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Original Article

Fluorescent Detection of Tooth Enamel Microscopic Damage Using a Silver Nanoparticle-Based Mixture

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ABSTRACT

Dental caries, as reported by the World Health Organization, affects about 60-90% of school-aged children and almost all adults worldwide. Early detection of micro-damage to tooth enamel has become an important issue in modern dentistry. Laser-induced fluorescence (LIF) diagnostics offers a promising solution by detecting caries through the analysis of the intrinsic fluorescence of microorganisms. This study presents a method for LIF detection of enamel micro-damage using a model mixture containing silver and polyvinylpyrrolidone nanoparticles. A total of 63 human tooth samples, collected for various clinical reasons, were analyzed. The findings showed that the fissure area and the cervical region of the tooth were the most informative zones for the detection of LIF. The fissure area, due to its anatomical structure, tends to accumulate pathogenic microflora and is highly susceptible to microcracks from chewing and other factors. Meanwhile, the cervical region is important for spectral analysis because it is the initial site for the formation of latent plaque and tartar. The optimal detection time for enamel was found to be 3 minutes after the application of the model mixture. Based on the ex vivo experimental results, it can be concluded that the silver nanoparticle and polyvinylpyrrolidone mixture is suitable for LIF diagnostics of tooth enamel in clinical settings, with few adjustments to the experimental conditions.

Keywords: Early diagnosis, Caries, Micro-damage to tooth enamel, Silver nanoparticles, Laser-induced fluorescent diagnostics, Polyvinylpyrrolidone

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Introduction

The ability to detect micro-damage to tooth enamel at an early stage plays a critical role in preventing the progression of dental caries. Detecting hidden bacterial accumulation, that can lead to plaque or tartar formation, helps in early intervention [1, 2]. Dental caries is a global health issue, affecting around 60-90% of children and nearly all adults, as reported by the World Health Organization [3-5]. Early identification of caries and prompt treatment are key to maintaining long-term dental health, ultimately improving the quality of life [6]. A variety of novel techniques are emerging worldwide to enhance the diagnosis of initial caries [7]. Among these, optical and laser-based methods have shown promise, including Optical coherence tomography, Raman scattering, fluorescence spectroscopy, and light scattering spectroscopy [8-11].

Laser-induced fluorescence (LIF) is a valuable diagnostic tool for identifying caries by analyzing the natural fluorescence emitted by microorganisms present in the enamel [12, 13]. However, this method can be limited in the early stages of caries development when bacterial populations are minimal, and enamel damage is subtle.

LIF techniques utilizing "red" wavelength radiation depend on the presence of sufficient porphyrins, byproducts of bacterial metabolism, to effectively detect caries [14-19].

The objective of this study is to develop a method for laser-induced fluorescence to diagnose enamel micro-damage, using a unique model mixture containing silver nanoparticles (Ag NPs). The diagnostic window is aimed to be within three to five minutes after applying the mixture to the enamel surface. A mixture of Ag NPs and polyvinylpyrrolidone (PVP) was prepared to achieve this goal.

This study utilized human tooth samples obtained for various clinical purposes, such as chronic periodontitis, third molar extraction, orthodontic treatments, and other indications, with a total of 63 samples collected from patients aged between 19 and 52 years. The samples were divided into 2 groups randomly for the tests: group 1 contained 12 samples to evaluate how the Ag NPs colloid interacts with the enamel surface, and group 2 consisted of 41 samples to assess the interaction of the model mixture, containing Ag NPs and polyvinylpyrrolidone (PVP), with the enamel surface for detecting micro-damage and bacterial accumulation.

Materials and Methods

The study investigated the interaction of a model mixture containing silver nanoparticles (Ag NPs) and polyvinylpyrrolidone (PVP) with tooth enamel to assess potential enamel damage. The model mixture consisted of a colloidal Ag NPs solution, where the concentration was maintained at 12 mg/l and the nanoparticle size ranged between 20 and 140 nm. To prepare the mixture, PVP (1.2% by volume) was incorporated, along with various standard toothpaste ingredients, such as carbopol, carboxymethylcellulose, methylparaben, titanium dioxide, sodium saccharin, sodium phosphate, and sorbitol.

