Original Article

FLUORESCENT DIAGNOSTICS OF MICROSCOPIC DAMAGE TO TOOTH ENAMEL USING AN INNOVATIVE MIXTURE OF SILVER NANOPARTICLES

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ABSTRACT

According to the World Health Organization, dental caries affects approximately 60-90% of schoolchildren and almost 100% of adults worldwide. An urgent task today is the early diagnosis of micro-damage to the tooth enamel. For this purpose, laser-induced fluorescence diagnostics can be used, which allows the identification of caries by analyzing the intrinsic fluorescence of microorganisms. In the framework of this work, a method of laser-induced fluorescence diagnostics of micro-damage to enamel was developed using a special model mixture with silver and polyvinylpyrrolidone nanoparticles. 63 samples of human teeth were examined, and removed for various clinical indications. During the experiments, it was shown that the most informative areas for laser-induced fluorescence diagnostics of tooth enamel are the fissure area and the cervical part of the tooth. In the area of fissures, pathogenic microflora usually accumulates the most due to its anatomical structure, and it is also most susceptible to the appearance of microcracks during the chewing process and other reasons. The cervical part of the tooth is an informative area for spectral analysis because the beginning of the formation of latent plaque and tartar occurs in this area. The optimal time for the diagnosis of enamel is 3 minutes after applying the model mixture. Based on the experimental results obtained ex vivo, it can be concluded that a model mixture with silver nanoparticles and polyvinylpyrrolidone can be used for laser-induced fluorescence diagnostics of tooth enamel in clinical conditions, with minor adjustments to the experimental conditions.

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

Introduction

The development of the carious process in the initial stages can be prevented by early diagnosis of micro-damage to the enamel of teeth and hidden foci of accumulation of bacterial microflora, which can lead to the formation of plaque or calculus [1, 2]. According to the World Health Organization, dental caries affects approximately 60-90% of schoolchildren and almost 100% of adults worldwide [3-5]. Early diagnosis of the disease and timely treatment allow you to maintain dental health for many years, which significantly improves the quality of life. New techniques are being developed around the world to effectively diagnose initial caries. For this purpose, laser radiation and optical methods are used, including the use of the Raman scattering effect, optical coherence tomography, light scattering spectroscopy, and fluorescence spectroscopy [6-9].

Laser-induced fluorescence diagnostics (FD) of enamel can be used to detect caries by analyzing the intrinsic fluorescence of microorganisms [10, 11]. However, the use of this method may be limited by a small amount of microflora in the early stages of the carious process and minor damage to the enamel.

Laser-induced fluorescence diagnostics using the "red" wavelength of radiation is possible only with a significant amount of porphyrins, which are a product of bacterial vital activity [12].

The purpose of this work is to develop a method for laser-induced fluorescence diagnostics of enamel micro-injuries using a special model mixture with silver nanoparticles (Ag NPs). The expected diagnostic time is 3-5 minutes after applying the mixture to the enamel surface. To achieve this goal, a model mixture consisting of Ag NPs and polyvinylpyrrolidone (PVP) was prepared.

Materials and Methods

To study the interaction of the drug Ag NPs with tooth enamel, samples of human teeth were used, and removed for various clinical indications (63 samples in total): chronic periodontitis, the third molar, orthodontic, and other causes. The age of the patients ranged from 19 to 52 years. To conduct experiments, biological samples were divided into

two groups in random order. Group 1 included samples for studying the interaction of the Ag NPs colloid with the surface of tooth enamel (12 pieces). Group 2 included 41 samples to study the interaction of the model mixture (Ag NPs and PVP) with the surface for the potential detection of micro-damage to the enamel of teeth.

When preparing a model mixture with Ag NPs and PVP, the colloid concentration was 12 mg/l, and the size of Ag NPs was 20-140 nm. The model mixture is a paste-like substance containing Ag NPs colloid at a concentration of 12 mg/l, PVP 1.2% by volume, and excipients that are commonly used for the preparation of toothpaste (methylparaben, carbopol, carboxymethylcellulose, titanium dioxide, sodium phosphate, sodium saccharin, and sorbitol).

The LSW-10 helium-neon laser (Jiangxi Liansheng Technology Co., Ltd, China), RIGOL RSA5065-TG laser electron spectroanalyzer (Rigol, China), and a fiber-optic probe were used for laser spectroscopic study of the interaction of the Ag NPs colloid as part of the model mixture.

