Violet and blue light-induced green fluorescence emissions from dental calculus: a new approach to dental diagnosis

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Abstract

Background: The purpose of this laboratory study was to explore the use of green fluorescence emissions elicited by violet and visible blue LED light excitation to assist in diagnosis of dental hard tissues, particularly to differentiate dental calculus from healthy tooth structure and dental caries. **Methods:** Microscopic digital photography of 100 teeth was undertaken using violet and blue LED illumination (405 and 455 nm wavelengths) using a custom-made stack of green compensating filters which removed the excitation light and imaged green fluorescence scores. Differences in green channel pixel values were analyzed using ANOVA. **Results:** Supra- and subgingival calculus showed moderately intense green fluorescence emissions. These emissions were stronger than dental caries ($P<0.026$), but less intense than those from sound tooth surfaces ($P<0.0022$). The presence of saliva on the surface did not significantly alter green fluorescence, while the presence of blood diluted in saliva depressed green fluorescence ($P<0.015$). **Conclusions:** Using violet or blue illumination in combination with green compensating filters may have potential application for dental hard tissue diagnosis, particularly for differentiating dental calculus from sound tooth structure and from carious lesions.

Keywords: Dental Calculus, Detection, Fluorescence, Optical Imaging, Green Luminosity

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Introduction

Deposits of dental calculus when present either above the gingival margin or within periodontal pockets retain pathogenic microorganisms and their products, and have been linked to gingivitis and periodontitis.[1,2] To ensure that clinicians are able to reliably detect calculus deposits, a range of special devices have been developed to augment traditional examination methods which rely on visual inspection for supragingival calculus, and tactile examination with a periodontal probe for subgingival calculus. Such methods include fluorescence in the near infrared region elicited by incident visible red light.[3-6] Their application includes both assessment of deposits present at baseline before debridement, and checking root surfaces for remaining deposits at the end of a debridement visit.[7-10] The use of near infrared fluorescence emissions (such as in the DiagnoDENT) requires phototransistors and other electronic sensors to be used, [11,12] since these wavelengths fall outside the visible spectrum. An enhanced clinical method for calculus detection using visible light would, in contrast, rely on what fluorescence emissions the clinician could see themselves.

Subgingival calculus present on root surfaces associated with deep periodontal pockets can be seen with direct vision is when a surgical flap is raised.[13] Another less invasive situation is when an intra-oral endoscopic camera is used for direct high magnification inspection of the subgingival environment. Such endoscopes (such as the Perioscope*) are shaped like periodontal probes or dental explorers, and contain solid glass rods or fiber-optic bundles within them, to both illuminate the object and then

transmit and magnify reflected images back to a sensor, to generate a video signal.[13-15]

There are already in clinical use a range of intra-oral cameras which employ fluorescence imaging to enhance detection of dental caries, with the excitation light being in the violet or blue range (400-470 nm wavelength), including the GC G-Cam†, Morita Penviewer*, and Dürr VistaCam◆. These devices use orange or red filters to improve the signal to noise ratio for red fluorescence emissions from lesions of dental caries, for positive fluorescence imaging.[6,16,17] The same devices could, however, also be used to view and capture images of supragingival calculus on tooth surfaces.

In a previous study, we showed that green fluorescence emissions are strong from normal tooth structure, but are reduced when dental caries is present, allowing lesions of dental caries to be differentiated from healthy tooth surfaces.[18] Following on from this previous work, the present study was designed to explore whether green fluorescence emissions could also differentiate between dental calculus and sound tooth structure as well as dental caries, using the negative fluorescence approach with violet or blue light as the excitation source.

