SPECTRAL LUMINESCENT PROPERTIES OF BACTERIOCHLORIN AND ALUMINUM PHTHALOCYANINE NANOPARTICLES AS HYDROXYAPATITE IMPLANT SURFACE COATING

Maklygina Yu.S.[1], **Sharova A.S.**[2], **Kundu B.**[3], **Balla V.K.**[3], **Steiner R.**[2,4], **Loschenov V.B.**[1,2]
[1]General Physics Institute of the Russian Academy of Sciences, Moscow, Russia
[2]National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Moscow, Russia
[3]Bioceramics and Coating Division, CSIR-Central Glass and Ceramic Research Institute, Kolkata, India
[4]The Institute for Laser Technology in Medicine and Measurement Technique, Ulm, Germany

Abstract

The development and the spectral research of unique coating as crystalline nanoparticles of IR photosensitizers were performed for the creation of hydroxyapatite implants with photobactericidal properties. It has been proved that by the interaction of nanoparticles covering implant with the polar solvent, which simulates the interaction of the implant with the biocomponents *in vivo* (fast proliferating and with immunocompetent cells), photosensitizers nanoparticles change the spectroscopic properties, becoming fluorescent and phototoxic. Thus, the developed coating based on crystalline photosensitizer nanoparticles with studied specific properties should have antibacterial, anti-inflammatory effect by the photodynamic treatment in the near implant area. This research opens the prospect of the local prevention of inflammatory and autoimmune reactions in the area of implantation. The results of the study suggest a promising this technology in order to create implants with photobactericidal properties.

Keywords: **nanophotosensitizers, bacteriochlorin, aluminum phthalocyanine, photoluminescence, hydroxyapatite implants, photobactericidal effect of photodynamic therapy.**

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Contacts: Loschenov V.B., e-mail: loschenov@mail.ru

Introduction

Study of the processes of integration of organic compounds and synthetic materials in biological tissue is of great importance for improvement of the quality of life of patients in need of regeneration or replacement of tissue defects [1-6]. Implant techniques have found application in many areas of medicine (orthopedics, neurosurgery, cardiac surgery, dentistry, traumatology, etc.). Surgery followed by implantation is a complicated process in terms of a post-operative recovery, prevention of inflammatory responses and implant rejection processes. When any foreign material is implanted into the body, the immune system induces the development of macrophage inflammatory and reparative response of the connective tissue, leading to the implant rejection [7-17]. This circumstance prevents the implant biointegration and leads to the development of related diseases.

Currently, the most promising material, which has been widely used in the clinical field of implantation, is hydroxyapatite featuring high stability, bioactivity and biocompatibility [18-23]. In addition, implants with antiseptic and anti-inflammatory properties are development due to the potential effectiveness of their application in order to accelerate the natural immune processes in biological tissues at early stages of healing, to minimize the inflammatory reactions of the body in the long-term post-implantation period, and to ensure the most comfortable conditions for biointegration and reliable attachment of implants [24-29]. Currently, the most promising methods to achieve the bactericidal effect are physical methods, in particular antimicrobial photodynamic therapy, having a pronounced photobactericide activity, anti-inflammatory effect, preventing the dystrophic and sclerotic processes, which can effectively reduce the risk of implant rejection and accelerate the biointegration [30-35].

Photosensitizers (PS) seem to be the most promising in the role of photobactericide substances. Of these, the most interesting are the PSs, which do not lose their bactericidal properties in contact with the implant surface, while sticking firmly enough to the porous hydroxyapatite surface to prevent its washing away over time. Thus, the PSs in the form of nanoparticles, i.e. nanophotosensitizers, which do not show their photodynamic activity in the absence of inflammatory agents (microbes, some immune cells), becoming phototoxic and photodynamically active in contact with specific biocomponents [36-38] are the most effective. The degree of nanoparticles activity is evaluated by the level of the photoluminescence signal intensity, which arises in contact with inflammatory agents and may be detected both in the monitoring and in the follow-up mode of study. In turn, the bactericidal and anti-inflammatory effect of photoactive implants is associated with the fact that the photosensitizer nanoparticles manifest specific properties under the influence of the laser radiation in the implantation zone. Hydroxyapatite-based implants have small size, but due to their porous structure they have a large surface area with the deposited layer of PS nanoparticles, which comes into direct contact with the biological tissue in vivo. The porous structure scatters the radiation exciting the photoluminescence well, which contributes to the effective activation of nanoparticles throughout the implant surface and ensures subsequent photodynamic effect.

