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Research article

A combination of near - infrared and fluorescence techniques for detecting early tooth lesions

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ABSTRACT

In dentistry, X-ray method has been considered as the gold standard for identifying different types of structural damage in teeth. However, X-rays not only have adverse effects on patients' health but also have failed in detecting small and early-stage lesions due to the low sensitivity of the method to these types of lesion. In order to overcome the disadvantages of X-ray method, in recent years, the infrared and fluorescence techniques have been developed strongly due to the fact that the optical properties of damaged tissue under infrared and ultraviolet lights are significantly different from those of sound dental tissue because of the demineralization of damaged tissue. The aim of the present study was to apply the fluorescence and infrared techniques to diagnosis of dental lesions at early stages. In this study, the optical systems with 850-nm LEDs and 365-nm LEDs which were applied for infrared imaging and luminescence imaging, respectively, were designed and used for diagnosing *in vitro* different types of early-stage lesions such as white spot lesions, dental plaque and lesions under restoration. A number of 68 tooth samples were assayed with infrared technique, fluorescence technique and clinical observation combined with radiography at the same time. Cohen's Kappa coefficient which was obtained with Statistical Package for the Social Sciences program was used to evaluate the agreement in diagnosis between the mentioned methods. The statistical evaluation showed that Kappa coefficients between traditional methods (clinical examination combined with X-rays) with optical techniques including infrared and fluorescence techniques were 0.147 and 0.235, respectively. These results suggest that all the lesions analyzed in this study were at the early stages and could not be detected by both visual inspection and X-rays, whereas the infrared and fluorescence techniques could be more sensitive to these types of lesion due to the fact that the optical techniques are able to detect early signs of demineralization of damaged tissue.

Key words: Dental lesions, fluorescence, infrared

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INTRODUCTION

Currently, the advanced imaging techniques used for diagnosis of dental lesions have become increasingly popular in the world. However, over 90% of Vietnamese suffer from dental diseases and 85% of Vietnamese children have tooth decay, according to National Hospital of Odonto - Stomatology. The lack of professional doctors as well as hesitation to go to see dentists in time are the main reasons for these high percentages. Instead of being treated at early stages of tooth decay, people usually go to see dentists when the damages become severe or even untreatable.

In dentistry, the most popular conventional diagnostic methods are clinical observation and radiography. The clinical inspection allows dentists to quickly examine all tooth surfaces with ease. However, this method only pays attention to significant lesions such as dental cavities and ignores early-stage lesions without holes or hidden caries. Although X-ray is considered the gold standard to identify structural lesions of teeth, the sensitivity of this method is only high for lesions that have developed into fairly obvious holes. Therefore, both mentioned methods are not really effective in the early diagnosis of dental lesions.

Compared with conventional techniques, infrared and fluorescence techniques are superior in detecting small or incipient lesions without affecting the patient's health. With regard to infrared technique, many researches have demonstrated that near-infrared (NIR) wavelengths from 780 to 1550 nm are effective in detecting early demineralization occurring beneath enamel layers [1–3]. The principle of infrared method is based on the difference in optical properties between healthy and damaged tooth tissue. In infrared light region, enamel scatters and absorbs quite weakly, in particular scattering coefficient strongly reduces to 2 – 3 cm^{-1} at 1310 nm and 1550 nm [3–6] and the absorption coefficient is low (μ_a < 1 cm^{-1}) [2]. As a result, healthy enamel becomes nearly transparent under NIR light. Meanwhile, damaged

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tissue or demineralized tissue appears dark in infrared images due to the fact that the low mineral density creates the porous structure in dental tissue, where illuminated NIR light is scattered many times and substantially attenuated, resulting in dark areas in infrared images.

In addition, the fluorescence technique has showed the ability to detect the presence of bacteria causing tooth decay on the enamel surface. The principle of this technique is that enamel emits blue fluorescence light when being excited by near ultraviolet light (UVA) or violet light[7]. However, Streptococcus Mutans bacteria presenting in dental plaque or lesions produce metabolites called porphyrins and porphyrins radiate red light under UVA and short-wavelength visible lights. The higher the concentration of bacteria, the higher the density of porphyrins and the stronger the red fluorescence is[8]. The presence of bacteria is one of the main causes of reducing mineral density in tooth tissue and increasing the risk of tooth decay. Therefore, combining fluorescence with infrared technology helps not only detect tooth damage at an early stage, but also monitor the process of lesion formation even when the lesion cannot be observed with traditional methods yet.

