ANALYSIS OF EFFICIENCY OF PHOTODYNAMIC TEETH BLEACHING WITH THE USE OF PHOTOSENSITIZER CHLORINE E_6

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Abstract

Teeth whitening is one of the most sought-after procedures in aesthetic dentistry. Discolorites that are difficult to whiten, caused by dentin changes or enamel defects, can be eliminated by oxidizing the chromogens with chemical agents that penetrate to the enamel and dentin. In recent years, the method of photodynamic bleaching (PDB) is considered to be minimally invasive. It does not use hydrogen peroxide that leads to increased sensitivity of teeth, and is relatively effective over time. A convenient solution for PDB would be to use chlorin e_6 as a photosensitizer, which has a high quantum yield of singlet oxygen generation, low phototoxicity, rapid elimination, on the one hand, and photobleaching capability, on the other. This paper presents quantitative data on the study of the effectiveness of PDB with chlorine e_6: color change for 100 teeth after the procedure, chlorine e_6 penetration into the tooth tissues, evaluation of the interstitial efficiency of the generation of singlet oxygen and photobleaching of chlorine e_6 during laser exposure. It has been statistically established that for one PDB procedure, the tooth color saturation (*C*) varies on average by 0.5 tones on the VITA scale, and the lightness of color (*L*) in some cases increases by more than 10 units.

Keywords: photodynamic teeth bleaching, photosensitizer, chlorin e_6, singlet oxygen generation.

For citations: Korshunova A.V., Makarov V.I., Ryabova A.V., Romanishkin I.D., Zorina O.A., Krechina E.K., Ponomarev G.V. Analysis of photodynamic teeth bleaching efficiency with the use of photosensitizer chlorine e_6, *Biomedical photonics*, 2019, vol. 8, no. 3, pp. 19–28. (in Russian) doi: 10.24931/2413–9432–2019–8–19–28.

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АНАЛИЗ ЭФФЕКТИВНОСТИ ФОТОДИНАМИЧЕСКОГО ОТБЕЛИВАНИЯ ЗУБОВ С ИСПОЛЬЗОВАНИЕМ ФОТОСЕНСИБИЛИЗАТОРА ХЛОРИН E_6

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Резюме

Отбеливание зубов является одной из самых востребованных процедур в эстетической стоматологии. Трудно поддающиеся отбеливанию дисколориты, вызванные изменениями дентина или дефектами эмали, могут быть устранены путем окисления хромогенов с помощью химических агентов, проникающих в эмаль и дентин. В последние годы фотодинамическое отбеливание зубов (ФДОЗ) рассматривается как минимально инвазивный и относительно эффективный по времени метод, при котором не используется перекись водорода, применение которой приводит к повышенной чувствительности зубов. Для ФДОЗ может использоваться фотосенсибилизатор (ФС) хлорин e_6, обладающий высоким квантовым выходом генерации синглетного кислорода, низкой фототоксичностью, быстрым выведением, с одной стороны, и способностью к фотообесцвечиванию, с другой. В настоящей работе

представлены количественные данные исследования эффективности ФДОЗ с хлорином e_6: изменение цвета для 100 зубов после процедуры, оценка проникновения ФС в ткани зуба, оценка внутритканевой эффективности генерации синглетного кислорода и интенсивности фотообесцвечивания ФС при лазерном воздействии. Статистически установлено, что за одну процедуру ФДОЗ насыщенность цвета зубов (*C*) изменяется в среднем на 0,5 тона по шкале VITA, а светлота цвета (*L*) в отдельных случаях повышается более чем на 10 единиц.

Ключевые слова: фотодинамическое отбеливание зубов, фотосенсибилизатор, хлорин e_6, генерация синглетного кислорода

Для цитирования: Коршунова А.В., Макаров В.И., Рябова А.В., Романишкин И.Д., Зорина О.А., Кречина Е.К., Пономарев Г.В. Анализ эффективности фотодинамического отбеливания зубов с использованием фотосенсибилизатора хлорин e_6 // Biomedical photonics. – 2019. – Т. 8, № 3. – С. 19–28. doi: 10.24931/2413–9432–2019–8–3–19–28.

