

# Silicon nanoparticles produced by femtosecond laser ablation in water as novel contamination-free photosensitizers

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## 1 Introduction

Suppression of pathogenic flora is one of the strategic goals in contemporary patient care in dermatology, dentistry, gastroenterology, and other areas of conservative and surgical therapy. A wide use of antibiotics in the second half of the twentieth century has led to the crisis of antibiotic and chemical resistance among pathogenic bacteria and viruses. Thus, bacterial isolates cultured from patients with skin wounds show an alarming trend toward antibiotic resistance even within four

**Abstract.** We report the synthesis of novel inorganic contamination-free photosensitizers based on colloidal silicon nanoparticles prepared by laser ablation in pure deionized water. We show that such nanoparticles are capable of generating singlet oxygen ( $^1\text{O}_2$ ) under laser irradiation with a yield estimated at 10% of that of photofrin, which makes them a potential candidate for therapeutics, antiseptics, or disinfectants. We also discuss a model of  $^1\text{O}_2$  generation and the possibility for optimizing its release. Potential advantages of such novel inorganic photosensitizers include stable and nonphotobleaching  $^1\text{O}_2$  release, easy removal, and low dark toxicity. © 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3086608]

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years of observation (between 1992 and 1996).<sup>1</sup> Intensive efforts are now placed on the advancement of alternative antimicrobial therapeutics, to which bacteria are not able to easily develop resistance. One of these may be a modality recently termed *photodynamic inactivation* (PDI),<sup>2</sup> *photodynamic disinfection*,<sup>3</sup> or *photodynamic antimicrobial chemotherapy* (PACT).<sup>4</sup> The most frequently used term is PACT and will be used here. PACT is based on a combination of photosensitizer (PS) chromophores and light, promoting a phototoxic effect on the treated microorganisms, in general via oxidative damage, known as *photodynamic therapy*.<sup>5</sup> Its use has been reported to treat localized infections *in vitro*, animal models, and patients, including dental<sup>6</sup> and periodontal disinfection,<sup>7,8</sup> antimicrobial therapy against *Helicobacter pylori* in the stomach,<sup>9</sup> pathogen inactivation in blood products,<sup>10</sup> wound healing and cosmetology,<sup>11</sup> acne treatment,<sup>12</sup> abdominal surgery,<sup>13</sup> and pulmonology.<sup>14</sup>

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The primary morbidity of PACT is the potential phototoxicity of normal light-exposed mammalian cells persisting after the treatment for days or even weeks. An ideal photosensitizer should be *removable* from tissue surfaces or solutions once the PACT session(s) is completed. Another significant handicap of PACT is the photobleaching of the PS. This effect is present for most organic PS, preventing the application of high treatment doses. Recent advances in the field of nanotechnology and the appearance of new classes of inorganic photosensitizers gave a promise for the solution of these problems.<sup>15</sup> Reducing the particles from micron-to nanosize scale leads to a greater specific surface area, potentially releasing more reactive oxygen species during PACT. For example, significant attention has been given to II-VI semiconductor-based nanoparticles such as CdSe and CdS, but the success was very modest, since these materials provide a relatively low quantum yield (QY) of singlet oxygen ( $^1\text{O}_2$ ) generation<sup>16</sup> and may release of  $\text{Cd}^{2+}$  ions yielding to a major intrinsic biotoxicity. Exempt of natural toxicity, nanoparticles of group IV semiconductors look much more promising for these tasks. A few recent studies reported the generation of  $^1\text{O}_2$  by porous silicon structures, fabricated by anodical etching of bulk silicon in hydrofluoric (HF) acid solutions followed by mechanical scratching to produce randomly size- and shape-dispersed elements.<sup>17–19</sup> Although the mechanism of  $^1\text{O}_2$  generation is not yet completely understood, the efficiency of its release was sufficient to cause a destruction of cancer cells.<sup>19</sup> Despite these first promising results, *in vivo* application prospects of such porous silicon-based photosensitizers look hardly realistic. Indeed, mechanical or other milling of porous silicon skeleton inevitably leads to a wide dispersion of both size and shape of formed elements, with the mean size reaching several  $\mu\text{m}$  (Ref. 19). In general, such structural characteristics are not consistent with tasks of photosensitizer delivery *in vivo*, as a plurality of uptake mechanisms may become involved due to different particulate sizes and shapes. Another potential problem consists in the contamination of Si surface with by-products (e.g., HF derivatives) during the anodical etching procedure, which can result in a secondary toxicity of Si-based photosensitizers. Moreover, attempts to solve the toxicity problem by applying alternative purely chemical methods do not look very promising, since these methods normally also employ undesirable nonbiocompatible surfactants or generate toxic by-products.<sup>20,21</sup>

