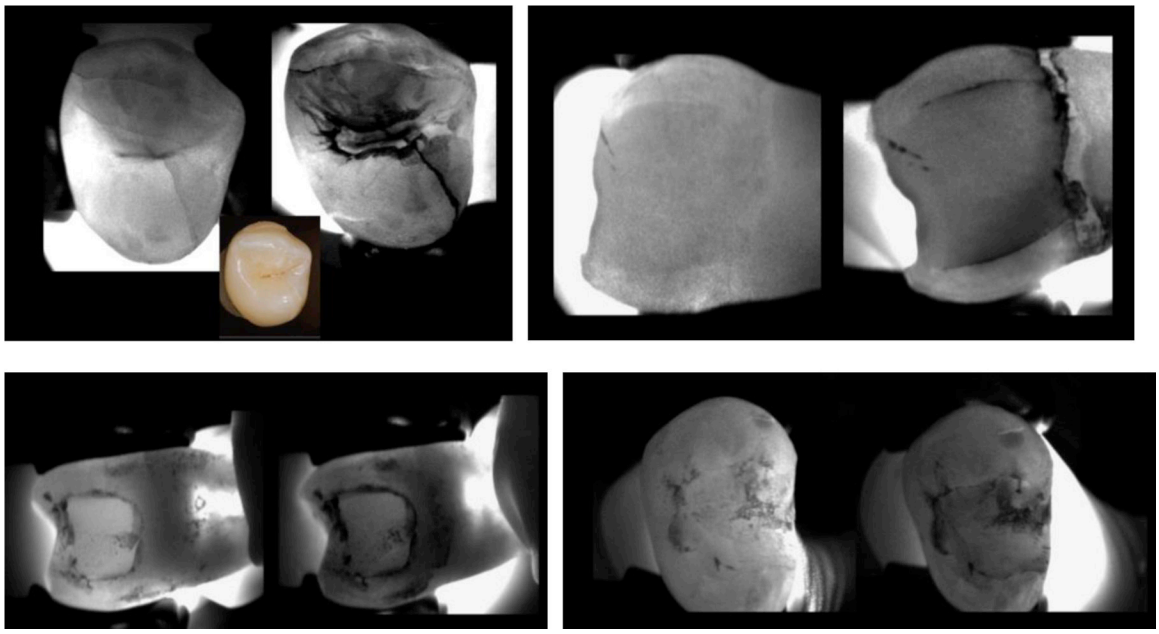

Fig. 7:

After the application of ICG fissures became significantly more visible (upper row). ICG liquid als0 enhanced the visualization of ill-adapted margins on near infrared images (lower row).

Table 1:

Statistical results (p values) obtained from one-way ANOVA and tukey's multiple comparison test. Bold values are significant. This table shows that ICG in thick gel form produced significant contrast changes when applied on different types of lesions. The contrast was measured on NIR images with no ICG on the lesion (NOICG), applying gel ICG (ICGGEL), after one week in water (W24), liquid ICG (ICGLIQ), three weeks in water (3WW) and thick ICG gel (ICGTG). Then the contrast was tested for significance between white spot lesions (WSL), brown spot lesions (BSL) and cavitated lesions (CAV).

Groups	NOICG	ICGGEL	W24	ICGLIQ	3WW	ICGTG
WSL VS. BSL	**0.046**	0.067	**0.025**	**0.036**	0.581	**0.034**
WSL VS. CAV	0.156	0.335	0.890	0.855	0.858	**<0.0001**
BSL VS. CAV	0.832	0.664	0.072	0.115	0.885	**0.015**