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Introduction

Every year, cosmetic dentistry is becoming more and more popular among the population. One of the main procedures to correct the aesthetic imperfections of teeth is their whitening [1]. Many types of discolorites affect the appearance of teeth. The causes of discoloration vary, as does the rate at which they are removed [2]. The discoloration of the teeth may be external or internal.

External stains usually occur as a result of the accumulation of chromatogenic substances on the external surface of the tooth. These stains are localized mainly in the acquired dental film and are generated either by the Millard reaction between sugars and amino acids (including chemical rearrangements and reactions between sugars and amino acids) or are acquired as a result of the retention of exogenous chromophores [3]. Chemical analysis of the stains caused by chromatogenic food demonstrates the presence of furfurals and their derivatives. The majority of external tooth stains can be removed using standard preventative procedures, of which there are a large number today. One such method is the use of pastes with a whitening effect and other abrasive methods [4]. However, over time, such spots darken and become more resistant, but, as a rule, they can still be bleached [5].

Internal stains are usually caused by deeper stains or defects in enamel. They are a consequence of aging, as well as the consumption of chromatogenic foods and drinks, smoking, taking tetracycline antibiotics, excessive fluoride taking, severe jaundice in infancy, porphyria, microcracks in enamel, physiological and pathological abrasion of teeth, tooth decay and restorations. As a result of aging and thinning of the tooth enamel, the underlying dentin layers tend to darken due to the formation of secondary dentin, which is darker and more opaque than the original, primary dentin. The combination of these processes leads to the darkening of the teeth.

Excess fluoride in drinking water above 1–2 ppm can cause metabolic changes in ameloblasts, which leads to defects in the matrix and improper calcification of the teeth [6].

Color changes when taking medication can occur both before and after the complete formation of the tooth. Tetracycline is incorporated into dentin during tooth calcification, probably by chelating with calcium to form tetracycline orthophosphate, which causes a discoloration. Also, internal stains are associated with hereditary conditions (for example, imperfect amelogenesis and imperfect dentinogenesis) [7]. Blood entering the dentinal tubules and metals released from dental restoration materials also cause discoloration of teeth.

Internal tooth pigmentation cannot be removed using regular preventative procedures. Nevertheless, it can be eliminated by bleaching with the help of chemical agents penetrating enamel and dentin to oxidize chromogenes [8]. Stains caused by aging, genetics, smoking or coffee respond better to whitening [9], blue-gray stains due to tetracycline — worse [10], and spots of brown fluorosis are moderately sensitive [11].

Techniques for chemical whitening, in which the color of enamel and dentin changes from dark to light due to the ability of active chemical components to pass through enamel and dentin and penetrate all parts of the tooth, causing oxidative breakdown of colored pigments are actively being developed [12]. However, this effect also has a negative effect on tooth enamel, pulp and periodontal tissue [13, 14].

To reduce bleaching time in the clinic, various methods are used to accelerate the decomposition of hydrogen peroxide, including chemical (alkaline pH), physicochemical (photooxidation), and physical (heating) methods [15, 16]. Hydrogen peroxide is optically transparent in the visible spectral range; however, it can absorb ultraviolet, medium infrared and far-infrared light, which leads to its decomposition. When bleaching with intense light sources, the addition of various dyes to the whitening gels leads to an improvement in the absorption of

light in the gel and, as a result, to a decrease in the heating of the tooth pulp. In addition to heating the gel (photothermic effect) [17], dyes can also cause photochemical reactions [18].

The sources of coherent and incoherent radiation used to catalyze the hydrogen peroxide bleaching process include quartz tungsten halogen lamps, plasma arc lamps, mercury lamps, light-emitting diodes (LEDs) and lasers with various wavelengths [19–21].

In general, pigments that give the color of a tooth are cyclic molecules with π-π conjugated electronic bonds. During bleaching, π-π bonds are destroyed due to oxidation (that is, loss of electrons) or other chemical reactions, and the molecules take the form of a broken ring, which leads to a loss of their light-absorbing properties. Although hydrogen peroxide is most widely used for teeth whitening, there are a large number of other oxidizing agents (O_2, $HO_2^{\cdot}$, NaClO, O_3, $HO^{\cdot}$). When choosing agents for whitening teeth, their oxidizing ability should be taken into account, that is, they must generate reactive oxygen species that can diffuse the easiest into dentin, and be non-aggressive and non-toxic [22]. Singlet oxygen ($^1O_2$) is a very strong oxidizing agent capable of decomposing organic molecules, however, due to its high reactivity, it cannot be stored, and its use is possible only when generated *in situ* as necessary [23].