The central idea of this study is that silicon nanoparticles prepared by laser ablation in liquids are able to act as photosensitizers producing  $^1\text{O}_2$  and resulting in microbe cell killing, opening potential antiseptics or disinfectant therapies. As a demonstration of the validity of this idea, we present preliminary results of photodynamic activity and generation of  $^1\text{O}_2$  using silicon nanoparticles in solution. Nanoparticles were produced by ultrashort laser ablation in liquids, which was originally introduced for the fabrication of plasmonic (noble metal) nanoparticles.<sup>22–27</sup> The femtosecond laser ablation regime offers several key advantages over conventional nanosecond or microsecond ablations, enabling a fine control of nanocluster parameters even in the absence of chemical surfactants during the ablation process.<sup>22</sup> However, the main advantage of this approach consists in the possibility of work-

ing in a clean, contamination-free environment, which eliminates the toxicity problem and provides complete sterilization of the resulting product.<sup>28</sup> The future steps of this study will include modification of nanoparticle design to improve photodynamic performance, enhancing both the quantum yield of the  $^1\text{O}_2$  generation and the increase of the desired spectral absorption.

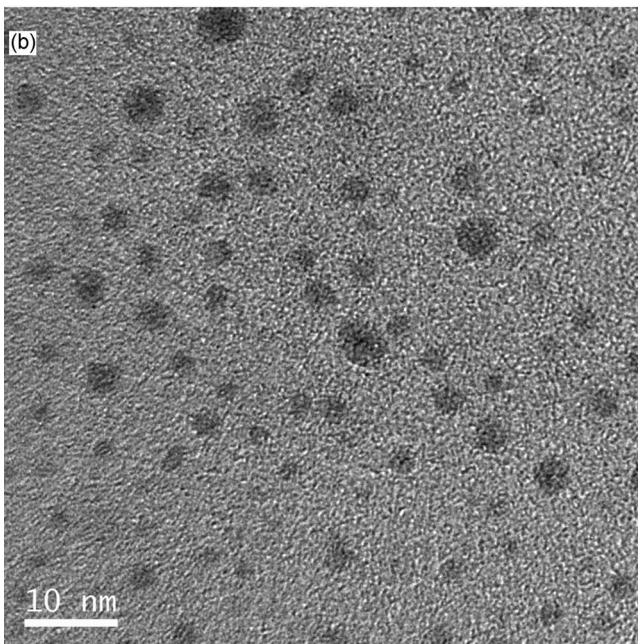
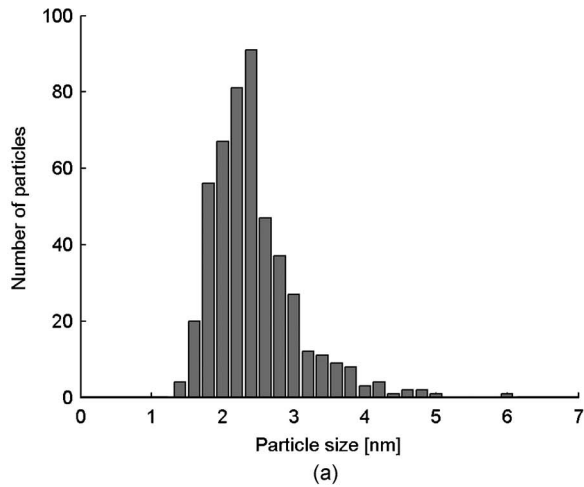
## 2 Materials and Methods