Materials and Methods

Sample Preparation

A total of 100 extracted human permanent teeth were selected from a large repository of extracted teeth collected from adults aged 18 years or more with the approval of the institutional ethics committee (Reference No: 2003000040) from a dental school exodontia clinic. A power analysis was undertaken to determine sample sizes in the various groups, based on data from a pilot study. The power analysis assumed α=0.05 and estimated β=0.2 (study power = 80%), giving N=20 per group for the three experimental groups: dental caries (group A), subgingival calculus (group B), and supragingival calculus (group C). There were a further 20 control samples included for both sound enamel (group D) and sound roots (group E), both which had been included in the previous study.[18] All teeth had been gamma sterilized and were stored in a solution of 0.1% thymol in distilled water to maintain hydration. All samples in group A had coronal cavities extending at least mid-way into the dentine, whilst samples in groups B and C had calculus deposits of between 5 and 20 mm^2 in area which were then used for analysis. To ensure they remained fully hydrated, teeth were removed from their storage media and placed on blotting paper to remove excess fluid from the surface. Imaging was completed within 10 minutes, so that dehydration did not occur.

Optical configuration

The arrangement for light sources and imaging was identical to the previous investigation.[18] In brief, two different excitation light sources were used, both of which were held at fixed positions 5 cm from the samples. The excitation LED light sources were as follows: for violet, the G-Light** (peak emission 405 nm, spectral range 390-420 nm), and for blue, the Tristar MR16†† (peak emission 455 nm, spectral range 430-490 nm). Samples were viewed through a custom filter stack to remove the excitation light but allow green fluorescence to pass,[18] and imaged first in the moist state with the surface free of excess fluid. To evaluate the impact of saliva and blood, samples were then imaged when covered by either a 7 µL or 14 µL drop of saliva (depending on the area to be covered), and finally when covered by anticoagulated blood. The saliva and blood were left on the surface for 3 minutes before imaging was undertaken. Both the saliva and blood were collected from a single healthy male volunteer. Their collection was approved by the institutional ethics committee (Reference No: 20060007701). The blood sample was collected in a heparinized tube and diluted into saliva to mimic the common clinical situation of gingival bleeding where blood becomes mixed into saliva. The ratio of the blood to saliva mix used was in the range of 0.57-0.71, which was the same as used in the previous study.[6]

For recording images of green fluorescence, a 3.3 megapixel digital camera‡‡ was fitted to a stereoscopic microscope◆◆. All images were taken in a dark room, using consistent low level ambient lighting and consistent exposure times for image series made using the same light source. The sample area imaged was 20 mm^2. A custom-made stack of four circular green gelatin filters (two CC40G and two CC50G colour compensating Wratten filters)*** was prepared, as described previously.[18] The stack of filters was

*Dental View, Lake Forrest, California, USA
†G-Cam ™, GC Corporation, Tokyo, Japan
*Morita Penviewer™, J. Morita Manufacturing Corporation, Kyoto, Japan
◆VistaCam™ , DÜrr Dental GmbH, Bietigheim-Bissingen, Germany

**G-Light, GC America, Chicago, USA
††Hi-Line Lighting Ltd, Kingston Upon Thames, UK
‡‡Coolpix 995, Nikon, Tokyo, Japan
◆◆U-PMTVC, Olympus, Tokyo, Japan
***Kodak, Tokyo, Japan