The method of photodynamic therapy (PDT), based on the generation of singlet oxygen by photosensitizers (PS) when they are irradiated with specific wavelengths, was initially presented in dentistry as an antimicrobial option — it was used to disinfect antibiotic-resistant microorganisms without causing resistance [24]. Now PDT is actively used in the treatment of dental caries and its complications, and is also widely used in periodontics, implantology, pathologies of the oral mucosa and maxillofacial surgery [25, 26]. The most commonly used photosensitizers in dentistry are phenothiazine-based dyes, such as toluidine blue, methylene blue and malachite green, they are photostable, and a slight change in tooth color can serve as a side effect of their use [27].

In recent years, the method of photodynamic bleaching (PDB) has been considered as minimally invasive and relatively effective in time, its use for bleaching one of the most difficult groups of tooth discolorites caused by taking tetracycline antibiotics is especially important. The most effective for PDB is the use of a potassium titanyl phosphate crystal laser ($KTiOPO_4$, KTP laser) in combination with a sulforhodamine B photosensitizer and a high concentration of hydrogen peroxide (Smartbleach® gel, Smartbleach International, Belgium) [28]. Moreover, PDB allows achieving a greater effect in a single procedure than can be expected from several months of application of bleaching mouth guards [29].

A convenient solution for PDB without the use of hydrogen peroxide, which leads to increased tooth sensitivity, can be the use of chlorin e_6 photosensitizer, which has a high quantum yield of singlet oxygen generation, low phototoxicity, rapid elimination, on the one hand, and the ability to photobleach, on the other [30]. In our earlier study, we studied the dynamics of chlorin e_6 accumulation in a 1% Geleofor gel compound in tooth tissues depending on the time of application [31]. To evaluate and select the optimal effectiveness of tooth PDB in this work, in addition to clinical observation of discoloration using a VITA spectrophotometer, quantitative characteristics of the PS penetration into the tissues of the extracted teeth were determined, interstitial efficiency of singlet oxygen generation and photobleaching of chlorin e_6 in the PDF were evaluated.

Materials and methods

Photosensitizer

Chlorin e_6, a natural derivative of chlorophyll, was used as a PS in the study as part of a 1% Geleofor gel (OOO "Lazer-Medcentr", Russia).

Tooth samples for the study

For laboratory studies, teeth of the frontal group, removed according to periodontal and surgical indications, were used. Before the research, the enamel surface was thoroughly cleaned with a brush and Detatrine-Z paste (Septodont, France). The teeth were stored in distilled water until the study. No more than two hours passed from the moment of tooth extraction to the start of the study. To assess the accumulation of chlorin e_6, both the extracted teeth and the cuts of the extracted teeth of the frontal group were obtained. In total, 100 extracted frontal teeth were used in the study. The distribution of the examined teeth shades on the Vita scale was as follows: 78 teeth - A shades (reddish-brown) and 22 teeth - B shades (yellowish-red).

To determine the optimal time for PS application, during which it penetrates the tooth tissue to the entire dentin depth necessary for effective PDB, a gel containing PS was applied to the entire vestibular surface of the extracted tooth using a dental brush for 1, 5, 10, 20 minutes. Next, the PS was washed off with running water and a tooth was cut along the vertical axis into thin cuts using a disk-shaped diamond dental bur.

