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Photo-bactericidal Properties of Hydroxyapatite Implant Surface Coating

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Abstract

Implant-associated infection is a common postoperative complication and remains a serious problem in the surgery. This work describes the development of hydroxyapatite implants with photo-bactericidal properties by the surface coating. The unique coating as crystalline nanoparticles of IR photosensitizers of implant was investigated using infrared spectroscopy. 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 immune-competent cells), IR photosensitizers nanoparticles change the spectroscopic properties, becoming fluorescent and phototoxic.

Thus, the developed coating based on crystalline IR 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 such technology application in order to provide the local inflammatory and autoimmune reactions prevention in the area of implantation that will improve implant antimicrobial effects and reduce potential long-term cytotoxicity. The results of the study suggest a promising this technology in order to create implants with photo-bactericidal properties.

Keywords: Aluminum; Phthalocyanine; Bacteriochlorin; Hydroxyapatite implants; Nano-photosensitizers; Photoluminescence; Photo-bactericidal effect of photodynamic therapy

Introduction

The research of the organic compounds and synthetic materials integration processes in biological tissue is crucial for improving 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.). Surgical intervention with subsequent implantation is a difficult process in terms of post-operative recovery, preventing inflammatory responses and of the implant rejection processes. During implantation, the immune system stimulates inflammatory and reparative response of the conjunctive tissue leading to the implant rejection [7-17]. This circumstance prevents implant biointegration and entails comorbidities.

At present the most perspective material, which is widely used in the field of clinical implantation, is hydroxyapatite (HAp). HAp is characterized by high stability, bioactivity and biocompatibility [18-23]. HAp was studied due to its crystallographical and chemical resemblance with the mineral part of the human hard tissue. It was proven that HAp has the ability to bond directly with bone tissue. Bacterial infections are associated with implant materials that may induce the local inflammation around the implant and eventually implant loss. This phenomenon is of major concern for both patients and health care providers because of the negative impact on patient quality of life and the economic cost of frequent implant replacements. Accordingly, there is a growing demand to inhibit bacterial colonization on implanted devices through the immobilization of an antibacterial agent on their surface that can both discourage initial bacterial adhesion and serve to kill them upon contact [24]. Porous bioactive ceramic such as HAp is one of the alternatives to be used as local drug delivery system. Furthermore, the inclusion of antimicrobial agents within HAp is expected to prevent or cure implant-associated infections via their direct release to local regions such as the implant-tissue interface. Antibiotics can also be applied on the implant surfaces [25] as photo-bactericidal substances, however, concerns exist regarding their efficacy considering the growing problem of antibiotic resistant bacteria and their short-term effect [26]. Thus, the leading developments are aimed at the implant design with antiseptic and bactericidal properties that will allow reducing of the immune processes duration and inflammatory reactions in biological tissues, ensuring the most comfortable conditions for implant biointegration [27-32].

Currently the most promising methods to achieve the bactericidal effect are physical methods particularly antimicrobial photodynamic therapy [33-38]. This method shows a pronounced photo bactericidal activity, anti-inflammatory effect and has gained more attention owing to its broad-spectrum antibacterial properties at very low concentrations, good biocompatibility, satisfactory safety profile, and inherent stability. Antimicrobial photodynamic therapy also prevents the dystrophic and sclerotic processes that can effectively reduce the risk of implant rejection and accelerate biointegration.

The photosensitizers (PS) seem the most promising as photo-bactericidal substances. The most interesting are the PS, which in contact with the implant surface do not lose their bactericidal properties and have enough grips with the HAp surface to prevent it from washing away by biointeraction. So the PS in nanoform are the

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most effective substances which do not exhibit their photodynamic activity in the absence of inflammatory agents (microbes, some immune cells) and become phototoxic and photoactive in contact with specific biocomponents [39,40]. The activity level of nanoparticles is evaluated by the intensity of the photoluminescence signal. This photoluminescence signal arises by interaction nanoparticles with inflammatory agents and may be detected as a monitoring and control study modes.

Bactericidal and anti-inflammatory effects of photoactive implants are associated with specific properties of photosensitizer nanoparticles by the laser radiation. HAp implants are of small sizes but have a large surface area due to its porous structure. Large surface area of the deposited PS nanoparticles layer directly comes into contact (interacts) with the biological tissue *in vivo*. The porous structure is well scatters excited photoluminescence radiation, which contributes to the effective nanoparticles activation over the implant surface and the extensive photodynamic effects.