Material and methods

The meso-tetra(3-pyridyl)bacteriochlorin (hereinafter bacteriochlorin, Bch) and non-sulfonated aluminum phthalocyanine (hereinafter phthalocyanine aluminum, PcAl) were studied as photosensitizers, which are able to provide the greatest depth of photodynamic treatment, having the absorption peak in the near infrared range corresponding to the region of the maximum optical transparency of biological tissues, which makes them the most promising of current photosensitizers [37-45].

The structural formula of *meso*-tetra(3-pyridyl)bacteriochlorin (FSUE «SSC «NIOPIK», Russia) is shown in Fig. 1.

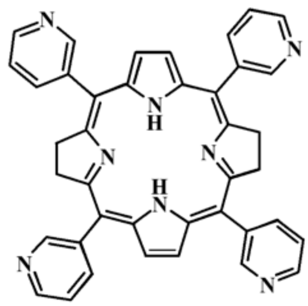

Fig. 1. The chemical formula of the *meso*-tetra(3-pyridyl)bacteriochlorin molecule

The structural formula of aluminum phthalocyanine (FSUE «SSC «NIOPIK», Russia) is shown in Fig. 2.

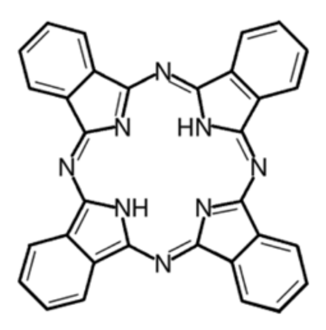

Fig. 2. The chemical formula of the aluminum phthalocyanine molecule

In the course of study the aqueous dispersions of molecular nanocrystals of bacteriochlorin (nBch) and aluminum phthalocyanine (nPcAl) were prepared. In both cases, the polycrystalline powder was added to distilled water to a concentration of 1 mg/ml. The resulting suspension was dispersed in Bandelin SONOPLUS HD2070 ultrasonic homogenizer with KE76 attachment (20 kHz, the amplitude of 165 microns). Using Photocor Complex (Russia) multi-angle spectrometer of the dynamic light scattering, allowing to obtain the nanoparticles distribution by size via the analysis of correlation function of the scattered light intensity fluctuations, it was found that the average particle diameter in the aqueous colloid was 220÷240 nm. Importantly, the resulting nBch and nPcAl colloids were not luminescent when excited by lasers at the wavelengths of 532 and 632,8 nm, respectively, i.e. the PS nanocrystals in a free form showed no photoactivity.

Hydroxyapatite-based implant was synthesized by a research group in India, led by professor V.K. Balla. By incubation (t = 30 min) of a porous implant in a colloidal solution, the nanoparticles were deposited onto the implant surface. The porous hydroxyapatite structure allows nanoparticles to penetrate deep inside, as evidenced by the color change in the implant thickness due to the characteristic color of colloidal solutions of photosensitizers.

The spectroscopic properties of the nanoparticles in the range of 0.4÷1.1 micron upon interaction with surface molecules of hydroxyapatite in different conditions were examined using LESA-01-«Biospec» fiber spectrometer (Biospec CJSC, Russia) [46,47].

Results and discussion

To study the dynamics of photosensitizer accumulation in the porous structure of hydroxyapatite composing the implant, a series of experimental studies was conducted. The implant biointegration process was simulated *in vitro* as follows:

stage 1: the spectral luminescent properties of nanoparticles were studied in the course of interaction with the surface hydroxyapatite molecules, given that the free nanocrystals in the aqueous colloid are not capable of luminescence;

stage 2: the spectral-luminescence properties of the nanoparticles have been studied in the conditions of implant surface (hydroxyapatite with a surface layer of PS nanoparticles) with a polar dimethyl sulfoxide (DMSO) solvent, which simulated the process of interaction with biocomponents (immunocompetent cells, bacteria, etc. *in vivo*);

stage 3: the spectral-luminescent properties were studied after washing off of the polar solvent from the surface of the implant porous structure by means of an aqueous dispersion in an ultrasonic homogenizer.