Study of the PS distribution in the thickness of the tooth using laser scanning confocal microscopy

The interstitial distribution of chlorin e_6 after the gel application on the enamel was studied through laser scanning confocal microscopy using LSM-710 microscope (Carl Zeiss, Germany). To obtain the images, we used a Plan-Apochromat lens with a magnification of 10x (0.3 aperture). Thin sections of teeth were placed on 0.17 mm thick coverslips and observed in the plane of cut. To excite autofluorescence of tooth tissues and fluorescence of chlorin e_6, an argon laser (Lasos Laser GmbH, Germany) with a wavelength of 488 nm was used; autofluorescence

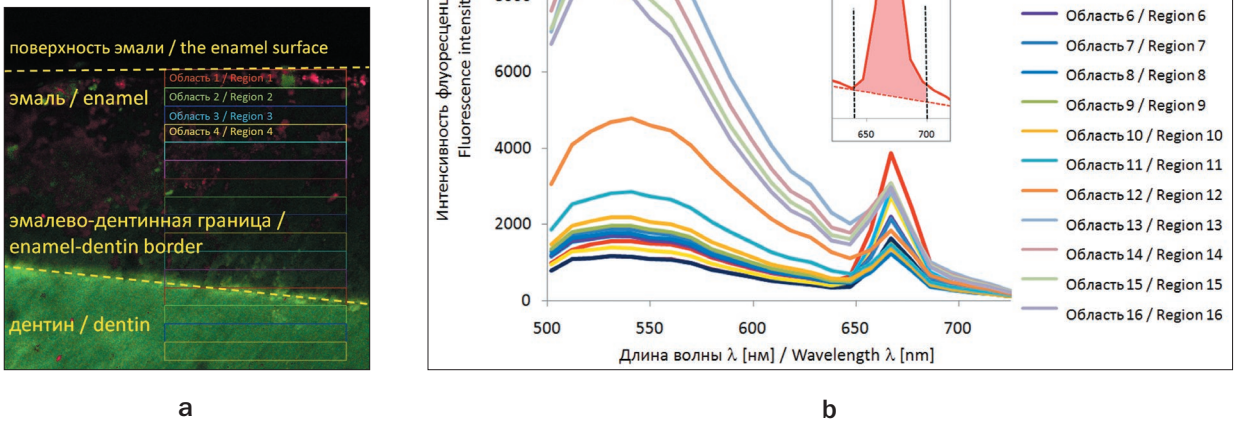

Рис. 1. Спектрально-разрешенное флуоресцентное изображение шлифа зуба, на эмаль которого был апплицирован ФС в течение 1 мин. Изображение получено при возбуждении 488 нм:
a – схема выделения прямоугольных областей на микроскопическом флуоресцентном изображении шлифа зуба для количественной оценки диффузии ФС;
b – спектры флуоресценции с выделенных прямоугольных областей шлифа зуба с шагом 40 мкм вглубь эмали от поверхности. На вкладке закрашена область под пиком флуоресценции хлорина e_6, которую использовали для построения зависимости интенсивности флуоресценции хлорина e_6 в тканях зуба на глубине от поверхности эмали

Fig. 1. Spectral-resolved fluorescent image of tooth section after 1 minute of photosensitizer application on the enamel. Image taken at 488 nm excitation:
a – a scheme for the selection of rectangular areas on a microscopic fluorescent image on the tooth section for the quantitative assessment of the photosensitizer's diffusion;
b – fluorescence spectra from selected rectangular areas of tooth section with 40 microns step into the depth of the enamel from the surface. On the inset an area under the chlorin e_6 fluorescence peak, which was used to plot the chlorin e_6 fluorescence intensity in the tooth tissue on the depth from the enamel surface, is highlighted

and fluorescence of PS were detected with 10 nm spectral resolution in the range of 500–750 nm. The result was a spectrally resolved fluorescence image of a tooth cut. Statistical and spectral analysis was carried out using ZEN software (Carl Zeiss, Germany). To quantify the PS diffusion deep into the tooth, the image of the tooth section was separated into rectangular regions at different depths from the enamel surface with a height of 40 μm and a width of 500 μm (Fig. 1*a*). Then we used the integrated fluorescence intensity of PS, averaged over the area of these rectangular regions (Fig. 1*b*). The integrated fluorescence intensity of PS was calculated from the total fluorescence spectrum by subtracting the "shoulder" of autofluorescence in the region of 640–700 nm and integrating the obtained signal over wavelengths (tab in Fig. 1*b*).

