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NANOSILICON-BASED PHOTOSYNTHESIS FOR CHEMICAL AND BIOMEDICAL APPLICATIONS

(PSY-NANO-Si)

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Thematic Priority: 3NMP

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Project coordinator organisation name: Universidad Politécnica de Valencia (UPV)

Organisation name of lead contractor for this deliverable: UPV

Draft 6

1. PROJECT EXECUTION

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Project WEB-site: http://www.mtm.upv.es/psy-nano-si

State of the art and Project objectives

The overall goal of this project is to explore the possible applications of porous silicon particles containing luminescent Si nanocrystals (nano-Si particles, or nano-Si) as efficient generators of singlet molecular oxygen ($^1O_2$) and develop novel biologically tolerable and environmentally friendly materials and prototype systems, able to compete with existing photo-sensitizers in photochemical, biological and medical applications.

Singlet molecular oxygen, $^1O_2$, is electronically excited molecular oxygen, which is extremely active towards chemical oxidation of organic molecules including those inside living cells. $^1O_2$ is usually produced due to excitation energy radiationless transfer to molecular oxygen, O_2, from a photosensitizer previously electronically excited by light. Such an approach is widely used in medicine for tumor cell destruction (Photodynamic Therapy, PDT) where organic dye molecules are used as a photosensitizer. Some of the bottlenecks in applications of organic dyes as a photosensitizer for PDT are:

1. High stability inside human body - that needs protecting the patient form direct sun light till the dye is completely removed from the body;
2. Side effects - dyes are complex organic molecules that might produce allergic reactions and other complications in patients.

Substitution of traditional dyes by nano-Si, which is known to be biologically tolerable material, can solve these problems: nano-Si will be passivated and/or dissolved very fast in the body and will lose its sensitizing ability in a controlled way without producing any side reactions.

In 2003 the joint team from Technical University of Munich, Moscow State University and Kobe University has discovered the generation of $^1O_2$ at the surface of nano-porous silicon layers under illumination [1]. This discovery is one of the fundaments of the PSY-NANO-Si Project. The second one is a nano-porous Si biodegradability [2]. However, many specific scientific and technological tasks of the Project had have to be solved to overcome the way from the fundamentals to practical materials and devices. The main objectives of the Project were:

- Research and development of technological processes for production of nano-Si materials in sufficient quantities;
- Research and development of technological processes for the formation of nano-Si materials with controllable size of porous particles;

- Comprehensive study of nano-Si as a photo-sensitizing material for generation of singlet oxygen in different media: organic solvents, water, physiological solutions;
- Modification of nano-Si surface to provide its hydrophylicity and protect it against fast dissolution in physiological solutions maintaining its sensitizing properties.
- Study of in vitro and in vivo cellular uptake and photo-cytotoxicity of nano-Si.

Here we report on the main technical achievements of the Project. The Report is organized via the following structure determined by pre-defined work packages (WP) of the Project:

WP1. Synthesis of nano-Si with size and properties adequate for sensitizing applications.
WP2. Modification of the composition and properties of nano-Si.
WP3. Kinetics and physical mechanisms of photo-stimulated generation of singlet oxygen by nano-Si.
WP4. Exploration of the possibilities to use nano-Si photo-sensitizers in Photodynamic Therapy of Cancer.
WP5. Standardization, management and control.

The work performed during the Project and the main achievements are described below.

References

1. D. Kovalev, E. Gross, N. Künzner, F. Koch, V. Yu. Timoshenko, M. Fujii, *Phys. Rev. Lett.* **89**, 137401 (2002)
2. L.T.Canham, C.L.Reeves, D.O.King, P.J.Branfield, J.Crabb, M.C.L.Ward. *Adv Mater.* **8**, 850 (1996).

1. Synthesis of nano-Si with size and properties adequate for sensitizing applications

1-1. Fabrication of nano-Si in quantities sufficient for its practical application

At the beginning of this project nano-Si was typically produced as nano-porous film by electrochemical etching of rather expensive mono-crystalline Si wafers [1]. The ordinary electrochemical process is carried out in an electrochemical cell, filled with diluted HF as an electrolyte, with a p-type doped c-Si wafer as anode and a Pt counter electrode. The anode etching is typically performed in galvanostatic regime (constant current trough the cell) and results in formation of porous silicon layer over the bulk silicon wafer (up to ~100 μm thick). At certain conditions – correlated HF concentrations and current densities – a layer of nano-porous Si is formed, which possess unique properties [1]. Such a layer is a porous matter with large internal surface, comprising of interconnected Si nano-crystals with typical sizes of only few nm, which are terminated by hydrogen atoms. In contrast with the bulk Si, nano-porous Si exhibits bright photoluminescence and can excite O_2 molecules to singlet state upon illumination. Thus, it can be used as photo sensitizer for generation of $^1O_2$. Photosensitizing nano-Si powder (needed e.g. for PDT) could be prepared by scratching and subsequent grinding of the porous layer. Very small quantities of photo sensitizer , micro-, or at the best, milligrams per wafer, can be prepared in this way. Such quantities are not sufficient for practical applications and are not enough even for the purposes of our research project alone. Thus, the first goal of the project has been to develop high output techniques for preparation of inexpensive nano-Si powder, which has the necessary physical and chemical properties to be used as an effective photo sensitizer. Two ways to achieve this goal have been explored:
- Chemical etching of poly-Si powder in HF/HNO_3 mixture (stain etching), and
- Electrochemical etching of large-size polycrystalline wafers with non-steady state current.

1-1.1. Study of stain-etching process for the production of nano-Si from standard poly-Si powders and wafers

Thin, luminescent nano-porous Si layers, referred to as “stain layers”, have been obtained in a chemical way by immersing c-Si wafers in etching solution containing HF, HNO_3 and water in certain ratios – typically at a HF excess [2, 3]. The chemical reactions, driving the stain-etching process, involve free carriers from the semiconductor, where the oxidant HNO_3 plays a role of free whole injector similarly to the positive electrode of an electrochemical cell. Thus, the mechanism of stain etching is quite similar to the anodic Si etching. Although the stain layers are thinner (only few μm), their morphology and photo-luminescent properties are similar to those of the thicker nano-porous layers formed electrochemically. On the other hand, the chemical (contact-less) nature of the stain-etching allows the process to be applied directly to silicon grains by simply mixing Si powder with the etching solution [4]. This approach has been followed by one of our teams (TUM) to develop a highly productive technique for preparation of photoactive nano-Si particles. Metallurgical grade polycrystalline silicon powder (~30 $/kg - commercially produced by Vesta Ceramics, LLC; mean particle size of 3-11 μm) has been used in this case as an initial material. Experiments with different modes of operation and various HF and HNO_3 concentrations have been carried out to establish a principal scheme for the process of Si powder stain etching [5] (see Fig. 1). Optimal results have been obtained by immersion of the Si powder in a solution of HF and water, followed by gradually adding HNO_3 until reaching HF:HNO_3:H_2O ratio of ~4:1:20 at the end of the etching process. Continuous mixing was required as the nano-structured Si particles become hydrophobic and float as a foam on the surface of the solution. The process has to be terminated in relatively small time window when a bright red-orange luminescence from the foam appears under UV excitation. The foam is then collected and dried under nitrogen flow. The so prepared product consists of nano-structured Si particles, described in more detail bellow.

The production of large quantities of nano-structured particles in that way is possible, but the control of the process become quite difficult if the mixing is performed manually. To make the stain-etching process more productive, less dangerous and more reproducible, an automated

machine has been constructed in TUM (Fig. 1). The machine is installed in a ventilated fume hood and consists generally of a rotatable polypropylene mixing drum (50 l volume) with Teflon ribs inside.

Figure 1. Machine for stain-etching of Si powder. The insert shows the wet foam of nano-Si particles as collected after etching in the machine.

The typical quantities of used chemicals and product, as well as the evolution of the etching process are illustrated in Figure 2. The machine can be loaded with up to 200 g of Si powder.

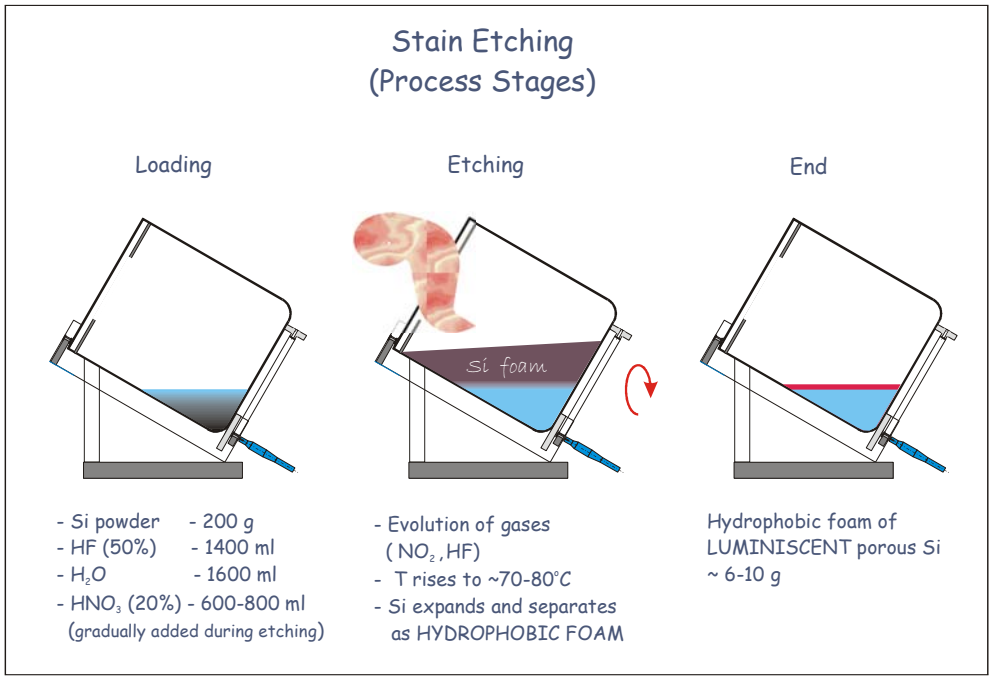

Figure 2. Scheme of the stain etching production of porosified silicon from crystalline Si-powder

After 30-40 min etching with intensive premixing, about 700 cm^3 of luminescent (wet) foam is produced from this Si quantity (insert in Fig 1). After drying in N_2 atmosphere and mechanical de-aggregation of the foam ~6-10 g of brightly luminescent nano-Si powder is obtained.

The mean size of the nano-Si particles, produced in this way, is reduced down to ~30% of the initial size of bulk Si grains and the particle surface is H-terminated, brightly luminescent and highly porous with an internal surface area about 250 m^2/g as measured via N_2 adsorption by standard Brunauer-Emmett-Teller method. The stain etched particles exhibit photo-luminescence with maximum near 670-700 nm that has been found to be optimal for energy transfer to molecular oxygen and corresponds to Si nanocrystal mean size about 2-4 nm. More

details about the structure and properties of stain-etched particles of various sizes can be found bellow (section 1-2). About 500 g of such nano-Si material has been produced during the Project implementation.

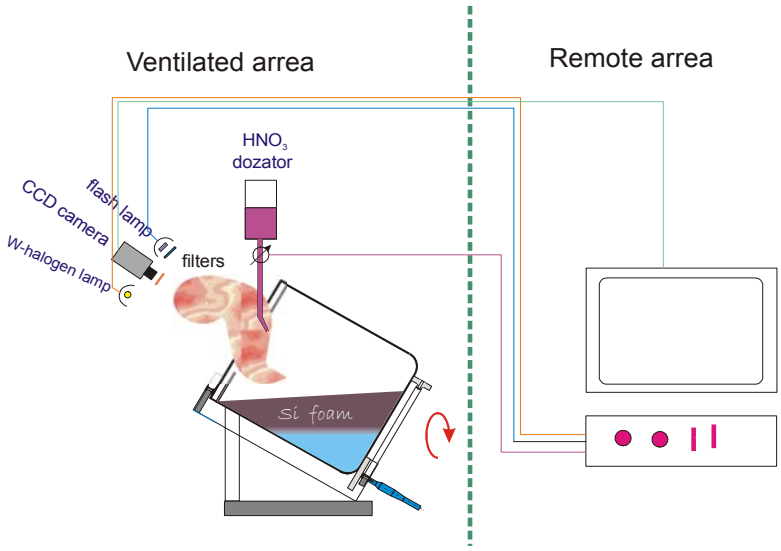

Figure 3. Completely automated system for production of nano-Si particles via stain etching

At the end of the project a completely automated system for production of nano-Si particles via stain etching has been designed. The available etching machine has been upgraded by adding a CCD camera, light sources and light filtering system. This allows the etching process to be monitored remotely in real time either directly or in a photo-luminescent mode. In the last case, the forming nano-Si foam is illuminated by blue light (powerful flash lams with blue filter) to excite luminescence from it while the luminescence evolution is observed by the CCD camera trough a red filter. Thus, the appropriate time window for termination of the process can be precisely determined. A remotely controlled HNO_3 dispenser has also been designed for improving the control of the etching process. The intensity of the etching and its termination can be varied remotely by controlling the rotation speed of the mixing drum. Actually, this system is a prototype of an industrial appliance for production of luminescent nano-Si powder.

1-1.2. Design of hardware and software for anodizing of large-size Si wafers in non-steady state regime

The existing up to now electrochemical etching technique does not provide an evidently scalable root for the production of Si nanocrystals. Indeed, estimated output of nanosilicon powder from one 4´´ Si wafer and etching time of 4 hours is about 0.5 gram. Therefore, the development of new experimental facility – powerful potentiostat with controllable and flexible choice of applied regimes - should open new perspective for production of nano-Si powder in quantities sufficient for practical application.

UPV team has proposed to use special pulse regimes for electrochemical treatment of large-size (4´´ and more) Si wafers to produce sub-micron luminescent porous Si particles with pre-determined and controlled sizes [6-8]. This regime requires both very short pulses of current with high intensity and quite long duration of a total treatment. To execute such a regime and analyze the results the very high number of data points (many millions) is needed for both signal generation and data acquisition. To the best our knowledge, such systems are not available in the market. To reach the necessary parameters, special original device (Potentiostat-Galvanostat MTM-400A), the corresponding software and thermo-stabilized electrochemical cell have been developed in UPV team (Fig. 4).

A B

Figure 4. (A) Potentiostat-Galvanostat MTM-400A; (B) Electrochemical cell and the system of its thermo-stabilization.

Table 1. The comparison of different electrochemical setups for pulse treatment

Hardware/ Software	Max Voltage, V	Max Current, A	Time Response	Number of experimental points	Number of pulses available
Power Supplies with home-made software	Large variety	Large variety	1sec	Software dependent	Software dependent
PAR-273A with standard software	100	1	1mks/0.3s	6144/Infinite	Appr.30
MTM-400A with VLAB software	200	9	3mks	Infinite	Infinite
PAR-273A + Upgrade kit and VLAB software	100	1	3mks	Infinite	Infinite

In the Table 1 some limits of the commercial and newly developed experimental setups are summarized. Hardware based on technology of pulse power supplies has too slow response for pulse treatment while traditional electrochemical research hardware has the limitation in output power and the number of data samples processed. New designed hardware MTM-400A overpasses both these limits and allows wafers processing in semi-industrial scale. We have developed also an upgrade kit for standard commercial galvanostat PAR-273A (Princeton Applied Research) which is used now in one of our laboratories (UPV) and allows to perform pulse regime experiments with small electrode areas.

1-1.3. Study of the formation of nano-Si powder by anodizing of large-area Si wafers

Besides the electrochemical hardware and sortware, we have developed and used a special electrochemical regime to introduce *a preferable grain size* into the entirely porosified Si material. This periodic pulse electrochemical regime permits layering of the entire porosified film and defines its preferable partition in the post anodization mechanical treatment. Thus, a preferable grain size is “hidden” in the grown porous Si film and is revealed only under additional mechanical treatment like milling. The pore dimension and total porosity are controlled by applied current density at the work pulse and prefabricated (hidden) grain size is

introduced during an application of the cut or/and relaxation pulses. We have applied the described procedure to produce a sub-micrometric grain porous Si powder and carried out SEM analysis of the material fabricated in different pulse regimes.

Four types of the electrochemical etching regimes have been used and compared: constant current regime I, two-level regime II (or III), and three-level regime IV (see the scheme below in Fig. 5).

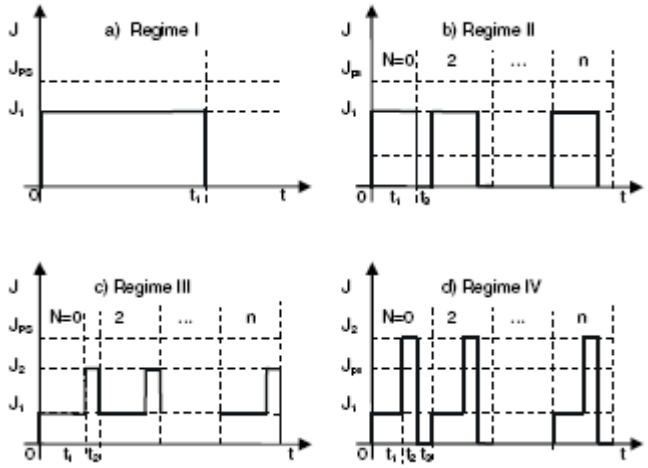

Figure 5. Description of the studied electrochemical pulse regimes.

The entire film produced under constant current conditions (Regime I) is always inhomogeneous in-depth regarding its pore size and porosity due to consumption of HF during anodizing and development of its concentration gradient. Samples produced under the periodic two-level regime II showed improved pore parameters due to smaller HF gradient along pore length. The best results were obtained with the regime IV: An additional cut/lift-off pulse introduces the well distinguished layering in the whole film formed on silicon wafer (Fig. 6).

The prepared porous Si material was finally scratched from a silicon substrate and milled in a ceramic mortar to reduce sizes of the obtained agglomerates. Due to employed two-level electrochemical regime, the porosified material has embedded moieties of controlled nanometric dimensions; they are more fragile and their crashing is easier. So, the obtained powder consists of nanoparticles which size is controlled in one dimension [9].

Yet more impressive results have been obtained when the above layered and then mortar-milled porous Si powder was dispersed in an organic solvent and additionally grinded in a special ball mill. After such a treatment the resulting nano-Si particle size (see Fig. 7) was indeed close to that predetermined by the electrochemical pulse treatment (about 400 nm in the case shown in Fig. 6) [9].

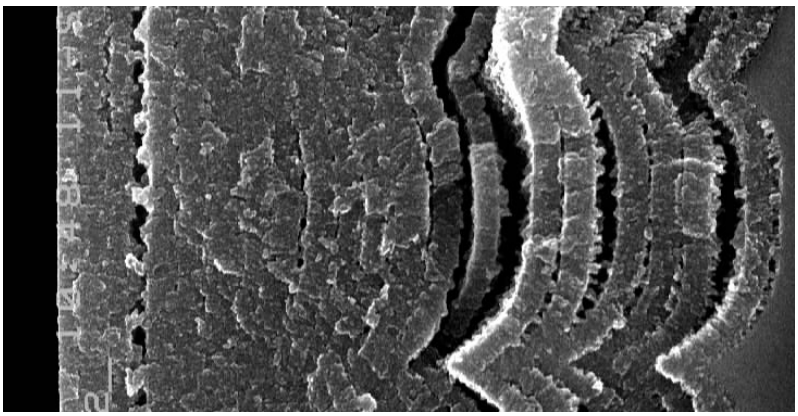

Figure 6. The SEM picture (cross-section) of a sample treated under a three pulse Regime IV: J_1 = 120 mA/cm^2, t_1 = 2 s, J_2 = 400 mA/cm^2, t_2 = 0.2 s, J_3 = 0, t_3 = 5 s, N = 20.