For the analysis, a laser spectroscopic system was utilized, comprising an LSW-10 helium-neon laser, the RIGOL RSA5065-TG spectro-analyzer, and a fiber-optic probe. These tools were essential to examining how the Ag NPs solution affected the enamel surface.

A detailed experimental protocol was designed to explore the interaction of the Ag NPs colloid and model mixture with enamel, aiming to identify micro-damage and bacterial buildup. The process began by calibrating the spectrometer using standard samples, ensuring proper signal intensity and wavelength readings. Before the application of any solutions, autofluorescence spectra of the enamel were recorded in various regions using both the RIGOL system and a video fluorescence unit.

Measurements were taken from three distinct regions of the tooth enamel: position 1 (the cutting edge of the tooth crown), position 2 (the center of the vestibular surface), and Position 3 (the cervical region of the crown). The fiber-optic probe was positioned at a slight angle (approximately 20°) to the surface to reduce interference from laser scattering and reflection. After each round of data collection, the probe was cleaned with 70% alcohol to avoid contamination.

The third phase of the experiment involved treating the enamel with either the Ag NPs colloidal solution or the model mixture containing both Ag NPs and PVP. After a 3-minute exposure, the enamel samples were rinsed with running water to remove the solutions.

During the fourth phase, fluorescence images of the enamel surface were captured, and fluorescence spectra of Ag molecules were recorded after applying the treatments. The equipment settings were unchanged throughout the measurements. Fluorescence spectra were collected from each area of the enamel both before and after treatment, with lesions or defects marked separately for later analysis.

Two experimental groups were studied. The first group focused on how the Ag NPs colloidal solution interacted with enamel surface microflora, while the second group examined the effects of the model mixture containing Ag NPs and PVP on enamel. Autofluorescence spectra were recorded for both groups, before and after treatment.

For all measurements, exposure times were standardized. The average spectra from three regions of each tooth were calculated before and after the application of the respective solutions.

Since the intensity of fluorescence can vary based on both tissue characteristics and technical factors like laser power, the analysis was based on the ratio of autofluorescence to the area under the laser peak, rather than on absolute fluorescence values.

To quantify the enamel autofluorescence, the area under the enamel fluorescence spectrum was divided by the area under the laser peak. These coefficients were then used for statistical analysis, which was conducted with the "Statistics 6.0" software.

The statistical analysis involved calculating the mean, standard deviation, and variance for the data, followed by an F-test to compare variances between the two groups. A Student's t-test was performed, with the results compared to tabulated values at a 95% confidence level to assess the reliability of the findings.

Results and Discussion

Laser spectroscopic analysis of the interaction between Ag NPs colloid and tooth enamel Ex vivo

The investigation into how the colloidal Ag NPs solution interacts with the tooth enamel ex vivo revealed that, after three minutes, only minimal fluorescence was detected, indicating a slight activation of the surface Ag NPs molecules. However, a significant increase in the fluorescence of the Ag molecules was observed 1 hour after the colloidal solution was applied. This delay suggests that the activation of Ag NPs surface molecules requires time and may be influenced by the presence of pathogenic microflora [20, 21].

Figure 1a presents the fluorescence spectra of Ag NPs collected 3 minutes after the application of the colloidal Ag NPs solution to the enamel surface. Diagnostic contrast coefficients for each sample were calculated at this 3-minute mark to facilitate further statistical analysis.

Laser spectroscopic analysis of the interaction between the model mixture and tooth enamel microflora Ex vivo

To shorten the laser-induced fluorescence diagnostic time to 3 minutes, PVP was introduced into the colloidal solution as an additional activator for the Ag NPs. The inclusion of these molecules in both free and semi-free states enables quicker fluorescence activation, thus reducing the diagnostic time for laser-induced fluorescence of the enamel.

The interaction between the model mixture (containing Ag NPs and PVP) and the tooth enamel surface was assessed using RIGOL equipment and a video fluorescence camera.

Figure 1a shows the fluorescence spectra of Ag NPs obtained after applying either the Ag NPs colloid (group one) or the model mixture (group two) to the enamel surface. Before the application of the experimental mixtures, only enamel autofluorescence was detected. Following the application of the colloid or the model mixture, fluorescence from the Ag NPs appeared, with the fluorescence intensity from the model mixture being nearly three times higher than that from the colloidal Ag NPs.

