Nanotechnology for biomedical applications – enhancement of photodynamic activity by nanomaterials

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Abstract. Over the last two decades nanotechnology has become one of the most dynamically evolving field of research. The unique properties of nanomaterials, not disclosing at macroscale, are examined and exploited to extend our understanding of the interactions taking place at atomic or molecular level. Those findings affect research in many areas, like e.g. alternative energy sources, electronics, physics and medicine. In this paper, the possibility of using nanomaterials for the enhancement of photodynamic activity, is discussed. A brief review on drug-delivery facilitating nanomaterials, regarding their characteristic features, is presented. An exemplary application of silver-doped nanomaterials for enhancement of photodynamic properties of two photosensitizers: Photolon and Protoporphyrin IX, is described. Influence of silver-doped nanomaterials addition on the fluorescence intensity of photosensitizers immobilized in silica-titania (SiO$_2$-TiO$_2$) sol was examined via VIS spectroscopy. Influence of sonication on the fluorescence enhancement was also investigated. It was demonstrated that the fluorescence enhancement of photosensitizers depends on the concentration of both: photosensitizer and silver-doped nanoparticles.

Key words: silver-doped nanomaterials, silica-titania (SiO$_2$-TiO$_2$), fluorescence enhancement of photosensitizers, photodynamic activity, photodynamic therapy.

1. Introduction

Nanomedicine, the application of nanotechnology in biomedical science, employs precisely engineered nanomaterials for novel therapeutic and diagnostic modalities [1–3]. Although no unambiguous definition of nanomaterials have been yet established, one can refer to them as to structures of size not exceeding 100 nm in at least one dimension. Materials designed at nanometer level gain novel properties, not occurring in bulk material of the same composition [4–6]. For example, increased surface-area-to-mass ratio of nanomaterials highly affects their reactivity. These new properties may be exploited to overcome some limitations of traditional therapeutic and diagnostic agents [7].

Over the past two decades many nanoscale systems have been proposed [8, 9]. Nowadays not only nanoparticles but also nanoparticles-based therapeutic products are commercially available [10, 11]. Many industrial and research institutions are working towards nano-functionalization and nano-structuring of materials’ surfaces, and applications of modified materials [12–14]. Modification of materials by coating them with nanoscale organic, ceramic or hybrid layers is a versatile way to add new value, advanced features and unique properties to conventional materials. Creating a nanofilm on material’s surface can significantly improve or even completely change its optical and electrical properties, as well as the wettability and biocompatibility. It may also introduce new unique properties, such as exceptional hardness or corrosion resistance [15].

Recently, new techniques to create 3-dimensional nanomaterials for biomedical engineering applications, have been proposed [16]. Ordered porous materials with exceedingly large surface areas are good candidates for highly efficient catalysts and sensors. Biomedical engineers are exploiting tailored nanofibers and patterned polymeric structures to develop tissue-engineered scaffolds, drug-delivery devices and microfluidic networks. A novel sensor based on gold nanoparticles may become the basis for non-invasive diagnostic tool for lung cancer [17, 18]. Nanotube-assisted protein deactivation may be used for the selective destruction of pathogens and cells [19]. Targeted nanotherapeutics are considered to be one of the most promising tools for therapeutic intervention in cardiovascular diseases [20].

2. Nanomaterials in photodynamic medicine

Photodynamic therapy (PDT) and diagnosis (PDD) belong to one of the most promising fields of contemporary medicine [21]. Laboratory research and clinical trials on photodynamic medicine are conducted worldwide, also in Europe [22], as well as in Poland [23]. To achieve satisfying therapeutic results of photodynamic procedure, applied photosensitizer must have certain characteristic. It should exhibit high selectivity in order to accumulate only in targeted tissue. Other essential features are lack of dark-toxicity and high PDT efficacy. Unfortunately, there are no PDT agents that meet all of these requirements [24]. Limited ability of many agents to reach the target tissue can lead to severe side-effects after exposure to light [25–27]. Activation energies of some agents extort prolonged exposure time. Hydrophobicity of some photosensitizers hampers their intravenous administration. Premature agent

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253
loss through rapid clearance and instability of photosensitizer in biological environment also cause problems [24].

Nanotechnology may help to overcome these difficulties, proposing nanomaterial-based agent delivery [28]. Coupling photosensitizer with nanoparticles may change the pharmacokinetic properties of the drug. It can be achieved by either surface binding of photosensitizer particles to a nanoparticle or confinement of photosensitizer in nanocapsules. In both methods therapeutic substance is in the form of nanoparticles.