Study of the generation of singlet oxygen in the depth of the tooth

To detect the generation of singlet oxygen ($^1O_2$), we used Singlet Oxygen Sensor Green reagent (SOSG, Molecular Probes®, USA), highly selective towards $^1O_2$ and not reacting to other reactive oxygen species, such as hydroxyl radical and superoxide anion [32]. SOSG has weak blue fluorescence with excitation maxima at 372 nm and 393 nm and emission maxima at 395 nm and 416 nm. In the presence of $^1O_2$, SOSG begins to fluoresce in the green range with excitation and emission maxima of 504 nm and 525 nm, respectively, which are easy to detect. Sections of teeth, on whose enamel PS was applied for a certain time, in saline with the addition of SOSG reagent were subjected to laser irradiation with a wavelength of 633 nm directly by a scanning laser (that is, PDB was carried out in the cut plane).

The power density produced by the scanning laser beam emerging from the objective lens in the object plane was calculated as follows. The spatial resolution of the confocal microscope is determined by the size of the illuminated spot limited by diffraction. The size of the focusing spot, assuming uniform illumination, is a function of the excitation

wavelength (λ_{EX}) and the numerical aperture (NA) of the objective:

$$S_{spotsize} = (1{,}22\lambda_{EX})/NA$$

Therefore, for a wavelength of 633 nm and a 10x lens with an aperture of 0.3, the spot size was 2.6 μm. Accordingly, the area of a circle with such a diameter is $5.2*10^{-8}$ cm^2, and the power density for 5 mW of laser output power is 96 kW/cm^2. To obtain one image, the laser scanned twice at a speed of 1.27 μs/pixel. Thus, during the acquisition of a single image, the radiation dose was 0.244 J/cm^2, and the time-averaged dose for a 1064 × 1064 pixels image was 170 mW/cm^2, which is comparable with the radiation power density of 100 mW/cm^2 in clinical conditions.

During a series of scans, fluorescent signals from PS and SOSG were recorded. By isolating the rectangular areas in the image of the tooth thin section at different depths from the enamel surface with a rectangle height of 40 μm, we obtained the time dependences of the photosensitizer bleaching and SOSG fluorescence rise.

The study of the PS concentration on the fluorescence spectra depending on the time of PS application and the dose of laser irradiation during PDB

The concentration of PS in tooth tissues was determined by measuring fluorescence intensity using LESA-01-Biospec fiber-optic spectrometer (OOO BIOSPEC, Russia). Collecting diffuse scattered light from tooth tissues when excited by laser radiation of 633 nm by a fiber optic catheter, fluorescence of PS was recorded in contact with the enamel and dentin surfaces on the tooth cut.

A study of the effect of the light dose on photobleaching of the PS was carried out using the Harmony LED device (OOO Laser-Medcentr, Russia) with a wavelength of λ = 400±10 nm. The laser power density was 100 mW/cm^2.

The total light dose was 180 J/cm^2, which corresponds to 30 minutes of exposure. After 10, 20, and 30 min from the onset of light exposure, the fluorescence spectra of PS were measured to evaluate the PS remaining in the tooth tissues, which was being bleached during the PDB.

Clinical evaluation of tooth color after PDB

The assessment of the color change of the frontal group of teeth before and after the PDB was determined using VITA Easyshade® V spectrophotometer (VITA Zahnfabrik, Germany). To determine the tooth color, the surface of the studied crown part of the tooth was cleaned and measurements were taken in the averaging mode before and after the PDB. The measuring tip was tightly applied at right angles to the surface of the tooth enamel covering the dentin. The tooth color characteristics such as lightness of color (L) and color saturation (intensity or purity) (C) were recorded. The lightness of color in comparison with the number of gray tones is determined in the range from black (L = 0) to white (L = 100). Color saturation (C) is the difference between the color and the gray tone of the same lightness, measured as the distance from the neutral axis. Based on the results, 2D histograms of saturation (C) and lightness (L) of the color of the teeth before and after the PDF were plotted. The results were processed in Python 3 by the method of kernel density estimation using matplotlib and seaborn graphic packages [33, 34].