Additional surface examination of the enamel was conducted using a video fluorescence camera, both before and after the application of the model mixture. **Figure 2** shows video fluorescence images of the enamel surface before the model mixture was applied and 3 minutes afterward.

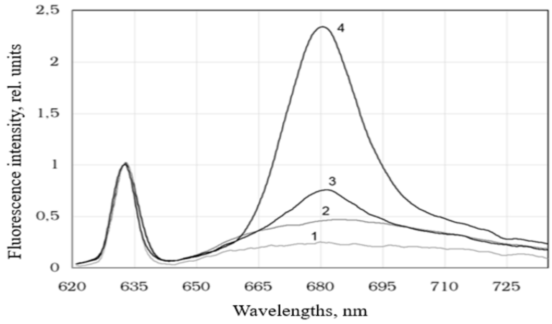
The autofluorescence spectra for enamel samples were analyzed before and after applying the model mixture. Initially, low levels of autofluorescence were observed from the enamel surface, indicating minimal microbial activity. However, after applying the model mixture containing silver nanoparticles (Ag NPs) and polyvinylpyrrolidone (PVP), a noticeable increase in fluorescence was observed. The fluorescence spectra were collected from different areas of the tooth, including the middle of the vestibular surface, the fissure area, and the cervical region of the tooth crown. The fissure and cervical areas showed the highest intensity of fluorescence, which can be attributed to their anatomical structure and susceptibility to plaque buildup.

In the pre-application phase, the fluorescence of surface microorganisms was weak. Once the model mixture was applied, the fluorescence of Ag molecules became apparent, indicating that these molecules were activated by the pathogenic microorganisms and some PVP. This enhancement in fluorescence served as a marker for detecting micro-damages in the enamel and the accumulation of microbial clusters on the surface of the tooth.

Statistical analysis of the data

For the first experimental group, the average fluorescence intensity was recorded as 1.375, with a standard deviation of 0.198 and a variance of 0.236. The second experimental group showed an average value of 2.127, with a standard deviation of 0.290 and a variance of 2.947.

The statistical analysis performed using the student's t-test confirmed that the difference between the two groups was statistically significant ($P < 0.05$). This result highlighted the importance of PVP in enhancing the activation of Ag NPs, facilitating a quicker and more effective response for enamel diagnostics.



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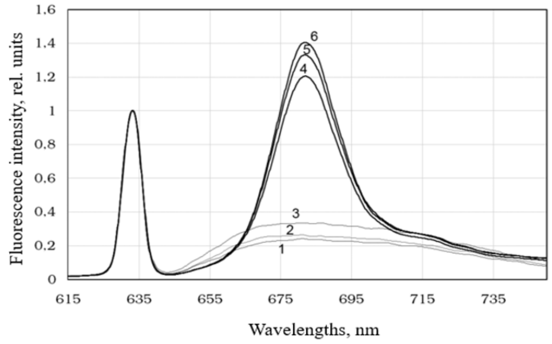

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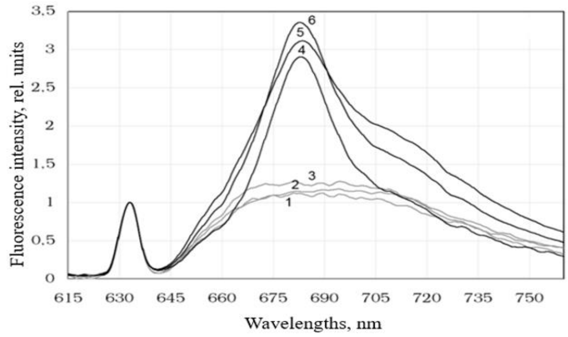

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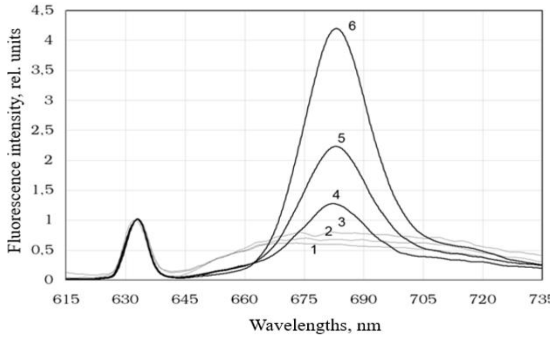