Synthesis of the colloidal silicon nanoparticles was carried out using a pulsed Ti:sapphire laser (Hurricane, Spectra Physics Lasers) operating at 800 nm, with a pulse width of 120 fs, 1-mJ max pulse energy, and 1-kHz repetition rate. An n-type  $\langle 100 \rangle$  silicon wafer target was placed at the bottom of a glass vessel filled with 5 mL of deionized water (18.2 M $\Omega$  cm) or  $\text{D}_2\text{O}$  (CDN Isotopes) and was irradiated for up to 30 min. Various pulse energies from 0.1 to 1.0 mJ were used, and the beam was focused onto a spot-size diameter of about 1 mm on the target using a lens with a focal length of 75 mm. The target was constantly moved in the focusing plane to obtain identical surface conditions for each ablation pulse.

To examine the generation of  $^1\text{O}_2$ , we used two systems, which were equipped with different pumping lasers (523 nm and 266 nm). Emission from the nanoparticle suspension placed in a quartz cuvette was collected at 90 deg to the excitation beam with a photomultiplier tube (PMT) in order to trace the time-resolved signal of the emission at different wavelengths. The 523-nm system, previously described in detail by Jarvi et al.<sup>29</sup> and Niedre et al.,<sup>30</sup> is composed of a 10-ns Nd:YLF frequency-doubled (Crystalaser QG-523-500) excitation laser. Emission from the sample is measured time resolved at five different wavelengths using filters centered at 1212 nm, 1240 nm, 1272 nm, 1304 nm, and 1332 nm ( $\sim 20$  nm full width at half maximum). The detector is a liquid nitrogen-cooled PMT (Hamamatsu R5509-42) with an extended spectral range from 300 to 1400 nm. This system was used for the singlet oxygen quantum yield estimations. The second system is composed of a 266-nm Nd:YAG laser (Surelite) for excitation. Emission from the sample is collected using a monochromator (Spectral Products) and fed into a NIR-enhanced PMT module (Hamamatsu H10330-75).

## 3 Results and Discussion

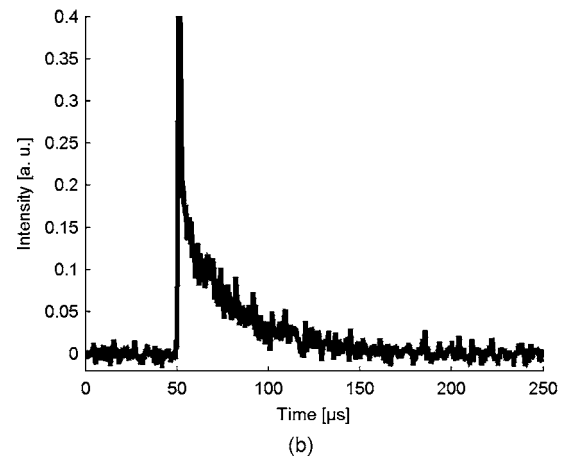
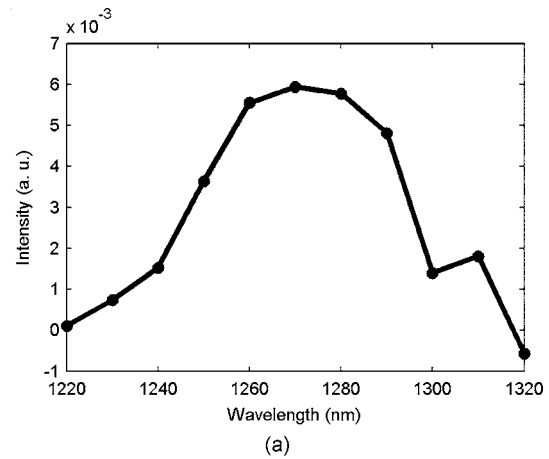
The exposure of the Si target to femtosecond radiation led to the ablation of the material and its release into the solution. The original clear solution became red-brown several seconds after exposure onset. The resulting colloidal solutions were very stable, and nanoparticles did not precipitate for several months after the preparation. To determine size characteristics of produced nanoparticles, we dropped a droplet of the solution on a carbon-coated copper grid of a transmission electron microscope (TEM) and dried it at room temperature. The grid was then placed into a TEM (Jeol JEM-2100F) to directly visualize the nanoparticles. Figure 1 shows a typical TEM image of Si nanoparticles (inset) produced by laser ablation at near-threshold conditions and a corresponding size distribution. One can see that the nanoparticles had a mean size around 2.4 nm. The increase of laser fluence led to an increase of nanoparticle size up to few tens of nm, with an