The results of the stepwise implant surface exposure are presented in the form of luminescence spectra in Fig. 3 and Fig. 4 for samples with the surface coating nBch and nPcAl, respectively.

The analysis of the luminescence spectra and luminescence peak dynamics at various conditions for both types of crystalline nanoparticles (bacteriochlorin and aluminum phthalocyanine) shows that the PS nanocrystals not initially possessing the photoactivity change their spectroscopic properties and acquire the luminescence ability in the course of interaction with hydroxyapatite surface molecules. This phenomenon probably can be explained by interaction of the surface molecules of nanoparticles with a complex and heterogeneous surface structure of hydroxyapatite. Depending on the location in the porous structure of hydroxyapatite and entourage of PS nanocrystals, their surface molecules may «lie», taking the para-position relative to the surface of the nanoparticle, or «rise up», taking the ortho-position, holding to the surface and showing the spectroscopic properties of PS solution shown in the conditions of interaction of the implant surface with DMSO.

Thus, the process of crystalline PS nanoparticle activation is confirmed by a sharp increase in the photoluminescence intensity of the signal (Fig. 3-4) when

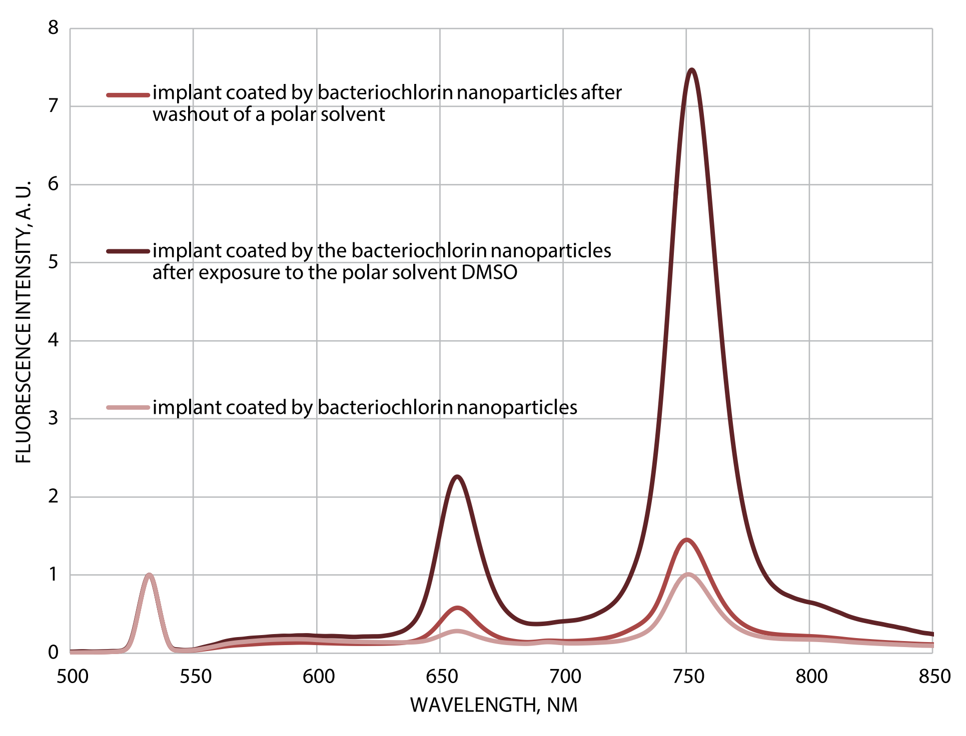

Fig. 3. The luminescence spectrum of the hydroxyapatite implant coated by bacteriochlorin nanoparticles under the different conditions (λ_{ex} = 532 nm)