Results and discussion

According to the fluorescence microscopy, the dependences of the PS fluorescence on the depth from the

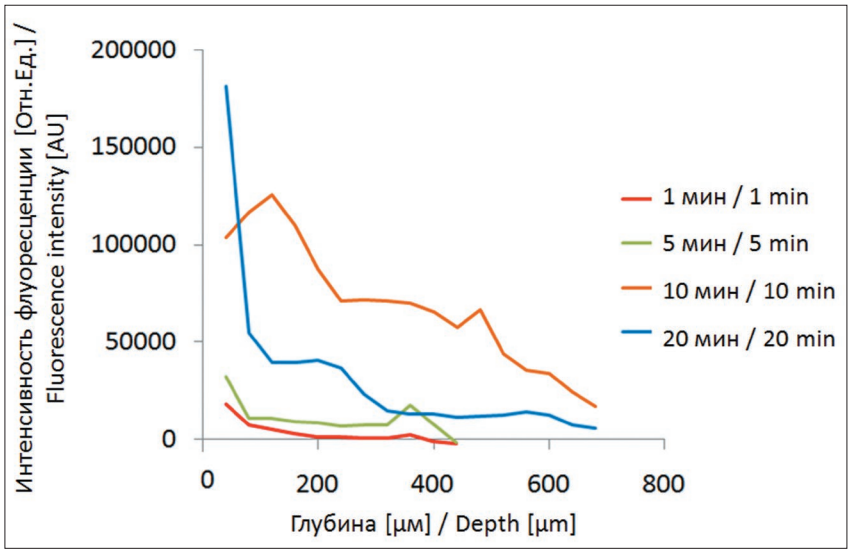

Рис. 2. Интенсивность флуоресценции ФС на глубине от поверхности эмали при различной продолжительности аппликации ФС
Fig. 2. The photosensitizer fluorescence intensity at the depth from the enamel surface for various application times

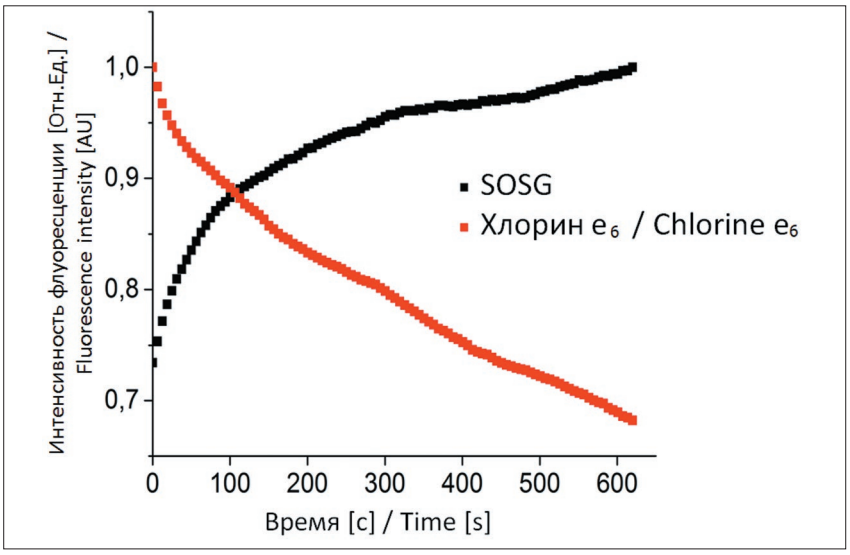

Рис. 3. Зависимость обесцвечивания ФС (красный) и разгорания флуоресценции SOSG (черный) во время ФДОЗ шлифа зуба, усредненное по всей толщине эмали
Fig. 3. The dependence of the bleaching of the photosensitiser (red) and the rise of SOSG fluorescence (black) during PDB of tooth section, averaged over the entire thickness of the enamel