To conduct a study on the interaction of the Ag NPs colloid and a model mixture with Ag NPs with the surface of tooth enamel to identify micro-injuries and foci of accumulation of pathogenic microflora, an algorithm for experimenting was developed, which includes several stages.

At the first preparatory stage, before the start of experimental studies, the RIGOL measuring system was calibrated in terms of wavelengths and signal intensity using standard samples. Images of the tooth enamel surface were recorded using a video fluorescence unit and the enamel autofluorescence spectra in various areas were measured using the RIGOL system before applying a colloid or a model mixture to the tooth enamel surface. During the experiment, several positions of the fiber-optic probe on the surface of the tooth enamel were selected: position 1 – the cutting edge of the tooth crown; position 2 – the middle of the vestibular surface of the tooth crown; position 3 – the cervical part of the tooth crown.

All measurements were carried out with light contact of the measuring probe with the surface of the fabric. The fiber-optic probe was positioned at a small angle (approximately 20°) to the surface under study, which made it possible to level the specular-reflected and diffraction-scattered components of the laser radiation. After each series of measurements of the tooth sample, the end of the fiber was wiped with 70% alcohol.

In the third stage, an Ag NPs colloid or a model mixture with Ag NPs and PVP was applied to the entire surface of the enamel and removed with running water after 3 minutes.

The fourth step was to obtain video fluorescent images of the tooth enamel surface and fluorescence spectra of Ag molecules after applying the formulations in the same mode and without changing the settings of measuring instruments. Thus, from each selected area of the tooth, a series of enamel autofluorescence spectra were obtained before and Ag NPs fluorescence spectra after applying an Ag NPs colloid or a model mixture with Ag NPs and PVP. Fluorescence spectra obtained from carious cavities and other visible lesions were separately labeled in the data array during the experiment.

Experimental studies were conducted using two groups of biological samples. In the first group, the interaction of a colloidal solution of Ag NPs (10 mg/l) with the surface microflora of tooth enamel was studied. In the second group, the interaction of a model mixture (with Ag NPs and PVP) with the surface of tooth enamel was studied. The autofluorescence spectra of enamel were measured before and after the application of the corresponding drug in each group.

All spectroscopic data were normalized for exposure time. Based on a series of spectra from three tooth regions, the average value of the enamel autofluorescence spectrum before and Ag NPs fluorescence after applying an Ag NPs colloid or a model mixture to the tooth enamel was calculated for each biological sample.

Since the fluorescence intensity is determined not only by the properties of tissues, but also by technical characteristics, in particular the intensity of laser radiation [13], it was not the absolute values of the autofluorescence area that were analyzed, but its ratio to the area under the laser peak.

For each sample, the value of the enamel autofluorescence coefficient was calculated as the ratio of the areas under the enamel fluorescence spectrum to the area under the laser peak. The diagnostic contrast coefficients of each sample from both groups were used for statistical processing of experimental results conducted using the computer program “Statistics 6.0".

An array of experimental data was used to calculate the mean, mean square deviation, and variance. Using these values, an F-test was calculated, which allows us to determine whether two samples have different variances, and a Student's t-confidence criterion, followed by comparing it with a tabular value for a given significance level p at a confidence interval of 95%-the probability of an error-free forecast.

Results and Discussion

Laser spectroscopic study of the interaction of Ag NPs colloid with tooth enamel ex vivo

The results of studies to identify the nature of the interaction of the colloidal Ag NPs solution with the surface of tooth enamel ex vivo showed that after 3 minutes, low fluorescence is observed due to slight activation of surface Ag NPs molecules, and a noticeable increase in fluorescence of Ag molecule occurs 1 hour after application of the colloidal solution. This suggests that it takes some time for the activation of Ag NPs surface molecules by pathogenic microflora [14, 15].

Figure 1a shows the fluorescence spectra of Ag NPs after applying Ag to the enamel surface, obtained 3 minutes after application. To carry out a statistical analysis of the results for this experimental group, diagnostic contrast coefficients were calculated for each sample (3 minutes after applying the colloid), which were used in further calculations.

Laser spectroscopic study of the interaction of a model mixture with the surface microflora of tooth enamel ex vivo

To reduce the time of laser-induced FD enamel to 3 minutes, PVP is added to the colloid as an additional Ag NPs activator. The presence of some molecules in the free state and surface molecules in the semi-free state makes them capable of relatively rapid fluorescence, which will reduce the time of laser-induced FD enamel.

The interaction of a model mixture with Ag NPs and PVP with the surface of tooth enamel was experimentally studied using RIGOL installations and a video fluorescent camera.

