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Aluminium Phthalocyanine Nanoparticles Application for Fluorescent Diagnostics and Photodynamic Therapy in Dentistry

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Abstract

Early diagnosis of tooth-enamel microcracks is of great importance in modern dentistry for caries prevention. It is known that the accumulation of the bacteria in the enamel microcracks can be reason of caries. A promising substance for early diagnostics of the accumulation of pathogenic microflora on the tooth enamel surface is aluminum phthalocyanine (AlPc).

It could be observed that AlPc does not fluoresce in the nanoparticles form but in the monomeric molecular form it does. This allows identification of local pathological microflora accumulation within microcracks of the tooth enamel because AlPc nanoparticles (nAlPc) can be activated by pathological microflora.

This paper describes the nAlPc application for fluorescent diagnostics (FD) of the enamel surface in vitro. To reduce the time from the beginning of interaction of nAlPc with the microflora to the appearance of nAlPc fluorescence, Protelan MST-35 surfactant was used as an additional activator.

For FD in dentistry a model compound with nAlPc, Protelan MST-35 and with the complementary components was prepared. The following components were used as complementary: the methylparaben, carbopol, carboxymethyl cellulose, titanium dioxide, sodium phosphate, sodium saccharin and sorbitol, which are commonly used in toothpastes.

Human teeth were used for the investigation of the interaction between nAlPc colloid and the model compound with nAlPc for the detection of microcracks and the areas of accumulation of pathogenic microflora on the enamel surface.

Statistical processing of the experimental results showed the effectiveness of the surfactant usage for the additional activation of nAlPc and a reduction in the FD time.

The application of nAlPc as a marker will make it possible to detect microcracks of the enamel tooth surface at the earliest stages and the areas of the pathogenic microflora accumulation, which can lead to the development of a caries.

Keywords: fluorescent diagnostics, nanoparticles, aluminum phthalocyanine, Protelan MST-35, surfactants, enamel microcracks, tooth enamel.

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1. Introduction

Applications of photodynamic therapy (PDT) and fluorescent diagnostics (FD) in dentistry become popular because these methods are noninvasive, nondestructive, inexpensive and enable real-time monitoring [1]–[4]. Likewise, in the PDT and FD methods the photosensitizers (PS) are used more often in the form of nanoemulsions and nanosolution (so-called nanophotosensitizers) due to the fact that photophysical substance properties are changed at the transition to nanoscale [5]. One of these is sulphonated aluminum phthalocyanine (commercial name Photosens, FGUP GNC"NIOPIK", Moscow, Russia) [4], [6]–[13].

In contrast to the molecular AlPc form the nAlPc does not have intrinsic fluorescence in a colloidal solution in free form. Fluorescence occurs when AlPc molecules become detached from an AlPc particle (e.g. on contact with bacteria, macrophages, etc.) [7], [9], [14]–[17]. Therefore, hydrophobic AlPc nanoparticles can be a "marker" for the detection of the hidden inflammation and places of pathogenic microflora accumulation during FD application.

It has been shown recently that some oral bacteria, including *Prevotella intermedia* [18], *Porphyromonas gingivalis* [18], [19], [20] *Streptococcus mutans* [21], [18], [22], *Streptococcus sanguis* [19], *Fusobacterium nucleatum* [20], *Actinobacillus actinomycetemcomitans* [19], [21] are susceptible to and can be killed by red light after sensitization with AlPc. Therefore, the usage of nAlPc for antimicrobial PDT and FD will allow us not only to detect the places of pathogenic microflora accumulation on the surface and in microcracks of tooth enamel but also to kill it.

Biologically compatible surfactant Protelan MST-35 was used as an additional activator of AlPc to shorten the duration and increase the effectiveness of the enamel FD. A fraction of AlPc molecules begin to separate from the AlPc particle surface, but do not separate completely, when Protelan MST-35 is added to the AlPc colloid. Without detaching from the particle surface these molecules can interact with the microenvironment and exhibit fluorescent and photodynamic properties of the molecular form of AlPc.