**Fig. 1** Typical size distribution of Si nanoparticles produced by laser ablation at low, near-threshold fluences ( $\sim 0.05 \text{ J cm}^{-2}$ ) and (b) corresponding TEM image.

increase of size dispersion. The nanoparticles exhibited the characteristic  $3.14 \text{ \AA}$  lattice spacing of the  $\langle 111 \rangle$  atomic plane associated with a crystalline state, although amorphous nanoparticles were also observed. The lattice structure was determined by measuring the distance between the atom rows visible on some of the particles in the TEM images. The atom rows are not visible in the TEM image from Fig. 1 because those particles are very small and probably completely amorphous.

The nanoparticle-based solution was then placed into a quartz cuvette and installed into optical systems for examining  $^1\text{O}_2$  release. Surprisingly, our experiments detected strong and stable  $^1\text{O}_2$  signal, which was monitored by the appearance of a phosphorescence peak at  $1270 \text{ nm}$ . An example of  $^1\text{O}_2$  signal under illumination of the samples with the  $266\text{-nm}$  system is shown in Fig. 2(a). Similar  $^1\text{O}_2$ -related phosphorescence signals were observed with the  $523\text{-nm}$  system. Since



**Fig. 2** Singlet oxygen phosphorescence spectrum and (b) time-resolved signal collected at  $1270 \text{ nm}$  upon excitation of the nanoparticles at  $266 \text{ nm}$ .

the lifetime of  $^1\text{O}_2$  is much longer in  $\text{D}_2\text{O}$  compared to water ( $55 \mu\text{s}$  compared to less than  $4 \mu\text{s}$ ),<sup>31</sup> the  $^1\text{O}_2$  experiments were mainly performed for nanoparticles produced in deuterated water ( $\text{D}_2\text{O}$ ). Time-resolved decay of the signals, shown in Fig. 2(b), yields a  $^1\text{O}_2$  lifetime of  $28 \mu\text{s}$ , which is lower than the expected  $55 \mu\text{s}$ . This is probably due to the quenching mechanism, which lowers the decay time and will be the subject of further investigation in future papers. We did not measure directly the  $^1\text{O}_2$  quantum yield of the nanoparticles, but rather the ratio of their quantum yield at  $1270 \text{ nm}$  in water with that of commercially available photosensitizer Photofrin estimated in the same irradiation conditions (wavelength of  $523 \text{ nm}$ , mean power density of  $110 \text{ mW/cm}^2$ , same fluence rate and fluence absorbed). Photofrin ( $2.5 \mu\text{g/ml}$ ) was irradiated for 90 minutes prior to singlet oxygen measurements, destroying potential aggregates and leaving Photofrin monomers.<sup>32,33</sup> Compared to Photofrin in the monomeric active form, the  $^1\text{O}_2$  production rate of the Si nanoparticles was estimated as 10% of the rate provided by Photofrin, which is an encouraging result taking into account that no optimization of the fabrication procedure was performed at this stage. Furthermore, Si nanoparticles subjected to a  $532\text{-nm}$  pulsed laser irradiation for 1 h at  $200 \text{ mW/cm}^2$  average intensity (for a total of  $720 \text{ J/cm}^2$ ) did not



show signs of  $^1\text{O}_2$  signal decay indicative for photobleaching, providing a decisive advantage over conventional organic photosensitizers.

