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NANOSILICON-BASED PHOTOSYNTHESIS FOR CHEMICAL AND BIOMEDICAL APPLICATIONS

Project no.: NMP4-CT-2004-013875

Contractors: Technical University of Munich, Germany, (Prof. Martin Stutzmann)
Katoliken University of Leuven, Belgium, (Prof. Peter De Witte)
Moscow State University, Russia, (Prof. Victor Timoshenko)
Technical University of Valencia, Spain, (Dr. Eugenia Matveeva)
University of Kobe, Japan, (Prof. Minoru Fujji)

Coordinator: Technical University of Valencia (Spain)
Chair person: Dr. Eugenia Matveeva;
Contact details: Technical University of Valencia, Institute for Nanophotonics technology, (former Centre of "Materials and Technologies of Micro fabrication"),
Cami de Vera s/n, 46071 Valencia, Spain,
Tel: +34 96 387 9622, Fax: +34 96 387 9729,
Email: eumat@upvnet.upv.es

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

State of the art and objectives of the entire Project and formulated for the reporting period

The overall goal of the project is to explore the possible applications of silicon nanocrystals (nano-Si) as efficient generator of singlet molecular oxygen ($^1O_2$), and develop novel biologically tolerable and environmentally friendly prototype systems, able to compete with existing photo-sensitizers in photochemical, biological and medical applications.
Five teams constitute a PSY-NANO-Si Consortium; three of them are the European leaders in major issues related with nano-Si from its fabrication to effective control of its structural and morphological properties. A Japanese partner, the Kobe University team, is a world-class center specialized in investigations of the optical properties of the

silicon nano particles. Inclusion into the consortium of the outstanding partner team from Catholic Leuven University dedicated to biological and therapeutic applications of singlet oxygen $^1O_2$, guarantees achievement of the tasks related to the planned bio-medical applications.

The Scientific and Technological objectives of the **entire Project** are:

- Research and development of technological processes for the formation of nano-Si materials with controllable shapes and sizes of particles;
- Comprehensive study of nano-Si as a photo-sensitizer material for generation of singlet oxygen in a gas and liquid phases;
- Study of delivery mechanism of nano-Si to local zones where its action is necessary and development of photodynamic therapy employing nano-Si

In a first year of the project implementation the principal objectives and achievements were:

- Development of two ways for nano-Si production (stain etching and electrochemical anodization). Determination of the more perspective one from the point of view of further downscaling and production quantities was postponed until more results are available;
- Analysis of the aging process of the obtained porous material and its effect on the surface and optical properties.
- Analysis of the possible strategies for fuctionalization of the porous materials through derivatization and/or impregnation to make it compatible with watery ambiences;
- Studying of the kinetics and mechanism of the singlet oxygen generation in gas phase.

All the objectives planned for the first year have been achieved. Based in those results the objectives for the **second year** are as follow:

- Further **downscaling** of the nano-Si particles prepared by the electrochemical and stain-etching techniques; the goal is to obtain the 50-100 nm mean size of nano-Si particles in sufficient quantities.
- Stabilization of the nano-Si particles against oxidation and degradation in aqueous ambient via **modification** of their surface through direct chemical derivatization and/or micelle formation.
- Study of the impact of the introduced modifications on **efficiency** of the singlet oxygen formation sensitized by nano-Si.
- In vitro study of nano-Si cellular uptake and **photo-cytotoxity**.

Work performed during the second year

Downscaling. Automated stain etching machine developed at TUM permitted a reproducible fabrication of porosified nano-Si. Downscaling of particles up to 100-300nm mean size using this technique is only possible if initial crystalline Si powder with 0.03-1mkm size grains is available. The achieved lots of silicon with suitable grain size were treated and possibility to produce nano-Si powder with average size of particles about 50nm (3g batch size) was confirmed, Fig.1.