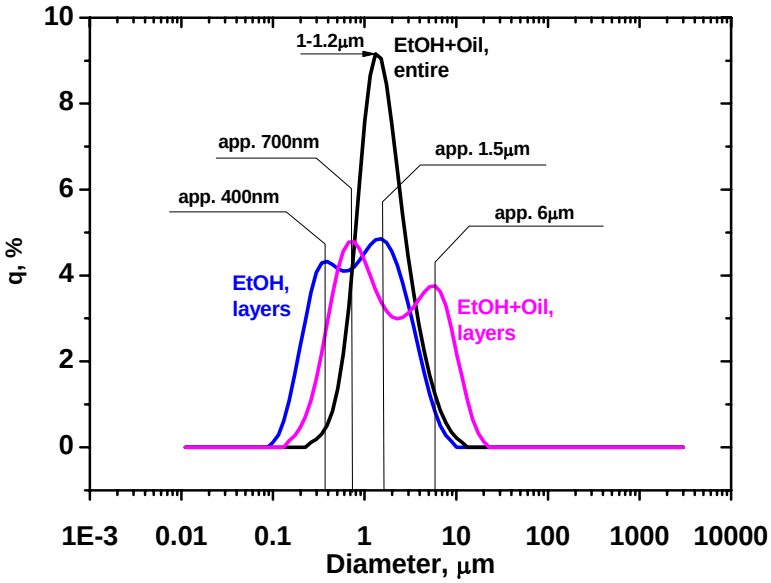

Figure 7. Size distribution of porous Si particles prepared by ball milling in different solvents. Blue and purple curves are obtained for the layered samples prepared by three-pulse electrochemical etching regime and milled in ethanol and ethanol/olive oil mixture, respectively. Black curve is obtained for the sample prepared by steady-state regime and milled in ethanol/olive oil mixture. Measurements are made by the Light Scattering Technique (Horiba instrument) [7].

1-2. Downscaling and standardization of the nano-Si particles

Various applications of nano-Si require particles with different sizes – some of them in the sub micrometer range. For instance, PDT implies transport of the photosensitizer to the treated cancer cells along the blood vessels. For the purpose, the photosensitizer particles should be smaller than 5 μm. A penetration trough the blood vessel walls requires particles smaller than 10 nm etc. Thus, the control of nano-Si particles size during preparation or by additional downscaling procedures is a task of our project.

The mean size of the porosified particles, produced by the stain etching method, depends on the initial size distribution of the Si grains in the silicon powder used as raw material. In principle, the machine, developed in TUM, can process reproducibly Si powders of any grain size. So, the downscaling of stain etched nano-Si particles is limited mainly by the availability of raw Si powder with micron and submicron mean size grains. In case of using the easily available powder with 3-11 μm grains, the finally obtained nano-Si particles has a broad size distribution in the 0.3-7 μm range as shown in Figure 8. The nano-porous layer on the surface of the particles can be clearly seen in the high magnification SEM images (Fig. 8b-8d). However the largest particles are incompletely porosified, containing a bulk crystalline core as can be seen in Figure 9. The reason for this peculiarity is that the stain layer cannot penetrate deeper than of 1-2 μm in the Si material. Thus, a preliminary ball milling of easy available Si powders (3-11 μm grain size) to grain sizes in the 1-4 μm range is enough for obtaining completely porosified nano-Si particles with sizes in the micrometer and near sub micrometer range. Further downscaling of the stain etched nano-Si material depends crucially on the availability of raw crystalline Si powder with grains of submicron size distribution. Such material is not available on the industrial market.

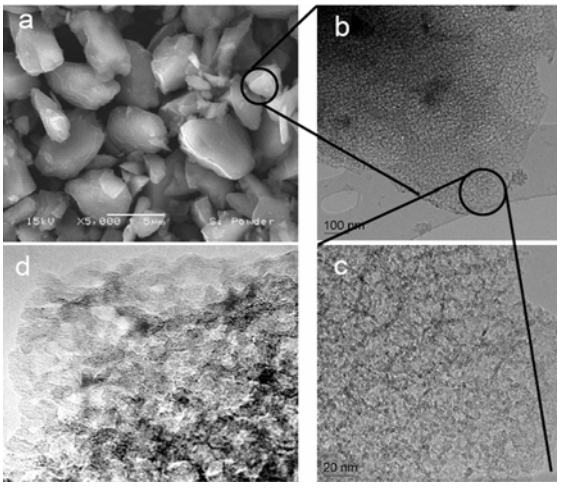

Figure 8. SEM (a) and HRTEM (b-d) images of the 0.3-40 μm size Si powder stain etched in solution: 7.5 ml of 49% HF + 4 ml of 18% HNO_3 per 1g of Si. Lattice fringes in (d) correspond to the (111) atomic planes of Si nanocrystals. Batch size of nano-Si can be up to 500 g.

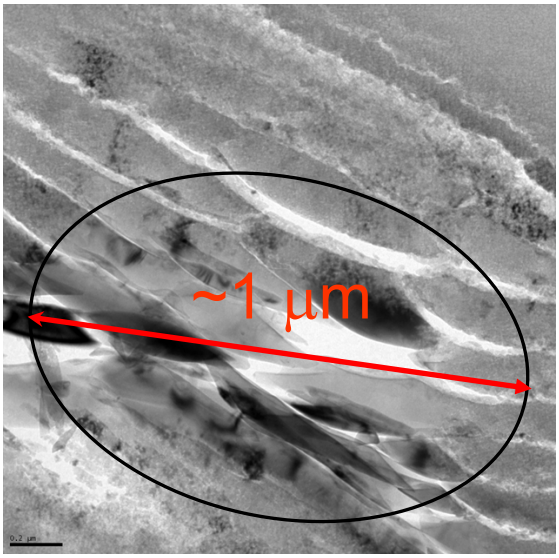

Figure 9. Cross-sectional TEM image of a single stain-etched particle of ~4 μm size. Ellipse in the center marks the non-porosified core of the Si grain.

Actually, the production of smaller (submicron to nanometer scale) nano-Si particles is quite challenging because (i) raw Si powders with grain sizes bellow 1 μm can not be prepared by milling of larger bulk Si grains and (ii) segregation of already prepared nano-Si powders by mechanical milling or ultrasonic fragmentation results in fast oxidation of nano-Si surface, thatv results in quenching the nano-Si exciton states responsible for $^1O_2$ formation (see below). In such a situation our Consortium has concluded that, to achieve production of smaller, but photoactive nano-Si particles, a new kind of raw Si material has to be explored. An interesting alternative has been found in using the experimental product, recently developed in the University of Duisburg, Germany (gift of Dr. Hartmut Wiggers). This product is brown to yellow powder, consisting of tiny silicon particles produced in the gas phase of a CVD reactor via decomposition of silanes either by infrared laser pulse or by microwave radiation. The first technique allows production of crystalline Si particles with mean size of ~ 200 nm and the second one of ~20 nm. The as prepared particles are rather porous themselves, but are not photoactive due to inappropriate internal structure and insufficient H-passivation. Therefore, we used these kinds of particles as row material, which has been further restructured by the stain-etching procedure developed previously. Indeed, stain-etching of the particles of ~200 nm mean size (actually having a broad size distribution in the 100-800 nm range) results in formation of brightly photo-luminescenting particles with size distribution in 0.05 – 0.5 μm range and with completely porosified bulk (without crystalline core) as can be seen in Figure 10A. The same treatment of the particles of ~20 nm mean size results in luminescent material consisting of almost ideal nano-silicon spheres with mean size down to ~ 5 nm (Fig. 10B).

It is important to mention that micrometer-size nano-Si particles can be produced by stain etching in large amount (batch size can be up to 500 g), while photoactive particles with nanometre sizes can only be produced in milligram quantities due to the limited amounts of raw silicon powder with ~ 200 nm and ~20 nm grains. Nevertheless, the presently available amounts of these materials were enough to perform first studies of their photosensitizing activity (see below).

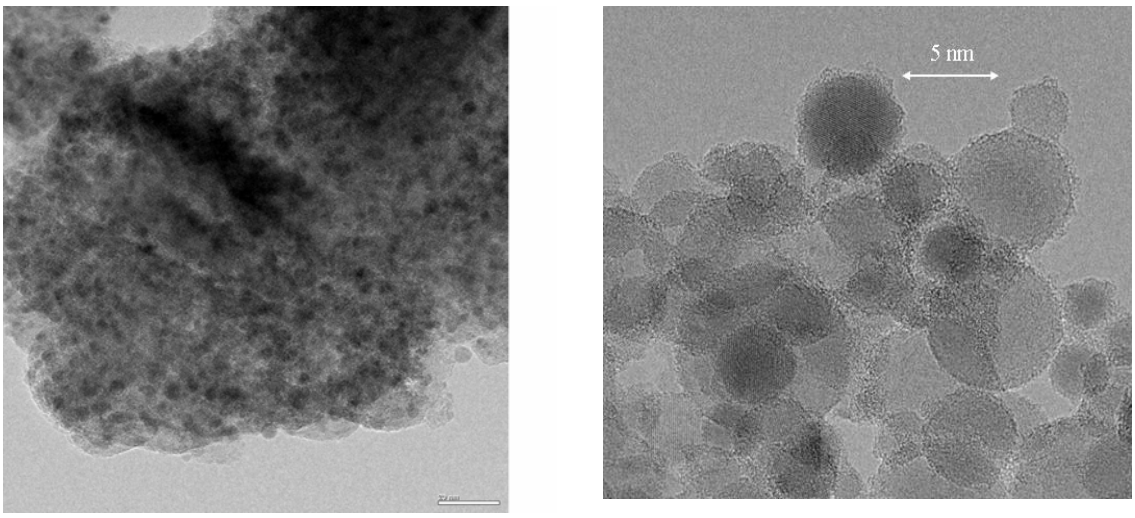

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Figure 10. (A) TEM image of the 0.1-1 μm size Si powder stain etched in solution: 7.5 ml of 49% HF + 4 ml of 18% HNO_3 per 1g of Si. (B) TEM image of the ~20 nm size Si powder stain etched in solution: 7.5 ml of 49% HF + 4 ml of 18% HNO_3 per 1g of Si.

1-3. Purification of the nano-Si particles for biomedical applications

1-3.1. Sterilization

Initially we applied standard sterilization procedures to the photoactive nano-Si particles intended to be applied in biomedical experiments. During these attempts we established that the heating of porous Si structures above 100°C in air atmosphere results in their fast oxidation by back-bonded oxygen incorporation (see Fig. 11), which significantly reduces the ability of the photosensitizer particles to generate singlet oxygen upon illumination. Thus, the sterilization of nano-silicon structures has to be performed in vacuum to preserve their photo activity and therefore the further experiments have been performed in high vacuum ($P<10^{-5}$ mbar) in a wide temperature range. Below we list the most important observations relevant to such sterilization procedures:

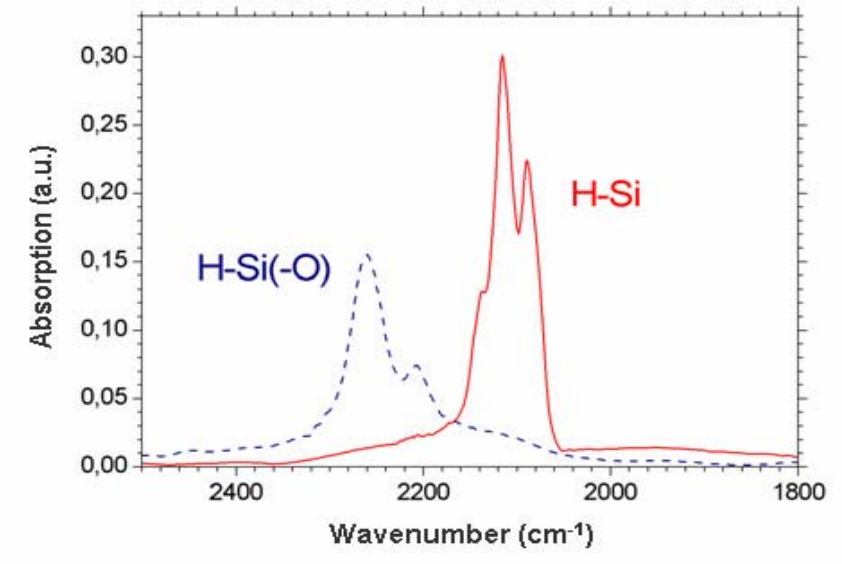

Figure 11. FTIR spectra, revealing the surface termination of Si nanostructures before sterilization (red) and after heating in air atmosphere at 200 ^{0}C for 30 minutes; Formation of monolayer of backbonded oxygen can be readily seen (blue curve).

1. Heating to 150-180^{o}C in vacuum does not influence significantly photoluminescence quantum yield of all tested Si nanostructures and their singlet oxygen generation rate. Heating above 200^{0}C gradually decreases both the photo-luminescence quantum yield and singlet oxygen generation rate. The photo-activity of the nano-structured materials is completely lost after heating above 300^{0}C as the hydrogen passivation is destroyed.
2. The presence of a water vapour during heating should be avoided because it induces fast oxidation of silicon nanocrystals that increases photoluminescence quantum yield but significantly decreases singlet oxygen generation rate.
3. Lipid-terminated surfaces are not stable under heating above 50^{0}C due to instability of lipids in this temperature range. Therefore surface termination of silicon nanocrystals by lipids should be performed after sterilization.

1-3.2. Cleaning from toxic residuals

The first IN VITRO experiments with biological cells have shown that most of the nano-Si particles, prepared by the project partners, induce a strong dark cytotoxic effect (before the active PDT phase) that very much depends on the particle sample. This unwanted effect is in contradiction with the initial project assumption that nano-Si is biocompatible. It also interferes with the observation of photo-cytotoxic effects, related to $^{1}O_2$ generation under illumination. Therefore, an intense search for the reasons behind the dark cytotoxic activity of the nano-Si material was set up. The team at TUM made a comprehensive investigation of the volatile species, released from stain-etched particles, by using mass-spectrometric measurements during a temperature ramp (thermal effusion spectra). A number of termo-induced signals were identified as fragments of HF and SiF_4 gases, which originate from HF and H_2SiF_6 residuals in the porous layer (Fig. 12). Both these substances are highly toxic and obviously are leftovers of the Si etching process (H_2SiF_6 is the major product of Si etching in HF containing solutions). Anomalous effusion of H_2O and OH fragments were also observed that can be explained by presence of partially polymerised silicates (non-volatile and non-toxic themselves), formed by H_2SiO_3 - another final product of Si etching. The major findings of the investigation can be summarised as follows:

- Immediately after preparation by stain-etching the nano-Si particles contain a significant amount of toxic impurities – HF and H_2SiF_6. These impurities are dangerous for biomedical applications. On the other hand, their presence preserves the nano-Si particles from oxidation and therefore the stain-etched photo-sensitizers (in contrast with the electrochemically produced) are stable under ambient conditions for months and even years. The later can be important for other (non-biological) applications of such sensitizers.
- The toxic impurities can reside in nano-Si in different forms: i) H_2SiF_6 and HF adsorbed within the pores of the particles (highly soluble state), ii) H_2SiF_6 and HF trapped by incompletely polymerized metasilicic acid within the pores (weakly soluble state) iii) H_2SiF_6 and HF encapsulated in completely polymerized silicate (non-soluble).
- The nano-Si particles, prepared by electrochemical etching (UPV), show much lower, but still significant, concentrations HF and H_2SiF_6 residua, residing mainly in absorbed state.

Following these findings we tried to develop an efficient procedure for detoxication of the stain-etched particles, without deteriorating their photo-activity. A series of samples - prepared at different etching conditions and post-treated in various ways – have been investigated by mass-spectrometric measurements, FTIR spectra (TUM) and simultaneously by IN VITRO cytotoxicity experiments (K.U.Leuven). Finally an appropriate three step cleaning procedure was established:

1) Washing of the wet particles with DI water immediately after the stain etching (before drying). Because the nano-Si particles are hydrophobic, this step is performed in a special vessel at intensive shaking and repeated several times till the PH-factor of the rest water approaches 6-7. It serves to remove the major quantities of toxic impurities and also to get rid of poly-silicate products, which can stabilize the toxic

substances during drying. A negative effect of this step is a partial surface oxidation of the np-Si layer (not wanted), which requires further surface modification.

2) Restoration of particles photo-activity by renovation of the Si-H surface termination via oxide etching in limited quantity of HF/alcohol solution at conditions of HF starvation. This step results in a minor recontamination of the particles.

3) Outgasing of the particles in vacuum at 180-200°C for at least 1 hour, which removes the last remainings of absorbed impurities. This step serves also as final sterilization of the particles.

Figure 13 illustrates the effect of the complete cleaning procedure on the effusion spectra originating from HF and H_2SiF_6 impurities. One can see that the quantity of toxic substances in the particles decreases by more than 3 orders of magnitude after complete purification process.

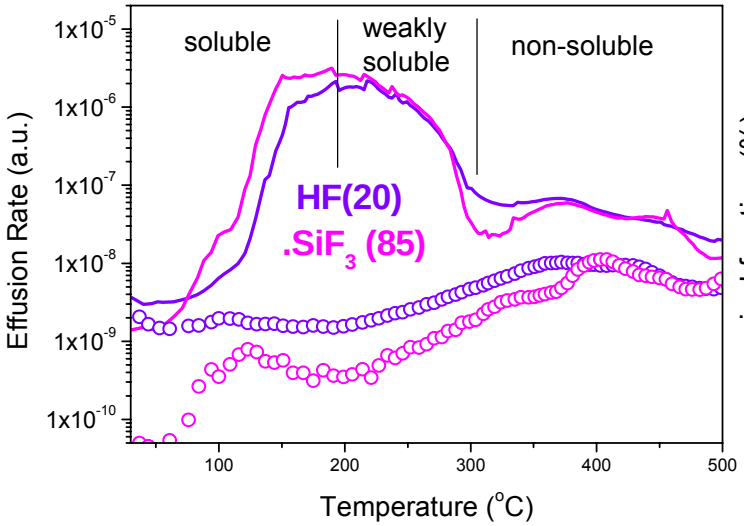

Figure 12. Thermal effusion spectra, originating from HF (violet) and H_2SiF_6 (magenda) impurities in stain-etched nano-Si particles, as measured immediately after particles preparation (lines) and after complete cleaning procedure (open circles).

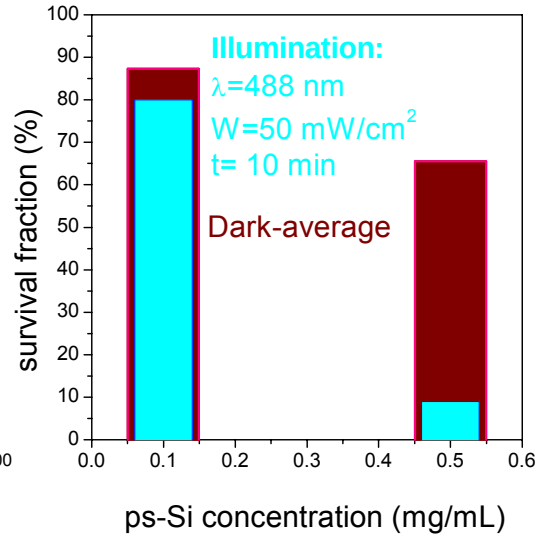

Figure 13. Cyto-toxic effects of stain-etched and purified nano-Si particles on living biological cells. The toxic effect in darkness (brown bars) and under illumination (cyan bars) are presented by the fraction of living cells that survive the respective treatment.

Figure 13 illustrates that the purification of stain-etched nano-Si particles results in a reasonably low particle toxicity in darkness, while preserving their photo-activity. More results on (photo)cytotoxicity investigations are presented in section 4 of this report. The details concerning the purification of stain-etched photo-sensitizers will be published elsewhere [10].

Conclusions

Nano-Si particles, suitable for photo-sensitizing applications, can be produced in large quantities by direct stain-etching of crystalline Si powders. The size of the photoactive particles produced in that way can be varied in the micrometer to nano-meter range and depends mainly on the grain size of the raw Si material. The stain-etching process and the equipment, developed in the course of this project, can be easily rescaled to industrial production.

Immediately after preparation by stain-etching the nano-Si particles contain a significant amount of toxic impurities – HF and H_2SiF_6. Thus, they are not appropriate for direct bio-medical applications. On the other hand, these impurities stabilise the photo-activity of the stain-etched material in air ambient for a prolonged time (up to years), which is an advantage for non-biological applications.

An efficient cleaning procedure for removal of the toxic substances from nano-Si particles was developed. The procedure results in a reduction of the concentration of HF and H_2SiF_6 residua in stain-etched particles by more than three orders of magnitude. Thus, the purified particles, initially produced by stain-etching, become acceptable for bio-medical applications too.

Ways to increase the productivity of electrochemical production of nano-Si via non-steady-state processes were demonstrated in the course of the project. A large flexibility in respect to the structure and properties of the obtained nano-porous material has been achieved by applying pulsed current regimes. A significant added advantage of these regimes is that particles with electrochemically predetermined sizes can be produced by segregation of the so prepared nano-porous layer.