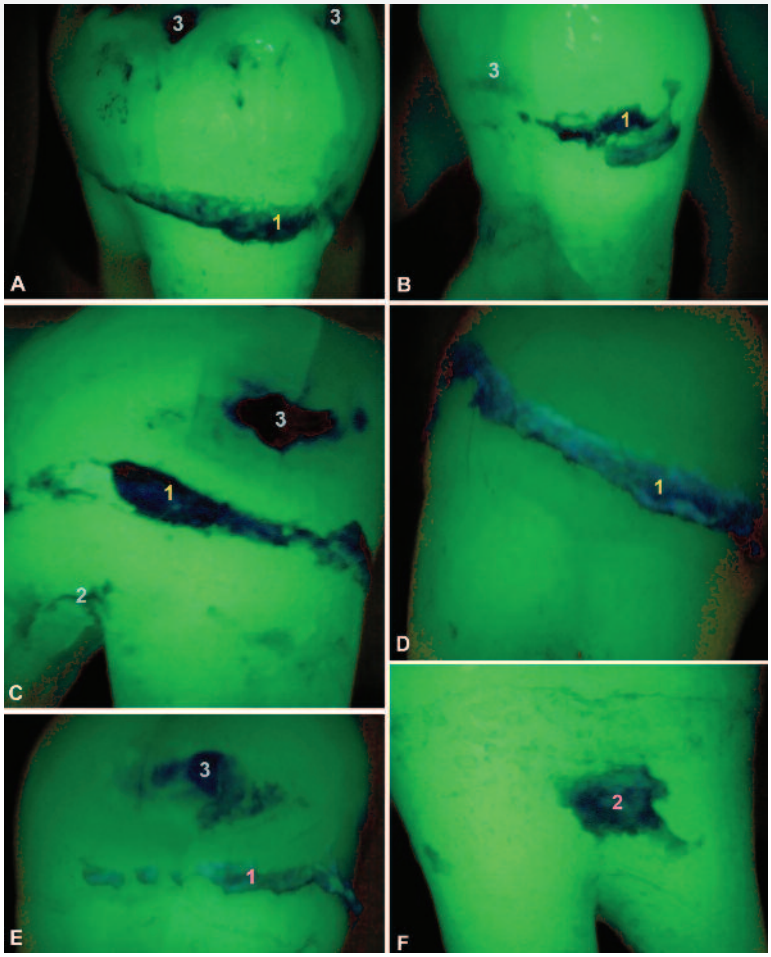

Figure: 1. Typical patterns of strong green fluorescence under 405 nm violet light excitation from sound tooth structure. Supragingival calculus (1), subgingival calculus (2) and dental caries (3) can all be distinguished because of their darker appearance. Lesions of caries include cavitations (panels A and C), white spot lesions (panel B) and both together (panel E).

attached to the objective lens of the microscope as a long pass filter, to attenuate ultraviolet and blue excitation wavelengths, but allow green fluorescence emissions to pass.

Data Analysis

From the digital images, the magnetic lasso tool in Adobe Photoshop CS2™ software was used to outline the sample target area, and the green channel data were computed using the histogram applet. As green channel data range from a minimum of zero (pure black) through to 255 (pure white), samples with greater green fluorescence show higher channel numbers. GraphPad Prism™ version 6 statistical software[†††] was used to compare green channel data from different sample types, under different conditions (free of fluid, saliva coated or blood coated). As data sets followed Gaussian distributions, ANOVA was used to analyze differences between groups, and repeated measures two-way ANOVA was used to compare samples under different surface conditions. The significance level was set as α=0.05.

[†††] GraphPad Software, La Jolla, California, USA

Results

In the images, the lower luminosity of supragingival calculus, subgingival calculus and dental caries (both white spot lesions and cavitations) could readily be distinguished from adjacent normal tooth structure which showed strong green fluorescence (Fig. 1 A-F).