Macromolecules accumulate in tumor cells as a result of increased permeability of endothelial barriers in tumor blood vessels (leaky vasculature) and diminished clearing. Therefore, intravenously administrated photosensitizer particles tend to reach and retain in tumor tissues [25, 29, 30]. This way of drug accumulation is called passive targeting.

In case of photosensitizer-nanoparticle complex, numerous ligands, such as monoclonal antibodies, phages or folic acid, can be coupled to nanoparticle’s surface in order to enhance the selectivity of accumulation. This approach is known as active targeting [31–33].

Size manipulation of photosensitizer-nanoparticle complex can also help avoiding extravasation into healthy tissues. Vascular pore size in majority of solid tumors varies from 100 nm to 780 nm. In contrast, in healthy tissue vascular pores have diameters varying from 2 nm to 6 nm. Therefore, optimal size of drug carriers is between 50–150 nm, which exceeds the defined size limit of 100 nm for nanomaterials [31].

2.1. Ceramic-based nanoparticles. Ceramic-based nanoparticles were designed as a carriers of hydrophobic photosensitizers [34]. Process of preparation of these nanoparticles is similar to sol-gel method, therefore it does not require elevated temperature conditions. The ceramic-based nanoparticles can be prepared with desirable size, shape and porosity. These nanoparticles may be extremely stable, so there is no swelling or porosity changes e.g. influenced by pH. Thus, the entrapment of the drugs in ceramic-based nanoparticles protects them against microbial attack or denaturation caused by extreme pH or temperature. Surface of these nanoparticles can be easily modified with a variety of ligands to perform active targeting. In addition, small size of nanoparticles slow down clearance of carried drugs [32, 35, 36].

In a study by Roy et al., 30 nm-diameter silica-based nanoparticles filled with 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH) were synthesized [34]. These carriers, due to stability at extreme conditions, did not release the entrapped drugs. However, their porous matrix was permeable to singlet oxygen, so the effect of photodestruction was maintained. Owing to encapsulation of photosensitizer, the loss of fluorescence in aqueous media was prevented. Synthesized complex was effectively taken up by the tumor cells in vitro and subsequently performed irradiation led to irreversible cell damage.

2.2. Polymeric nanoparticles. Depending on preparation method, polymer-based nanoparticles may have either a form of nanospheres (with photosensitizing agent uniformly dispersed on their surface) or nanocapsules (with the drug entrapped inside the polymer membrane) [37–40]. Although ceramic-based nanoparticles show greater stability, polymeric carrier systems have other advantages. For example, polymeric nanoparticles can be synthesized from biodegradable materials, which would allow sustained release of anticancer drug over a period of days or even weeks [41].

The biodegradable PLGA (poly (dl lactic-co-glycolic acid)) nanoparticles loaded with zinc (II) phthalocyanine were prepared by Ricci-Junior et al. Besides of satisfying photoactive properties, the complex showed sustained drug release rate [42].

Scientist from University of Michigan proved that the encapsulation of photosensitizer protects its photodynamic effectiveness [43]. They embedded methylene blue in 30 nm nontoxic polycryamide-based platforms and performed a PDT procedure in vitro on rat C6 glioma cells. Experiments proved that polymeric nanocapsules protected the entrapped photosensitizer from enzymatic or environmental degradation and at the same time, permitted light-generated singlet oxygen to diffuse out of nanoshells and to perform cytotoxic reactions. Potential toxicity of encapsulated drug was reduced, which means that only illuminated cells suffered from severe damage, while others remained intact, although all of them were in contact with the photosensitizer-nanoparticle complex. Other important advantage of this approach was shortening the incubation time in PDT procedure. Since these nanoparticles were designed to deliver only singlet oxygen, its internalization to cells was not essential, the external contact with the cell membrane should be sufficient enough to maintain good phototheraphy effectiveness.

2.3. Polymeric micelles. Amphiphilic co-polymers in water solution tend to form spherical nanosized (10–100 nm) molecules [31, 32, 44]. The assembly takes place when concentration of co-polymers exceeds the critical micelle concentration (CMC). Hydrophobic segments gather in the micellar core and hydrophilic segments form the surface. Outer hydrophilic layer provides stable dispersion in aqueous media (which facilitates intravenous administration), while inner core can be loaded with relatively large amounts of hydrophobic photosensitizer. Therefore, block-copolymer micelles have been used as carriers of hydrophobic photosensitizer entrapped in their interior [45–47].