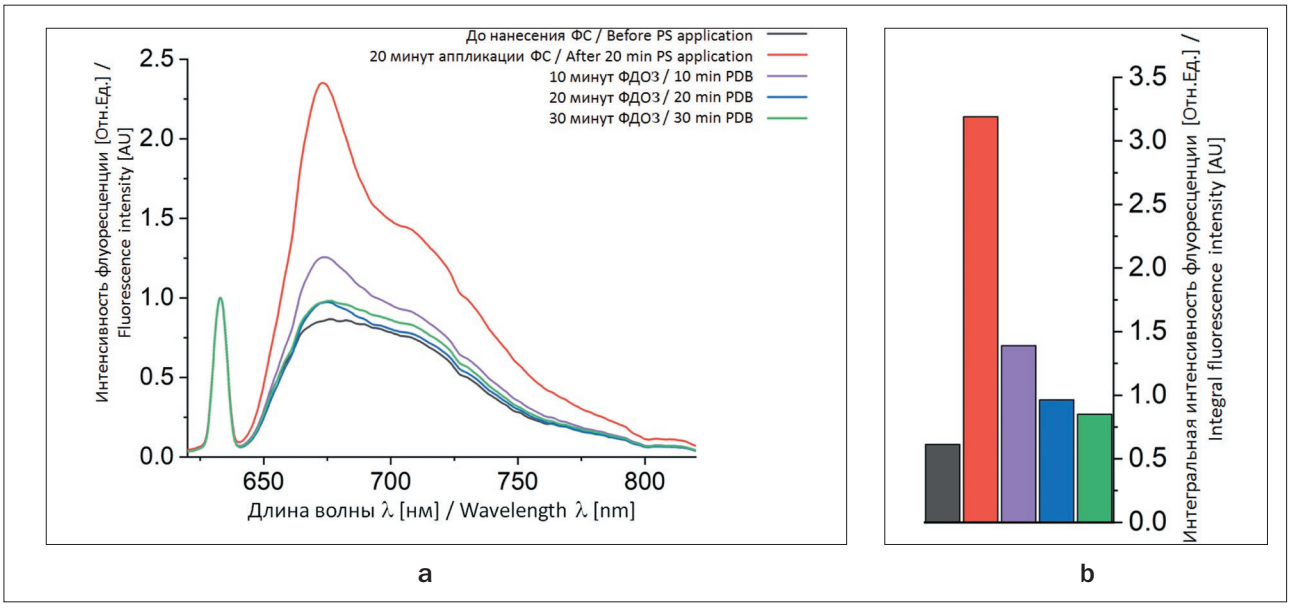

Рис. 4. Спектры флуоресценции зуба до аппликации ФС, сразу после, и спустя каждые 10 мин облучения (*a*). Интегральная интенсивность флуоресценции от зуба, нормированная на пик лазерного рассеяния (*b*)
Fig. 4. Fluorescence spectra of the tooth before the photosensitiser application, immediately after, and after every 10 minutes of irradiation (*a*). Integral fluorescence intensity from the tooth, normalized on the peak of the scattered laser (*b*)

enamel surface were plotted for different times of the PS application on the enamel surface (Fig. 2).

The highest concentration of PS is observed at the enamel surface. It then gradually decreases, which corresponds to the physical diffusion of substances. However, at the border of enamel and dentin, there is a small relative increase in the PS concentration (in different teeth, the border of dentin and enamel was at different depths). The tendency for the total PS presence increase with time of the PS application is also clearly observed.

The dependences of PS bleaching and SOSG singlet oxygen sensor flare-up are obtained depending on the depth of the enamel surface on the tooth cut during the PDB. The averaged values of the PS bleaching

Рис. 5. Визуальная оценка цвета удаленных зубов до и после процедуры фотодинамического отбеливания в соответствии с классической шкалой Вита
Fig. 5. Visual color matching of the extracted teeth before and after the procedure of PDB using classical Vita scale