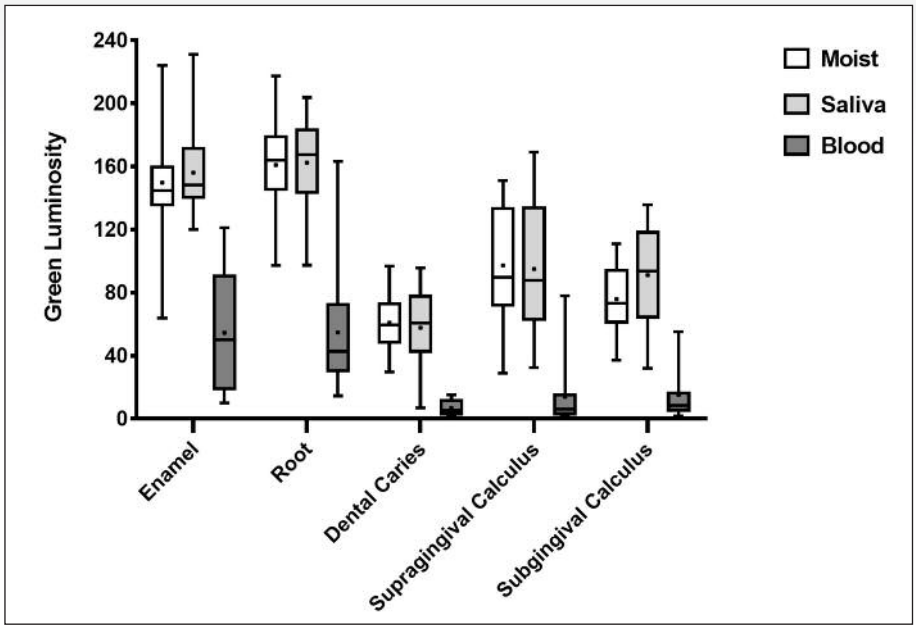

Figure 2: Boxplots showing green fluorescence elicited by violet light excitation (405 nm) under different surface conditions. The middle dot indicates the group mean, whereas the box plot itself indicates from top to bottom the maximum, 75% quartile, median, 25% quartile and minimum. Data for sound enamel and sound roots are taken from Ref. 18.

The boxplots for green fluorescence emitted from samples under violet or blue light excitation are shown in Figures 2 and 3, respectively, while Table 1 presents statistical comparisons of sample types.

Considered as a group, calculus samples in the moist state or when covered by saliva consistently gave luminosity scores up to 170 for violet light excitation, and up to 200 for blue light excitation. The differences between supragingival calculus and sound enamel and between sound roots and subgingival calculus, were both statistically significant ($P<0.0023$ and $P<0.0001$, respectively).

Regardless of whether surfaces were free of fluid or were coated with fluids, both types of calculus gave significantly stronger green fluorescence emissions than dental caries when excited by either violet or blue light ($P<0.026$). In contrast, there were no significant differences between supra- and subgingival calculus under violet light ($P=0.7001$) or blue light ($P=0.2125$).

In terms of the effect of surface conditions under violet or blue light excitation (Table 2), there were no statistically significant differences between moist or saliva-coated surfaces for any sample type ($P>0.05$). In contrast, the presence of blood diluted into saliva significantly depressed the green channel fluorescence emissions for all samples, compared to the same materials in a moist state ($P<0.003$) or when coated with saliva ($P<0.015$).

Discussion

In a previous investigation, the potential usefulness of green fluorescence emissions for aiding in caries detection was shown, applying the principle of negative fluorescence where the target of interest appears dark against the surrounding healthy tissue.[18] In this previous study, a custom-made green filter stack was assembled to serve as a long pass filter, blocking reflections from the violet or blue light sources but allowing green fluorescence emissions to pass through to the camera. Using the same optical configuration, the present experiments extend the findings by exploring variations in green fluorescence between dental calculus and sound tooth structure, and between dental calculus and dental caries. While the strong green fluorescence emissions from sound tooth structure and corresponding lack of these from lesions of dental caries is a phenomenon that has been well described in the literature,[18-20] the differences between dental calculus and dental caries have not hitherto been reported.

Table 1. P values for differences between dental caries and calculus samples

Sample Comparison	Violet Lighting	Blue Lighting
Caries vs Supragingival Calculus	0.0003	<0.0001
Caries vs Subgingival Calculus	0.0107	0.0004
Supra- vs Subgingival Calculus	0.4042	0.1384

In terms of overall rankings, the results from the present study show the strongest emissions from sound tooth structure (200–250 in luminosity), followed by dental calculus deposits (170-200), and then by dental caries (100-140). While the numerical values shift according to whether violet or blue light is being used (being higher for blue than for violet), the ranking remains the same nonetheless. The use of intense 455 nm LED or diode laser light could therefore have application for differentiation between calculus deposits and other surfaces. Such an approach could be used in endoscopy of periodontal pockets deposits,[15] where the instrument is used within narrow and confined periodontal pockets.[14,21]