The nano-Si particles, prepared by electrochemical etching show much lower, but still measurable, concentrations HF and H_2SiF_6 residua. A simplified purification procedure – outgasing in vacuum at 150-180^oC - can be recommended for successful bio-medical applications of electrochemically prepared photo-sensitizers.

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2. Modification of the composition and properties of nano-Si

2-1. Ageing effects in nano-Si powders and films

Freshly formed luminescent nano-Si is hydrogen passivated. While exposed to the air environment, hydrogen atoms are gradually substituted due to interaction with oxygen that results in formation of more stable chemical bonds with oxygen participation (Si-O-Si and other groups). The process of gradual change of nano-Si surface due to interaction with environment is known as “ageing” and is found to be even helpful from the point of view of the nano-Si luminescence intensity increase and stabilization. However, intuitively it is clear that oxidation of nano-Si surface may protect the surface from electronic contacts with adsorbed oxygen molecules and, therefore, prevent singlet oxygen production. Therefore, the objectives to reach were to study ageing effects in nano-Si (to be able to control the sensitizer properties) and develop different surface chemistries, if needed, to preserve luminescence and singlet oxygen formation by nano-Si in narural environments.

2-1.1. Ageing effects in powders and films of nano-Si in natural environment

Electrochemically prepared nano-Si films and powders. We have studied an influence of nano-Si ageing effect on the luminescence spectra and on the efficiency of energy transfer from nano-Si excitons to O_2. As a characteristic related to the energy transfer efficiency (and mechanism, see below), we have proposed to use the spectra of nano-Si photoluminescence (PL) reversible quenching by oxygen, $Q_{O2}(\lambda)$, determined as:

$$Q_{O_2}(\lambda) = \frac{I_{N_2}(\lambda)}{I_{O_2}(\lambda)} \qquad (1),$$

where $I_{N2}(\lambda)$ is the PL spectrum measured in non-quenching N_2 environment and $I_{O2}(\lambda)$ is the PL spectrum in quenching O_2 atmosphere (Fig. 1A). Evolution of the Q_{O2} parameter over ageing time allows for studying of the energy transfer mechanism and correlates it with the changes occurred in chemical composition of the materials within the same period of time.

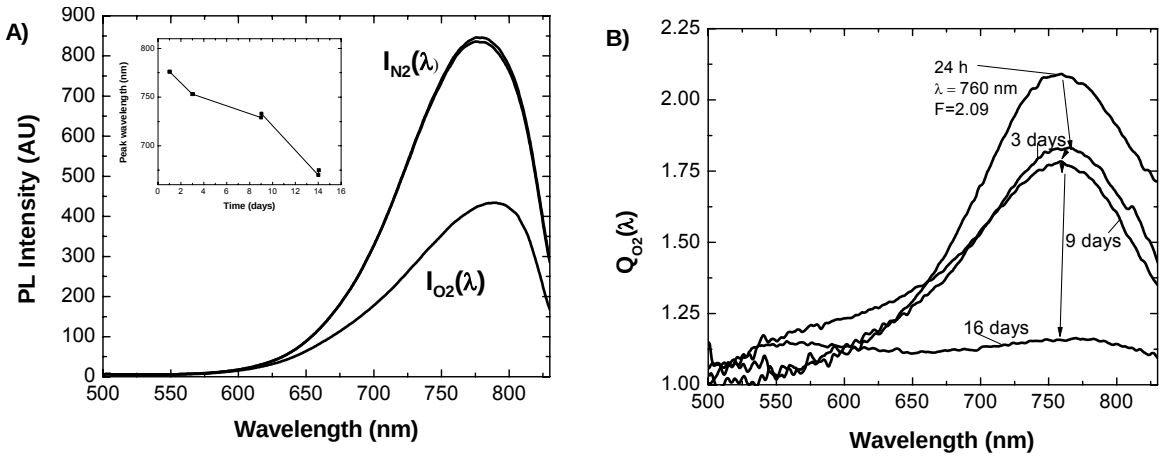


Figure 1. A) PL emission from the electrochemically prepared nano-Si material (layer on a wafer) in different ambient: nitrogen and oxygen gases. Inset: evolution of the PL peak wavelength with ageing time. B) “Quenching spectra” (see text) and their evolution during natural aging in air environment.

The main results of the “natural ageing” are the following: (i) the PL spectrum experiences monotonic short wavelength shift during natural ageing, from initial maximum position in red - near IR (780 nm in Fig. 1) to final (stabilized) position around 600 nm after ~30 days of ageing; (ii) The PL intensity remains approximately same during this time (or even increases, see below); (iii) the efficiency of PL quenching by O_2 dramatically drops during the first 2 weeks of ageing. The latter property is well demonstrated in the Fig. 1B: after 16 days of ageing nano-Si PL is almost insensitive to oxygen.

Especially important are found to be the data concerning the details of the property (iii), namely the profile of the quenching spectrum and its changes with time. Indeed, initially it has a maximum near 760 nm that corresponds to the energy of the second excited ($^1\Sigma$) state of molecular oxygen and evidences in favour of energy transfer from excited Si nanocrystals to O_2 as a mechanism responsible for the luminescence quenching. After 2 weeks in air atmosphere quenching efficiency becomes very low and a new shoulder appears in the quenching spectrum at ~580 nm that indicates about an appearance of a new PL centres or new quenching mechanisms in this system (see below).The decrease of efficiency of PL quenching by O_2 is accompanied by an appearance and growth of additional absorption bands in the 2200-2300 cm^{-1} region of the FTIR spectrum, which are related to vibrations of (O)-Si-H_x groups containing back-bonded oxygen atoms. Therefore, we conclude that formation of the layer of back-bonded oxygen atoms during nano-Si natural ageing is a reason of fast (~2 weeks) lost of photosensitizing activity of naturally (in air atmosphere) aged nanoSi.

We showed also that physical (via phisisorption) covering of nano-Si surface by a protective layer of unsaturated hydrocarbons (see below about nano-Si functionalization) does not slow down the ageing effect, but decreases an initial value of quenching efficiency in the quenching band maximum (760 nm) from 2.1 to 1.7 (not shown).

We studied also the nano-Si ageing effect in “neutral” organic solvents, such as hexane. We found that, similarly to the case of air atmosphere, short wavelength shift of the maximum of PL spectrum takes place, and the maximum position stabilizes after 30 days of ageing. However, contrary to air atmosphere, PL intensity does not remain during ageing and decreases 3-5 times during 30 days (not shown).

Stain-etched nano-Si powder. Contrary to the electrochemically prepared nano-Si, stain-etched nano-Si maintains constant both PL intensity and efficiency of PL quenching by O_2 during months and even years of the material contact with air atmosphere. Being so, only one quenching band at 760 nm is observed in quenching spectrum with a maximum efficiency of quenching about 2.2 (not shown). FTIR measurements have shown also a full absence of Si back-bonded oxygen. This “ageingless” stain-etched nano-Si powder has attracted our attention at the initial stage of the Project implementation as a very promising material for applications as photosensitizer for $^1O_2$ production. However, as it has been shown later (see Part 3), this feature of the material is due to a presence of residual HF in nano-pores, which continuously remove Si oxides and refreshes Si-H covering. The underside of this feature is an increased dark cytotoxicity of the stain-etched nano-Si powder.

2-1.2. Stability of nano-Si powders and films towards heat treatment in environment and in vacuum

Except natural ageing, nano-Si surface modification at elevated temperatures in air atmosphere and in vacuum has been studied with the aim to elaborate the method of nano-Si sterilization. It is found that annealing in air at temperatures between 120^{o}C and 270^{o}C results in formation of back-bonded oxygen (similar to oxidation process at room temperature). At temperatures above 300^{o}C hydrogen atoms are removed from the surface due to effusion and a layer of Si-O is formed at the surface (not shown).

Heating in vacuum does not affect surface composition of Si nanocrystals up to ~ 270^{o}C but afterwards a gradual effusion of hydrogen from the surface is detected. This result gives us an idea about a sterilization method of Si nanocrystals that would a thermal treatment in high vacuum (10^{-5} bar) in the temperature range below 250^{o}C.

2-1.3. Stability of nano-Si powders and films towards oxidation in aqueous solutions

The process of Si nanocrystal oxidation can be performed in wet conditions. Apparently, a wet oxidation is more favorable than dry oxidation because of the rate. In wet oxidation, the main oxidizing agent is water, and the process is governed by the following reaction resulting in hydrogen generation:

$$Si\ (solid) + 2H_2O\ (vapor) = SiO_2\ (solid) + 2H_2$$

However, in presence of water not only Si oxidation, but also Si etching (dissolution) can take place. Indeed, Si etching implies the presence of both oxidant and reducer. For instance, etching rate of bulk Si by hydroxide in concentrated KOH solution (pH 14) is of about 1 μm/min. Since water contains both oxidant and reducer components it is easy to show that water can provide etching of porous Si. Indeed, because the surface of porous Si (10^7 cm^2/g) is about 10^7 higher than that of bulk Si (1 cm^2/g), the concentration of OH^- anions can be 10^7 times lower than that at pH 14 to provide similar etching effect. Therefore, one can expect that in water at pH 7 efficient etching (dissolution) of porous Si can take place. Indeed, we found that mixing of nano-Si (Si nanocrystals) with water results in their complete oxidation in a time scale of an hour that is accompanied by efficient generation of hydrogen. This effect is even more efficient under illumination.

It became clear after above mentioned experiments that special consortium efforts are required to develop novel methods towards tailoring of surface properties of Si nanocrystals. This work has been done and is described below.

2-2. Derivatization of nano-Si to supply it with necessary physical-chemical properties

Since as-prepared luminescent Si nanocrystals (SiNCs) are H-terminated and therefore hydrophobic, the first step to create a prototype of SiNC-based photosensitizer should be a modification of SiNC surface with the aim to make it hydrophilic and able to work in biological ambient. Such surface modification by surfactants should not, however, result in a decrease of the NC durability that may, in principle, take place due to surfactant-induced weakening of surface tension and consequent increase of interaction with H_2O molecules. Surfactants should not also decrease an ability of the nanocrystals to transfer the excitation energy to acceptors (first of all molecular oxygen) on their surface. These rather contradictory tasks have been largely solved in the framework of the present project by means of porous Si (porSi) surface modification by hydrocarbons, both physically (adsorption) and chemically (formation of Si-C bond). The modification procedures as well as physical-chemical properties of modified surfaces will be described below.

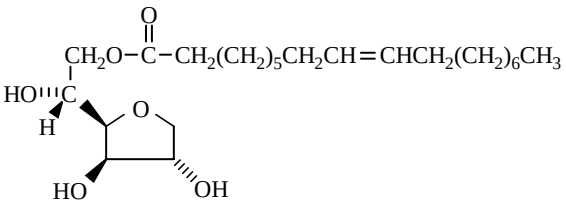
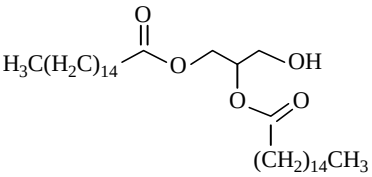
2-2.1. Derivatization of stain-etched porSi powder by means of physical adsorption of non-ionic surfactants

First experiments for covering nano-Si powder by a hydrocarbon layer with polar end groups have been carried out with luminescent nano-Si powder produced by applying stain etching process (pure chemical treatment) to the metallurgical grade polycrystalline silicon powder (commercially produced by Vesta Ceramics, LLC; mean particle size of 3-11 μm, the mean particle size after stain etching was ~1-4 μm).

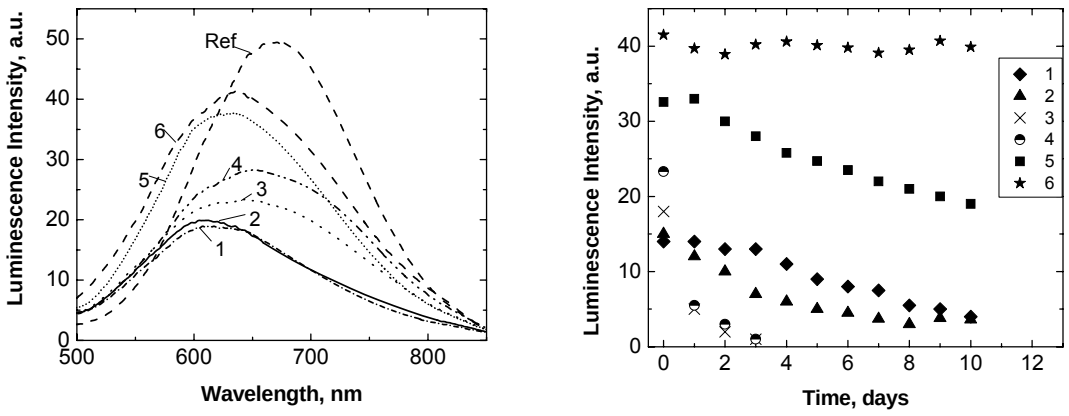
An endowment with the wettability on the surface of nano-Si was provided in the present work by physical adsorption of *nonionic surfactants* since it has been shown in the literature that use of more traditional ionic surfactants can result in nano-Si PL quenching. Chemical formulas of the ionic surfactants are summarized in Table 1.

As one can see, hydrocarbons of different length and with different end polar groups have been used. It has been found that after ultrasound treatment of the mixtures of nano-Si powder with hexane solution of the above hydrocarbons the powder surface becomes hydrophilic and the PL intensities of such hydrocarbon-covered nano-Si powders are of comparable value, although suspensions of the samples **5** and **6** show the highest (1.5-2.0 times higher than for other samples) PL intensity (see Fig. 2A).

Table 1. Names and formulas of the surfactants used

Number	Name	Full formula
1	Oleyl ether glycolic acid ethoxylate	$CH_3(CH_2)_xCH_2CH{=}CHCH_2(CH_2)_6CH_2O(CH_2CH_2O)_yCH_2{-}C(=O){-}OH$, x=4-6; y ~ 2
2	Sorbitan monooleate (Span 80)	
3	Monoglyceride (DL-α-laurin)	$H_2C(OH){-}CH(OH){-}CH_2O{-}C(=O){-}CH_2(CH_2)_9CH_3$
4	Diglyceride (1,2-palmitin)	
5	Oleyl alcohol	$CH_3(CH_2)_6CH_2CH{=}CHCH_2(CH_2)_6CH_2OH$
6	Undecylenic acid	$H_2C{=}CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2C(=O){-}OH$

We have studied also a stability (durability) of all suspensions as a function of time elapsed after suspension preparation (Fig. 2B). The best results are again obtained for the samples **5** and **6** (especially for **6**). Therefore, the surfactants **5** (oleyl alcohol) and **6** (undecylenic acid) at best satisfy the requirements of the nano-Si surface wettability and long time stability in water without substantial quenching of nano-Si PL.



A B

Figure 2. (A) PL spectra of aqueous suspensions of the nano-Si powder covered by different surfactants. Full names of the surfactants used are shown in Table 1. Excitation was at 350 nm. (B) Time evolution of the PL intensity of aqueous suspensions of the surfactant-covered nano-Si powders.

It is worth to note that for all samples studied a drop of the PL intensity as a function of time that the modified powders contact with water was accompanied by a shortwavelength shift of the luminescence band maximum. It implies that the nano-Si surface oxidation by water, which is accompanied by a decrease of the crystalline phase of luminescent nanoparticles and corresponding blue shit of the PL spectra due to quantum confinement effect, is responsible for a drop of nano-Si luminescence intensity.

Our preliminary measurements have shown that for all these suspensions a sensitivity of their PL to oxygen remains to be rather high even after several days after preparation (Fig. 3A). One could suggest that the proposed physical (i.e. via physisotbtion) covering of the stain-etched nano-Si powders by linear unsaturated hydrocarbons, such as oleyl alcohol or undecylenic acid, solves all the problems and creates an organic layer protective against nano-Si oxidation/dissolution and, on the other hand, penetrable for molecular oxygen to produce singlet oxygen. However, more detailed study of the interaction between the hydrocarbon-covered nano-Si surface and molecular oxygen enabled us to find evidences in favour of a change of the mechanism responsible for the PL quenching.

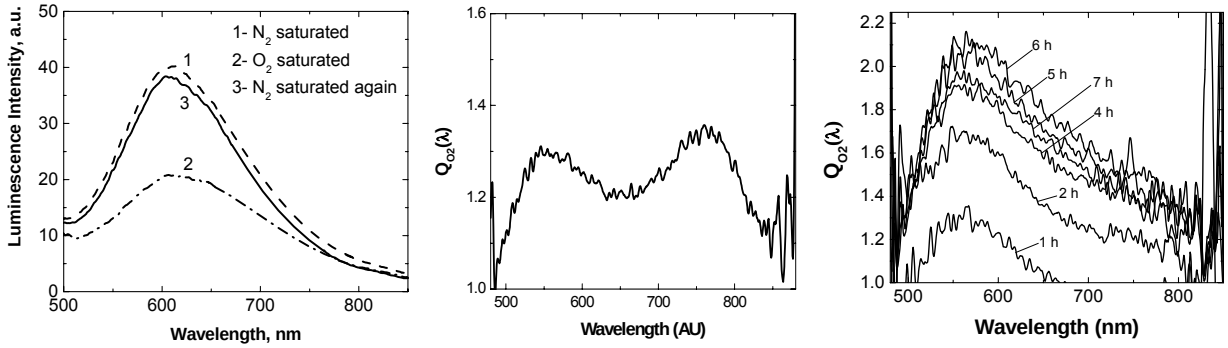


Figure 3. (A) PL spectra of a water suspension of the stain-etched nano-Si powder covered by undecylenic acid after purging the measurement cell with N_2 (1), O_2 (2) and again N_2 gas (3). (B) Spectrum of quenching of the PL of the same powder (in dry state) by O_2 measured in gas phase. (C) Spectra of quenching of the PL of the same powder by O_2 in water suspension measured with different delay (in hours) after exposure the powder to water.

Indeed, even before exposure to water, immediately after physical covering by a hydrocarbon layer, quenching spectra of the nano-Si powder exhibit not only the band at 760 nm corresponding to the excitation energy transfer to molecular oxygen, but an additional band at 550 nm (Fig. 3B). After exposure to water the second quenching band becomes dominant and its intensity growth with time (Fig. 3C). As our analysis has shown, this additional band in the 550-580 nm region corresponds to quenching of the PL related with new luminescent surface states formed due to nano-Si oxidation and containing SiO groups, most likely in the form of Si=O. FTIR measurements unambiguously show that appearance of these luminescent surface states is not related to back-bonded oxygen in (O)Si-H_x groups. In this connection the question arises if the nano-Si PL quenching related with ~550 nm quenching band results in formation of singlet (excited) oxygen or different mechanisms are responsible for this quenching. The question will be discussed below, after presentation of the data obtained with electrochemically-prepared nano-Si.

2-2.2. Durability and photophysical properties of surfactant-covered particles of electrochemically-prepared porous silicon in aqueous suspensions

It has become clear after first experiments on photo-cytotoxicity of different types of nano-Si particles that electrochemically prepared nano-Si shows much higher photoeffect in living cells as compared to stain-etched nano-Si. As a result, our further efforts were directed to functionalization of electrochemically prepared nano-Si and detailed study of photophysical properties of this hybrid material. Two alternative methods of nano-Si surface functionalization were applied, physical adsorption or chemical binding of undecylenic acid.

Silicon wafers of both *n*+-(0.01-0.02 Ohm·cm) and p^--type (10-20 Ohm·cm) were used for electrochemical preparation of bright luminescence nano-Si layers. In both cases wafers were etched in 1:1 HF: ethyl alcohol electrolyte solution under current density of 50-150 mA/cm^2. For *n*+-type Si illumination by the light of the 100 W halogen lamp during 25 minutes was employed. For powder preparation nano-Si layer was scratched from the surface and grinded mechanically.

For *physical* modification of nano-Si samples a suspension was prepared by dipping 20 mg of nano-Si powder in 10 ml of hexane containing 1-2 mg of undecylenic acid. Short sonication was employed to facilitate penetration of the surfactant molecules into the pores; then the organic solvent was completely evaporated. The total time for coverage procedure was as short as 1 hour. For *chemical* modification of the obtained wafers or powders, the stainless steel reactor was employed: the nano-Si powder (or a layer on a wafer) with a surfactant (undecylenic acid) was introduced and heated in an oven at 125ºC during 16 hours. Upon cooling the powder was washed with chloroform to eliminate an excess of non-reacted undecylenic acid.