Materials and Methods

Meso-tetra (3-pyridyl) bacteriochlorin and non-sulfonated phthalocyanine PcAl aluminum were used as PS which can provide the greatest penetration depth of photodynamic treatment. Test compounds have the absorption peak in the near infrared range corresponded to the region of maximum optical transparency of biological tissues. Optical properties of test compounds give reason to consider them as the most promising PS especially regard to monitoring of pathological processes with deep localization [41-44].

Nanoparticles of bacteriochlorin (nBch) and Aluminum phthalocyanine (nPcAl)

Meso-tetra(3-pyridyl)bacteriochlorin (hereinafter referred to as "bacteriochlorin") produced by the Organic Intermediates and Dyes Institute (Russia) (Figure 1a). Coarse crystals of aluminum phthalocyanine produced by the Organic Intermediates and Dyes Institute (Russia) (Figure 1b).

The aqueous colloid of molecular nBch and nPcAl were prepared as follows. For both samples, polycrystalline powder was added into the distilled water to obtain a concentration of 1 mg/ml. The resulting suspension was dispersed in ultrasonic homogenizer Bandelin SONOPLUS HD2070 with nozzle KE76 (20 kHz, 165 μm amplitude).

By means of multi-angle dynamic light scattering spectrometer Photocor Complex (Russia), allowing to obtain the nanoparticle size distribution through analysis of correlation function of the dispersed light intensity fluctuations, it was determined that mean particle diameter in the aqueous colloid is 220÷240 nm. It is important to note that the resulting nBch and nPcAl colloids not luminesce by laser excitation with 532 and 632.8 nm wavelengths, respectively. Thus, free forms of the PS nanocrystals show no photoactivity.

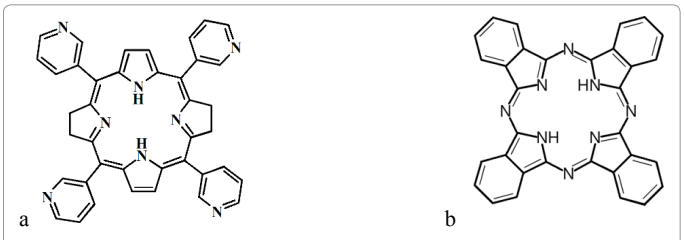


Figure 1: The chemical formula of the a) meso-tetra(3-pyridyl)bacteriochlorin molecule b) aluminum phthalocyanine molecule.

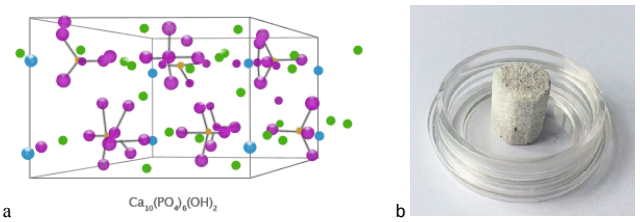


Figure 2: a) Crystalline hydroxyapatite packaging with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$; b) Digital photo of hydroxyapatite implant.

Hydroxyapatite (HAp)

Coralline derived HAp is the most perspective biomaterial for implantation. HAp implants were synthesized by the research group of Professor Balla Vamsi Krishna in India. [45].

The tailor-made HAp implants were fabricated from the HAp powder. The HAp powder was prepared by precipitation between $Ca(OH)_2$ and H_3PO_4 according to the following reaction:

$$10\ Ca(OH)_2 + 6H_3PO_4 = Ca_{10}(PO_4)_6(OH)_2 + 18H_2O.$$

An aqueous solution of H_3PO_4 was added very slowly to a suspension of $Ca(OH)_2$. During reaction, temperature of the suspension was maintained at 80°C. The resulting precipitate was aged, dried and calcined at 800°C. The calcined powder was ground in a planetary mill (Figure 2).

During the fabrication, the powder was intimately mixed with appropriate quantity of naphthalene powder by repeated sieving. The powder mix was inserted into rubber bag and compacted at a pressure of 160 MPa by cold-isostatic pressing (EPSI NV; SO 10036, Belgium) to form cylindrical shape. By heating at 80°C, the naphthalene was driven off from the green implant and great care was taken at this stage to prevent cracking. Finally, the implant was sintered at 1250°C for 3 h to improve the mechanical properties.