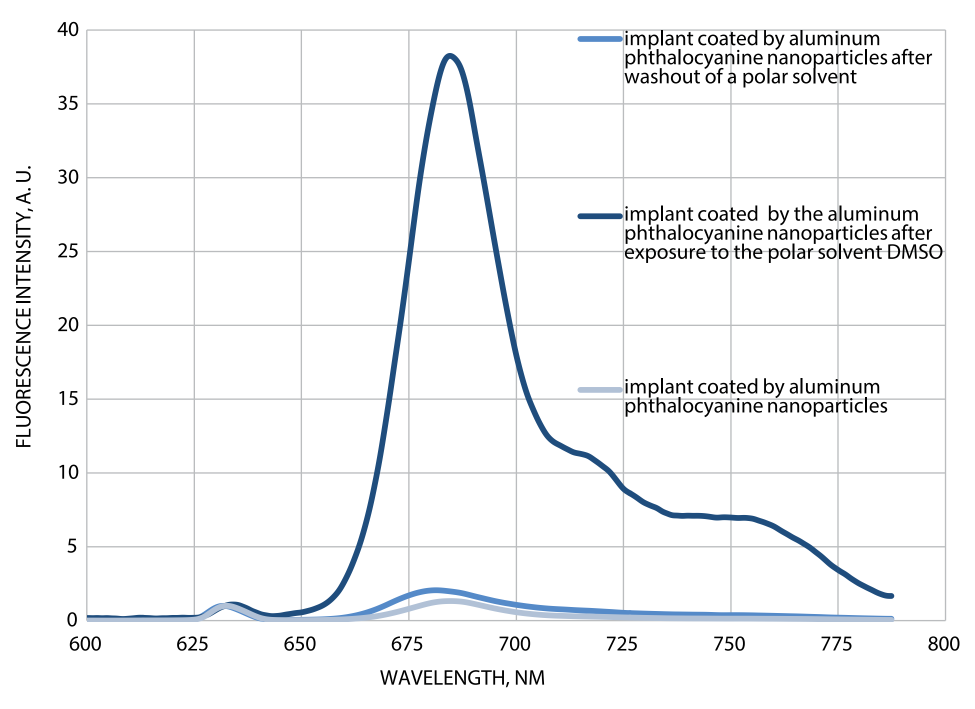

Fig. 4. The luminescence spectrum of the hydroxyapatite implant coated by aluminum phthalocyanine nanoparticles under the different conditions (λ_{ex} = 632.8 nm)

interacting with a polar solvent, which allowed simulating the process of implant interaction with the surface layer of PS nanoparticles featuring the biocomponents *in vivo*.

In the course of study it was found that the surface molecules of PS nanoparticles have a sufficiently strong interaction with the surface structure of hydroxyapatite, since even aqueous dispersion failed to achieve the nanocrystal elution from the porous structure of the implant, which confirms the presence of the luminescence signal for both nanoparticles types after the third stage of exposure (Fig. 3-4).

The work also involved a study of the temporal dynamics of the luminescence spectra on the surface of the hydroxyapatite-based implant coated with PS nanoparticles. The analysis of the dynamics of the luminescence spectra for both types of crystalline nanoparticles (nBch and nPcAl) showed that the initially photoinactive PS nanocrystals in the course of interaction with the surface hydroxyapatite molecules acquire the ability to luminescence, but the luminescence peak intensity varies over time under the influence of the exciting laser radiation with the appropriate wavelength (nBch: λ_{ex} = 532 nm, nPcAl: λ_{ex} = 632.8 nm).

The range of the surface luminescence of hydroxyapatite-based implant coated with nBch, when excited by laser radiation (λ_{ex} = 532 nm), has two peaks of luminescence: λ_{em} = 758 nm and λ_{em} = 654 nm. At constant continuous exposure to the exciting laser radiation the intensity of luminescence peaks varies over time, while the luminescence peak signal intensity corresponding to λ_{em} = 654 nm increases over time, whereas the luminescence peak signal intensity corresponding to λ_{em} = 758 nm decreases over time (Fig. 5).