For aqueous suspension preparation both types of surface modified nano-Si powders were simply suspended in de-ionized water and then the suspensions were studied with respect to their luminescent and other properties.

For the study of nano-Si luminescence reversible quenching by oxygen, bubbling of aqueous nano-Si suspensions alternatively by nitrogen and oxygen gases during 5-10 minutes was carried out followed by PL spectra measurements.

Fig. 4A shows a typical time evolution of PL spectra of the physically modified nano-Si aqueous suspension (measured under N_2 atmosphere). The changes observed well conform to the known scheme according to which nano-Si surface interaction with water results in fast Si NCs oxidation. It implies a decrease of the Si-NC core size and, following the quantum confinement effect, a short wavelength shift of the PL spectrum. Somewhat surprising, however, remains a considerable (up to 50-fold in some cases) increase of the PL intensity of nano-Si powder aqueous suspension after one-two day keeping in water.

Fig. 4B shows typical quenching spectra $Q_{O2}(\lambda)$ obtained simultaneously with the corresponding luminescence spectra shown in Fig. 4A. One can see that the initial $Q_{O2}(\lambda)$ spectrum obtained 30 minutes after suspension preparation contains mainly one band centered at ~780 nm, but three hours later a much more intensive band around 570 nm prevails in the $Q_{O2}(\lambda)$ spectrum. During the next four days (96 hours) this maximum remains to prevail, although its maximum magnitude fluctuates between 1.5 and 2.0. During the next several days both quenching bands decrease their amplitude and then disappear at all (Q_{O2} ~1.0 over the all spectrum) that corresponds to the absence of quenching action of oxygen, although luminescence itself continues to be fairly intensive (not shown). Therefore, maximum amplitude of the quenching band at 570 nm is reached after ~0.5-2.0 day of PSi keeping in water and has a magnitude of ~2.0 vs ~1.25 for the maximum amplitude of the quenching band in the 760 nm region.

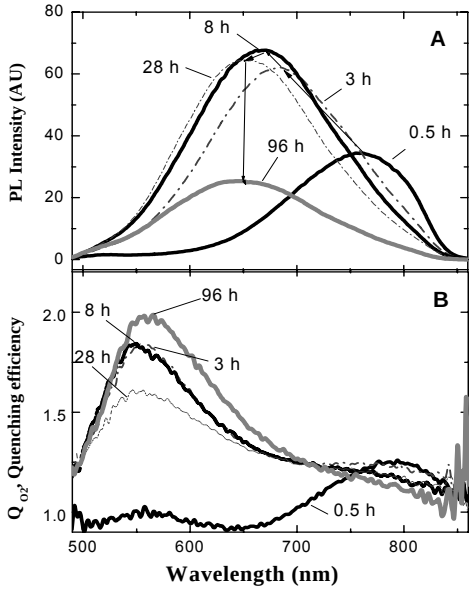


Figure 4. PL (A) and PL quenching efficiency (B) spectra measured for aqueous suspension of physically modified nano-Si powder at different times after the powder immersion in water.

To describe an integral effect of nano-Si PL quenching by molecular oxygen, the two new characteristics will be introducedm sucy as:

- an integral (averaged over the PL spectrum) efficiency of nano-Si PL quenching by O_2,

$$Q_{O2-INT} = \frac{\int I_{N2}(\lambda)d\lambda}{\int I_{O2}(\lambda)d\lambda}, \quad (2)$$

- relative number of quenched nano-Si excitons normalized on the first measurement value,

$$N_{1O2} = \left[\int I_{N2}(\lambda)d\lambda - \int I_{O2}(\lambda)d\lambda\right]_{NORM}. \quad (3)$$

As one can see in Fig. 5, $Q_{O2\text{-}INT}$ increases from 1.1 up to 1.3-1.4 during the first 4 hours after suspension preparation and remains at this level during ~4 days, whereas N_{1O2} experiences 6.5-fold increase during the first 8 hours of nano-Si interaction with water. This is rather interesting result for possible practical applications of PSi as a photosensitizer in aqueous environment since, if we accept that energy of each Si-nc exciton quenched in the 570 nm band of the PL quenching spectrum is transferred to O_2, then this huge increase of N_{1O2} is equal to the growth of a number (concentration) of $^1O_2$ molecules generated by nano-Si.

Since the initial growth of $Q_{O2\text{-}INT}$ is accompanied by (i) total increase of the PL intensity, and (ii) a shift of the PL spectrum to the short wavelength side of the spectrum where intensive quenching band near 570 nm appears at the same time, a magnitude of N_{1O2} increases. In our case (Fig. 5) the increase of N_{1O2} is 6.5-fold. One can suggest that in case of 30- or even 50-fold build-up of the luminescence intensity of nano-Si aqueous suspensions, that has been observed earlier, similarly large increase can be expected for N_{1O2}.

Therefore, there are good grounds for saying that concentration of $^1O_2$ generated by nano-Si powder in aqueous environment may considerably increase after the powder immersion in water and reach its maximum not earlier than several hours after beginning of nano-Si interaction with water. Certainly, new investigations should be done to obtain final conclusions. First of all, the above mentioned suggestion should be confirmed or discarded that the PL quenching in the quenching band at ~570 nm follows the mechanism of energy transfer with $^1O_2$ formation. Such investigations are in progress in our groups.

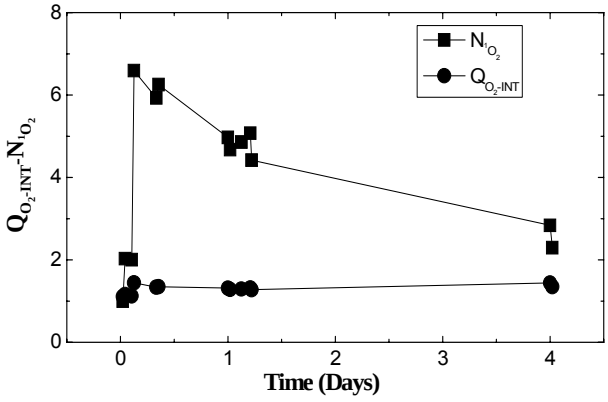


Figure 5. Time evolution of the integral efficiency of nano-Si PL quenching by O_2, $Q_{O2\text{-}INT}$, and of the relative number of quenched nano-Si excitons, N_{1O2}.

The most interesting fact revealed in the above data is an observation of a new quenching band in the vicinity of 550-570 nm of the quenching spectrum $Q_{O2}(\lambda)$ which can be explained by O_2-induced quenching of PL of **Si=O-related surface states** localized in this spectral region.

It is worth to note that the above mentioned SiO-related surface states should serve as quenchers and energy acceptors for Si-ncs quantum-confined excitons having energies higher than that of the surface states (≈2.2 eV). Similar value of energy has been earlier obtained theoretically for excited SiO-related surface states of Si-ncs, the states being ascribed to Si=O groups on Si-nc surface. A presence of such states well explains the above mentioned fact that the short-wavelength shift of PSi luminescence spectrum, observed in course of PSi oxidation, stops when maximum of the spectrum reaches a position around 590 nm (2.1 eV).

We have found also that chemical binding of nano-Si surface with surfactant molecules strongly decreases, but does not eliminate completely the surface interactions with molecular oxygen and water molecules that results in build-up of the band near 570 nm in the quenching spectrum, so that Q_{O2}(570 nm) reaches a magnitude of 1.4 in 24 hours after wafer immersion in water.

2-2.3. Durability and photophysical properties of surfactant-covered particles of electrochemically-prepared porous silicon in Simulated Body Fluids (SBF)

We have found that, contrary to de-ionized water, in physiological solutions (electrolytes) the physically adsorbed hydrocarbon layer is not more a protection against nano-Si fast (minutes) dissolution that is accompanied by hydrogen evolution and full quenching of luminescence. As our observations showed, practically same results are obtained for non-covered (as-prepared) H-terminated nano-Si layers (not shown).

We found, however, that the objectives (1) to protect nano-Si surface against corrosion in physiological solutions, and (2) preserve an ability of nano-Si to be photosensitizer for $^1O_2$ formation can be succeeded if the standard method of temperature-initiated chemical functionalization is applied with the only modification that the chemical reaction is carried out not in Schlenk system under argon atmosphere, but in simple reflux flask under surrounding air atmosphere that means that no O_2 and H_2O traces are removed from the hydrocarbon protector (undecylenic acid) and these reagents take part in the surface modification reaction.

Fig. 6 shows the PL and PL quenching spectra of nano-Si layer chemically covered by undecylenic acid at 95°C during 16 hours in presence of O_2 and H_2O traces. The spectra were measured in SBF at different delays after immersion of the layer in the electrolyte solution. One can see that a stay of nano-Si layer in SBF results in considerable short-wavelength shift and marked build-up of the luminescence intensity. Quite different is also behaviour of the PL quenching spectrum: it changes from almost complete absence of sensitivity to oxygen during the first 3 hours to the multiple increase of the quenching efficiency (up to ~2.50 in maximum) during the next 5-6 days followed by its slow decrease. The observed growth of nano-Si sensitivity to oxygen can stem from erosion-induced detachment of a part of the protective hydrocarbon layer. However, the erosion is not observed by nicked eye and likely has a local character.

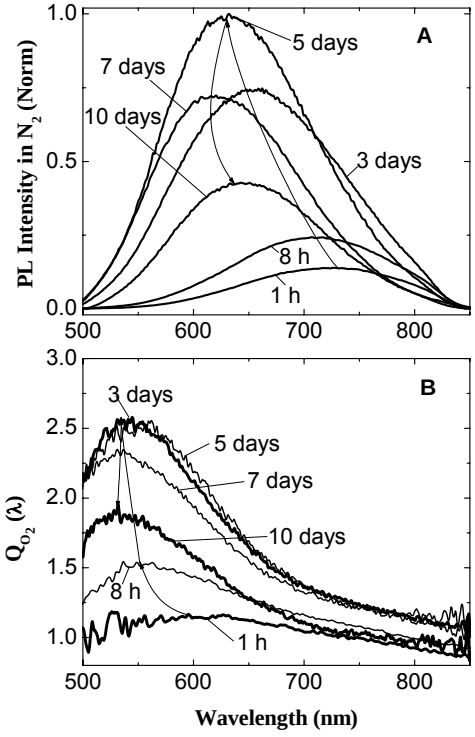


Figure 6. PL (A) and PL quenching (B) spectra of the nano-Si layer modified by undecylenic acid at 95°C measured at different delays after the layer was immersed in SBF (time delays are indicated near corresponding curves).

Analysis (not shown) of the integral quenching values $Q_{O2\text{-}INT}$ and N_{1O2} determined by formulas (2) and (3) demonstrates that during the first 6 days $Q_{O2\text{-}INT}$ rises from 1.0 (no quenching) up to 1.70. On the other hand, the N_{1O2} magnitude increases by 110 times during the first 5 days that is mainly a result of the luminescence intensity growth.

We suggest that the PL build up is a result of formation (due to oxidation in wet conditions) of a large number of luminescent SiO-related surface states, which exhibit PL over the whole visible region with the maximum near 580 nm and manifest themselves in the PL quenching spectra obtained after nano-Si stay in SBF (Fig. 6B). An average number of such surface states per one nanocrystal may considerably exceed 1.0 and they can serve as very effective traps for electronic energy of initially excited excitons confined in nano-Si. Supposing that the electronic energy transfer from exciton to the surface state is much faster than the rates of radiationless processes in nano-Si, one can easy explain the observed increase of the luminescence intensity (about 10 times in maximum) as an increase of nano-Si luminescence quantum yield due to re-directing electronic excitation energy from radiationless deactivation channels to emitting surface states.

The found considerable (about 110 times) growth of the "normalized quantum yield of quenching", N_{1O2}, may imply the corresponding growth of singlet oxygen generation efficiency, if the mechanism of the quenching is still an excitation energy transfer to molecular oxygen, as in case of O_2 direct interaction with nano-Si confined exciton. The second possible mechanism of quenching may be an electron transfer to O_2 and formation of superoxide anion radical O_2^-. Experiments are now in progress to identify the mechanism responsible for quenching of the or Si surface states by O_2.

Therefore, a partial chemical modification of nano-Si surface is realized by thermal reaction of undecylenic acid, containing molecular oxygen and water traces, with Si-H terminated nano-Si matrix. As FTIR measurements show (not presented here), besides Si-C bond formation and surface covering by the hydrocarbon layer, some part of the surface remains Si-H terminated, whereas other part becomes oxidized that is accompanied by appearance of luminescent SiO-related surface states. Interaction of the surface with SBF solution results in slow (time scale of a few days) and considerable build up of nano-Si PL that can be accounted for by an increase of the number of luminescent surface states, which effectively capture an energy of confined excitons. The surface states can be efficiently quenched by molecular oxygen, so that the apparent quantum yield of quenching increases by 110 times during the first 5 days. The latter property can be helpful for efficient formation of reactive oxygen species (singlet oxygen or superoxide anion radical) in physiological solutions.

2-3. Impregnation of nano-Si with reagents possessing photo-sensitizing properties

One of the ideas of this part of the Project is to use nano-porous silicon (nano-Si) as a platform to carry an immobilized organic photosensitizer, which is able to generate singlet oxygen and exhibits intensive absorption of light in the red–near IR region, in which a skin and tissues have the "transparency window". We have suggested that combination of well known photosensitizing activity of the porphyrin-type photosensitizers with bio-degradability of nano-Si and its ability to form solid particles of different size can result in interesting applications of such a hybrid material in photodynamic therapy. Another idea is to evaluate the principal possibility to use nano-Si as a carrier for efficient molecular photosensitizers with the aim to release in a controllable way the adsorbed photosensitizers in physiological conditions. To make such an evaluation, an interaction of these photosensitizers with different forms of nano-Si has been investigated.

In this work we carried out a comparative study of the fluorescence and photosensitizing properties of both water-soluble and hydrophobic porphyrins immobilized in oxidized (i.e. non-luminescent) and as-prepared (i.e. luminescent) nano-Si films.

Porphyrins in oxidized nano-Si matrix. Fig. 7 shows the porphyins used for filling porous Si pores: water-soluble cationic porphyrin 5,10,15,20-tetrakis-(N-methyl-4-pyridyl)porphyrin tetrachloride (TMPyP4), water-soluble anionic porphyrin 5,10,15,20-tetrakis-(4-

sulfonatophenyl)porphyrin, Na salt (TPPS), hydrophobic porphyrin mesoporphyrin IX dimethyl ester (MP), and amphiphilic chlorine-type tetrapyrrolic compound chlorin e_6. Thermal oxidation of as-anodizide nano-Si samples was performed at 500 °C in air ambient for 1.5 hours. Porphyrin concentration in impregnation solutions was $2–3 \cdot 10^{-3}$ M.

The following were obtained in the experiments on nano-Si impregnation with organic photosensitizers (porphyrin-type molecules):
- Oxidized porous silicon may serve as an inert matrix to accumulate molecular photosensitizers such as porphyrins. Among several investigated hydrophobic and hydrophilic porphyrins, the best results were obtained with cationic porphyrin TMPyP4, which was accumulated in porous silicon at concentration ~5 times higher than highest possible concentration in solution.
- Oxidized porous silicon matrix had no any quenching effect on porphyrin fluorescence states. However, some concentration quenching of fluorescence was observed for investigated immobilized porphyrins. The minimum concentration quenching effect (losses ~40%) was found for the fluorescent state of TMPyP4. The porphyrin triplet state is found to be rather long-lived and it is completely (more than 99%) quenched by molecular oxygen of air at 1 atmosphere pressure. Therefore, efficiency of $^1O_2$ formation should be rather high (tens percents) for all adsorbed porphyrins.
- Direct measurements of 1.27 μm luminescence of singlet oxygen generated by the adsorbed porphyrins suggest that strong quenching of luminescence of $^1O_2$ takes place induced by oxidized porous silicon surface, most likely due to energy transfer to high-frequency (Si)O-H vibrations.
- In presence of molecular oxygen the combination of immobilized porphyrin (as photosensitizer of singlet oxygen formation) and luminescent nano-Si cannot be used to improve photosensitizing ability of Si nanoparticles because of fast oxidation of nano-Si by singlet oxygen generated by excitons and/or by porphyrins.
- On the other hand, our evaluations show that the combination of immobilized porphyrin (as photosensitizer of singlet oxygen formation) and luminescent (non-oxidized) nano-Si chemically modified by hydrocarbon layer can be effectively used for controllable release of the photosensitizer into physiological solution because the rate of the nano-Si dissolution (and therefore of the photosensitizer release) can be easy governed by conditions of nano-Si chemical modification (see Part 2-2.3).

Conclusions

Modification of nano-Si surface through physical adsorption of non-ionic surfactants renders the surface hydrophilic. In de-ionized water the surface remains stable against erosion during many days if the modification is realized with linear unsaturated hydrocarbons such as undecylenic acid, oleic alcohol. In physiological solutions the nano-Si surface modified by physical adsorption of non-ionic surfactants is not protected against fast erosion and dissolution.

Chemical (thermal) modification of nano-Si by same unsaturated hydrocarbons via Si-C bond formation results in hydrophylicity of the surface and protects it against fast dissolution. Partial oxidation of the modified nano-Si surface during modification can result in formation of such a specific protective layer which allows effective interaction between excited Si nanocrystals and molecular oxygen (that is a basis for photosensitizing activity) without visible corrosion/dissolution of nano-Si during many days. On the other hand, chemical modification of nano-Si surface in oxygen- and water-free conditions results in full protection of the excited Si nanocrystals against interaction with O_2 that well stabilizes nano-Si luminescence but excludes a use of such material as a photosensitizer.

It is found that in case of both physical and chemical modification of nano-Si surface by hydrocarbons with polar end groups specific luminescent surface states are formed on Si nanocrystal surface as a result of its contact with water. These surface states but not confined excitons are then effectively quenched by molecular oxygen.

Classical molecular photosensitizers used for singlet oxygen production, such as porphyrin-type molecules, adsorbed on oxidized nano-Si matrix can effectively generate $^1O_2$ but, at the same time, the generated $^1O_2$ is effectively quenched by the same surface.

Combination of immobilized porphyrin and luminescent (non-oxidized) nano-Si chemically modified by unsaturated hydrocarbons and simultaneously oxidized by water can be effectively used for controllable release of the photosensitizer into physiological solution because the rate of the nano-Si dissolution (and therefore of the photosensitizer release) can be easy governed by conditions of nano-Si chemical modification.

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4. V. Chirvony, A. Chyrvonaya, J. Ovejero, E. Matveeva, B. Goller, D. Kovalev, A. Huygens, P. de Witte, *Surfactant-modified hydrophilic nano-structured porous silicon for photosensitized formation of singlet oxygen in water, Advanced Materials* **2007**, *19*, 2967–2972.
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6. E. Pastor, J. Salonen, V.-P. Lehto, E. Matveeva, *Influence of porous silicon oxidation on its behaviour in simulated body fluid,* Phys. Stat. Sol. (c) **4**, No 6, 2136-2140 (2007)
7. E. Pastor, J. A. Ayúcar, J. Curiel-Esparza, E. Matveeva, J. Salonen, V. P. Lehto, Electrochemical Oxidation of Mesoporous Silicon: Structural and Morphology Properties of the Obtained ox-porSi Material. ECS Transactions **6** (No 11, Nanoporous Materials), 23-34 (2007)
8. L. Bychto, M. Balaguer, E. Pastor, V. Chirvony, E. Matveeva, *Influence of preparation and storage conditions on photoluminescence of porous silicon powder with embedded Si nanocrystals*, *J. Nanoparticles Research* **2008**, *10* (8) 1241-9.
9. E. Pastor, M. Balaguer, L. Bychto, J. Salonen, V.-P. Lehto, E. Matveeva, V. Chirvony, *Porous Silicon for Photosensitized Formation of Singlet Oxygen in Water and in Simulated Body Fluid: Two Methods of Modification by Undecylenic Acid, J. Nanosci. Nanotechnol.* **2008**, *8*, No. 12, doi:10.1166/jnn.2008.BBE19.
10. M. Balaguer, E. Pastor, L. Bychto, P. Atienzar, M. A. Miranda, E. Matveeva, V. S. Chirvony, *Durability and photophysical properties of surfactant-covered porous silicon particles in aqueous suspensions, Phys. Stat. Sol. (a)* **2008**, *205*, No 11, 2585-2588.
11. L. Bychto, Y. Makushok, V. Chirvony, E. Matveeva, *Pulse electrochemical method for porosification of silicon and preparation of porous Si dust with controllable particle size distribution, Phys. Stat. Sol.(c)* **2008**, *5*, No 12, 3789–3793.
12. E. Pastor, J. Salonen, V.-P. Lehto, V. Chirvony, E. Matveeva, *Physical adsorption vs. chemical binding of undecylenic acid on porous silicon surface: a comparative study of differently functionalized materials, Phys. Stat. Sol. (a)* **2008***,* submitted.
13. E. Pastor, J. Curiel-Esparza, J. Salonen, V.-P. Lehto, E. Matveeva, *Electrochemically induced bioactivity of acetylene derivatized porous silicon,* Phys. Stat. Sol. (a) **2008**, submitted
14. M. Balaguer, E. Pastor, E. Matveeva, V.S. Chirvony, *Undecylenic-acid modified luminescent porous silicon nanostructures for the photosensitized formation of singlet oxygen in corroding physiological solutions, Advanced Materials*, **2008***,* submitted.
15. M. Balaguer, E. Matveeva, *Luminescence Quenching in Porous Silicon Materials Prepared by Different Methods by Molecular Oxygen In Gas and Water Ambient and Formation of Singlet Oxygen and Other Reactive Oxygen Species, J. Nanoparticles Research* **2008**, submitted.