The aim of the present study was to apply fluorescence and infrared techniques to *in vitro* diagnosis of dental lesions at early stages and evaluate the agreement in diagnosis between the optical techniques and conventional methods.

MATERIALS AND METHODS

Samples

Tooth samples were collected regularly from many sources such as Odontology Department of National Hospital of Odonto – Stomatology and Odonto - Maxillo - Facial Hospital. After being collected, tooth samples were cleansed of food, bacteria, blood and periodontal ligaments without losing dental plaque or damaging the intrinsic lesions of samples. Each tooth sample had a separate base that was made of denture adhesive powder in order to keep it in balance during diagnostic imaging. All the samples were preserved in physiological saline solution in order to keep samples from dehydrating during the study process as well as to ensure the sterilization.

The optical systems

The infrared imaging system

With regard to infrared imaging, the tooth samples were captured under 850-nm illumination with the scattering system (also known as occlusal transillumination) and the transmission system (or approximal transillumination) showed in Figure 1.

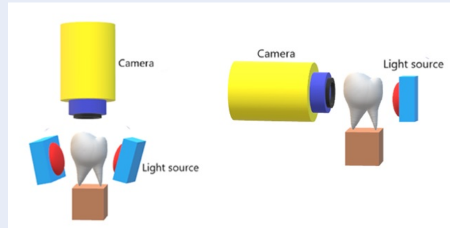

Figure 1: Schematic diagrams of scattering (left) and transmission (right) infrared imaging systems.

The scattering system consists of two symmetric 850-nm LEDs and a NIR camera to capture NIR images of occlusal regions. The camera position was calibrated to get the optimal distance from tooth samples to guarantee the best image quality. The LEDs were put close to the sample so that the light did not hit the camera directly, preventing flares in the image. The LEDs had wavelength of 850 nm and power of 1 W. The NIR light emitted from 850-nm LEDs went through the sample right on top of the gum line, then was scattered and absorbed inside the tooth before reaching the occlusal surface and getting out of the tooth. Therefore, this method was used to observe the lesions on the chewing surface of the tooth samples.

With regard to approximal transillumination technique, the transmission system was designed simpler than the scattering one. It consists of one 850-nm LED put next to the lateral surface of the tooth and a NIR camera placed on the opposite side. All the components of the system were placed coaxially. With this technique, the LED light travelled through the tooth and reached the camera. The captured image was the result of optical interaction between NIR light with both enamel and dentine. Therefore, this technique was applied to the inspection of structure damages placed on the lateral surface of teeth.

The fluorescence imaging system

The fluorescence imaging system is showed in Figure 2. A 365-nm LED (1) was used to excite the sample (3) to emit fluorescence. The shortpass filter (2) was placed between the sample and the LED to eliminate visible light from the LED as well as noise from the surroundings. A longpass filter (4) was put between the sample and the dental camera (5) to allow the fluorescent light emitted by the sample to reach the camera while all the noise was reduced. The capturing process was conducted in normal lighting conditions to test the system's capacity for clinical application in the long run.

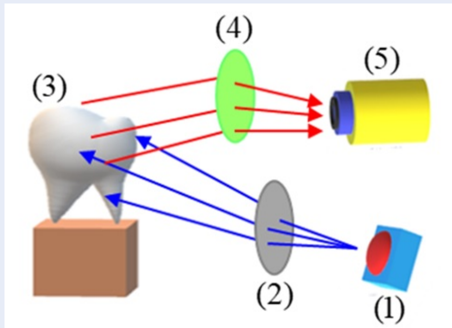

Figure 2: Schematic diagram of fluorescence imaging system.

Evaluation of the effectiveness of infrared and fluorescence techniques

In order to evaluate the effectiveness of the infrared and fluorescence techniques compared to clinical - X-ray combination in diagnosis, we designed the assessment procedure showed in Figure 3. Due to the fact that the major shortcoming of clinical visual inspection is the low sensitivity in detecting early dental lesions, in this study the clinical examination was carried out separately by the two dental experts. The experts used both visual observation and X-ray images to come to the conclusion of the existence of the lesions (Figure 3, data obtained from X-rays combined with clinical examination).