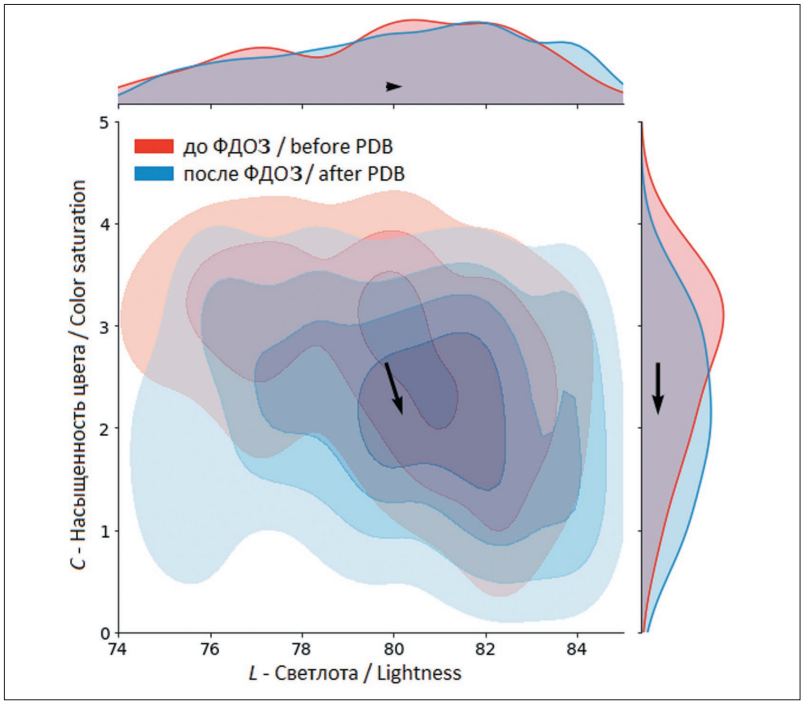

Рис. 6. Статистическое распределение цвета 100 зубов до (розовый) и после (голубой) процедуры фотодинамического отбеливания. Оценка цвета зубов выполнена с использованием спектрофотометра VITA Easyshade® V Вита, приведены данные в координатах насыщенности цвета (C) и светлоты цвета (L). По осям отложены распределения значений C и L до и после процедуры фотодинамического отбеливания. Черной стрелкой представлено усредненное изменение цвета зубов в эксперименте
Fig. 6. The statistical distribution of 100 teeth color before (red) and after (blue) the PDB procedure. Evaluation of the teeth color was performed using a VITA Easyshade® V Vita spectrophotometer; data in the coordinates of color saturation (C) and color lightness (L) are presented. Along the axes, the distributions of C and L values are plotted before and after the photodynamic bleaching procedure. The black arrow represents the average discoloration of the teeth in the overall experiment

over the entire thickness of the enamel and the singlet oxygen sensor flaring are presented in Fig. 3. Based on these data, 10 min after the start of irradiation, the PS is bleached by a third of the initial concentration, and the fluorescence of the SOSG singlet oxygen sensor reaches a plateau.

The nature of the PS bleaching in the tooth tissue during PDB is confirmed by fluorescence spectroscopy. Thus, tooth fluorescence before application of the PS gel, immediately after, and after every 10 min of irradiation, repeats the dependence of the PS fluorescence intensity obtained on the tooth cut under a confocal microscope (Fig. 4).

The teeth appearance before and after the PDB compared with the classic Vita scale is presented in Fig. 5. Also, it was found that PDB can be more effective if the enamel is periodically moistened during the procedure. Similar results of *in vitro* studies on enhancing the photobleaching effect of resin-based restorative materials in water were obtained in [35].

In addition to the classic Vita score, tooth color data were obtained before and after the PDB using VITA Easyshade® spectrophotometer. It was statistically established that in one FDB procedure, the average change in lightness of color (L) was 0.36 units, with a maximum bleaching reaching 11.4 units, and a minimum of 4.7. At the same time, the average change in tooth color saturation (C) was -0.5 units, the maximum bleaching occurred by –2 tones, the minimum – 0.

The results are comparable with the review comparing conventional tooth bleaching and photobleaching [36].

Conclusion

The PS (chlorin e_6) used in the study penetrates deep into the tooth tissues over time (enamel and dentin), which makes it possible to conduct PDB throughout the entire tissue depth. After 20 min PS application to the tooth surface, the fluorescence of chlorin e_6 was detected both in the depth of the enamel and in dentin. Although the average interstitial concentration of PS decreases with depth, it still reaches values sufficient to conduct effective PDB.

To achieve a significant effect from PDB, the singlet oxygen generation over the entire thickness of enamel and dentin is necessary, which was experimentally proved using the SOSG singlet oxygen sensor and photobleaching of the PS.

To determine the effectiveness of PDB, objective data on tooth color were obtained before and after PDB using the Vita scale and VITA Easyshade® spectrophotometer. It was shown that the average change in lightness of color (L) was 0.36 units, and the maximum bleaching reached 11.4 units. Also, the average change in tooth color saturation (C) was 0.5 units, for individual teeth achieving a 2-tone change. Thus, the use of the PDB allows effective aesthetic correction of tooth color.

This work was carried out within the state assignment of the Federal Agency for Scientific Organizations of Russia ("Physical methods in medicine and biology", No. 0024–2019–0003).

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