There are several possibilities of composition of the core, however the corona almost always consists polyethylene glycol (PEG) [48]. Hydrophilic surface made of PEG prevents the opsonisation and ensures the systematic drug circulation. Depending on what the core is made of, the size and pharmacokinetics of the photosensitizer-nanoparticle complex may slightly differ [45, 46]. For example, polyester micelles are biocompatible and biodegradable. Poly(L- amino acid)-based nanoparticle on the other hand are sensitive to pH. That feature makes them a potential controlled release anti-cancer drug carriers due to decreased pH in tumor tissue. Some of synthesized micelles are thermosensitive [47–50].
Another important characteristic of micelles is that they show higher stability than low molecular weight surfactants, because usually critical micelle concentration – CMC – for polymer-based micelles is very low [46–48]. Furthermore, these nanoparticles have a polarity gradient extending from hydrophobic core to hydrated surface, which allows them to associate hydrophobic particles of varying polarities within different regions of a micelle [51]. Another advantage of copolymer micelles is rather narrow diameter distribution [35, 46, 48].

Recently a novel nanocarrier system based on micelles have been proposed [52]. Micells, synthesized by Y. Li et al., were composed of linear PEG (forming a hydrophilic corona) and flexible two-arm oligomer of cholic acids (forming a hydrophobic core). Developed nanocarrier was size-tunable by means of modification of the length of linear PEG chain as well as the centrifugation of cholic acid oligomer. Other tunable properties of those carriers were the value of CMC and drug-loading capacity.

### 2.4. Liposomes

Liposomes consist of bilayer lipid membranes that encapsulate a fraction of solvent. Their aqueous core can be exploited as a carrier of photosensitizing agents [36]. There are uni- and multimellar liposomes with diameters varying from 30 nm to several micrometers [53]. Choice of bilayer components and preparation method determines size, surface charge, stability and permeability of liposomes. For example, unsaturated phosphatidylcholine species form permeable but not very stable bilayers, whereas bilayers formed by dipalmitoylphosphatidylcholine are rather impermeable, however rigid and stable [35].

To improve the functionality of liposomes as drug carriers, their surface can be decorated with PEG to prolong their circulation time in the bloodstream [35]. Also attaching ligands to their surface would increase the drug accumulation selectivity. But in the case of liposomes, active targeting can be achieved simply by changing an overall surface charge to positive [31, 54, 55]. Cationic liposomes electrostatically bind themselves with negatively charged phospholipid headgroups that are preferentially exposed on tumor endothelial cells.

Unfortunately, liposome-based drug delivery systems suffer from certain shortcomings. For example, poor drug encapsulation efficiency, as well as increased aggregation of photosensitizer particles in the entrapped state (aggregating photosensitizers exhibit poor PDT efficacy) [34, 56]. Liposomes also have a tendency to opsonisation and immunology system eliminates them quite quickly from the blood. Therefore, attaching polyethylene glycol to their surface, is essential. Another disadvantage of liposomes is their tendency to dissociate in aqueous solutions [57].

### 2.5. Dendrimers

Dendrimers are polymeric symmetric monodisperse complexes that comprise of well-defined branches around a small molecule, called core [32, 57, 58]. The core is determined as generation zero (G0) and next generations are synthesized as layers between each focal point. Dendrimers can be synthesized either in divergent or convergent manner. Divergent synthesis begins in the core and moves toward the periphery. Convergent approach of synthesis starts at the peripheral branches of the final molecule and ends at the core [59, 60]. Since dendrimers consist from AB-n type monomers each, generation of branching units doubles or triples.

Photosensitizing agents can be attached to dendrimer surface, its core or to interior layer surrounding the core. The two latter domains protect entrapped drug from the outside environment. However, interactions of these drug carriers with the surroundings is mainly determined by the terminal groups [35]. Dendrimers with hydrophilic corona and hydrophobic interior (and vice versa) can be obtained simply by modifying their termini [35]. The particles size could be well controlled in the nanometer range [61].

Conformation of dendrimers is pH-dependent. At extreme pH values (e.g. 4 or 11) electrostatic repulsion is forcing branches apart, while at more neutral pH the conformation is tighter [57, 62]. Solvent dependant conformation changes are also observed. Namely, for polar dendrimers in a non-polar solvent density at the core is higher, while in a polar solvent density at the surface is higher [57].