Cohen's Kappa coefficient is a statistic that helps evaluate the agreement between qualitative components. In medicine, this index is used to evaluate the consensus between two tests or the diagnostician when evaluating a certain disease condition after excluding random factors. Therefore, in this study, Cohen's Kappa coefficient was used to analyze the agreement in diagnosis between fluorescence technique, infrared technique and the combination of X-ray and visual methods.

RESULTS AND DISCUSSION

The study was carried out on 68 tooth samples. The diagnostic results of typical tooth samples were analyzed as followings.

White spot lesions

Currently, diagnosis of white spot lesions by infrared technology has become more potential than examination with radiology because X-rays are less sensitive in detecting early lesions. Meanwhile, the mineral density of damaged tissue is lower than that of sound tissue, NIR light can be scattered many times in demineralized tissue and significantly attenuated before reaching the NIR camera. As a result, NIR images of lesions appear darker than that of sound tissue. Similar to infrared technique, using fluorescence technique is also able to observe early white spot lesions due to the fluorescent properties of the demineralized tissue and porphyrins. Many studies have shown that damaged or demineralized tooth tissue has the fluorescence intensity significantly weaker than healthy tooth tissue [4,5]. In addition, the high concentration of Streptococcus Mutans at lesion areas produces porphyrins which emits red fluorescence under UVA excitation [6].

Figure 4 shows a tooth sample with a white spot lesion. According to the clinical inspection by two dental experts, this was a sound tooth (Figure 4A), in which the red circled area was diagnosed as enamel hypoplasia (MIH).

The fluorescence image of the sample (Figure 4B) shows the appearance of red luminescence at the MIH area which is related to the existence of porphyrins. Due to the fact that Porphyrins is the product of the Streptococcus Mutans bacteria, the fluorescence signal of this sample indicated that besides MIH, the density of Streptococcus Mutans bacteria was high, probably leading to the tooth decay in the near future. Based on the observation under near-infrared light, a distinct dark area appeared at the MIH area, indicating that demineralization had occurred here. Besides, when combining the infrared images captured by transmission (Figure 4C) and scattering (Figure 4D) infrared imaging systems, we could determine the location, size and depth of the demineralization area in detail. This result showed a high agreement between infrared and fluorescence techniques in detecting white spot lesions.

Unfortunately, based on the X-ray film (Figure 4E), the experts concluded that there was no sign of injury. This result could be related to the low sensitivity of X-ray method to white spot lesions.

Dental plaque

Dental plaque or calculus is considered not damage tissue, but it is one of the main causes of tooth damage. Plaque which is not removed at early stages could

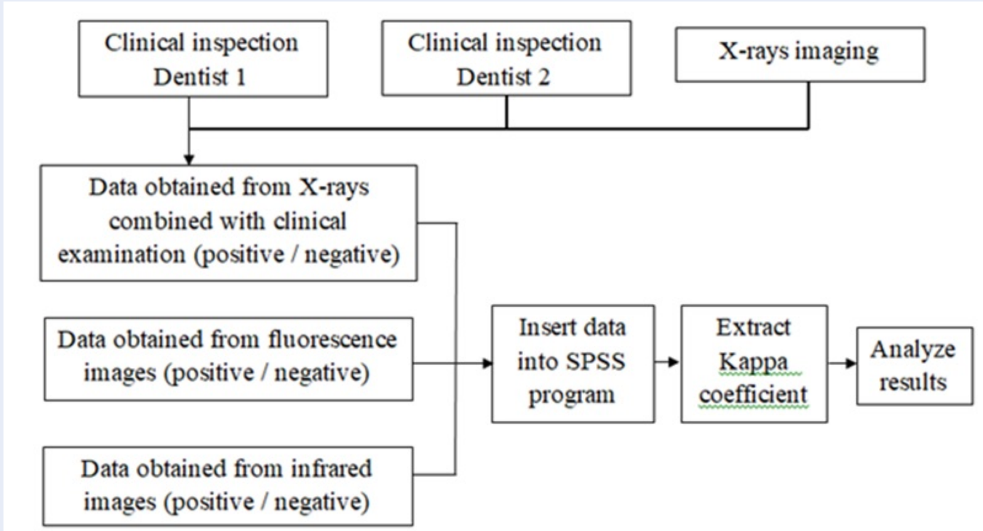

Figure 3: Procedure of evaluating the effectiveness of infrared and fluorescence techniques.