3. Kinetics and physical mechanisms of photo-stimulated generation of singlet oxygen by nano-Si

3-1. Study of nano-Si as a photo-sensitizing material for efficient generation of singlet oxygen in the gas phase

The objectives of this part of the project were to study the efficiency of $^1O_2$ generation as a function of nanocrystalы size distribution, morphology as well as to study the kinetic of $^1O_2$ generation and recombination for different nano-Si systems in the gas phase. The singlet oxygen generation efficiency was monitored by means of the photoluminescence (PL) spectroscopy and electron-paramagnetic resonance (EPR) technique [1-17]. Kinetics of the $^1O_2$ generation and recombination for different nano-Si systems are investigated by means of the time-resolved PL spectroscopy. In order to prevent the photo-induced oxidation of nano-Si the PL was excited by short laser pulses and PL transients at 1.63 eV were measured and analyzed. The experiments were carried out at room temperature for the samples of nano-Si powder prepared from electro chemically produced nano-Si. The partial pressure of molecular oxygen was 1 Bar. The time-resolved measurements showed a shortening of the PL lifetime of different nano-Si systems (electrochemically prepared porous Si layers and powder, stain-etched Si powder, aqueous suspensions of Si nanocrystals) in oxygen ambient. The strongest decrease of the lifetime occurs at the photoluminescence energy of 1.63 eV, which corresponds to the spectral maximum of the $^1O_2$ generation due to the energy transfer from excitons confined in Si nanocrystals to O_2 molecules adsorbed on Si nanocrystal surfaces. The energy transfer time of about 50 microseconds is typical for the electrochemically prepared porous Si powder with the maximal efficiency $^1O_2$ generation at room temperature.

From the time-resolved PL data we estimate the energy transfer time, τ_{tr}, and the singlet oxygen photosensitization efficiency, η, by using the following expressions [1]:

$$\tau_{tr} = \left[\left(\tau_{PL}^{ox} \right)^{-1} - \left(\tau_{PL}^{vac} \right)^{-1} \right]^{-1}, \qquad (1)$$

$$\eta = 1 - \int_0^\infty I_{PL}^{ox}(t)dt \Big/ \int_0^\infty I_{PL}^{vac}(t)dt, \qquad (2)$$

where τ_{PL}^{vac} and τ_{PL}^{ox} are the PL lifetimes for nano-Si in vacuum and in oxygen ambient, respectively; $I_{PL}^{vac}(t)$ and $I_{PL}^{ox}(t)$ are the PL signals in vacuum and in oxygen ambient, respectively. It should be noted that τ_{tr} and η give the lower and upper limits of the corresponding energy transfer time and photosensitization efficiency because the PL quenching is not completely reversible.

Figure 1 shows τ_{tr} and η as functions of the porosity of nano-Si. One can see that the energy transfer time decreases and the efficiency increases with increasing porosity. The minimal value of τ_{tr} and maximal value of η are inherent in the samples with highest porosity. The $^1O_2$ generation efficiency (more exactly, an efficiency of excitation energy transfer from nano-Si to molecular oxygen), which is defined accordingly Eq. 2, increases strongly for nano-Si samples obtained from electrochemically prepared porous Si samples when their porosity increases from 60 to 90 % [3].

The samples with the maximal efficiency of the singlet oxygen generation exhibit bright photoluminescence in the spectral range 1.6-2.0 eV, which is a result of the radiative recombination of excitons confined in Si nanocrystals with hydrogen-terminated surfaces and size distribution in the range from 2 to 4 nm. The concentration of photogenerated $^1O_2$ molecules is estimated to be about $3 \cdot 10^{18}$ cm^{-3} for porous Si powder of 87% porosity in oxygen atmosphere at 1 bar pressure under illumination with photon energy of 2.5-3 eV and photon flux of about 10^{20} cm^{-2}.

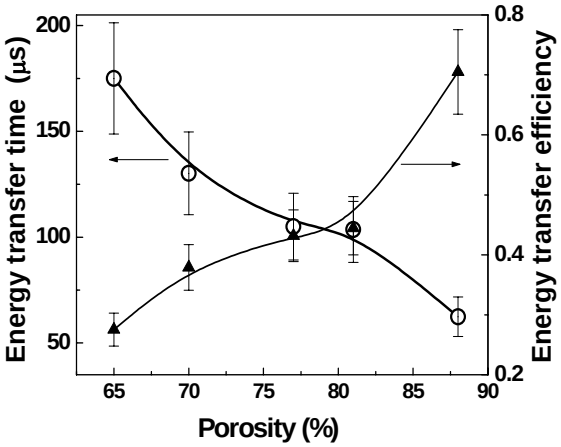

Figure 1. Energy transfer time (circles) and photosensitization efficiency (triangles) *vs* porosity [3].

The EPR spectroscopy was used to determine the concentration of excited (singlet) molecular oxygen photosensitized by nano-Si [1]. The singlet oxygen generation in nano-Si powder is studied quantitatively at various oxygen pressures and exciting light intensities with the use of the technique proposed. The experimental results showed that nano-Si as the photosensitizers of singlet oxygen are promising for biomedical applications.

Typical EPR spectra of the nono-Si samples measured at a high level of the microwave power are shown in Figure 2. The EPR signal is characterized by the effective g-factor of 2.0055±0.0005 and the line width of 12±0.5 G. The corresponding paramagnetic defects are usually attributed to so-called P_b-like centers, which are Si DBs at Si/SiO_2 interface. The EPR signal intensity (I_{EPR}) of the sample in vacuum at high microwave power is the weakest one because of the saturation effect [1, 2]. This effect results from relatively long times of the spin relaxation of P_b centers. We found that I_{EPR} was larger for the sample in O_2 atmosphere in darkness. Furthermore, I_{EPR} for the sample under illumination in O_2 atmosphere decreased reversibly. Note, that prolonged intensive illumination led to an irreversible increase of I_{EPR} due to the defect formation under photo-oxidation of the nono-Si surfaces [4-8].

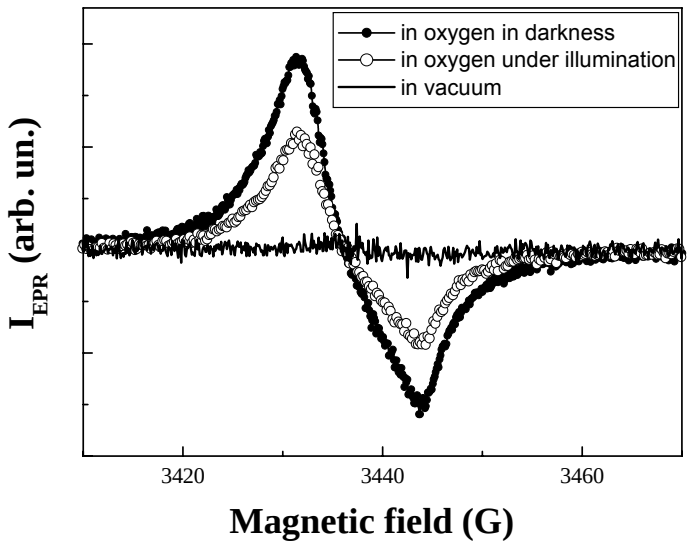

Figure 2. EPR spectra of porSi powder in vacuum and in oxygen ambient (P_{ox}=760 Torr) in darkness and under illumination (from Ref. [1]).

In general, I_{EPR} is determined by both the number of paramagnetic centers (Si DBs in our case) and their relaxation time (T_r). The latter can be expressed as: $T_r=1/(1/2T_1+1/T_2)$, where T_1 is the longitudinal relaxation time (spin-lattice relaxation) and T_2 is the transverse relaxation time,

associated with spin-spin relaxation. Usually, for Si DBs in nc-Si in vacuum: $T_1 << T_2$ and then $T_r \approx 2T_1$. While the value of T_1 for bulk Si at room temperature lies typically in a range of several microseconds, this time can be longer in small Si nanocrystals because of a low density of phonon modes. Indeed, I_{EPR} for the sample in vacuum exhibit the strong saturation even at relatively low microwave power (see Figure 3). Note that according to our experiments the microwave power dependence of the EPR signal for the sample in vacuum does not change under illumination.

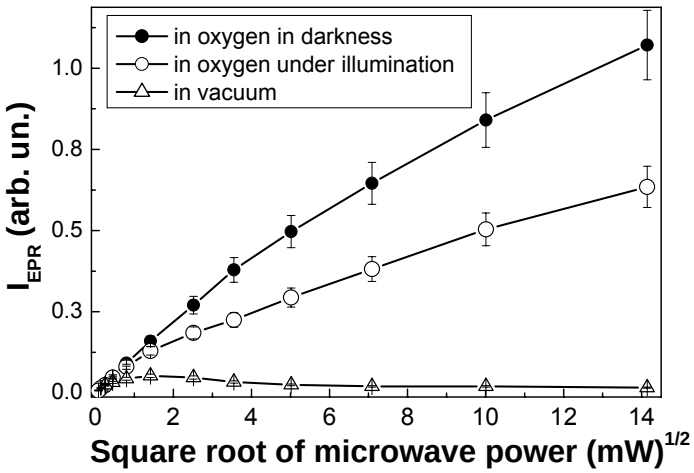

Figure 3. Microwave-power dependence of the EPR signal of por-Si powder in vacuum and in oxygen ambient (P=760 Torr) in darkness and under illumination (from Ref. [1]).

The value of T_2 is inversely proportional to the average density of paramagnetic centers and it can be controlled by the amount of paramagnetic molecules as triplet O_2 molecules adsorbed on nano-Si surface. Some of the molecules can disturb Si DBs via the magnetic dipole-dipole interaction, and it should result in a decrease of T_2 and then T. Thus, the saturation effect can be overcome by a significant shortening of T. In fact, this effect is observable for both the EPR spectra (Fig. 2) and the microwave power dependence (Fig. 3). In particular, I_{EPR} for the samples in O_2 atmosphere at P_{ox} =760 Torr decreases by 40 % under illumination with photon flux of about 10^{20} cm^{-2}. It indicates that the concentration of the triplet O_2 molecules around nc-Si decreases by $\Delta N \approx 0.4 N_0$, where N_0 is the initial O_2 concentration. The latter number at P_{ox} =760 Torr is about 10^{19} cm^{-3} (the Avogadro number divided by the molar volume). From the comparison of I_{EPR} in O_2 atmosphere in darkness and under illumination one can estimate the $^1O_2$ molecule concentration to be equal to $\Delta N = 4 \cdot 10^{18}$ cm^{-3}. The latter value is comparable with the number of nono-Si and it is by 2 orders of magnitude larger than the Si DBs concentration in the samples investigated. This fact demonstrates that Si DBs are only probes to monitor the $^1O_2$ photosensitization process.

Thus, the EPR and PL studies give evidences of the efficient energy transfer from excitons confined in nano-Si to oxygen molecules adsorbed on the nanocrystal surface. The observed photosensitization of the singlet oxygen generation is accompanied by an increase of the relaxation time of Si DBs on the nanocrystal surface because of the significant depletion of the ground (triplet) state of the adsorbed molecular oxygen. This fact allows us to measure quantitatively the concentration of generated singlet oxygen, which can be of the order of 10^{18} cm^{-3}. The observed remarkable photosensitizing property of nano-Si is obviously promising for biomedical applications.

3-2. Nano-Si for efficient generation of singlet oxygen in the liquid phase

Silicon nanocrystals were prepared using the electrochemical etching of crystalline Si wafers in hydrofluoric solutions. The obtained free-standing porous silicon (porSi) films were dried in air and then milled to get a powder with the maximal grain size of the order of 10 microns.

According to the XRD and Raman scattering data, the mean sizes of Si nanocrystals (nc-Si) in the initial porSi layers and in the powder formed from them were equal to 3–4 nm. The porSi powder was dispersed in pure water bubbled with oxygen to get a homogeneous aqueous suspension. Photoluminescence (PL) was excited by a nitrogen-laser radiation, the PL spectra were detected using a CCD chamber, the PL transients were registered using a photomultiplier and recorded by a digital oscilloscope.

Typical PL spectra of the as-prepared aqueous suspensions of PSi powder are shown in Fig. 4. The PL spectra are characterized by a broad band centered at 1.5 eV and it can be interpreted as the radiative recombination of excitons confined in nc-Si with sizes distributed from 2 to 4 nm. The PL intensity in oxygen-saturated water decreases in comparison with that in oxygen-free water. Inset of Fig. 4 shows the spectral dependence of the photoluminescence quenching degree, which was determined as the ratio of the photoluminescence intensity (I_{PL}) of the suspension without dissolved gas to the intensity (I_{PL}^{oxyg}) after its saturation with O_2. It is seen that the maximum suppression of the photoluminescence of silicon nanocrystals occurs near 1.63 eV, which is close to the energy of the $^3\Sigma \rightarrow {}^1\Sigma$ transition in O_2. This fact indicates that the quenching of photoluminescence of silicon nanocrystals occurs due to the transfer of energy to oxygen molecules, i.e., in the process of photosensitizing the generation of singlet oxygen. Our analysis of the difference between the spectrally integrated intensities of the photoluminescence of silicon nanocrystals in suspensions with dissolved oxygen and after its exhaust indicates that near 40% of the energy of excitons is transferred to the excitation of O_2 molecules. This number, which corresponds to the energy transfer efficiency η=0.4, is about 1.5-2 times smaller than η for the singlet oxygen photosensitization in gas phase. It is obvious that the interaction of nano-Si with water molecules results in a decrease of the photosensitization properties of nano-Si.

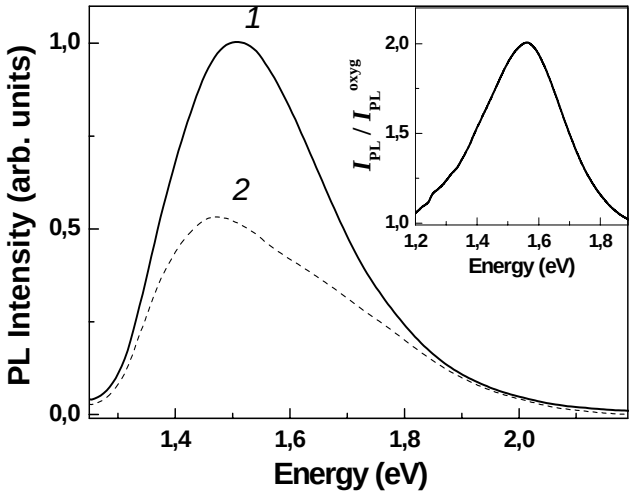

Figure 4. PL spectra of por-Si dispersed in oxygen-free water (1) and in water, saturated by oxygen at 1 bar (2). Inset shows the PL quenching degree after the saturation of the suspension with O_2.

Additional information on the efficiency of singlet oxygen photosensitisation was obtained by analyzing the kinetics of the PL of nano-Si excited by laser pulses. The Fig. 5 depicts the exciton PL transients at 1.63 eV for PSi dispersed in oxygen-free water and in oxygen ambient. As one can see the exciton lifetime decreases for the PSi in oxygen-saturated water. The relaxation time of the exponential part of the photoluminescence kinetics in the solution without O_2 is $\tau \approx 85$ μs, which is close to the natural radiative lifetime of excitons confined in Si nanocrystals emitting at the photon energy near 1.6 eV. The saturation of the solution with gaseous O_2 at a pressure of 760 Torr leads to a decrease in the photoluminescence relaxation time to $\tau_{ox} \approx 50$ μs. The ratio $\tau/\tau_{ox} \approx 1.6$ is close to the measured quenching degree of stationary photoluminescence (see inset in Fig. 4). These properties indicate that the decrease of the lifetime of the photoluminescence of silicon nanocrystals in the aqueous suspension saturated with oxygen is caused by the interaction of excitons confined in the nanocrystals with the surrounding O_2 molecules, i.e., by the singlet oxygen generation.

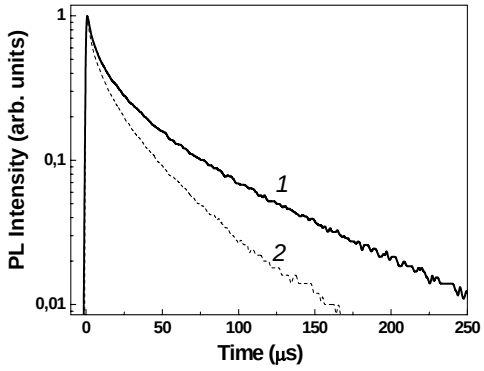

Figure 5. PL transients at 1.6 eV measured in aqueous suspensions of nan-Si (1) without dissolved oxygen and (2) after saturation with oxygen at 760 Torr.

3-3. Choice of biocompatible liquid carrier for DPT

Dynamic cancer phototherapy (DPT) involves transportation of a photo- sensitizer and oxygen to the tumour cells by a liquid carrier via the blood vessel system. The active phase of the phototherapy –photo-generation of the curing agent singlet oxygen ($^1O_2$) - is also carried out within the same liquid. Hence, the liquid carrier plays a role of temporary blood substitute and has to fulfil a number of requirements:

(i) The liquid must form a stable and uniform suspension with the photo sensitizer particles, thus, enabling their smooth transportation without unwanted effects of separation, particle coalescence, clogging of blood vessels etc.

(ii) The solubility of oxygen in the liquid has to be reasonably high

(iii) The liquid has to be biocompatible and must be able to leave the body via natural processes of metabolism and breathing.

(iv) The photo-activity of the sensitizer must not be inhibited by the liquid carrier.

(v) The liquid carrier has to be chemically inert with respect to excited $^1O_2$ molecules.

The performance of nano-Si photosensitizer in a number of liquids has been investigated in the course of the project. However, most of these liquids have specific drawbacks when compared with these requirements. Bellow are presented our considerations concerning the suitability of several major liquid groups as nano-Si carrier. We also suggest an uncommon approach to the problem and initial result in support of it.

a) Aqueous suspensions of nano-Si particles

An conventional assumption of the project have been that during a PDT treatment the nano-Si photosensitizer must operate in an environment similar to the natural blood plasma – i.e. it have to be applied as a suspension of nano-Si particles in aqueous solutions. On the other hand, there is a major problem of mixing nano-Si particles with water or aqueous solutions (natural blood, physiological saline solutions etc.) due to the fact that the photoactive nano-Si is very hydrophobic. This is in conflict with requirement (**i)** because the photo-sensitizer tends to separate from the liquid carrier causing transport complications. Even if forcibly mixed, the transfer of oxygen (in both normal $^3O_2$ and excited $^1O_2$ forms) between the liquid and the active nano-Si surface occurs via liquid/gas interface (the pores of the nano-Si particles cannot be filled by liquid due to the capillary forces). This can limit the net yield of $^1O_2$ – in conflict with requirement **(iv).** In order to overcome these limitations, many efforts have been done to modify the surface of the nano-Si, so that it acquires hydrophilic properties, but without deterioration of its photo-activity. Although some progress has been achieved in this respect (see section 2 of this report) a new specific problem was bought fourth by these investigations. Actually, an improvement of the contact between nano-Si and water results in very fast oxidation of the active internal surfaces of the sensitizer and corresponding deterioration of its photo-activity. Surface modification with compounds, which protect the nano-Si surface from oxidation on the other hand prevent the free exchange of energy between excitons and O_2 molecules, hence,

blocking the sensitizer activity (section 2). The IN VITRO (photo)cytotoxic experiments also did not found significant improvements, related to the surface modified nano-Si photosensitizers (see section 4).Thus, the formation of an aqueous suspension of nano-Si particles, appropriate for PDT application, is still a matter of compromise between many contradicting factors. The relatively low solubility of O_2 in water can also be considered a shortcoming of the water-based liquid carriers.

b) Organic solvents

In contrast with the water many organic solvents are wetting well the surface of nano-Si and hence, can penetrate within its pores and can form stable suspensions with nano-Si particles. In fact, many of our investigation of the performance of the photosensitizer particles in a liquid environment were carried out by using organic solvents because of these reasons. Unfortunately, almost all organic solvents are not biocompatible and hence they could not be considered as appropriate liquid carriers for PDT.