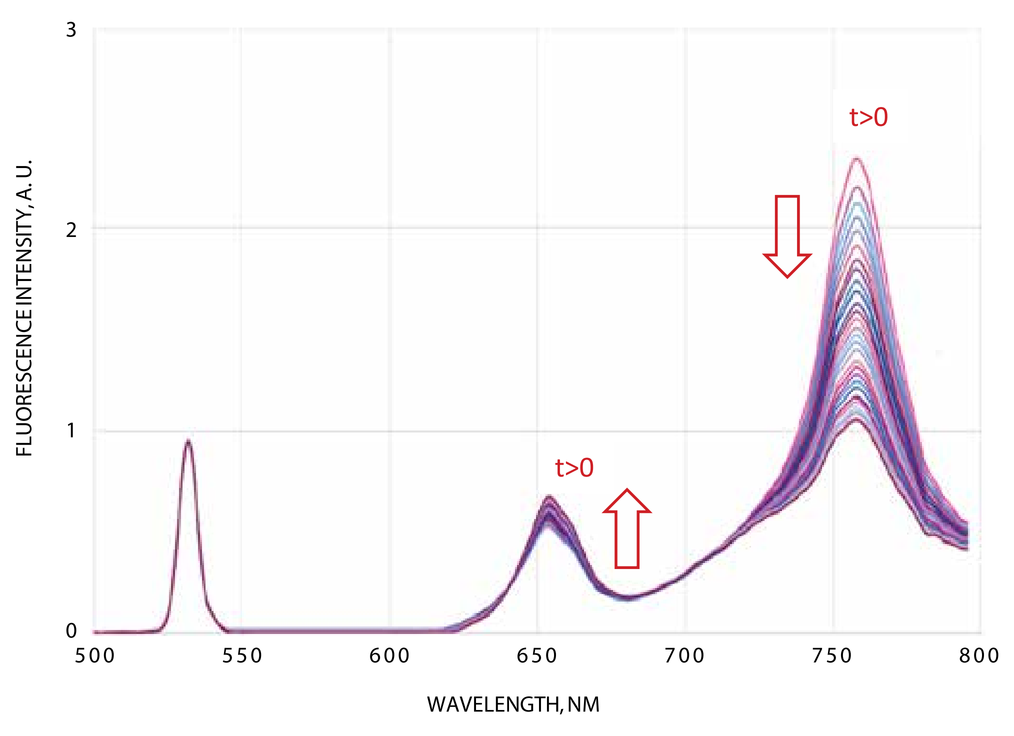

Fig. 5. The dynamics of the luminescence spectrum time transforming of the implant based on hydroxyapatite coated by bacteriochlorin nanoparticles (λ_{ex} = 532 nm)

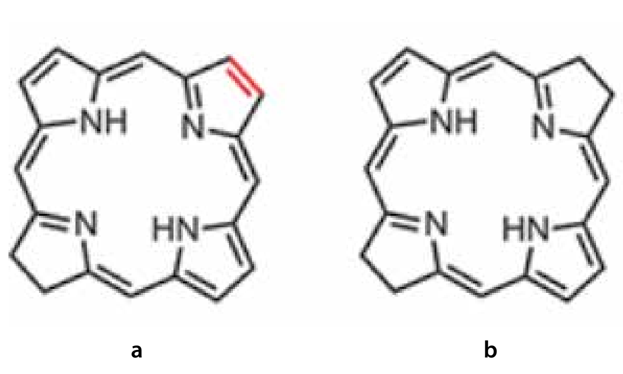

Fig. 6. The chemical formula of: a – chlorin molecule; b – bacteriochlorin molecule

Apparently, depending on the exposure duration, which corresponds to a change in the level of energy imparted to the system the surface molecules change their position relative to each other and surface structure of hydroxyapatite, showing the spectroscopic properties of bacteriochlorin (λ_{em} = 758 nm) or chlorine (λ_{em} = 654 nm) solution.

The reversibility of the process should be noted, which indicates the impossibility of structural change of the photosensitizer molecule itself, namely the formation of an independent chlorine molecule. Fig. 6 shows that the difference in structure between chlorine and bacteriochlorin molecules is the presence of one more double bond in the structural formula of chlorine. Therefore, probably under the influence of the exciting laser radiation, the interaction between two adjacent bacteriochlorin molecules may occur, forming a common temporary double bond between them, which collapses in the absence of the exciting radiation. In this case, one of the molecules may manifest the chlorine spectroscopic properties of the molecule. Based on these results, it is concluded that the bacteriochlorin nanoparticles interact both with each other in pairs,