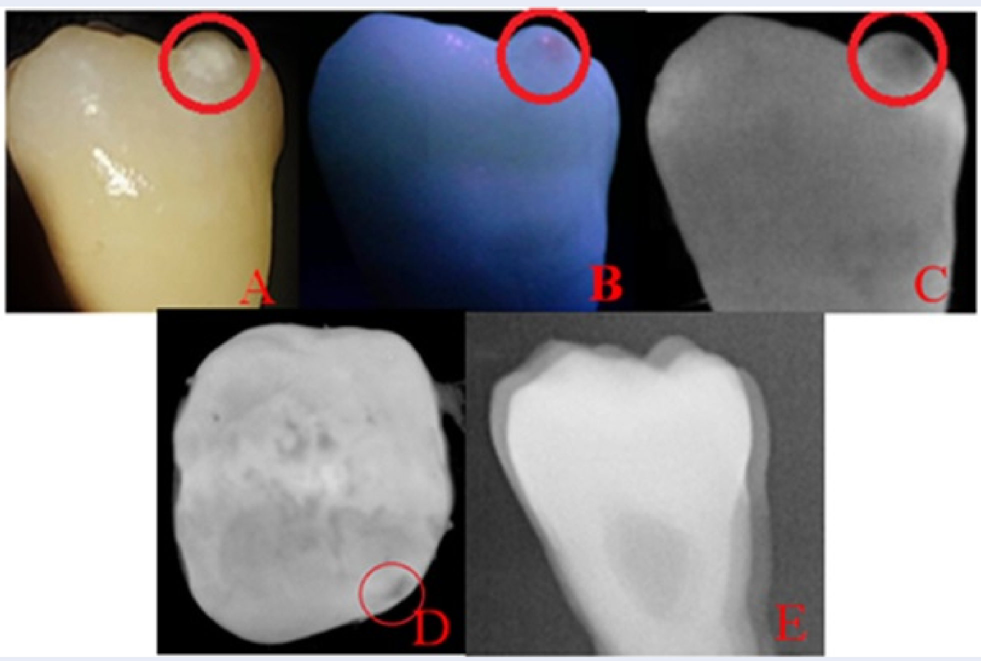

Figure 4: Sample with a white spot lesion: visible image (A), fluorescent image (B), transmission NIR image (C), scattering NIR image (D), X-ray film (E).

gradually calcify, leading to mineral loss and more severe damages in the long run.

In clinical practice, dentists might pay attention to dental plaque not enough to recognize the possibility of demineralization in enamel tissue beneath the plaque. Many studies[4,7,8] have proven that infrared technique helps dentists determine whether the demineralization is occurring in enamel tissue due to the fact that dental plaque at early stages does not cause demineralization in tissue and also does not absorb NIR light, resulting in appearing as sound tissue in NIR images, whereas calculus combined with demineralized enamel becomes darker in NIR images. Besides, the fluorescence technique is able to detect the existence and quantity of porphyrins in plaque.

Occlusal plaque

During the clinical diagnosis of the sample with occlusal plaque (Figure 5), two dental experts independently evaluated and reached the different conclusions. Based on the international caries assessment and detection system - IDCAS, the first expert considered the sample as sound tooth. According to the second expert this sample had visible external signs of damage when the tooth was dried (Figure 5A), but it had no sign of lesion based on the X-ray film (Figure 5D).

In the fluorescence image (Figure 5B), the interstitial plaque (red circled area) showed the red-orange fluorescence indicating the presence of porphyrins. This fluorescence region coincided partially with the dark lesion in the NIR image (Figure 5C), indicating the presence of demineralization on the occlusal surface of the tooth sample. The partial coincidence between fluorescence and NIR images means the interstitial plaque appeared in the fluorescence image was larger than in the infrared image, because, in general, some plaque areas are demineralized not enough to be showed up in infrared images although they could be observed in fluorescence images due to the presence of porphyrins. This result suggests that infrared technique is more precise than fluorescence in observing demineralization beneath the plaque. The infrared technique helps to determine whether the presence of bacteria in plaque is enough in terms of time and concentration to cause demineralization.