At the end of the project we realised that there is a class of liquids, which could fulfil very well the requirements for an appropriate nano-Si carrier – the perfluorocarbons. Here we briefly present also the major findings concerning the photo-activity of nano-Si particles in this class of liquids.

c) Photoactivity of nano-Si in liquid perfluorocarbons

Perfluorocarbons (PFC) are compounds in which all H atoms from a corresponding hydrocarbon are substituted by F atoms. The liquid PFCs possess unique properties. They are chemically inert, thermally stable, inflammable, non-toxic and biocompatible. The Liquid perfluorocarbons can dissolve approximately 20-50 times more oxygen than blood plasma or any other water-based electrolyte. Hence, their capacity for oxygen delivery approaches the one of red blood cells [R1]. Therefore, PFC liquids have been used as oxygen carriers in “artifical blood substitutes”. The PFCs are lipophilic (i. e. hydrophobic), similarly to the nano-Si particles. Hence, they can form homogeneous suspensions with photoactive nano-Si particles. Thus requirements (i)-(iii) and (v) are perfectly met by PFC. However, the photoactive behavior of nano-Si in such an environment have not been studied until now. Here, we present experimental results on the quenching of the photoluminesce (PL) of nano-Si suspension in PFC, induced by saturation of the liquid by O_2, which is indicative for the generation of $^1O_2$ in this environment. The experiments are carried out in Perfluoroocthyl Bromide (PFOB) - a perfluorocarbon derivative which is the most common ingredient of artificial blood substitutes.

Figure 6 shows the time variations of PL signal, excited at λ = 488 nm and monitored at λ=760 - the characteristic wavelength for photo-excitation of O_2 molecules to their singlet state, $^1O_2$. The investigated PSi/PFOB suspension was kept under constant boubling with either N_2 or O_2 during two separate measurements.

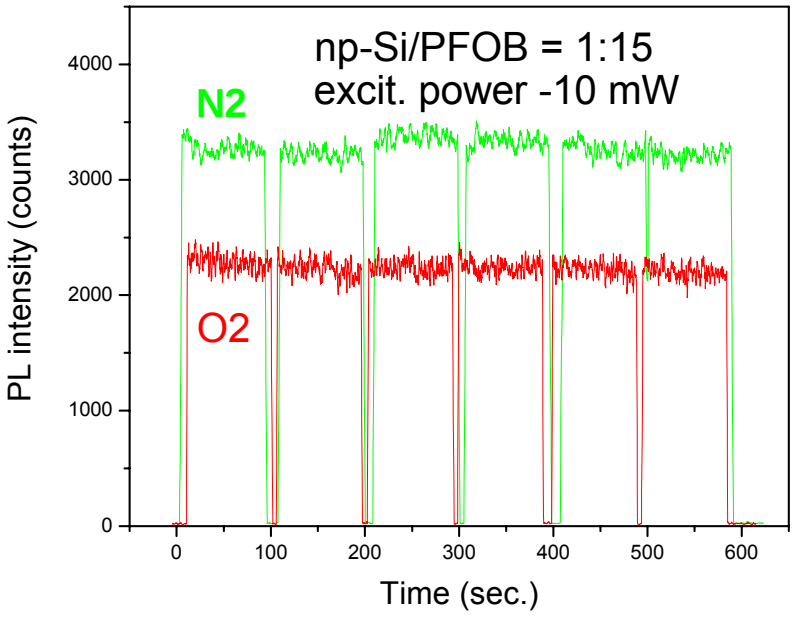

Figure 6. PL intensity at λ=760 nm versus time for a PSi/PFOB suspension under constant bubbling with N_2 (green) and with O_2 (red) gases.

The figure clearly shows a quenching of the PL in the O_2 saturated suspension. The corresponding PL quenching strength (~1.4 at RT) is comparable to that of nano-Si particles in air or in gaseous O_2. So, it can be concluded that the photo-sensitizer is quite active in producing $^1O_2$ in PFOB surrounding. Furthermore, the PL intensity does not show any significant variation during the experiments under, both, N_2 and O_2 saturation conditions. This indicates the absence of a significant degradation of PSi/PFOB suspensions on a time scale of 10 minutes – the typical duration of the active phase of a DPT treatment.

d) Perfluorocarbon as a nano-Si carrier ?

As shown above the nano-Si particles perform quite well as photosensitizers in O_2 satturated PFC environment. On the other hand, perfluorocarbons are hydrophobic themselves and thus cannot be used directly as liquid carrier of such particles. This problem, however, can be solved in a manner similar to the way PFCs are used in artificial blood substitutes. The nano-Si particles can be incorporated in lipid vesicles, filled with PFC as shown in Figure 7. Such vesicles have hydrophilic outer surface and can be mixed with aqueous solutions (e.g. physiological saline solutions), thus, forming an emulsion of "photosinsetizer cells" that can be transported along the blood vessels. This "last minute approach" seems to be very promising, but it has not been explored experimentally yet.

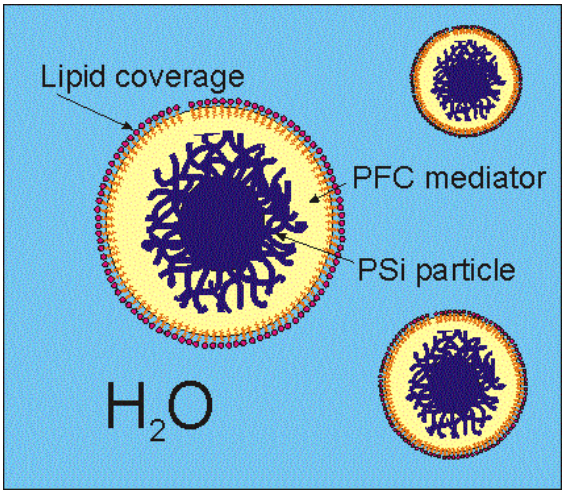

Figure 7. Nano-Si photosinstizer cells

Related publications of the Consortium

1. E. A. Konstantinova, V. A. Demin, A. S. Vorontzov, Yu. V. Ryabchikov, I. A. Belogorokhov, L. A. Osminkina, P. A. Forsh, P. K. Kashkarov, V. Yu. Timoshenko, *Electron-paramagnetic resonance and photoluminescence study of Si nanocrystals-photosensitizers of singlet oxygen molecules,* Journal of Non-Crystalline Solids **352** (9-20), 1156-1159 (2006).
2. E. A. Konstantinova, V. A. Demin, V. Yu. Timoshenko, P. K. Kashkarov, *EPR diagnostics of the photosensitized generation of singlet oxygen on the surface of silicon nanocrystals.* JETP Letters **85** (1), 59-62 (2007)
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4. Exploration of the possibilities to use nano-Si photosensitizers in photodynamic therapy (PDT) of cancer

4-1. Introduction

Photodynamic therapy (PDT) of tumors involves the systemic or topical administration of a photosensitizer (PS) to patients followed by irradiation of the tumor mass with light of an appropriate wavelength. In the presence of oxygen, photoactivated sensitizers generate highly reactive oxygen species, especially singlet oxygen ($^1O_2$). The oxidative damage to various cellular organelles and functions induced by singlet oxygen leads to direct cytotoxicity on tumor cells. Indirect effects, however, have also been shown to contribute to tumor destruction. These effects originate from the PDT-induced severe vascular damage and result in the collapse of the entire microcirculation system. Consequently, persistent tumor ischemia contributes to the overall tumor ablation. Moreover, an indirect antitumoral-specific immune reaction may also be induced by PDT as a result of the activation of inflammatory cells. It is likely that this phenomenon plays a role in long-term tumor control [1].

A number of PS are already in clinical practice. The first PS approved was *Photofrin®,* a porfimer sodium, followed by *Visudyne*® (benzoporphyrin derivative), *Levulan*® (5-aminolevulinic acid), and most recently *Foscan*® (m-tetra hydroxyphenyl chlorin) and *Hexvix*® (hexyl-5-aminolevulenic acid). 5-Aminolevulinic acid (5-ALA) and its hexyl-ester are precursors of protoporphyrin IX (PpIX), a PS which is formed especially in targeted malignant tissue [2]. All these compounds have a long triplet state lifetime ($\tau_T \geq$ 500 ns) which is considered to be a requirement for efficient photosensitization [3].

In the framework of the present Project the first solid-state PDT photosensitizer is developed, which is a powder of micro- and submicrometer-size particles of the luminescent porous silicon (hereafter nanosilicon, or nano-Si) that can produce singlet oxygen when illuminated in O_2 ambience [4]. Compared with the above-mentioned molecular photosensitizers, nano-Si can prove to be a more efficient source of $^1O_2$ as the life times of photo-created excitons in it are longer (about 100 μs) [4,5]. It also has an extremely large inner surface, where exciton-to-O_2 energy transfer can occur. And finally, nano-Si is biocompatible and can be easy and controllably decomposed in physiological solutions down to simple innocent compounds of silicic acid [6].

4-2. Main results

To increase the chance of success the consortium partners were solicited to investigate time and money in finding different nano-Si preparations, and over the entire project period, a total of **64** samples were received and investigated into detail by the K.U.Leuven partner on their (photo)cytotoxic characteristics. As PDT efficacy might depend on certain conditions used, specific attention was paid to the following parameters: a) cell culture related issues (extracellular concentration, dark cytotoxicity, intracellular accumulation, cell type, incubation time/conditions, presence/absence of serum constituents), b) nano-Si particle related issues (preparation method, size, coating or not, chemical stability), and c) light related issues (fluence rate, fluence, activation spectrum).

The results presented here highlight the major findings obtained in K.U.Leuven (the samples investigated here were named as EUXX) and MSU teams. The MSU team investigations were carried out in parallel with measurements performed in K.U.Leuven team and included measurements of nano-Si in vitro photocytotoxicity and in vivo toxicity. Necessity for this parallel study was arisen because the MSU was the only group which could fabricate nano-Si particles by own forces and then perform phototoxicity experiments immediately after nano-Si preparation, without its surface modification, grinding and storage. Thus we could check the idea that porous Si after-preparation procedures can considerably worsen photosensitizing ability of nano-Si.

4-2-1. Experiments with singlet oxygen traps

As a proof of principal, nano-Si samples were investigated upon receipt on their singlet oxygen production capacity using chemical singlet oxygen traps. This procedure was also necessary since the biological activity was not assessed directly by the labs producing the particles, which had to be shipped to the consortium partner at the K.U.Leuven. The generation of singlet oxygen by nano-Si critically depends on the presence of Si-H surface functionalization, and an inadvertent oxidation taken place during transport could inactivate the photo-activity of the particles. All samples received were stored at 4°C, and some under inert gas (argon).

Overall the results show that not all nano-Si particle batches obtained proved to be efficient singlet oxygen producers. However, most samples showed a singlet oxygen production that was quantifiable.

4-2-2. Experiments with cultured cells

As PDT effects on cultured cells depend on the conditions used, and disappointedly the first experiments pointed out a lack of photo-activity of nano-Si, a very broad evaluation was set up modifying all possible parameters affecting the cellular PDT outcome.

To make sure that the lack of photoactivity or the presence of dark cytotoxic effects was not due by chance to a specific malignant cell line, a range of cells (T24 (human TCC), AY-27 (rat TCC), A431 (human SCC), 3T3 (human fibroblasts)) was used in most of the experimental work. Overall no major differences were seen among the different cell lines.

Besides, nano-Si samples received were investigated using different extracellular concentrations and different incubation times in the presence/absence of serum constituents, and in dark and light conditions. As far as the light conditions are concerned, a range of fluence rates and fluences were used. Besides, by changing the light source the activation spectrum was altered. These experiments were of interest since nano-Si particles have, in contrast to most photosensitizers, the unique characteristic to possess a continuous absorption spectrum over the UV and visible region. To start off 4 different light sources were used: a halogen lamp, a low pressure sodium lamp, a mercury lamp and an Argon laser. Since it turned out that some photo-activity was seen especially in case of the laser light source, most of further cellular irradiations were performed with this light source.

The results presented here highlight the major findings.

In order to test the (photo)cytotoxicity of the nano-Si particles, a new cell proliferation quantification method was conceived, since most common tests were incompatible with the reductive capacity of nano-Si. A methylene blue staining of the cells present was found to be most suitable for the task.

In Fig. 1 typical results are shown. Hypericin, used as control photosensitizer, induces a 100 % cell killing after irradiation, whereas no activity is observed under dark conditions. Light on its own does not induce any cellular effects. The nano-Si particles affect cell viability in dark conditions, an effect which is concentration-, and particle-dependent, but no additional photo-effects could be seen. Changing conditions as described above (among others, e.g. using undecylic acid coating of nanoSi particles) basically did not change the overall outcome of these in vitro experiments.

Two conclusions can be drawn from these results:

1) Although previous chemical trap experiments showed the generation of singlet oxygen by irradiated nano-Si particles, the cellular experiments did not show any evidence of such an activity. The reasons responsible for the above contradiction may be the following. The chemicals used as traps for singlet oxygen are not water-soluble so that these trap experiments were performed in organic solvents (hexafluorobenzene, methanol). Lifetime of excited (i.e. singlet) oxygen in organic solvents amounts up to tens of milliseconds as compared to ~3 μs in water. Besides, chemical trap molecules easy penetrate to pores of nano-Si particles so that each Si nanocrystal participating in singlet oxygen generation is encompassed by a solution of chemical trap molecules in organic solvent. So, one can suggest that a majority of generated singlet oxygen interact with the chemical trap molecules.

However, the situation is quite different in case of cell experiments where morphology of the heterogeneous system is such that a majority of target molecules (i.e. cell membrane molecules) are spatially well separated from silicon nanocrystals embedded in walls of nanoSi pores. Besides, the lifetime of $^1O_2$ generated by the nanocrystals is rather short to assure an escape of $^1O_2$ from the pores and its diffusion-controlled encounter with the cell target molecules.

2) Unexpectedly, most of the nanoSi particles are capable of inducing a (strong) dark cytotoxic effect that very much depends on the particle sample.

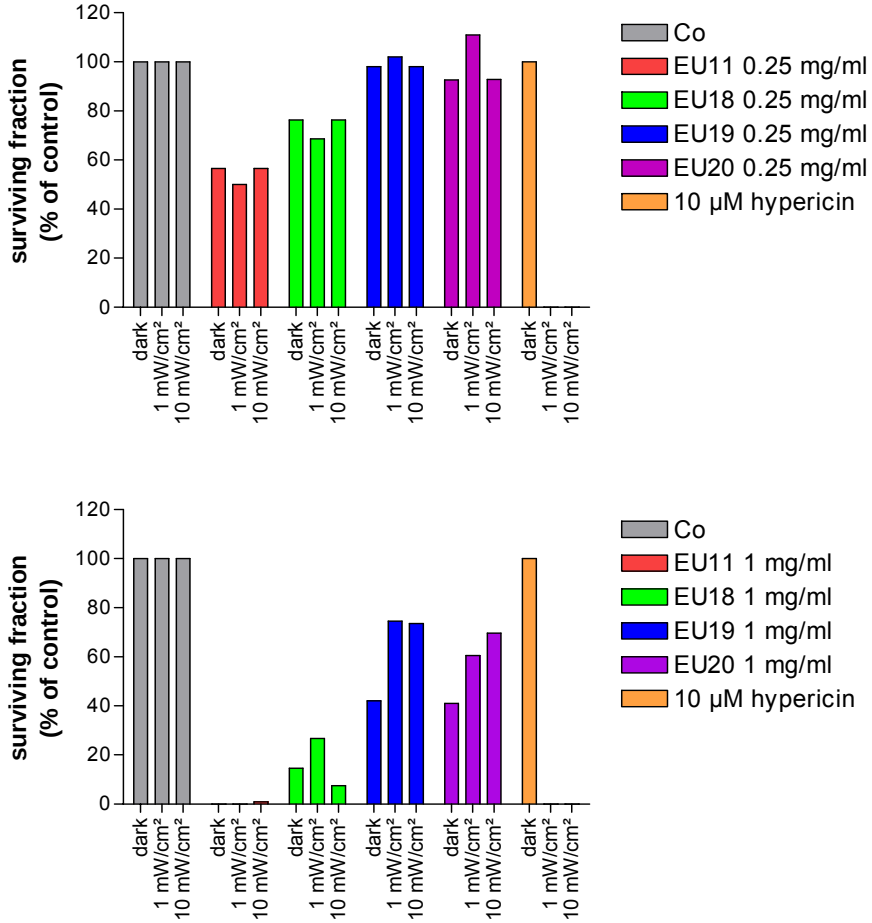


Figure 1. Cell surviving fractions as a function of treatment

From the previous results it is clear that nano-Si particles show very limited photocytotoxic effects, that is very small amounts, if any, of singlet oxygen in a water environment can reach cell target molecules. In the next experiments the excitation fluence rates used were dramatically increased to 50 and 100 mW/cm^2. Although these fluence rates are very high for *in vitro* conditions, and it is difficult to envisage how this type of light treatment could be translated into *in vivo* conditions, it was of interest to boost the PDT activity of the particles in somewhat extreme light conditions.

For the first time in a long series of cellular experiments, an interesting sample (EU30) of electrochemically etched nano-Si that showed very low dark cytotoxicity even at a concentration of 2.5 mg/ml indeed showed a pronounced photocytotoxic effect. From Fig. 2 it can also be seen that light conditions as 100 mW/cm^2 for 10 min induced a pronounced cell killing effect, and therefore the fluence rate window that can effectively be used is narrow.

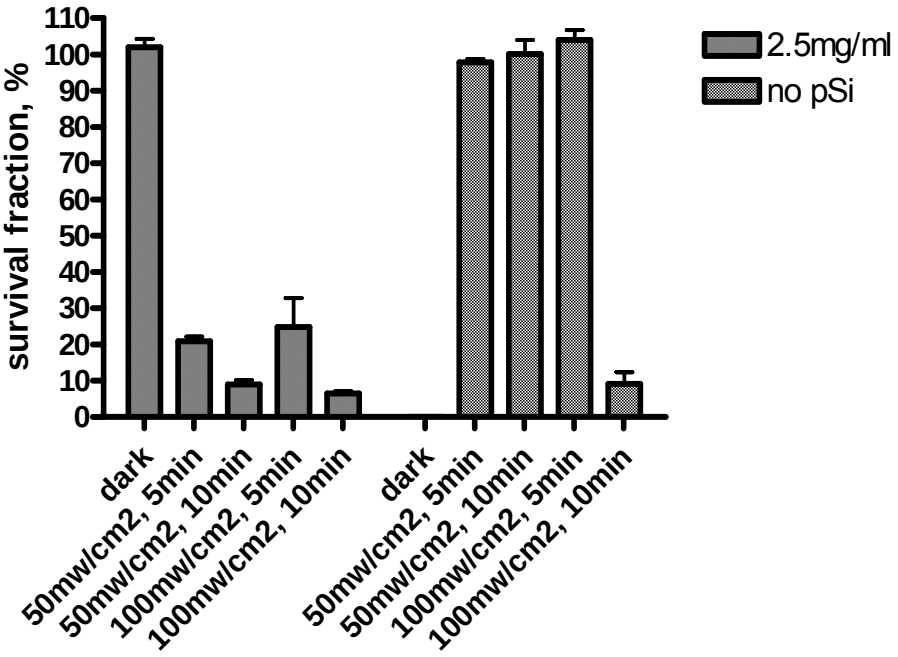


Figure 2. Cell surviving fractions after treating cells with sample EU30 in different conditions

It should be mentioned, however, that comparable experiments in high light intensity conditions with many other nano-Si samples did not result in similar interesting results. Nonetheless, the previous experiment likely learned that a combination of a somewhat higher concentration of nano-Si particles showing low dark cytotoxicity with a high light fluence rate is cardinal for inducing evident photocytotoxicity.