To achieve the photobactericidal effect in the area of implantation, the photosensitizers in nanoform were applied on the HAp implant surface. Porous implant surface was coated with relevant nanoparticles by incubation (t = 30 min) of the implant in a colloidal solution of nBch and nPcAl. Porous HAp structure allows nanoparticles to penetrate deep inside as evidenced by the color change in the implant thickness due to the characteristic color of colloidal photosensitizer solutions.

Spectroscopic properties of the nanoparticles were examined with the use of a fiber spectrometer LESA-01 "BIOSPEC" (in the range of 0.4 ÷ 1.1 μm) in various conditions including the interaction with surface molecules of HAp [46].

Results and Discussion

Series of repeated and then averaged manipulation were carried out to study the dynamics of photosensitizer accumulation in the HAp porous structure. Implant biointegration process was simulated in the *in vitro* conditions as follows:

• **Stage 1:** The study examined the spectral properties of fluorescent nanoparticles by interaction with surface molecules of HAp, provided that free nanocrystals in aqueous colloid can't exhibit the luminescence;

• **Stage 2:** The study examined the spectral-luminescence properties of the coated implant in the interacting implant surface (HAp with the surface layer of nanoparticles) with a polar solvent (dimethyl sulfoxide - DMSO), which mimicked the interaction with biocomponents (immunocompetent cells, bacteria, etc. *in vivo*);

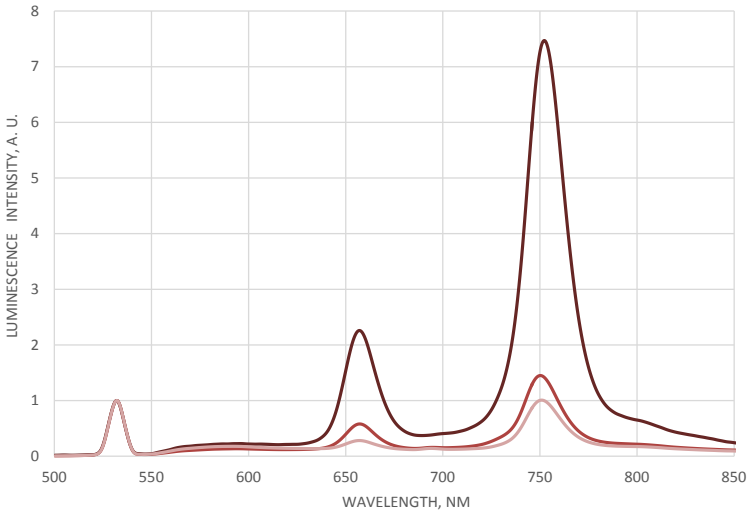


Figure 3: The luminescence spectrum of the HAp implant coated by nBch under the different conditions (λ_{ex} = 532 nm): implant coated by nBch, implant coated by nBch after exposure to the polar solvent DMSO, implant coated by nBch after washout of a polar solvent.

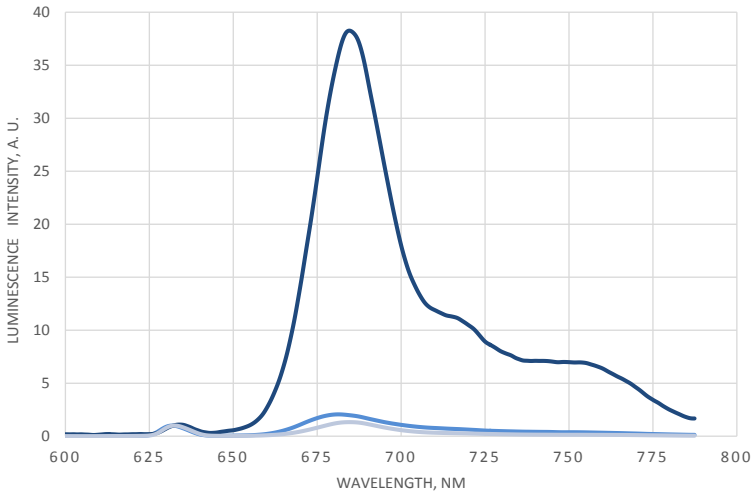


Figure 4: The luminescence spectrum of the HAp implant coated by nPcAl under the different conditions (λ_{ex} = 632.8 nm): implant coated by nPcAl, implant coated by nPcAl after exposure to the polar solvent DMSO, implant coated by nPcAl after washout of a polar solvent.