Approximal plaque

Figure 6 is the test result of tooth sample with approximal plaque (circled area), whose the lateral surface showed the white-yellow plaque under visible light. Both experts considered this sample as sound tooth with both clinical inspection (Figure 6A) and X-ray image (Figure 6D). However, when the plaque was excited by ultraviolet light, it emitted red fluorescence, showing the existence of porphyrins metabolites (Figure 6B). At the same time, the plaque under infrared light became darker than the surrounding enamel area, indicating that there was demineralization occuring beneath the plaque (Figure 6C).

In order to verify the diagnostic results by fluorescence and infrared techniques, we scraped off the white-yellow plaque and then observed it again under visible, infrared and ultraviolet light (Figure 7). In the visible image (Figure 7A), the initial plaque became a opaque white area which no longer emitted red fluorescence (Figure 7B), indicating that the plaque layer had been completely taken off. It also did not show the blue fluorescence as sound tissue, proving that the enamel tissue was demineralized. More importantly, although the plaque was cleaned off, the dark area on the infrared image remained unchanged. This result suggests that infrared technique could detect demineralization of enamel even located under the plaque.

Lesions under the restoration

Dental fillings are not a permanent technique. In some cases such as sudden change in temperature on tooth surfaces, strong bite force, poor filling technique, etc., the filling material (restorative material) might be deformed, creating fissures between the tooth surface and the filling material, leading to the formation of lesions under restoration. In particular, recurrent caries under restoration are more difficult to be observed and diagnosed than regular caries because the restoration overlaps the lesion. Infrared technique could be useful for detecting this type of lesions because sound enamel is almost transparent whereas lesions with demineralization appear as the dark spotted under NIR light.

Figure 8 shows a sample with the lesion under restoration. During the clinical evaluation (Figure 8A), the experts recognized the presence of the fissures between the tooth surface and the filling material but they could not come to the final conclusion of demineralization. Radiography was also not helpful in this case (Figure 8D) because it did not record any signs of lesion. However, in the fluorescence image (Figure 8B) the red fluorescence was detected at the edge of the restoration. Similarly, in the infrared image (Figure 8C), the boundary of the restorative material appeared dark, related to the demineralization after restoration. The diagnostic results of this sample by using fluorescence and infrared techniques suggests the potential tool for monitoring and diagnosing the appearance of lesions under restoration.

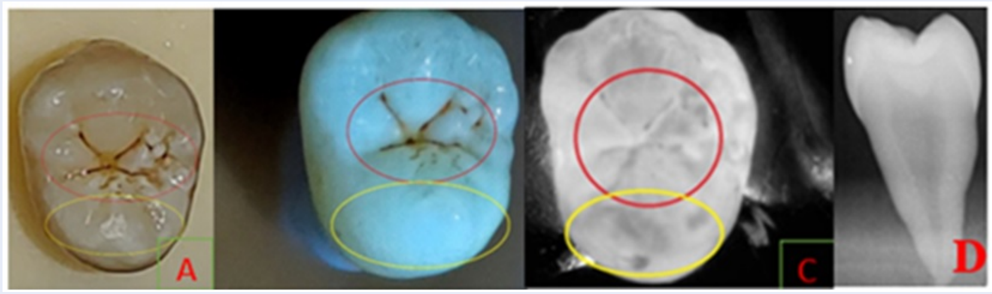

Figure 5: Sample with issure plaque: visible image (A), fluorescent image (B), scattering infrared image (C) and X-rays film (D).

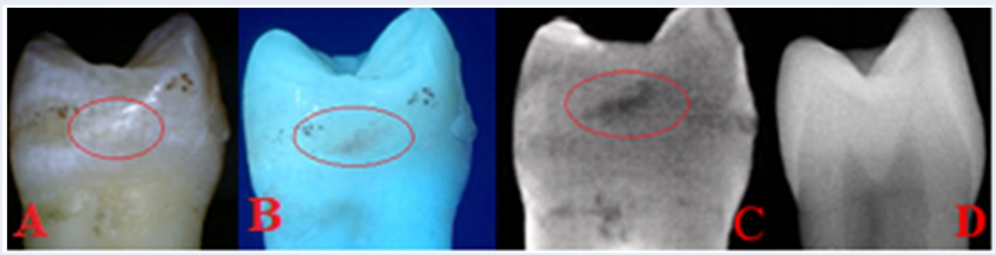

Figure 6: Sample with approximal plaque before scaling: visible image (A), fluorescent image (B), infrared image (C), X – rays film (D).