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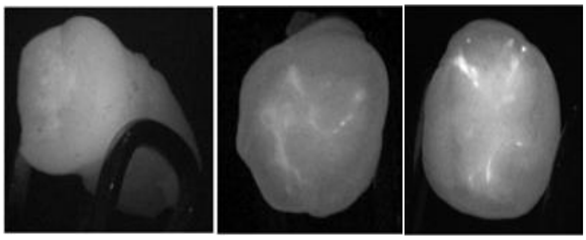
Figure 1. Fluorescence spectra; a) Ag NPs fluorescence spectra after the application of the Ag NPs colloid (group 1) and the model mixture (group 2), b-d) fluorescence spectra of tooth enamel after the application of the model mixture to various regions: the cutting edge of the tooth crown (b), the middle of the vestibular surface of the tooth crown (c), and the cervical part of the tooth crown (d), respectively; note: the numbers correspond to the spectral groups, with further details provided in the article text.



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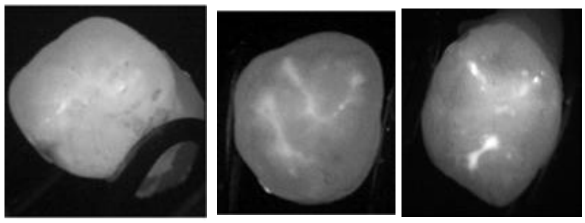

b)

Figure 2. Video fluorescent images of tooth enamel surface; a) before applying the model mixture, b) three minutes after applying the model mixture to the cutting edge of the tooth crown, the middle of the vestibular surface of the tooth crown, and the cervical part of the tooth crown, respectively.

The study led to the creation of a new laser-induced fluorescence diagnostic technique designed for detecting enamel micro-damage, using a specialized mixture containing silver nanoparticles (Ag NPs) and polyvinylpyrrolidone (PVP).

Our experiments revealed that certain areas of the tooth enamel, particularly the fissure and cervical regions, provided the most valuable data when subjected to laser-induced fluorescence analysis. The fissure region is prone to gathering pathogenic microorganisms due to its structure and is more vulnerable to microcracks during various activities like chewing. Similarly, the cervical part of the tooth is critical because it is where latent plaque and tartar begin to form. These areas, therefore, offer the best locations for spectral analysis [22-26].

For optimal results, the laser should operate within a power range of 2 to 5 mW, with a wavelength of 633 nm. In combination with the model mixture containing Ag NPs at a concentration of 12 mg/l, the optimal time for conducting the enamel diagnostic is approximately 3 minutes after the mixture is applied. This time frame ensures both high-quality data and efficient procedure duration [27-31].

In conclusion, the findings suggest that the Ag NPs and PVP-based model mixture holds promise for practical use in clinical settings to conduct laser-induced fluorescence diagnostics on tooth enamel, with only slight adjustments needed from the experimental conditions tested in the lab.

Conclusion

The technique developed for laser-induced fluorescence to detect enamel micro-damage, using a specialized mixture containing Ag NPs, provides a reliable means of measuring the fluorescence intensity on the enamel's surface. This fluorescence increase is likely an indication of the presence of pathogenic bacteria or microcracks in the enamel structure.

By capturing video fluorescence images of the enamel before and after the application of the model mixture, it is possible to visually identify areas with the highest fluorescence intensity. These regions are crucial for identifying the early stages of plaque accumulation, tartar formation, or the emergence of enamel microcracks.

The experimental outcomes confirm that the formulation of the diagnostic mixture with Ag NPs is appropriately chosen, facilitating efficient fluorescence detection of tooth enamel within just three minutes following its application.

Statistical analysis of the two experimental groups demonstrates the effectiveness of adding PVP as a secondary activator to Ag NPs. This addition enhances the diagnostic process by increasing fluorescence response and reducing the overall time required for the procedure.

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Conflict of Interest: None

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Ethics Statement: None

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