In the study reported by El-Sayed et al. it has been proved that dendrimer size affects its extravasation to interstitial tissue [63]. PAMAM (poly(amidoamine)) dendrimers from size 1.5 nm (G0) to 4.5 nm (G4), were synthesized. Experiments showed that with increasing dendrimer size extravasation across the endothelium increased exponentially.

Unfortunately, dendrimers suffer from the same disadvantages as other cationic macromolecules (like liposomes, micells). Positively charged surface tends to destabilize cell membranes and to initiate cells lysis [64, 65]. It has been found that that higher generation dendrimers are more toxic, while lower generation dendrimers with carboxylate surface groups showed no cytotoxicity [66].

### 2.6. Metal nanoparticles

In this approach anticancer drug is usually covalently bound to the surface of carrier through a chemical linker. Pharmacokinetics of such molecule depends on the metal nanoparticles. For example, Wieder et al. have shown that hydrophobic photosensitizer phthalocyanine can be attached to the surface of gold nanoparticles via a thiol moiety [67]. Obtained complex was soluble in polar solvents. Also a great improvement of PDT efficiency (due to 50% enhancement of singlet oxygen quantum yield) was observed in comparison to free phthalocyanine.

Photodynamic effect depends on light dose, oxygen concentration, the interval between administration of the photosensitizer and light exposure, and, of course, the type and solubility of photosensitizer [68]. There is a great interest in the synthesis and research of new non-porphyrin photosensitizers (e.g. from plants) due to their properties [69]. Photosensitizer named Photolon is a complex of chlorin e6 (Ce6) and polyvinylpyrrolidone (PVP). Solubility of Ce6 and their complexes with PVP was evaluated by a few scientific groups [70, 71]. It was proved that complexation with PVP prevents Ce6 aggregation or even disrupts pre-existing Ce6 aggregates, thus...
preserving the monomeric form of the photosensitizer [70]. It should be emphasized that PVP prevents aggregation of Ce6 molecules at lower pH, which can potentially enhance the PDT activity of the complex. In vivo biodistribution data indicated that the tumor uptake of Ce6 in the PVP formulation was enhanced compared to Ce6 alone [71]. Ce6-PVP has a faster elimination rate in the normal human skin compared to other porphyrin-based photosensitizers. Moreover, this complex has a higher sensitivity and specificity for tumors.

It was already demonstrated that chlorin class photosensitizer as Photolon immobilized in the silica sol-gel matrix exhibits better photochemical and photophysical properties in comparison to Protoporphyrin IX [72]. The synthesis of mentioned materials is simple and results in the defined form with the ability to optimise photophysical properties (e.g. to prevent sensitizer aggregation) [73]. Photolon was examined due to its biological activity and light induced photodynamic effect against bacteria [74].

Due to the physical properties, silver nanoparticles are often used for fluorescence enhancement [75, 76]. The phenomenon of fluorescence enhancement may be exploited in photodynamic diagnostics.

3. Examination of the influence of silver-doped nanoparticles on Photolon and Protoporphyrin IX fluorescence

The aim of this study was to check, whether the addition of Ag-doped nanoparticles may enhance the fluorescence of examined photosensitizers entrapped in the SiO2–TiO2 sol, thus improving the photodynamic activity that may be exploited for better diagnosis. It was also examined, whether the sonication of components solutions affects on the fluorescence intensity.

Generally, the sol-gel method is well known as a promising method of producing bioactive materials for various biomedical applications. The growing interest towards sol-gel materials is based on possibility to form bioactive sol-gel derived coatings on implants in low processing temperature. It has been shown that the addition of the silica into the materials structure has a positive effect on osteoblast response on medical implant surfaces sol-gel titania films. Exploration of the biological response of the TiO2–SiO2 coatings proved their ability to form a tight contact with the surrounding tissues, providing a strong chemical bond with them [77–81].

3.1. Materials preparation. The sol-gel method is a cost-effective and convenient route to prepare mono- and multi-component glasses and ceramics. Silica-based sol-gel materials are often modified by metal oxides to improve physical and optical properties. SiO2-based materials are often used as carriers of metal nanoparticles or serve as a support for organic molecules immobilization. In a typical reaction, sol-gel material is produced from silica precursor in acidic (or base) hydrolysis process. In order to decrease the materials refractive index and to get high quality visible-range-transparent materials, tetraethoxysilane (TEOS) and tetraethyl orthotitanate (TEOT) are often used as precursors. Sols used in these study were prepared from TEOT, TEOS and 96% ethyl alcohol (C2H5OH pure, for analysis). The molar ratio of TEOT:TEOS:C2H5OH material was 0.02:1:20. 36% hydrochloride acid HCl was added as the catalyst to ensure the acidic hydrolysis (pH 2). The mixture was stirred for 2 hours on a magnetic stirrer with the speed 400 rpm at room temperature. All solvents and reagents were obtained from commercial sources and used without further purification: (a) chemicals: TEOS (Aldrich), TEOT (Aldrich), ethyl alcohol (Polish Chemicals); (b) photosensitizers: Protoporphyrin IX (PP IX, Sigma), Photolon (Ph, Belmedpreparaty).