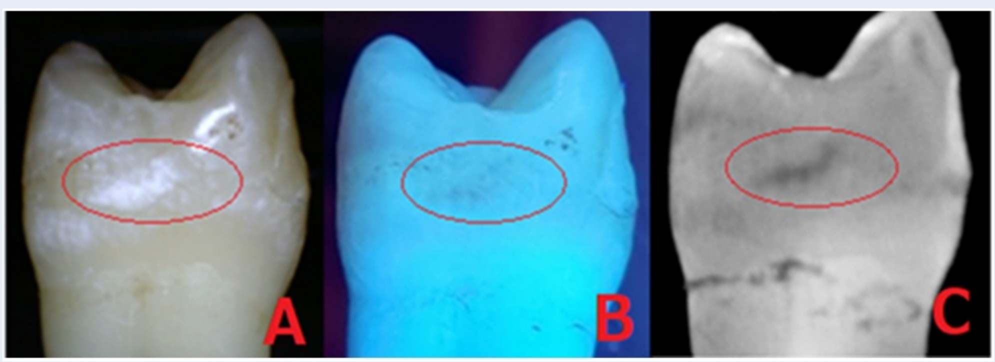

Figure 7: Sample with approximal plaque after scaling: Visible image (A), fluorescent image (B), infrared image (C).

Evaluation of the effectiveness of infrared and fluorescence techniques

The statistical evaluation of infrared technique

With regard to the assessment of the effectiveness of infrared and fluorescence techniques, Cohen's Kappa coefficient was used to analyze the agreement in diagnosis between fluorescence technique, infrared technique and the combination of X-ray and visual methods. First, based on the diagnostic results of dental lesions using infrared and fluorescence techniques which have been published in a number of researches [1,2,4–7], we analyzed the infrared and fluorescence images of 68 *samples*. Second, the two dental experts combined visual inspection and X-ray images to diagnose these samples and come to the final results. Next, all the diagnostic results were inserted into the data table in SPSS program according to the convention: 0 = Negative = Sound sample, 1 = Positive = Lesion. Finally, Statistical Package for the So-

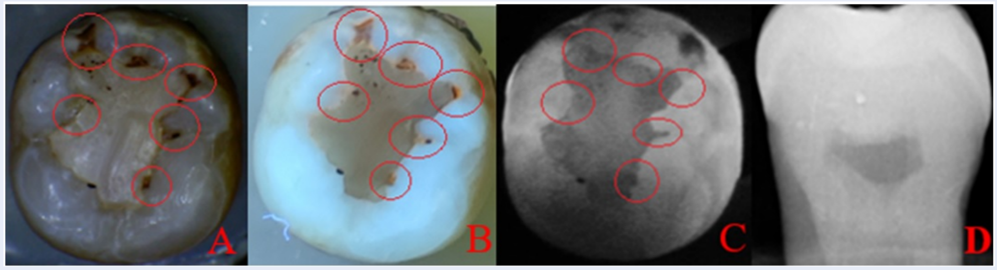

Figure 8: Sample with lesion under the restoration: visible image (A), fluorescent image (B), infarared image (C), X – rays film (D).

cial Sciences (SPSS) software output the results as the following tables.

According to Table 1, the infrared and clinical-X-ray examinations reported the same diagnostic result on 39 out of 68 samples (57.4%), and Kappa coefficient was 0.147, indicating the low consensus between clinical – X-ray assessment and infrared technique. This result could be related to the low sensitivity of conventional methods to early lesions such as white spot lesions or plaque.

The statistical evaluation of fluorescence technique

According to Table 2, the fluorescence and clinical-X-ray examinations gave the same conclusion on 42 out of 68 samples (61.8%). Even though Kappa coefficient of fluorescence was 0.235 which is a little higher than that of infrared technique, this result also reflects the significant difference in diagnostic results between fluorescence technique and traditional methods, because fluorescence technique is sensitive to lesions at early stages, whereas the conventional methods are not.