• **Stage 3:** The study examined the spectral luminescence properties of the coated implant after polar solvent washing by aqueous dispersion in an ultrasonic homogenizer to avoid the possibility of decontamination of the nanoparticle surface layer under the action of biocomponents (*in vivo*).

Results of the stepwise exposure on the implant surface are presented in the form of luminescence spectra in Figures 3 and 4 for samples with a surface coating of nBch and nPcAl, respectively.

Analysis of the luminescence spectra dynamics at various conditions for both types of crystalline nanoparticles (bacteriochlorin and aluminum phthalocyanine) shows that initially inphotoactivity PS nanocrystals by the interaction with HAp surface molecules change their spectroscopic properties and acquire the ability to luminesce. Probably this phenomenon can be explained by the interaction between the surface molecules of nanoparticles and complex surface structure of HAp. Surface molecules of nanocrystals may «lie» relative to the surface of the nanoparticle (taking parallel position) or may "wake up" relative to the surface of the nanoparticle (taking orthogonal position). Hence, the surface molecules show spectroscopic properties of PS solution (with the use of DMSO) depending on their location and position.

Thus, the process of photosensitizer crystalline nanoparticles activation is confirmed by a steep increase of the photoluminescence signal intensity (Figures 3 and 4) with the use of polar solvent, which permite to simulate the biocomponents interaction with the implant coated with the surface layer of PS nanoparticles *in vivo*.

During the study it was also found that the surface molecules of PS nanoparticles interact with the surface structure of HAp sufficiently strong, whereas the nanocrystals washout from the porous structure of the implant was not achieved by the way of aqueous dispersion. This fact is confirmed by the presence of the luminescence signal for both nanoparticle types after 3 stages of exposure (Figures 3 and 4).

Moreover, the luminescence spectra' dynamics of HAp -based implants surface coated with PS nanoparticles were experimentally investigated in the study.

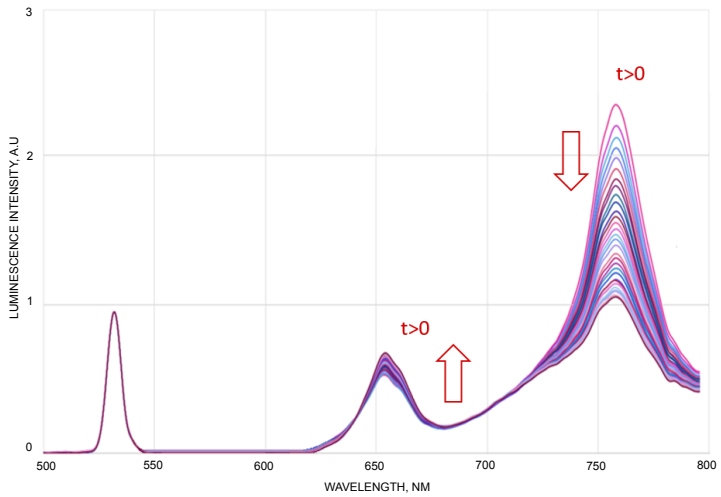
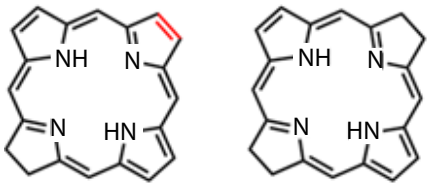


Figure 5: The dynamics of the luminescence spectrum time transforming of the implant based on hydroxyapatite coated by nBch (λ_{ex} = 532 nm).



Chlorin Bacteriochlorin

Figure 6: The chemical formula of chlorin and bacteriochlorin molecules.

The analysis of the luminescence spectra dynamics for both types of crystalline nanoparticles (nBch and nPcAl) shows that initially nonphotoactive PS nanocrystals acquire the ability to luminescence in contact with HAp surface molecules, though the intensity of luminescence peak varies with time under the influence of the exciting laser radiation (nBch: λ_{ex}=532 nm, nPcAl: λ_{ex}=632.8 nm).

Bacteriochlorin nanoparticles (nBch)

The luminescence spectrum of the implant surface coated by nBch excited by laser radiation λ_{ex} = 532 nm has two luminescence peaks λ_{em} = 758 nm and λ_{em}=654 nm. At constant exposure to the excitation laser radiation, the luminescence intensity peak changes over time. The intensity of the luminescence peak corresponding λ_{em} = 654nm increases over time, whereas the intensity of the luminescence peak corresponding λ_{em} = 758nm decreases over time (Figure 5). Apparently depending on the exposure time surface molecules change their position relative to each other and the surface structure of HAp, showing spectroscopic properties of bacteriochlorin solution (λ_{em} = 758nm) or chlorin (λ_{em} = 654nm).