The photoactive agents, like Protoporphyrin IX (PP IX) and Photolon (Ph), when dissolved in liquids, are oxygen and light sensitive. Therefore, the stock solutions of photosensitizers in ethyl alcohol were freshly prepared by short sonication, immediately after the sols preparation. 1 ml of SiO2–TiO2 sol and suitable volume of the photosensitizer’s stock solution were mixed in order to get following photosensitizer concentrations: 0.8 µmol/dm3, 4 µmol/dm3, 8 µmol/dm3, 16 µmol/dm3, 24 µmol/dm3, 32 µmol/dm3, and 40 µmol/dm3.

Ag-doped silica nanoparticles were prepared by Tollens’ method [82]. Silica spheres were dispersed in the distilled water and the solution of silver ammonia complex Ag(NH3)2⁺[0.2 M] was added. Glucose solution was used as the reducing agent [69, 83]. Finally the colloidal suspension of Ag-doped nanoparticles, in which silver concentration was equal 32.4 mg/dm3, was prepared.

![TEM image of prepared Ag-doped silica nanoparticles](Image)

Figure 1 shows a TEM image of obtained Ag-doped silica nanoparticles. According to the micrograph, the size of silver nanoparticles was less than 5 nm.

Fluorescence signals were recorded directly after the samples preparation, then after the addition of 50 µl of silver nanoparticles suspension (silver concentration in sol-gel samples equal 1.62 µg/ml) and finally after the addition of the next 50 µl of silver suspension (total silver concentration in samples equal 3.24 µg/ml). In order to inhibit the photosensitizers aggregation and to improve the silver distribution in SiO2–TiO2 sol, sonication was applied. Samples were placed in the ultrasonic bath for 5 minutes after photosensitizer’s addition into sol, and each time directly after the addition of the colloidal suspension of silver-doped nanoparticles, as well. For comparison, a set of samples remained non-sonicated.
3.2. Method. The spectra were recorded with AvaSpec-3648 spectrometer (Avantes Inc.). Spectroscopic measurements of sols were performed in standard cuvettes (path length 10 mm). As an excitation light source, pulsed semiconductor laser $\lambda = 415$ nm (TopGaN) was applied. Generally, porphyrines are characterized by the strong absorption band at about 400 nm, known as a Soret band. This feature might be used as a marker for trace amounts of porphyrines e.g. in clinical diagnostics of porphyria. Untreated whole blood samples are often analyzed for autofluorescent porphyrins that emit intense red fluorescence ($>620$ nm) upon excitation at around 400 nm [84, 85].

3.3. Results. First, the absorption spectra were recorded in the transmission mode (see: Figs 2 and 3). Figure 2 presents absorption spectra of undoped and silver doped SiO$_2$-TiO$_2$ sol. Maximum of the absorption band for undoped SiO$_2$-TiO$_2$ sol was observed at 340 nm and it indicates the presence of titanium dioxide. Low absorption signal at about 430 nm is determining the presence of silver nanoparticles in sol-gel materials. It was stated that with the increased concentration of silver nanoparticles, the higher absorbance at 430 nm is observed.

Following, absorption spectra of photosensitizers in SiO$_2$-TiO$_2$ sols were recorded (see Fig. 3). The Soret band and additional absorption bands at the wavelengths range 500–700 nm (Q-bands) are observed. Two prominent absorption bands, at 403 nm (Soret band) and at 655 nm (chlorine-type band) in case of Photolon-doped sol and at 564 nm in case of PPIX-doped sol, were visible. Other peaks, at 536 nm for Photolon and at 601 nm for PPIX, were observed as well.

Fluorescence spectra of Protoporphyrin IX and Photolon in SiO$_2$-TiO$_2$ sols were recorded under the excitation at 415 nm (Fig. 4). Fluorescence maxima at $\lambda_{em} = 665$ nm and $\lambda_{em} = 670$ nm for Protoporphyrin IX and at $\lambda_{em} = 611$ nm for Photolon (see Fig. 4), were observed.