2. Material and methods

2.1. Preparation of the AlPc colloid

An aqueous colloidal suspension of nAlPc at a concentration of 10 mg/l was obtained from a large-dispersed water-insoluble AlPc powder (FGUP GNC"NIOPIK", Moscow,

Russia). The powder of AlPc in distilled water was subjected to ultrasonic dispersion for 30 minutes with Bandelin SONOPLUS HD2070 (Germany). The hydrodynamic radius of the obtained nanoparticles, measured with the Photocor light-scattering spectrometer complex (Russia), was ~152±60 nm (70% of the total volume). It is significant that the colloidal suspension of the nanoparticles does not fluoresce when it is irradiated at 633 nm. To study the interaction of nAlPc with the surface microflora of enamel a colloid of nAlPc at a concentration of 10 mg/l was used.

2.2. Preparation of the model compound

For performing of the tooth enamel FD, the model compound containing nAlPc (10 mg/l), Protelan MST-35 (1% by volume) and the complementary components was prepared. The following components were used as complementary: the methylparaben, carbopol, carboxymethyl cellulose, titanium dioxide, sodium phosphate, sodium saccharin and sorbitol, which are commonly used in toothpastes. Protelan MST-35 is the surfactant, which was obtained from 100% natural materials and it does not harm the human body and the environment.

Before preparing the model compound, the structure and concentration of the components were optimized. Sample of the model compound were examined immediately after preparation and after being in a special thermostat (42 °C, 1 month), which is equivalent to being at room temperature for 12 months. Special mixing of components to produce the model compound (with nAlPc and Protelan MST-35) and thermostating was carried out by Cosmeceutical Incubator Ltd (Russia).

To investigate the spectroscopic properties of nAlPc, the additional samples were used: the basis of the model compound without the nAlPc and surfactant and the model compound with nAlPc. Additional samples of the model compound were prepared to test the hypothesis that the surfactant can activate the surface molecules of a nanoparticle like a solvent. The difference between a nanoparticles solvent and a surfactant is that the solvent transfers the surface molecules to the completely free state, while the surfactant makes them more mobile and capable of rapid interaction with the microflora without detaching them from the nanoparticle surface. Protelan MST-35 is the surfactant, which was obtained from 100% natural materials and it does not harm the human body and the environment.

Human teeth were used for the investigation of the interaction between nAlPc colloid and the model compound with nAlPc for the detection of microcracks and the areas of accumulation of pathogenic microflora on the enamel surface. Human teeth

were extracted for different reasons: 53% (n = 22) - chronic periodontitis, 28% (n = 11) - third molars ("wisdom teeth"), 9.5% (n = 4) - orthodontic reasons, 9.5% (n = 4) - other reasons (41 samples in total). After extraction, all samples were placed into tubes with a 0.9% sodium chloride solution.

During the experiments the nAlPc colloid or the model compound was applied to the surface of the tooth enamel and in 3 minutes was removed by water.

2.3. Experimental setup

For the fluorescence measurements the fiber-optic spectrometer LESA-01 (BIOSPEC Ltd, Moscow, Russia) was used (Fig. 1). A helium-neon laser with a wavelength of 633 nm and an output power of 2-4 mW at the fiber probe was used as an irradiation source for fluorescence excitation. A fiber-optic probe with one irradiation and six receiving fibers (each of 200 μm in diameter) was attached to the spectrometer. Detailed information about the experimental setup can be found in the paper [23].