Evidently, an intense search for nano-Si material lacking dark cytotoxic activity was set up. Of interest, porous silicon can be prepared either by anodic etching of Si electrode (usually Si wafer) in HF electrolyte or by chemical stain-etching of Si in HF:HNO_3:H_2O solutions. Although the latter technique results in a higher production yield, many more toxic reagents have to be used in the Si etching process, and therefore electrochemically etched nano-Si samples might be easier to detoxify from its impurities.

As EU 30, an electrochemically etched nano-Si sample, was endowed with a low dark cytotoxicity, the preparation method (Prof Timoshenko, Moscow) was shared with the consortium partner at the University of Valencia (Prof. Chirvony, Prof Matveeva). Consequently a series of accordingly prepared nano-Si samples (EU50-EU61) were tested on their (photo)cytotoxic effects according to the previously mentioned protocol. Photo-activity was tested using 50 mW/cm^2 of Argon laser light. Concentrations from 1 mg/ml on started to exhibit dark cytotoxicity. Lower concentrations were free of this unwanted side-effects but unfortunately did not show any photo-activity as well.

In case of stain-etched nano-Si a study was set-up to better understand the origin of the dark cytotoxic effects. It was concluded that the toxic impurities can reside in nano-Si in different forms:

i) H_2SiF_6 and HF adsorbed within the pores of the particles,

ii) H_2SiF_6 and HF trapped by incompletely polymerized metasilicic acid within the pores, and

iii) H_2SiF_6 and HF encapsulated in completely polymerized silicate.

Consequently, different washing procedures were tried to remove most or all impurities prior to bio-testing. To further evaluate the clearance of residual cytotoxic impurities from nano-Si, the different particle preparations (EU32-EU48) were checked for their dark cytotoxic characteristics. Figure 3 illustrates the dark effect of some of the different nano-Si particles (EU41-43, EU45-47), on the cell proliferation.

Although extensively washed till the oxidation of the porous layer occurred, which was observed by diminished hydrophobicity and determined by FTIR, in general the "immediately washed" particles still contained cytotoxic compounds trapped in the pores. Being more hydrophilic than other types of nano-Si particles used in this study, "immediately washed" particles released the cytotoxic compounds in the water based medium and caused the significant decrease in the survival rate of the cells even at the lowest concentrations used in the study. On the contrary, due to their hydrophobicity, the "refreshed" particles showed slightly lower impact on the cell proliferation than "immediately washed" ones although it was determined by the thermal effusion experiment that the concentration of the toxic species was the same (HF) or higher (H_2SiF_6) in the refreshed particles. The lowest cytotoxicity was observed with the "outgassed" particles which after "refreshment" in HF:IPA solution were purified from residual toxic species by outgassing in vacuum at high temperatures, preserving their H-termination of the Si surface and consequently their hydrophobicity while diminishing the contents of the toxic HF and H_2SiF_6 .

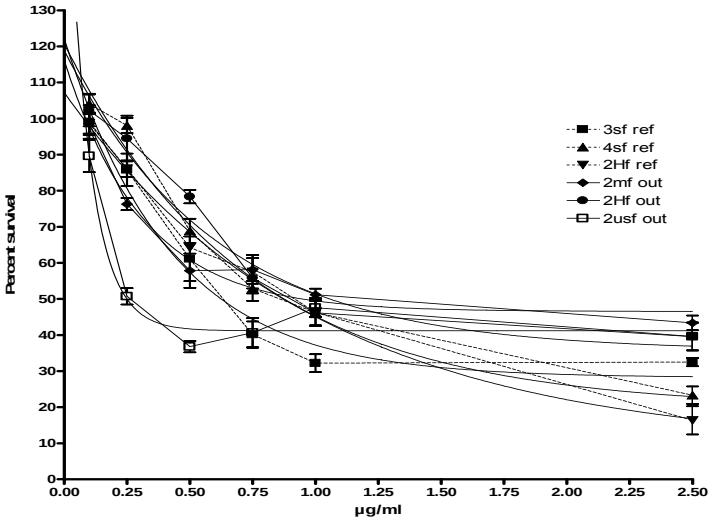


Figure 3: Dark cytotoxicity of pSi particles EU41-43, EU45-47

The (photo)cytotoxicity (under 50 mW/cm^2 conditions) of two luminescent nano-Si samples (EU45 (3sf)); EU46 (4sf)) and one non luminescent (EU41 (2mf)) was further tested. From Fig. 4 it can be seen that EU41 and EU46 showed photo-dependent activity. Although these results looked very promising, it should be noted that the marked difference between dark cytotoxicity and photocytotoxicity was no longer present when the samples were re-tested after a couple of weeks, even when they were kept under an inert (atmosphere (Argon). The reason of the instability is unknown.

The same nano-Si samples were tested after keeping the particles for two days in water and then making the PSi suspension in medium. The results show an overall increase of dark cytotoxicity, and a loss of photokilling capacity.

Similar results were obtained with sample EU42, that was kept for up to 8 days in water. Likely the partial oxidation of the particle surface resulting in an increased hydrophilicity allows the particles to associate more closely with the cells, and/or leads to an increased release of toxic impurities, resulting in an increased dark activity.

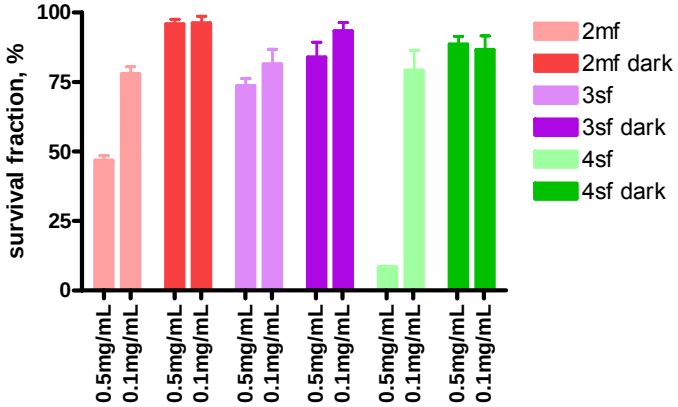


Figure 4: (Photo)cytotoxicity of np-Si particles EU41, EU45 and EU46

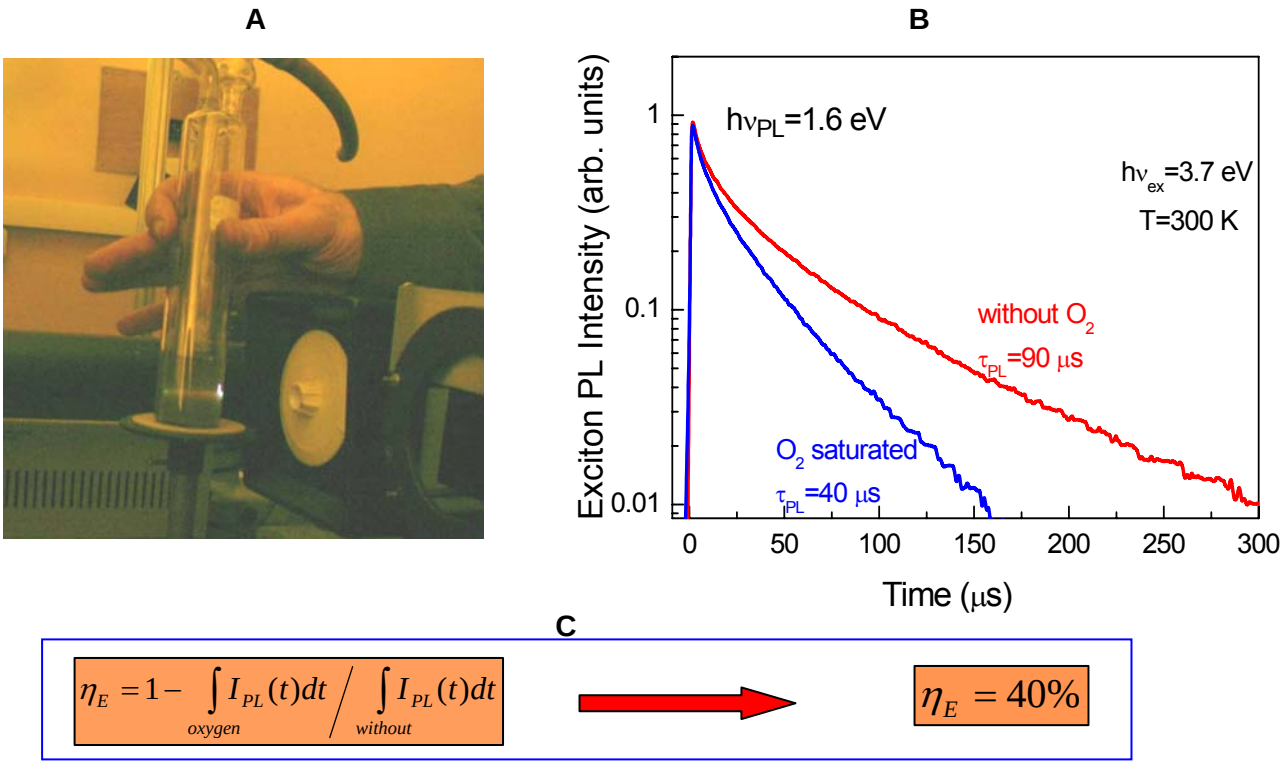


Figure 5. (A) Luminescence of the nano-Si aqueous suspension excited by UV laser beam. (B) Luminescence decay kinetics of the nano-Si suspension in oxygen-free (red curve) and oxygen-saturated (blue curve, 760 Torr pressure) conditions. Excitation was with 3.7 eV photons and luminescence detection at 1.6 eV. (C) Formula to evaluate quenching effect (decrease of the luminescence intensity due to oxygen influence) that results in efficiency η_E of the energy transfer to O_2.

The biomedical experiments performed by MSU team were preceded by preliminary photochemical measurements, namely by investigation (by the luminescence methods) of nano-Si interaction with molecular oxygen in conditions analogous to the conditions used in the further in vitro cell experiments. For this purpose, suspension of powder of freshly produced electrochemical porous Si in de-ionized water has been prepared (1 mg of nano-Si was

dispersed in 3 ml of H_2O) by intensive stirring by magnetic stirrer. The stirring resulted in formation of homogeneous suspension that evidences about hydrophylicity of nano-Si surface that may be a result of partial nano-Si surface oxidation. The suspension gave a bright reddish luminescence under UV laser beam excitation (Fig. 5A). Then PL spectra (not shown) and decay kinetics (Fig. 5B) of the nano-Si suspension were measured in oxygen-free and oxygen-saturated (760 Torr pressure) conditions. As one can see, saturation of the water suspension by oxygen results in considerable shortening of the PL decay kinetics. The shortening was found to be completely reversible and previous longer lifetimes were measured when oxygen was removed again. Calculation of the areas under decay curves results in conclusion that efficiency of nano-Si exciton quenching by O_2 is about 40% (Fig. 5C).

The kinetic data unambiguously evidence that the PL quenching is due to dynamical interaction of nano-Si excitons with oxygen, whereas a characteristic profile of the PL quenching spectrum demonstrating a maximum near 1.63 eV evidences that the quenching results in energy transfer from nano-Si localized excitons to molecular oxygen. It is worth to note that the quantum yield of the PL of nano-Si aqueous suspension was evaluated in these measurements and it turned to be about 1%.

After the above preliminary measurements, in vitro cytotoxicity experiments have been carried out by MSU team with analogous nano-Si suspensions prepared in de-ionized water and then added to living cells. Fig. 6 shows a dependence of the normalized number of living cells of the mouse fibroplasts on nano-Si concentration after standing during 1 hour in darkness or with illumination. One can see that for nano-Si concentrations about 0.5 g/L and higher illumination results in considerable decrease of the number of survived cancer cells. The death of more than 85% of cells is registered under 2.5 g/L nano-Si concentration. At the same time in darkness an influence of nano-Si on fibroplast cells survival is practically absent in all used concentration diapason. Therefore, a conclusion can be drawn that (1) the suppression of cancer cells reproduction is a result of action of singlet oxygen generated by excited nano-Si, and (2) absence of influence of nano-Si presence on fibroplast cells survival in darkness indicates that nano-Si is rather biocompatible.

It has been also shown that the phase composition of the cells changes, as it shown in Fig. 7. The changes evidence that nano-Si action to the cells is not limited by possible changes of the nutritive medium during the experiment (for example, due to its oxidation or adsorption on nano-Si), but is related to initiation of serious perturbations in intracellular processes. The results can be interpreted in favor of apoptosis as a mechanism responsible for the cell death.

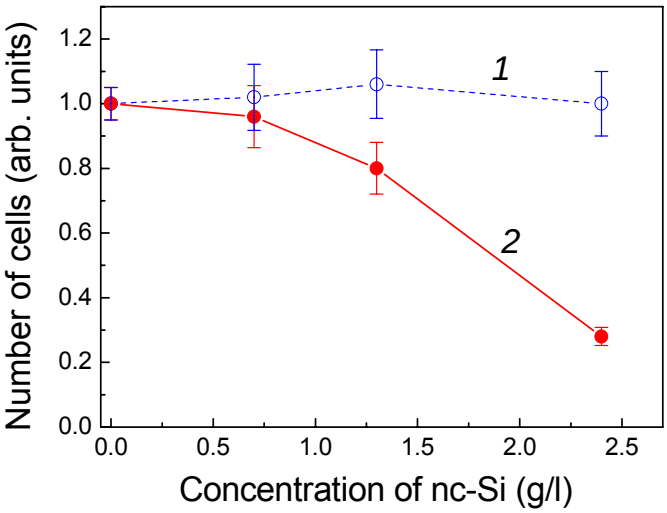


Figure 6. Cancer cell number *vs* nano-Si concentration in the dark (blue symbols) and after illumination (red symbols) determined by changes of the sample optical densities and normalized by the values in the control group where nano-Si was not added. Illumination was realized during 1 hour by the light of 250 W mercury lamp transmitted through cut-off water filer (to eliminate heating IR light) and band-pass 350-600 nm glass filter. Radiation power on the sample was ~1 mW/cm^2.

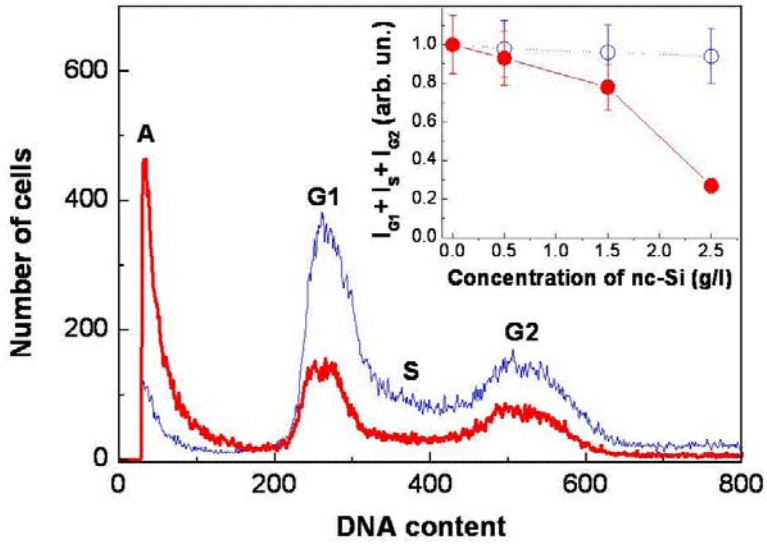


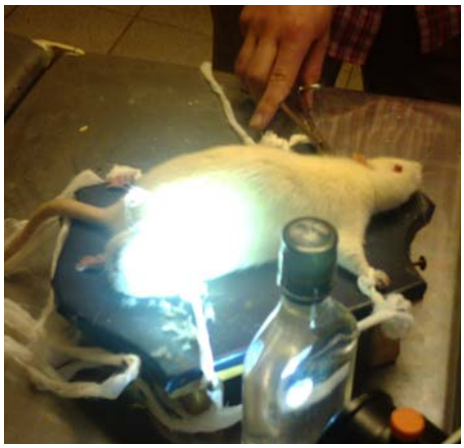
Figure 7. Histogram of DNA content for the cancer cells kept in the nutrient solution with dispersed nano-Si (1.5 g/l) in darkness (blue curve) and after illumination (red curve). Symbols G1, S, and G2 mark different cycles of the cell proliferation. The apoptotic cell region is marked by A. Inset shows the relative contribution of G1, S and G2 regions *vs* nc-Si concentration in the dark (blue symbols) and after illumination (red symbols).

4-2-3. In vivo experiments

The original project plan supposed the implementation of *in vivo* work after selecting suitable nano-Si particles in *in vitro* conditions. As can be learned from the above text, a total of 64 nano-Si samples were tested in K.U.Leuven on their singlet oxygen generation capacity and *in vitro* dark and photo-dependent cytotoxic activity, taking into account and investigating all possible parameters influencing/improving the outcome. Although evidence was borne that the nano-Si particles are able to produce singlet oxygen in organic solvents, the general *in vitro* photo-killing effects (in an aqueous environment !) was very poor or non-existing, and when present, not reproducible. So a major amount of time within the bio-part of the project was spent by K.U.Leuven team to find defined nano-Si particles endowed with reproducible high photocytotoxic and low cytotoxic properties, but evidently without having achieved the intended purpose. Being confronted with the poor *in vitro* results and without having a reasonable chance of success, no extensive *in vivo* work (biodistribution, PDT efficacy) was performed in K.U.Leuven team. From our long-standing experience with photosensitizers we known that at least a good but preferably an excellent *in vitro* PDT activity has to be present, before an interesting *in vivo* outcome can be expected. Besides, limited *in vivo* work with intravenously injected nano-Si particles showed that some of the animals were killed instantaneously, likely by generating gaseous hydrogen or releasing toxic impurities by contact of the particles with water (in blood).

MSU team used an experience obtained in their *in vitro* measurements to carry out *in vivo* experiments. The experiments were carried out on rats with tumors of alveolar cancer infected into legs. The same methodology of nano-Si preparation was applied as in case of the *in vitro* experiments: the fresh electrochemically prepared nano-Si powder was suspended in de-ionized water by intensive stirring. Then it has been injected into animal tumor and illuminated with some delay (Fig. 8, A). The obtained results are summarized in the Table (Fig. 8, B). As one can see, injection of the nano-Si suspension was certainly resulted in the PDT effect, and the effect value depended first of all on the time delay after the nano-Si injection and before illumination. Detailed analysis of the obtained *in vivo* data is yet in progress.

A



B

Rat's No	Time from injection of nano-Si to illumination (h)	Time from illumination to analysis (h)	Destruction of tumor (%)	Nano-Si penetration into cells: 0 – no, 1 – yes
1	0.5	24	30	0
2	0.5	4.5	50	0
3	0.75	24	55	0.5
4	0.4	72	60	0.5
5	4	48	70	1

Nano-Si concentration 0.5 g/l; illumination time 20 min.

Figure 8. (A) A typical view of the *in vivo* PDT experiments and (B) Table of the summary of experimental results obtained by MSU team.

4-3. Conclusions

In investigations carried out by K.U.Leuven team sixty-four nano-Si samples were tested on their singlet oxygen generation capacity and *in vitro* dark and photo-dependent cytotoxic activity, taking into account and investigating all possible parameters influencing/improving the outcome. Although evidence was borne that the particles are able to produce singlet oxygen in organic solvents, the general photo-killing effects (in an aqueous environment !) was very poor or non-existing, and when present, not reproducible. The best results on both detection of singlet oxygen by chemical traps in organic solvents and in vitro photocytotoxicity in physiological solutions were obtained with electrochemically prepared nano-Si powders.