Figure 1a shows the fluorescence spectra of Ag NPs after applying the Ag NPs colloid (for the first group) and the model mixture (for the second group).

It can be seen from the graphs that only autofluorescence of enamel is present before applying the experimental compositions. After applying an Ag NPs colloid or a model mixture to the surface of the tooth enamel, Ag NPs fluorescence appears, and the intensity of Ag fluorescence after applying the model mixture is almost 3 times higher than after applying the colloid.

Studies of the enamel surface were also carried out using a video fluorescent camera before and after applying the model mixture. **Figure 2** shows video fluorescent images of the enamel surface of the teeth before applying the model mixture and 3 minutes after.

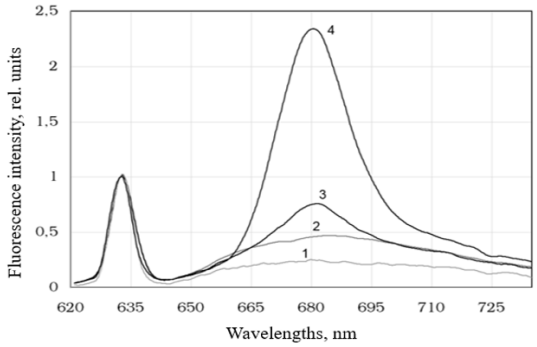
Figures 1b-1d displays the corresponding autofluorescence spectra of the enamel before applying the model mixture (spectrum group 1-3) obtained from various areas of the teeth. The fluorescence spectra of Ag NPs (spectrum group 4-6) were obtained after applying the model mixture to the surface of the tooth enamel. Spectra 1 and 4 were obtained by placing the fiber optic probe in position 2 (the middle of the vestibular surface of the tooth crown), spectra 2 and 5 in position 1 (the area of the tooth fissure) and spectra 3 and 6 in position 3 (the cervical region of the tooth crown). It can be seen from the graphs that the areas of fissure and the cervical part of the tooth have the highest fluorescence intensity, due to their anatomical structure and the nature of the formation of latent plaque and tartar.

As can be seen from the presented figures, autofluorescence of the surface microflora is poorly expressed before applying the model mixture. After applying the model mixture, the fluorescence of Ag molecules appears on the enamel surface, which was activated by pathogenic microflora and in a small amount of PVP. Thus, by the appearance and enhancement of the fluorescence of Ag molecules, it is possible to judge possible micro-damage to the enamel and foci of accumulation of pathogenic microflora on the surface [16, 17].

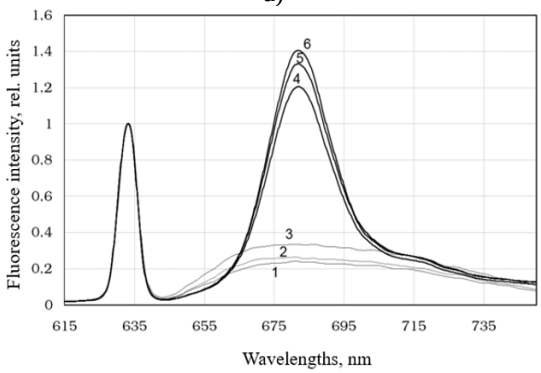
Results of statistical data processing

For the first experimental group, an average value of 1.375, a mean square deviation of 0.198, and a variance of 0.236 were calculated. For the second group, these values are respectively 2.127; 0.290, and 2.947.

Statistical processing of two groups of measurements on human teeth ex vivo (using a colloidal solution of Ag NPs and a model mixture with Ag NPs) using the Student's t-test of reliability showed that the calculation results were statistically significant ($p<0.05$) and the use of PVP a for additional activation of Ag NPs.



a)



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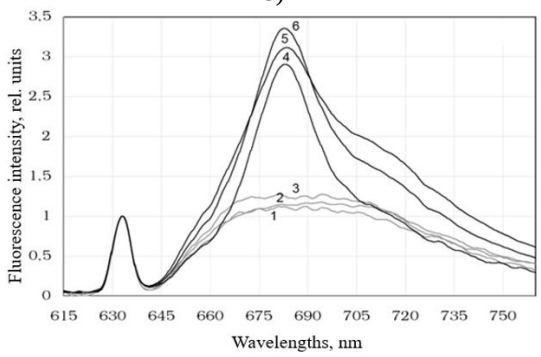