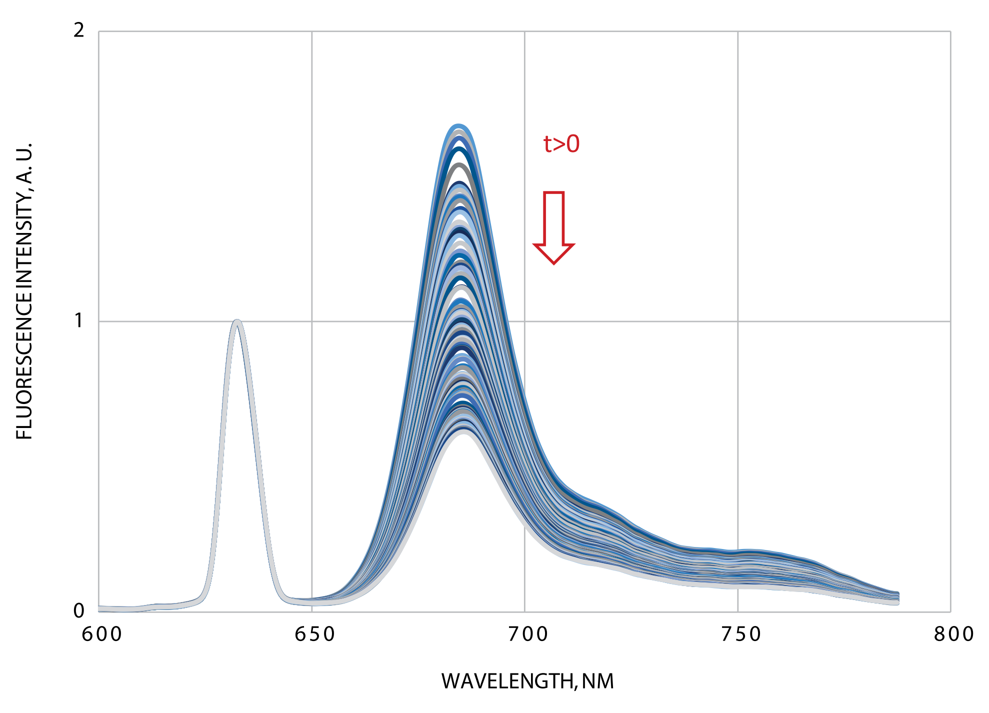

Fig. 7. The dynamics of the luminescence spectrum time transforming of the implant based on hydroxyapatite coated by aluminum phthalocyanine nanoparticles (λ_{ex} = 632.8 nm)

and with a complex porous structure of hydroxyapatite. Depending on the nature and strength of interaction, as well as location and environment of nanoparticles, they can take different positions relative to each other and the surface structure of hydroxyapatite, while changing the spectroscopic properties, but without undergoing irreversible changes in the molecular structure.

Fig. 7 shows the dynamics of the luminescence spectrum of the hydroxyapatite-based implant surface coated with nPcAl. The luminescence spectrum of the hydroxyapatite-based implant surface coated with nPcAl, when excited by laser radiation λ_{ex} = 632,8 nm, has a luminescence peak λ_{em} = 682 nm. At constant continuous exposure to the exciting laser radiation over some time, the peak intensity of the luminescence decreases over time.

Apparently, like in the case of nBch, during irradiation, the surface molecules change their position relative to each other and the surface of hydroxyapatite structure, which leads to spectral changes. The reversibility of the process should be noted, which indicates the absence of structural changes in the nanophotosensibilizer molecule itself or dye burnout.

Conclusion

The work proved the possibility of activating the nanoparticles of bacteriochlorin and aluminum phthalocyanine, which was estimated by the level of photoluminescence signal in the control study mode. The presence of a strong interaction between the surface molecules of the photosensitizer nanocrystals and surface structure of hydroxyapatite was also revealed, which will further eliminate the possibility of leaching of the surface layer of nanoparticles.

The analysis of the dynamics of the luminescence spectra for two types of crystalline nanoparticles showed that the initially photoinactive PS nanocrystals, in the course of interaction with surface hydroxyapatite molecules, acquire the ability to luminescence, but the luminescence peak intensity varies over time under the influence of the exciting laser radiation. Based on these studies, it was concluded that the PS nanoparticles interact both among themselves and with a complex porous structure of the implant. Depending on the interaction nature and forces, as well as location and own environment of nanoparticles, they may take various positions with respect to each other and the surface structure of hydroxyapatite, changing the spectroscopic properties. Meanwhile the structure of molecules does not undergo irreversible changes.

The study suggests that this technology is promising for creation of implants with photobactericide properties.

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