Let us consider one properties and mechanism of  $^1\text{O}_2$  generation. Photosensitizing molecules are normally optically excited into an active singlet state, and after the intersystem crossing process, they accumulate in the long-lived triplet state. Interaction of this excited triplet state with the  $\text{O}_2$  triplet ground state results in energy and spin exchanges followed by a relaxation of the PS to the ground state, while  $\text{O}_2$  is raised to an activated singlet state. High efficiency of energy transfer requires (1) a longer lifetime of the excited triplet state of the PS compared with the energy-transfer time, (2) matching of allowed energies of the PS and the singlet states of  $\text{O}_2$ ; and (3) small spatial separation between  $\text{O}_2$  and the PS. Kovalev<sup>17,18</sup> showed that all these requirements can be fulfilled in the case of porous silicon, which is a surface-supported nanoporous structure composed of nanocrystal-based skeleton. In his model, Kovalev considers exchange interaction between electrons and holes that have a certain mutual spin orientation. When the size of the crystallite approaches the bulk exciton Bohr radius, the upper and lower exciton states are assumed to be an optically active spin singlet ( $S=0$ ) and an optically passive spin triplet ( $S=1$ ), respectively. Kovalev also showed that at room temperature, 75% of the excitons are permanently in the triplet state, while the exciton decay time is controlled by the indirect singlet exciton lifetime, which is several microseconds to several hundreds of microseconds long, depending on the size of the Si nanocrystals. Such a long exciton lifetime makes porous silicon assemblies suitable for energy—or charges-transfer interactions. The transfer of energy from excitons confined in Si nanocrystals to oxygen molecules is indicated by the characteristic phosphorescence emission at 1270 nm (Refs. 17 and 18). The model also implies the most efficient excitation of  $^1\text{O}_2$  under the H passivation of dangling bonds, whereas the presence of oxide layer in the nanocrystal composition is considered as a detrimental process decreasing  $^1\text{O}_2$  release.

Although laser-synthesized silicon nanoparticles present a rather different object in terms of morphology, composition, and surface passivation, we believe that the emission of  $^1\text{O}_2$  can be attributed to the same mechanism. It should be noted that even without a detailed study of Si-based properties and optimization of the fabrication procedure, our nanoparticles manifested very promising photosensitizer characteristics. In future, we expect to substantially increase  $^1\text{O}_2$  release by optimizing the laser synthesis procedure. In particular, we anticipate that the efficiency of  $^1\text{O}_2$  generation must depend on the size of nanocrystals, their composition, and passivation. In contrast to the porous silicon fabrication procedure, laser synthesis offers an unprecedented flexibility for the manipulation of both the size of nanoparticles and the ambient chemistry. In particular, size characteristics can be tuned by changing the laser fluence during the experiments, while the surface chemistry and passivation of nanoparticles can be controlled by both the minimization of oxygen content (e.g., by pumping nitrogen or argon in the solvent prior to ablation) and the addition of some reactive chemical components during the fabrication procedure. In the latter case, we plan not only to engineer the surface composition and passivation in a desir-

able way, but also to functionalize the surface for some particular applications (e.g., for tumor targeting). We also expect to engineer the absorption band toward the infrared (IR) range, which is not forbidden for semiconductor-based quantum dots,<sup>17</sup> by optimizing synthesis procedure. In particular, such engineering can be done by a proper size and surface chemistry selection, as well as by the creation of complex structures such as alloys (e.g., Si-Ge alloy). The shift of the absorption band toward the NIR range will significantly simplify *in vivo* cancer therapy applications, since mammalian tissues become more transparent within the 0.8 to 1.1 micron band.<sup>34</sup>

## 4 Conclusion

We show that colloidal silicon nanoparticles of well-defined dimension can be produced by femtosecond laser ablation in a clean, contamination-free environment (water), which eliminates the toxicity problem of alternative fabrication techniques and provides complete sterilization of the resulting product. The Si nanoparticles exhibit  $^1\text{O}_2$  release upon excitation at various wavelengths and present no photobleaching, which makes them very interesting as potential photosensitizers for therapeutics, antiseptics, or disinfectants tasks. Further experiments optimizing the quantum yield of  $^1\text{O}_2$  generation by the nanoparticles and their uptake in various cells are on the way.

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