Finally, the present study is informative with regards to the potential influence of ambient fluids which may cover the tooth surface. In line with previous reports,[16-18] there was no difference between moist surfaces and those coated with saliva. This is to be expected since water has very poor absorption of visible green, blue and violet light. As an extrapolation, one would not expect gingival crevicular fluid to absorb any of these same colours of light. There was, in contrast, a significantly reduced luminosity when blood was present, which can be explained by the strong absorption of all three colours of light into haemoglobin. The practical application of this finding is that for a device such as a periodontal endoscope, water irrigation could be used to remove blood from a periodontal pocket without impairing the green fluorescence readings.

Conclusion

As a proof of concept study, this laboratory investigation shows that applying violet or blue light can elicit green fluorescence from healthy tooth structure, and that such emissions are reduced significantly where dental calculus is present, regardless of whether this is supragingival or subgingival calculus. The reduced green fluorescence is even greater for dental caries. Such an approach may have clinical utility to augment existing diagnostic methods, especially when blood on the surface is removed immediately prior to imaging.

Table 2. Differences due to surface conditions (dry versus saliva versus blood)

Sample	DRY vs SALIVA	DRY vs BLOOD	SALIVA vs BLOOD
VIOLET LIGHT			
Dental Caries	0.4043	<0.0001	<0.0001
Supragingival Calculus	0.7846	<0.0001	<0.0001
Subgingival Calculus	0.0620	<0.0001	<0.0001
BLUE LIGHT			
Dental Caries	0.7920	0.0001	<0.0001
Supragingival Calculus	0.0742	0.0028	0.0143
Subgingival Calculus	0.6093	<0.0001	0.0003

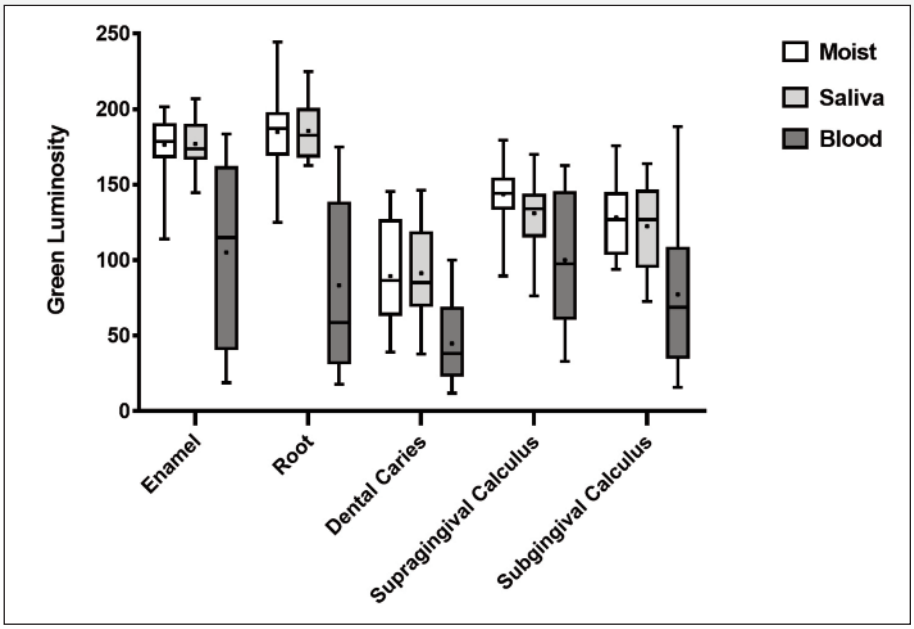

Figure 3: Boxplots showing green fluorescence elicited by blue light excitation (455 nm) under different surface conditions. Data for sound enamel and sound roots are taken from Ref. 18.

Conflict of interest

The authors declare that they have no conflict of interests.

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