More reproducible positive in vitro results were obtained by MSU team with analogous electrochemically prepared nano-Si samples, and the main difference between samples of these two groups is that those of MSU team were freshly prepared, non-covered by any protective layers and minimally subjected to de-integration procedures. Only these freshly prepared and immediately used samples showed in vivo photokilling effect, although nano-Si concentrations and illumination intensities used were considerably higher than those which can be acceptable for clinical applications. These data imply that surface modification and storage procedures only worsen photocytotoxicity activity of nano-Si samples, likely due to formation of Si nanocrystal surface states which serve as effective traps of energy of initially excited excitons and compete with energy transfer to moleculr oxygen. Besides, from the physical investigations done by the consortium partners, it became evident that although the nano-Si particles, which are in fact micro- and submicrometer size pieces of a porous Si layer containing photochemically active Si nanocrystals, can rather effectively generate singlet oxygen that is well documented in experiments with singlet oxygen chemical traps, these active oxygen molecules likely effectively deactivate on the nano-Si pore walls and do not escape outside to reach cell targets. In case of photochemical trap experiments chemical traps for singlet oxygen detection easy penetrate into nano-Si pores and effectively interact with the short-lived singlet oxygen molecules just near Si nanocrystals where the singlet oxygen was produced. Taking into account the low initial quantum yield of luminescence of nano-Si suspensions used in our experiments (about 1%) one can conclude that it very much looks like the present generation of nano-Si particles are not ready yet to be tested preclinically in *in vivo* conditions. Much more *in vitro* work has to be performed including development of the methods of down-up synthesis of Si nanocrystals which will exhibit much higher quantum yield of luminescence, the quantum yield should be at least comparable with the triplet state quantum yields of organic compounds (about 90%) used as photosensitizers in traditional PDT practice.

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Related publications of the Consortium:

7. V. Chirvony, A. Chyrvonaya, J. Ovejero, E. Matveeva, B. Goller, D. Kovalev, A. Huygens, P. de Witte, *Surfactant-modified hydrophilic nano-structured porous silicon for photosensitized formation of singlet oxygen in water,* Advanced Materials **19**, 2967–2972 (2007)
8. Timoshenko VY, Kudryavtsev AA, Osminkina LA, Vorontsov AS, Ryabchikov YV, Belogorokhov IA, Kovalev D, Kashkarov PK., *Silicon nanocrystals as photosensitizers of active oxygen for biomedical applications,* JETP Letters, **83** (9). pp. 423-426. (0021-3640), (2006)
9. S. Koynov, R. Pereira, M.Stutzmann, I. Crnolatac, A. Huygens, P. de Witte, *Purification of nano-porous silicon for bio-medical applications*. In preparation (2008)

5. Standardization, management and control

5.1. Design of Central Server for the Project Control

Structural design, development of software, connection protocols via internet, anti-theft measures, testing of the Central server and its launching in the internet have been fulfilled in time and, during all the time of the PSY-NANO-Si project implementation, all materials of the Project were completely available at the WEB site:
http://www.mtm.upv.es/psy-nano-si

WEB-Based project control system was designed for the following purpose:

- Simplify dynamic access to the basic project documents with current modification.
- Present the set of work packages, tasks, subtasks, deliverables, etc as the tree organized data structure with easy access to the all items.
- Organize the selection of subsets of tasks and task elements for each team and individual person.
- Visualization the progress of project performing.
- Organize the reporting process.
- Management of research teams and personal activity.
- Communication between project participants.
- Automatic e-mail reminding about deadlines of tasks and task elements.
- Management of the scientific data knowledge base.

There were four levels of access to the site, depending on the rights given, that provide users with information on the Project corresponding to their level of involvement:

- Coordinator
- EC Stuff
- Team leader
- Researcher

WEB-Based project control system has been realized as the set of ASP files and ISAPI DLL files. The pages are dynamically generated using the information from the database. It is working on the Microsoft Internet Information Server platform.

5.2. Standardization of materials, documents and deliverables

In order to organize regular and uniform mechanism of data uploading to the PSY-NANO-Si web knowledgebase and an easy search and access to these data the standard system was implemented. This knowledgebase is realized in PSY-NANO-SI web-based control system. The knowledgebase can be accessed after login to the project web site and choosing ‘Shared Scientific Data’ section. It would be filled step by step by participants during project realization. Scientific data like different type 2D plots (f.e.kinetics), spectra, SEM or AFM images can be uploaded to the server. In order to respect privacy of the commercially important data each data item has one of the following access statuses: public, consortium only, team and coordinators. Each data item has the corresponding database record that allows organizing and easy structuring. The structure of this database record is the following:

- Data ID
- Data Name
- Type of data (2D plot, 3D plot, image)
- Access right (public, consortium only, team only, private)
- Quality control (Checked, bad, temporary)
- Research task in tree-like style “Goal1\Subgoal2”
- Research subtask in tree-like style “Subtaskl2\Details3”
- Material. Material details stored in the separate table as a set of components: different compounds, multilayer structure, etc.

- Environment - electrolyte, chemical solutions, gases, etc. Environment details stored in the separate table as a set of chemical components with resprective concentration.
- Responsible person
- Date

Additionally, each data item can contain one or some experimental parameters of the following format:

- Parameter name
- Parameter unit
- Value

By using this structure it is possible to extract from the knowledgebase the data for specific conditions split by some parameter: temperature, concentration, etc, or data on different types of material or treatment, or get data for the selected task, or estimate the personal input from the team or researcher. To standardize documentation accompanied the project and facilitate to all the members an easier access to the corresponding templates available at the EC WEB, these documents were put into the PSY-NANO-Si Server for downloading by the Consortium members.

5.3. Financial and Technical Management of the Project

This task was regularly performed by all the teams through the corresponding central services of each University involved into the Project.

6. Summary

On the basis of comprehensive physico-chemical model investigations, photocytotoxic and PDT activities of the nano-Si materials prepared and functionalized by the different methods have been studied. The conditions are found at which nano-Si exhibits *in vitro* photocytotoxic and *in vivo* PDT activities, but at the same time no any toxicity is observed without special illumination. Those conditions are, however, yet far from the demands placed on approved PDT drugs, first of all from the point of view of maximum admissible drug concentration and intensity of illumination that makes one to conclude that the present generation of nano-Si particles is not yet ready to be tested preclinically in *in vivo* conditions and that much more *in vitro* work has to be performed including development of new (and not existing at the moment) methods of (semi-)industrial down-up synthesis of a few-nanometer size Si nanocrystals as a new nano-Si material.

Different methods of porous silicon particles (nano-Si material) fabrication, such as stain etching of initial metallurgical-grade Si powder and electrochemical anodization of polycrystalline Si wafers, as well as corresponding equipment have been developed to produce from tens milligrams to hundreds grams of different porous nano-Si material. Various methods of nano-Si surface modification and functionalization have been also applied to make the surface hydrophilic and stable in de-ionized water as well as in physiological solutions (electrolytes). An ability and efficiency of nano-Si materials to produce singlet molecular oxygen ($^1O_2$) in different environments, from gas phase to organic liquids and then aqueous suspensions, have been studied by direct optical detection of $^1O_2$ as well as by use of $^1O_2$ chemical traps.

The work performed enabled us to develop the methods of scalable production of the luminescent porous-Si based nano-Si materials as a new type of solid-state photosensitizers as well as a new carrier for drug delivery, to investigate transformation of nano-Si surface and its photosensitizing ability as a result of interaction with water, to develop new methods of full and partial protection of nano-Si surface against corrosion in physiological solutions. These new possibilities are developed now in the Consortium laboratories and will be used for practical applications.

2. DISSEMINATION AND USE

Participation in Scientific Congresses:

1. E-MRS Spring Meeting, Strasbourg, France, May 29-June 2, 2005

C1-1. V.Parkhutik, T.Makushok, L.T.Canham, *Deposition of calcium phosphate phases onto porous silicon substrates under a.c. bias*

C1-2. V.Chirvony, V.Parkhutik, E.Matveeva, O*ptical properties of porphyrine molecules immobilized in nano-porous silicon matrix*

2. Nanomeeting 2005, Minsk, Belarus, June 2005

C2-1. D. Kovalev, *Silicon nanocrystals: photosensitizers for molecular oxygen*

3. ICANS-21 (21 International Conference on Amorphous and Nanocrystalline Semiconductors), Lisbon, Portugal, September 2005

C3-1. P.K. Kashkarov, A.A. Kudryavtzev, L.A. Osminkina, Yu.V. Ryabchikov, I.A. Belogorokhov, A.S. Vorontzov, V.Yu. Timoshenko, *Silicon nanocrystals as efficient sensitizers of singlet oxygen molecules for biomedical applications*

C3-2. Yu.V. Ryabchikov, A.S. Vorontzov, I.A. Belogorokhov, V.A. Demin, L.A. Osminkina, E.A. Konstantinova, P.A. Forsh, P.K. Kashkarov, V.Yu. Timoshenko, *Photoluminescence and EPR investigation of silicon nanocrystals as photosensitizers of singlet oxygen molecules*

4. International Symposium on Application of Quantum Beam, Kobe, Japan, October 18-19, 2005

C4-1. D. Kovalev, *Nanosilicon: new properties-new functionality*

5. Nanosecurity 2005: from basic research to applications, Halle, Germany, October 24-25, 2005

C5-1. D. Kovalev, *Porous Silicon: from quantum effects to its production in industrial scale for explosives, hydrogen sources and biomedical application*

6. Porous Semiconductors Science and Technology, March 12-17, 2006, Sitges, Barcelona, Spain

C6-1. M. Fujii, N. Nishimura, H. Fumon, S. Hayashi, B. Goller, D. Kovalev, *Singlet oxygen formation by porous silicon in solution;*

C6-2. B. Goller, D. Kovalev, J. Diener, S. Limaye, S. Subramanian, *Scaleable synthesis route for silicon nanocrystal assemblies*

C6-3. V.Yu. Timoshenko, A.A. Kudryavtzev, L.A. Osminkina, A.S. Vorontzov, I.A. Belogorokhov, A.I. Efimova, E.A. Konstantinova, T.Yu. Bazylenko, D. Kovalev, P.K. Kashkarov *Luminescent porous silicon as efficient sensitizer of singlet oxygen for biomedical applications*

C6-4. P.K. Kashkarov, L.A. Osminkina, E.A. Konstantinova, A.V. Pavlikov, A.S. Vorontzov, V.Yu. Timoshenko, *Control of charge carrier density in mesoporous silicon by adsorption of active molecules*

C6-5. Yu.V. Ryabchikov, I.A. Belogorokhov, A.S. Vorontzov, L.A. Osminkina, V.Yu. Timoshenko, P.K. Kashkarov, *Dependence of the singlet oxygen photosensitization efficiency on morphology of porous silicon*

C6-6. V.Yu. Timoshenko, E.A. Konstantinova, V.A. Demin, Yu.V. Ryabchikov, A.S. Vorontzov, I.A. Belogorokhov, L.A. Osminkina, P.A. Forsh, P.K. Kashkarov, *EPR and Photoluminescence Investigation of Silicon Nanocrystals as Photosensitizers of Singlet Oxygen Molecules*

C6-7. V. Chirvony, V. Bolotin, E. Matveeva, V. Parkhutik, J.M. Albella, *Photophysical properties of water-soluble porphyrin molecules immobilized in oxidized nano-porous silicon matrix*

C6-8. V. Chirvony, E. Matveeva, J.M. Albella, V. Parkhutik, *Quenching of photoluminescence from porous silicon by porphyrin molecules*

C6-9. E. Pastor, J.Curiel-Esparza, M.C. Millan, E. Matveeva, V.Parkhutik, *Influence of porous silicon oxidation on its behaviour in simulated body fluid*

7. IX Congreso Nacional de Materiales, 20th-22th June, 2006, Vigo, Spain.

C7-1. E. Pastor, E. Matveeva, J. Curiel, M. C. Millan, V. Parkhutik, *Comportamiento del silicio poroso oxidado en fluido corporal simulado.*

C7-2. E. Pastor, E. Matveeva, V. Parkhutik *Aplicaciones del silicio poroso nanoestructurado como biomaterial.*

8. Nanostructures: Physics and Technology, St. Petersburg, Russia, June 26-30, 2006

C8-1. D. Kovalev, *Silicon nanocrystals: photosensitizers for molecular oxygen*

9. 211th Springs Electrochemical Society Meeting: Chicago, USA, May 6-10, 2007

C9-1. E. Pastor, J. A. Ayúcar, J. Curiel-Esparza, J. Salonen and E. Matveeva, *Electrochemical oxidation of mesoporous Silicon: structural and morphology properties of the obtained ox-porSi material*

C9-2. E. L. Pastor, L. Bychto, A.A. Chyrvonaya, M. Balaguer Ramirez, J. Salonen, V.S. Chirvony, E. Matveeva, *Nano powders of porous silicon for photosensitized formation of singlet oxygen in water: two methods of preparation and modification by surfactants*

10. 3rd Ukrainian scientific conference on physics of semiconductors (USCPS-3), Ukraine, Odessa, 17 – 22 June 2007.

C10-1.D. Kovalev, *Nanosilicon: new properties - new functionality*

11. 2nd International Conference on Surfaces, Coatings and Nanostructured Materials (NanoSMat 2007): Algarve, Portugal, July 9-11, 2007

C11-1. E Pastor, J. Curiel, E. Mäkilä, J. Salonen, V.-P. Lehto, and E. Matveeva, *Electrochemical Oxidation of Chemically Derivatizated Porous Silicon Samples as a Method to Increase Their Bioactivity*

C11-2. E. Pastor, L. Bychto, M. Balaguer, J. Salonen, V.P. Letho, V.S. Chirvony, E. Matveeva,

Porous Silicon for Photosensitized Formation of Singlet Oxygen in Water and in Simulated Body Fluid: Method of Preparation and Modifications by Undecylenic Acid

C11-3. V.Yu. Timoshenko, A.S. Vorontsov, L.A. Osminkina, P.K. Kashkarov, A.A.Kudryavtzev, D. Kovalev, S.S. Sukharev, I.V. Reshetov, *Porous Silicon as Singlet Oxygen Photosensitizer for Biomedical Applications*

12. 1st Scanbalt Biomaterials Days, 26th - 28th of September 2007, Turku (Finland).

C12-1. E. Pastor, J. Salonen, V-P. Leptho, J. Curiel, E. Matveeva. *Porous Silicon derivatization treatments in order to obtain biocompatible structures.*

13. 3rd International Conference on Micro-Nanoelectronics, Nanotechnology & MEMs, Athens, Greece, November 18-21, 2007

C13-1. M. Balaguer, E. Pastor, P. Atienzar, M. Miranda, V. Chirvony, E. Matveyeva, *Durability and photophysical properties of surfactant-covered porous silicon*

C13-2. L. Bychto, Y. Makushok, V. Chirvony, E. Matveeva, *Pulse electrochemical method for porosification of silicon and preparation of porSi powder with controllable particles size distribution*

C13-3. P. K. Kashkarov, L.A Golovan', S.V. Zabotnov, D.A. Mamichev, V.Yu. Timoshenko. *Novel Photonic Media Based on Nanostructured Semiconductors and Dielectrics*

14. NATO Advanced Study Institute "Sensors for Environment, Health and Security: Advanced Materials and Technologies", Vichy, France, September 16-27, 2007

C14-1. V. Yu. Timoshenko*, Singlet oxygen generation and detection for biomedical applications*

15. 6th International Conference "Porous Semiconductors-Science and Technology", Mallorca, Spain, March 2008

C15-1. V. Chirvony, M. Balaguer, E. Pastor, L. Bychto, E. Matveeva, *Luminescence of Si nanocrystals in aqueous environment*

C15-2. E. Pastor, J. Salonen, V.-P. Lehto, V. Chirvony, E. Matveeva, *Physical adsorption vs. chemical binding of undecylenic acid on porous silicon surface: a comparative study of differently functionalized materials*
C15-3. V. Chirvony, M. Balaguer, E. Matveeva, *Quenching of Si nanocrystal luminescence by O_2 as a probe to distinguish between free exciton and surface state emission*
C15-4. M. Balaguer, E. Pastor, V. Chirvony, E. Matveeva, *Surfactant-covered porous silicon particles: preparation and durability in aqueous environment*
C15-5. E. Pastor, J. Curiel-Esparza, J. Salonen, V.-P. Lehto, E. Matveeva, *Electrochemically induced bioactivity of acetylene derivatized porous silicon.*
C15-6. L. Bychto, Y. Makushok, E. Matveeva, *The overwiew of electrochemical regimes for porous silicon powders preparation*
C15-7. D. Kovalev, *Silicon nanocrystals: universal spin-flip activators?*
C15-8. B. Goller, S. Polisski, D. Kovalev, *Spin-flip excitation of organic molecules mediated by photoexcited silicon nanocrystals*
C15-9. A.I. Efimova, E.A. Konstantinova, A.S. Vorontsov, I. Leuhin, A. Pavlikov, V.Yu. Timoshenko, P.K. Kashkarov, *IR and EPR Study of Ammonia Adsorption Effect on Silicon Nanocrystals*
C15-10. E.A. Konstantinova, V.A. Demin, M.B. Gongalskiy, Yu.V. Ryabchikov, P.K. Kashkarov, *EPR diagnostics of singlet oxygen generation on porous silicon surface*
C15-11. I.A. Belogorokhov, Yu.V. Ryabchikov, A.S. Vorontsov, L.A. Osminkina, V.Yu. Timoshenko, P.K. Kashkarov, *The investigation of singlet oxygen photosensitization process in semiconductor heterosystems based on silicon nanocrystals in aqueous solution*

16. International Workshop "On the way to NanoTechnological Revolution", Marcelle, France, October 5-10, 2008.

C16-1. V. Chirvony, *Silicon nanocrystals as possible photosensitizer for singlet oxygen production.*

17. 214th ECS Meeting - *Honolulu*, Hawai, 2008

C17-1. E. Matveeva, Y. Makushok, L. Bichto and E. Pastor. *A Three Pulse Electrochemical Regime as a Fabrication Platform for Nano-structured Multifunctional Silicon Based materials and Their Primary Prototypes*

Publication of full-size articles:

2005

1. D. Kovalev and M. Fujii, *Silicon nanocrystals: photosensitizers for oxygen molecules,* Advanced Materials **17,** No 21, 2531-2544 (2005)
2. M. Fujii, D. Kovalev, B. Goller, Sh. Minobe, Sh. Hayashi, V. Yu. Timoshenko, *Time-resolved photoluminescence studies of the energy transfer from excitons confined in Si nanocrystals to oxygen molecules,* Physical Review **B72**, 165321 (2005)
3. D. Kovalev, E. Gross, J. Diener, V. Timoshenko, M. Fujii, *Photoluminescence fatigue effect in luminescent porous silicon induced by photosensitized molecular oxygen,* Phys. Stat. Sol (c) **2**, 3188 (2005)
4. M. Fujii, M. Usui, S. Hayashi, E. Gross, D. Kovalev, N. Kuenzner, J. Diener, V.Yu. Timoshenko, *Singlet oxygen formation by porous Si in solution.* Physica Status Solidi A **202,** No 8**,** 1385-1389 (2005)

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5. Timoshenko VY, Kudryavtsev AA, Osminkina LA, Vorontsov AS, Ryabchikov YV, Belogorokhov IA, Kovalev D, Kashkarov PK., *Silicon nanocrystals as photosensitizers of active oxygen for biomedical applications,* JETP Letters, **83** (9). pp. 423-426. (0021-3640), (2006)

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9. V.S. Chirvony, V.L. Bolotin, J. Ovejero, E.S. Matveeva, B.M. Dzhagarov, J. Albella, V.P. Parkhutik, *Luminescence properties of the porphyrin/porous silicon composites,* Phys. Stat. Sol. (a) **204**, #5, 1523-1527 (2007)
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11. Limaye, S.; Subramanian, S.; Goller, B.; Diener, J.; Kovalev, D. *Scaleable synthesis route for silicon nanocrystal assemblies,* Physica Status Solidi A **204** (5), 1297-1301 (2007)
12. V. Parkhutik, V. Chirvony, E. Matveeva, *Optical properties of porphyrin molecules immobilized in nano-porous silicon,* Biomolecular Engineering **24,** 71-73 (2007)
13. V. Chirvony, A. Chyrvonaya, J. Ovejero, E. Matveeva, B. Goller, D. Kovalev, A. Huygens, P. de Witte, *Surfactant-modified hydrophilic nano-structured porous silicon for photosensitized formation of singlet oxygen in water,* Advanced Materials **19**, 2967–2972 (2007)
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