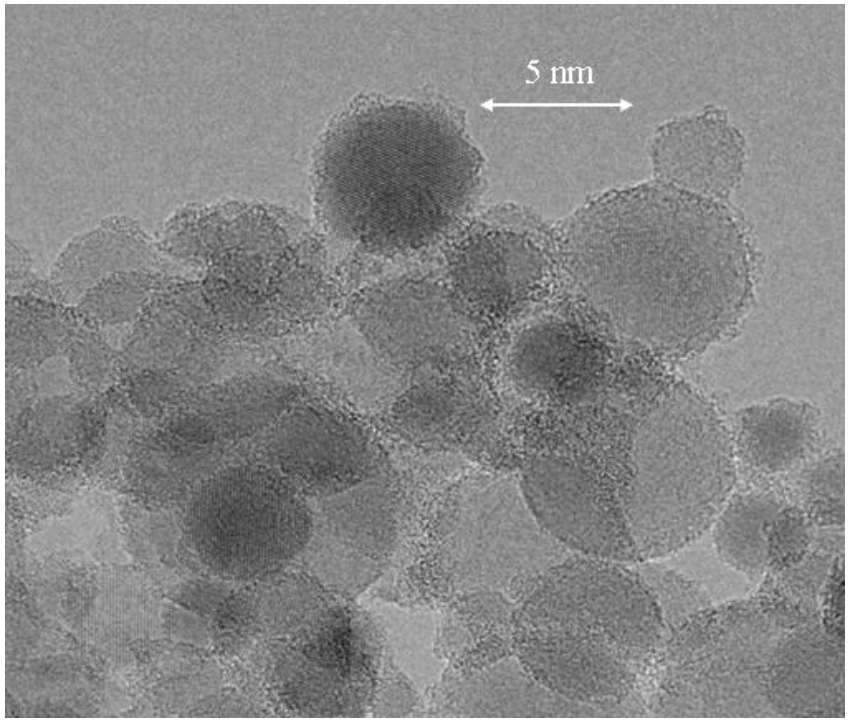

Fig. 1. **TEM** images of the >20nm size nano-Si powder stain etched in solution: 7.5 ml of 49% HF + 4 ml of 18% HNO3 per 1g of Si. Batch size of nano-Si is up to 3g.

The second method – electrochemical – at the beginning of the project was not applied widely enough for producing a sufficient quantity of nano-Si. It was due to absence of the corresponding experimental facilities – adequate programmable power source. Designed at UPV a powerful MTM-400 potentiostat for treatment of larger area Si wafers (up to 10cm diameter, or 80 cm^2) was fabricated and tested during the reporting period. The experimental results achieved resulted in a patent application on an electrochemical Method for production of nano-porous silicon particles with controllable size. This method based on pulse electrochemical regimes permits introducing desired morphology into the developed porous layer in-situ simultaneously with its growth.

At the first glance the stain-etched material seems to be a better candidate for using it as a material for developments of a new photosensitizer. Nevertheless its initial luminescence resulted to be ten times lesser than that of the electrochemically prepared one. As well, the contradictions between ageing, initial luminescence level and long-living time of the material in bio ambience make the final conclusion on suitability of different type of nano-Si materials for PDT of essential importance. Consortium has already postponed its final choice and is still very cautious about making the ultimate decision.

Modification. A modification of nano-Si is necessary to make the material compatible with bio-ambiences. Miscibility with water was set-on by encapsulation of the nano-Si particles into surfactant micelles. Different organic compounds with dual liophylic/ hydrophylic functionalities were used to form a chemical barrier between nano-Si particles and bio-ambience similar to used by the living cells in bio-systems. The proposed approach demonstrated good results and applicability: hydrophobic nano-Si materials were successfully dissolved/ suspended in water and other water based solutions (acellular body fluids and simulated body fluids with other bio components).

A physical adsorption method was applied to attach different organic molecules to the hydrophobic H-terminated silicon surface. The physical adsorption mechanism in contrast to the chemical adsorption one permits a dynamic exchange with molecules in solution and, on the other hand, allows keeping the physical properties of the semiconducting material unchanged. Indeed, the optical properties of the studied nano-Si water suspensions (luminescence and singlet oxygen generation) were preserved for the period of time from 1 to 14 days depending on chemical nature of the used surfactant, Fig.2.