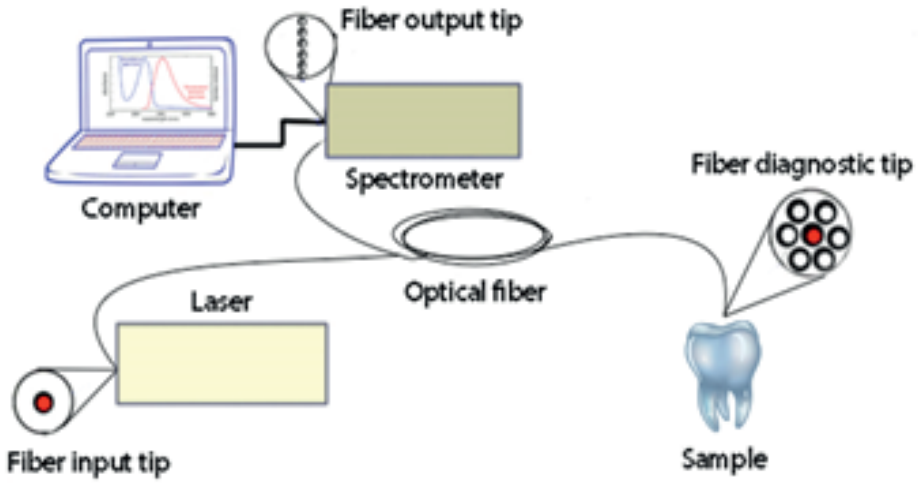


Figure 1: Experimental setup.

A video fluorescent system was used to visually control the increase of the nAlPc fluorescence. The system consists of a laser radiation source (635 nm), an optical filter with a transmission range of 650-1500 nm and a sensitive black-and-white camera. The original fluorescent images are presented, weren't subjected to additional processing.

2.4. The processing of the experimental data

For each sample from different regions on the tooth surface the fluorescence spectra before and after the nAlPc colloid/model compound application were obtained using LESA-01.

The autofluorescence coefficient K_{afl} of enamel was calculated as the ratio of the areas under the enamel autofluorescence spectrum to the area under the laser peak ("Uno Momento" software). Then the values of the enamel autofluorescence from all researched regions on the enamel surface were averaged across a single sample. Similar calculations were performed to compute the fluorescence coefficient k_{fl} after the application of the nAlPc colloid/model compound of a single sample. The normalization to the laser peak makes it possible to quantitatively compare the results of measurements that were obtained from different samples and to exclude the dependence of the results of spectrometry on the power of the laser radiation.

To estimate the differences in fluorescence from the enamel surface before and after applying the experimental compositions with nAlPc the coefficient of diagnostic contrast k_{DC} was calculated for each sample:

$$k_{DC} = \frac{k_{fl}}{k_{afl}}$$

Statistical processing of the experimental results was carried out using the computer program "SPSS statistics v23.0". To compare two groups of teeth (at application nAlPc colloid or model compound with nAlPc) Student's t-test was used.

3. Results and discussion

3.1. The results of interaction of the AlPc colloid and the model compound with the surface microflora of the tooth enamel in vitro

For FD of the tooth enamel surface the model compounds containing nAlPc, surfactant and the complementary components were prepared. The fluorescence spectra of various samples of the model compounds are shown in Fig. 2.

On the graph, the peak at 633 nm corresponds to the backscattered laser radiation from the surface of the sample, which was used to normalize the fluorescence spectra (670 nm) and to quantitavely estimate the concentration of the fluorescent components. As can be seen from the graphs the basis of the model compound (without