CONCLUSION

Based on the data obtained from our study, we suggest that near-infrared and fluorescence techniques are effective for the diagnosis of early-stage lesions including white spot lesions, dental plaque and lesions under restoration. The combination of both optical techniques could eventually substitute radiographic bitewings and support dentists in clinical inspection due to the fact that infrared technique helps observe demineralized lesions located even beneath enamel while fluorescence technique is useful for detecting the presence of bacteria on the tooth surface. In the future research, cutting tooth samples into slices and histological examination of tooth tissue under microscope are required in order to study more deeply the interaction between NIR – UVA light and teeth.

ACKNOWLEDGMENT

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LIST OF ABBREVIATIONS

LEDs: *Light-emitting diodes* .
NIR: Near-infrared.
UVA: Ultra-violet.
MIH: Molar incisor hypomineralisation.
IDCAS: International *Caries* Detection and Assessment System.
SPSS: Statistical Package for the Social Sciences.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

Pham Thi Hai Mien contributed in designing the ideas and optical systems, supervision and editing the manuscript.

Nguyen Tran Kim Hoang participated in diagnosing the tooth samples by clinical examination and X-ray images.

Le Phu Duong contributed in manufacturing the optical systems, capturing and analyzing the infrared and fluorescence images, collecting experiment data and writing original drafts.

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Table 1: Comparison of infrared technique and clinical-x-ray methods

		Clinical + X-ray		Total
		0	1	
Infrared	0	7	2	9
	1	27	32	59
Total		34	34	68
Kappa coefficient			0.147	

Table 2: Comparison of fluorescence technique and clinical-x-ray methods

		Clinical + X-ray		Total
		0	1	
Fluorescence	0	8	0	8
	1	26	34	60
Total		34	34	68
Kappa coefficient			0.235	

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Bài nghiên cứu

Kết hợp kỹ thuật hồng ngoại và huỳnh quang trong phát hiện các loại tổn thương răng

Phạm Thị Hải Miền[1,*], Nguyễn Trần Kim Hoàng[2], Lê Phú Dương[1]

TÓM TẮT

Kỹ thuật X quang trong nha khoa là tiêu chuẩn vàng để xác định tổn thương về cấu trúc răng. Tuy nhiên, X - quang ảnh hưởng xấu đến sức khỏe bệnh nhân do sử dụng bức xạ ion hóa, cũng như có độ nhạy không cao trong việc phát hiện các tổn thương giai đoạn sớm trên bề mặt lẫn bên trong cấu trúc răng. Nhằm khắc phục những nhược điểm của X-quang, kỹ thuật hồng ngoại và huỳnh quang đã được nghiên cứu và phát triển mạnh mẽ trong những năm gần đây dựa trên thực tế là tính chất quang học của mô răng tổn thương trong vùng ánh sáng hồng ngoại và tử ngoại có sự khác biệt lớn so với mô răng khỏe do có sự khử khoáng ở mô tổn thương. Mục đích của nghiên cứu này là ứng dụng kỹ thuật huỳnh quang và hồng ngoại trong phát hiện tổn thương răng giai đoạn sớm. Chúng tôi đã thiết kế và chế tạo hệ quang học chụp ảnh hồng ngoại sử dụng LED 850 nm và hệ quang học chụp ảnh huỳnh quang sử dụng LED 365 nm để chẩn đoán *in vitro* các loại tổn thương răng khác nhau như tổn thương đốm trắng, cao răng hay tổn thương dưới miếng trám. Tổng cộng 68 mẫu răng đã được phân tích đồng thời bằng kỹ thuật hồng ngoại, huỳnh quang, quan sát lâm sàng kết hợp X-quang. Hệ số Kappa được tính bằng phần mềm Statistical Package for the Social Sciences để đánh giá mức độ đồng thuận trong kết quả chẩn đoán giữa các phương pháp trên. Kết quả thống kê cho thấy hệ số Kappa trong so sánh giữa phương pháp truyền thống với kỹ thuật hồng ngoại và huỳnh quang lần lượt là 0,147 và 0,235. Kết quả này cho thấy các tổn thương sớm tuy không thể phát hiện bằng phương pháp truyền thống nhưng các kỹ thuật quang học lại khá nhạy trong phát hiện chúng bởi vì kỹ thuật quang học có thể phát hiện được sự mất khoáng xảy ra ở các tổn thương sớm.

Từ khoá: Tổn thương răng, huỳnh quang, hồng ngoại

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