Next, silver doped nanoparticles were added into the photosensitizers in SiO$_2$-TiO$_2$ sols. The fluorescence intensity of Protoporphyrin IX at 611 nm and Photolon at 665 nm was analyzed. The influence of components sonication on the fluorescence intensity, was examined, as well.

It was stated that the increase of silver concentration caused an increase of fluorescence intensity in case of sonicated, as well as non-sonicated samples (see Figs. 5–8). It was also proved that the fluorescence intensity strongly depends on sonication.
A. Ulatowska-Jarża, J. Pucińska, K. Wysocka-Król, I. Hołowacz, and H. Podbielska

Fig. 6. Influence of addition of silver nanoparticle's on the fluorescence intensity at $\lambda = 611$ nm of Protoporphyrin IX in SiO$_2$-TiO$_2$ sol, sonicated sample.

Analyzing Fig. 5, one may see that fluorescence of Protoporphyrin IX increases with the increase of nanosilver concentration. The even more visible increase of fluorescence intensity after addition of silver nanoparticles was observed in case of sonication for certain Protoporphyrin concentrations (see Fig. 6). Maximal fluorescence intensity of Photolon-containing samples increased 10 times after sonication.

Figures 7 and 8 depict the results of the same measurements in case of Photolon.

3.4. Discussion. The sonication of multicomponent system containing silver-doped nanoparticles enhances the fluorescence, therefore it is possible to use less photosensitizing agent and still being able to record the fluorescence spectrum (compare Fig. 6 and Fig. 8). In the presence of silver-doped nanoparticles the fluorescence intensity for certain photosensitizer's concentrations increases due to sonication: 10-fold increase was observed in the case of PP IX in concentration $15 \mu\text{mol/dm}^3$ and 3-fold increase in the case of Photolon in concentration $5 \mu\text{mol/dm}^3$.

Sol-gel colloid consists of two separate phases: a dispersed phase (SiO$_2$-TiO$_2$) and a continuous phase (solvent). In silica-titania sol, photosensitizer molecules were separated by dispersed phase, what inhibited the aggregation process. It was already proved that sol-gel materials protect certain porphyrine derivatives against aggregation, simply by physical immobilization [86]. Aggregation of porphyrine molecules is an undesired effect that may diminish the efficiency of photodynamic diagnosis. The creation of aggregates not only decreases fluorescence intensity, but also diminishes photosensitizer’s photodynamic activity [87].

The sonication and addition of silver nanoparticles to photosensitizers increases the fluorescence intensity, thus lowering the photosensitizer concentration. This may be exploited for enhanced photodynamic diagnosis, decreasing the risk of potential side effects, e.g. skin photosensitivity and systemic toxicity.

4. Conclusions

There is a great interest in applying nanotechnology in biomedicine. It has been already shown that cellular-uptake may be increased by encapsulation of hydrophobic photosensitizers in water soluble nanocarriers. Selectivity of drug accumulation can be enhanced through active targeting or carrier size manipulation. Encapsulation of photosensitizer protects it from enzymatic degradation and rapid changes in intracellular environment. Entrapping drugs in nanocapsules also reduces its cytotoxicity. It was demonstrated that platforms to deliver only singlet oxygen, while conserving the embedded photosensitizer, may be synthesized [88]. Moreover, nanocarriers can contain not only photosensitizers particles, but also MRI contrast agents [89]. Optimal time of drugs systematic circulation can be ensured by modifying nanoparticles size (e.g. for nanoparticles range between 121 nm and 343 nm clearance becomes more rapid with increasing nanoparticle size) [90]. Therefore, nanocarriers may significantly improve pharmacokinetics of photodynamic agents. However, one has to
remember that nanotechnology may cause new toxicological risks and even the tests performed on the same nanomaterials can produce different results. Therefore, the safety caution in nanomaterials applications, is recommended [91].

Our study confirmed that SiO$_2$-TiO$_2$ sol-gel materials inhibit aggregation of certain porphyrine derivatives by physical immobilization. Therefore high fluorescence signal of photosensitizer is maintained. We have also proved that the addition of Ag-doped nanoparticles into photosensitizer-containing SiO$_2$-TiO$_2$ sol increases the fluorescence of the photosensitizer. We have found that at certain photosensitizer concentrations sonication of the multicomponent system causes further fluorescence enhancement. Therefore, the same photodynamic effect could be achieved with lower concentration of photosensitizer. The patient’s benefit would be the reduction of negative side-effects caused by photosensitizer application.

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