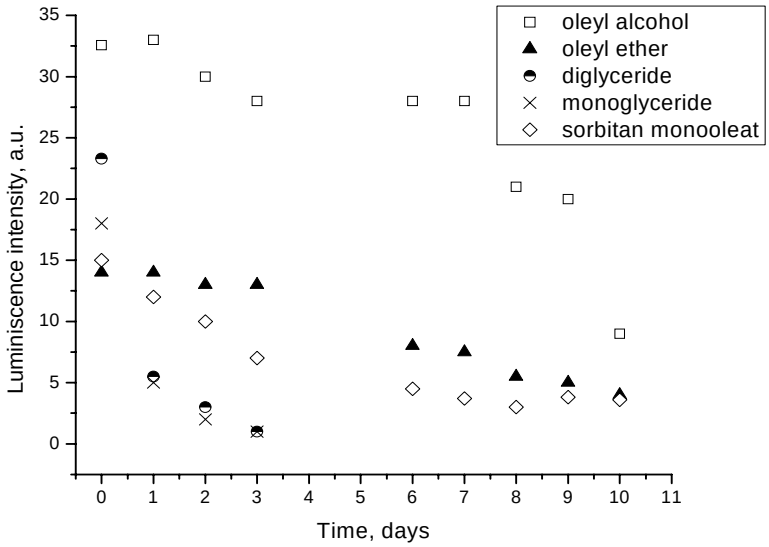

Fig.2. Long time deactivation of luminescence in studied porous silicon suspensions modified by different surfactants

Efficiency. Studies on singlet oxygen generation mechanism in liquid phase were started with materials electrochemically prepared and modified (or not) by surfactants. Works using liquid media like organic solvents, pure water or acellular body fluids are in progress. A direct evidence of singlet oxygen generation by nano-Si particles in liquid phases was obtained. The studies were performed with numbers of materials prepared by different partners and employing different methods. The efficacy was compared with the well known molecular system, namely hypericine. Results on singlet oxygen generation and its capture by organic traps confirmed that some types of nano-Si powders are competitive with the reference photosensitizer. Electrochemically prepared porous silicon lifted up from the Si wafer by ultrasound and then modified by undecelenic acid demonstrated the best results. These data definitively confirmed that the type of nano-Si material plays a key role in the global effectiveness of nano-Si in PDT.

The results also demonstrated that photoactivity of the nano-Si material and the subsequent energy transfer to molecular oxygen is lesser intensive in liquid medias (the photoluminescence yield, for instance, decays in 2-3 times after surfactant coverage of nano particles). Luminescence of suspensions is quenched quite fast: from hours for unprotected material to few days for encapsulated micelles. A decay rate is correlated

with the rate of oxidation of the particles by water ambience. The life-time of the active protected particles is quite enough to obtain a possible therapeutically effect in cellular systems. The requirements formulated by the KUL partner responsible for cytotoxicity study are: at least 24 hours for active/ useful live-time of nano-Si powder in bio-medias.

Citotoxicty. Both types of nano-Si have been studied in the assays concerned the cellular uptake. The stain etched nano-Si material demonstrated higher dark toxicity and could not be washed out from the surface of plates with cancer cells after the experiments. That could be a response of a system to small permeability of material into the cells because of a huge size of particles (supplied to the Leuven partner and studied up to the date) in comparison with cell dimensions. Electrochemically prepared nano-Si showed more rewarding behavior and lesser dark toxicity, most probably due to a finer particle size.

Work planned, objectives and expected results for the third year

The Consortium has decided to continue the works with both types of nano-Si and accumulate more detailed data on the bio activity for both materials. For that an extension of the contract for 6monhs has been asked (on 30 of March 2007). In the extended version of the Project the Consortium accepted additional works on preparative and downscaling methods to produce nano-Si, studying of both materials in bio-systems as effective generators of singlet oxygen and as pharmaceutical agents. Some of the already concluded tasks are going to be renewed.

For WP1 – synthesis of nano-Si:
Further downscaling of the Si nano-particles prepared by electrochemical techniques; the goal is to find conditions to obtain the 50-100 nm mean size of nano-Si particles in a controllable way and in sufficient quantities.

For WP2 – surface modification:
Stabilization of the Si nano-particles against oxidation and degradation in simulated biological ambient is to be continued via modification of particles surface through direct chemical derivatization and micelle formation. Optimal ratio between physical and chemical type of binding of surfactant molecules into surface of nano-Si will be studied.

For WP 3 – Mechanism of singlet oxygen generation:
Impact of the introduced modifications on efficiency of the singlet oxygen generation by nano-Si in simulated and real bio-ambient will be analyzed for new prepared materials. Alternative routs for energy transfer from nano-Si to components of watery surrounding will be analyzed in details; namely the possibility to formation of other reactive oxygen species (ROS) is going to be considered. The reactivity of singlet oxygen and other ROS toward different organic molecules in watery solutions will be studied to explore the possibility of using new nanostructured system for alternative chemical applications.

For WP 4 – Application of nano-Si as a novel photosensitizer:
In vitro and in-vivo studies of nano-Si cellular uptake and photo-cytotoxity are going to be extended to more nano-scaled materials prepared by stain etching and electrochemical methods.