It should be noted that the process is reversible therefore structural change of the PS molecule or self-formation of chlorin molecules are impossible. Figure 6 shows that the difference in structure of bacteriochlorin and chlorin molecules is the presence of one more double bond in the chlorin compound. So the interaction between two neighbor molecules of bacteriochlorin may occur under the influence of the exciting laser light. This interaction is probably based on the formation between two molecules of common temporary double bond, which collapses in the absence of the exciting radiation. In this instance, one of bacteriochlorin molecules may show chlorin spectroscopic properties. Thus, the nBch interact both pairs between themselves and with the complex porous structure of HAp. Depending both on the strength of interaction and own nanoparticle localization environment, PS molecules can take different positions relative to each other and the HAp surface. Variation of the PS molecule position leads to the spectroscopic properties changes but not at the expense of the molecular structure changes.

Aluminum phthalocyanine nanoparticles (nPcAl)

The luminescence spectrum of the implant surface coated by nPcAl has a luminescence peak λ_{em} = 682 nm excited by laser radiation λ_{ex} = 632.8 nm.

At constant exposure to the excitation laser radiation, the peak intensity of luminescence changes over time namely the intensity of the luminescence peak decreases over time (Figure 7). Apparently, depending on the exposure time surface molecules change their position relative to each other and the surface structure of HAp, leading to spectroscopic properties changes.

It should be noted that the process is reversible therefore structural change of the PS molecule or dye burnout are impossible.

Thus, the interaction of the nanoparticles with surface structure of the implant takes place through the surface molecules. In its turn, the surface molecules may take a different position with respect to the surface structure of HAp. Therefore, the surface covered with PS nanoparticles changes spectroscopic properties depending on the interaction forces, location and environment of the surface PS molecules, but not because of molecular structure changes.

Conclusion

Thus, the research has proved possibility to activate the nanoparticles covered the implant surface. Activity level of nanoparticles was estimated by the control of photoluminescence intensity. There was also found the strong interaction between the surface molecules of PS nanocrystals and HAp surface structure providing a strong grip.

The analysis of the luminescence spectra dynamics for the both types of crystalline nanoparticles showed that initially in photoactive PS nanocrystals acquire the ability to luminescence by reaction with

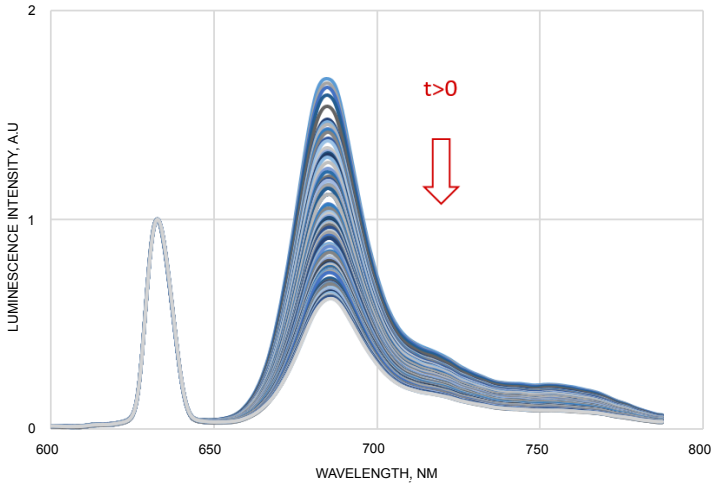


Figure 7: The dynamics of the luminescence spectrum time transforming of the implant based on hydroxyapatite coated by nPcAl (λ_{ex} = 632.8 nm).

HAp surface molecules. But the luminescence intensity varies over time under the influence of the exciting laser radiation. Based on this research it was concluded that the PS nanoparticles interact both among themselves and with a complex porous structure of the implant. Depending both on the interaction strength and own nanoparticle environment localization, PS molecules can take different positions relative to each other and the HAp surface. Variation of the PS molecule position leads to changing of the spectroscopic properties but not at the expense of the molecular structure changes. The findings of the study suggest this technology promising in order to create implants with photo-bactericidal properties.

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