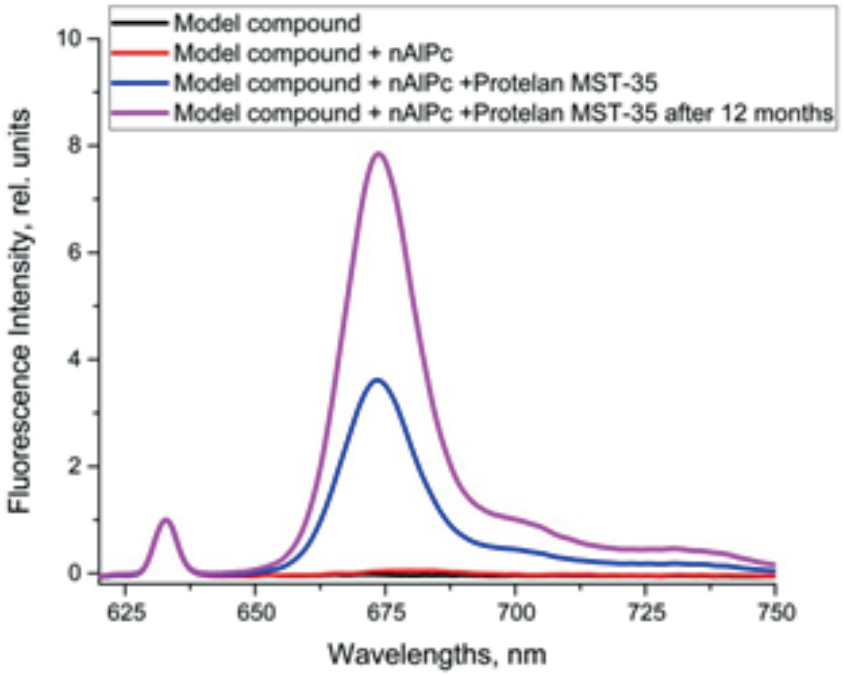


Figure 2: The fluorescence spectra of various samples of the model compounds: the basis of the model compound (without the nAlPc and surfactant), the model compound with nAlPc and the model compound (with nAlPc and Protelan MST-35), obtained immediately after preparation and after simulated 12 months.

the nAlPc and surfactant) and the model compound with nAlPc are an almost entirely 'black body' from the spectroscopic point of view.

It should also be noted that the spectroscopic properties of the model compound measured immediately after preparation and after 12 months have changed. The fluorescence intensity of nanoparticles in the model mixture increased 2-fold after 12 months. Thus, the model mix can be used for diagnostics after 12 months.

It was also found that in the model compound in the activated state there is 1-2% of AlPc molecules from their total number, which corresponds to a certain part of the surface molecules that are in the active state. The hydrogen index (pH) of the model compound was 6.27. Enterobacteriaceae, Pseudomonas aeruginosa, Staphylococcus aureus, mold fungi and yeast were absent in the model compound. Thus, the model compound has microbiological purity, homogeneous and thick consistency, which makes it suitable for using in the clinical application.

Then, the interaction of the model compound (with nAlPc and Protelan MST-35) with the surface microflora of the tooth enamel was investigated. For each sample, the diagnostic contrast coefficient was calculated, as described in the materials and

methods. In Figure 3, the enamel autofluorescence spectrum before and the nAlPc fluorescence after application of the model compound are presented.