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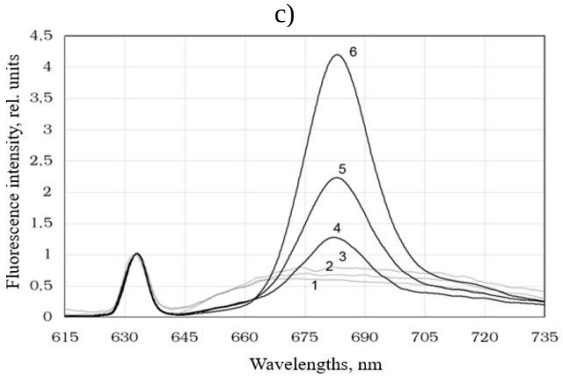
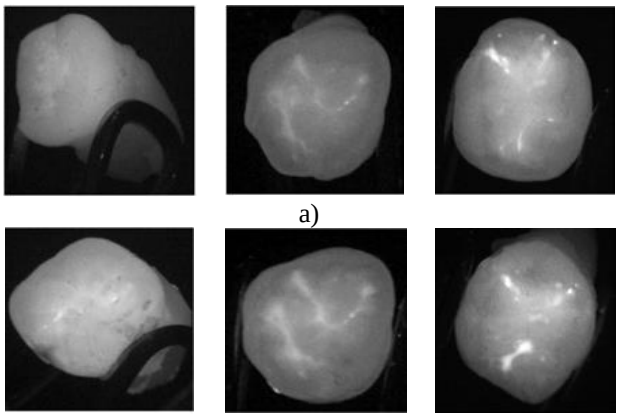


d)

Figure 1. Fluorescence spectra: a) Ag NPs after applying the Ag NPs colloid (group 1) and the model mixture (group 2); b-d) tooth enamel, when applying the model mixture to the cutting edge of the tooth crown, to the middle of the vestibular surface of the tooth crown, to the cervical part of the tooth crown, respectively. Note: the numbers show the groups of spectra, the description is given in the text of the article



a)

b)

Figure 2. Video fluorescent images of the tooth enamel surface before applying the model mixture (a) and 3 minutes after (b). when applying the model mixture to the cutting edge of the tooth crown, to the middle of the vestibular surface of the tooth crown, to the cervical part of the tooth crown, respectively.

Based on the experimental results obtained, a method of laser-induced fluorescence diagnostics of tooth enamel was developed using a model mixture containing Ag NPs and PVP to detect micro-damage to the enamel surface.

During the experiments, it was shown that the most informative areas for conducting laser-induced FD tooth enamel are the fissure area and the cervical part of the tooth [18-20]. The fissure area usually accumulates the most pathogenic microflora due to its anatomical structure, and it is also most susceptible to the appearance of microcracks during the chewing process and other causes [21, 22]. The cervical part of the tooth is an informative area for spectral analysis because the beginning of the formation of latent plaque and tartar occurs in this area [23-25]. The optimal laser power (at a wavelength of 633 nm) for conducting laser-induced FD enamel is 2-5 MW at an Ag NPs concentration of 12 mg/l in the model mixture. The optimal time for the diagnosis of enamel is 3 minutes after applying the model mixture, including in terms of the quality of the data obtained and the duration of the entire procedure as a whole.

Based on the experimental results obtained ex vivo, it can be concluded that the model mixture with Ag NPs and PVP can be used to perform laser-induced FD tooth enamel in clinical conditions, with minor adjustments to the experimental conditions.

Conclusion

The developed method of laser-induced fluorescence diagnostics of potential micro-injuries of tooth enamel using a model mixture with Ag NPs allows us to quantify the intensity of Ag NPs fluorescence on the enamel surface, the growth of which may be associated with the presence of pathogenic microflora on the enamel surface or in microcracks.

Video fluorescence images of the tooth enamel surface before and after applying the model mixture make it possible to qualitatively assess the places of the highest intensity of Ag NPs fluorescence, which will help in identifying potential areas of formation of latent plaque, tartar, or microcracks.

Experimental results have shown that the composition of the diagnostic model mixture with Ag NPs is optimally selected and allows for FD of tooth enamel in 3 minutes after application.

Statistical processing of two groups of measurements showed the effectiveness of using PVP as an additional Ag NPs activator to increase the efficiency of laser-induced FD to detect micro-damage to the enamel and reduce the time of the procedure as a whole.

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Conflict of interest: None

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Ethics statement: All patients and their attending physicians have given voluntary, informed consent to the use of extracted teeth as a research object.

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