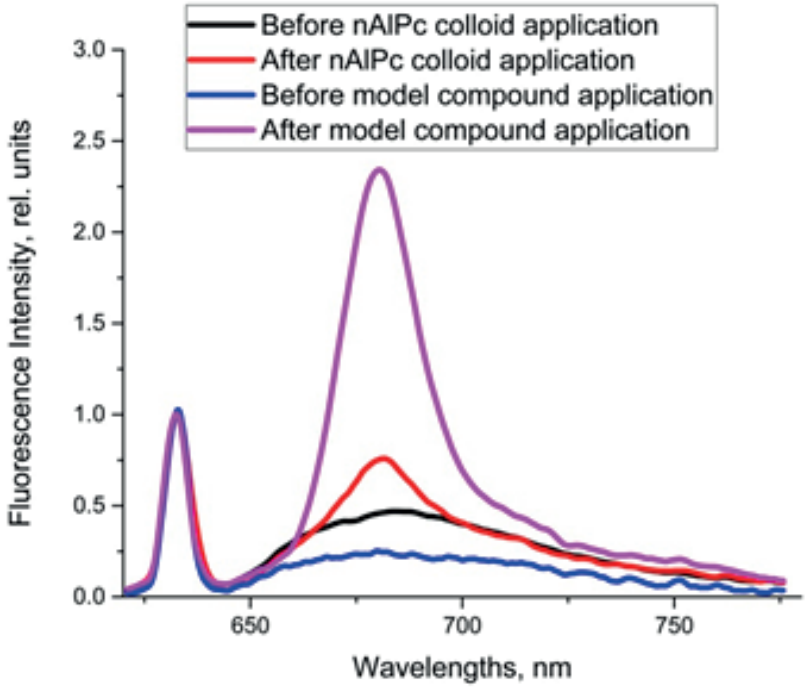


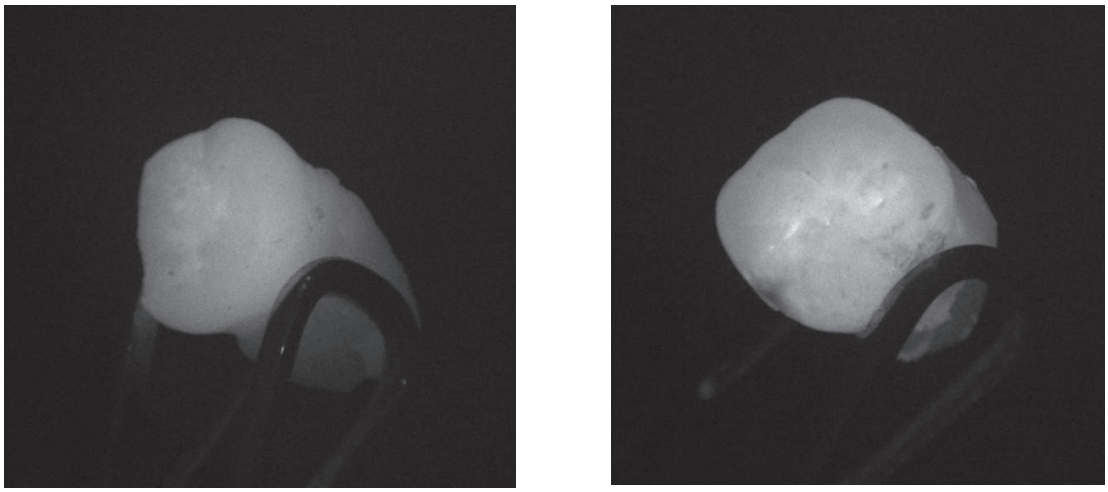
Figure 3: The example of fluorescence increases of nAlPc after application of the model compound with surfactant compared to the application of the nAlPc colloid on the enamel surface. Measurements were made after 3 minutes after application.

Figure 4 shows the video - fluorescent images of the tooth enamel surface before and in 3 minutes after the model compound application.

Statistical analysis by Student's t-test has shown that the average k_{DC} of the model compound (with nAlPc and Protelan MST-35) is significantly greater than the average k_{DC} of the nAlPc colloid ($p < 0.05$). Thus, the usage of Protelan MST-35 for additional activation of nAlPc is justified.

4. Conclusions

The results of experimental studies demonstrated the promise of using Protelan MST-35 as an additional activator of nAlPc to reduce the time and increase the effectiveness of FD. Also, in vitro studies have shown that the application of the model compound with nAlPc and Protelan MST-35 allows to perform the FD of tooth enamel surfaces in 3 minutes after application. It is also possible to use a model compound for detecting the plaque, dental calculus and hidden places of pathogenic microflora accumulation in the oral area. This fact speaks about promising prospects of using the developed



(A) (B)

Figure 4: Figure 4 shows the video - fluorescent images of the tooth enamel surface before (A) and in 3 minutes after (B) the model compound application.

model compound in dentistry as a convenient tool for FD in real time and at a low cost of the procedure.

In the future joint using of nAlPc and surfactants can allow to increase the sensitivity and effectiveness FD and PDT methods in dentistry for detection not only change of enamel surface but also inflammation processes in periodontal tissue.

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