Effect of Model Biological Media of Stability of Complex of Silver Nanoparticles Applied onto Silicon Nitride Substrate

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 3, pp. 176-180, July, 2010
Original article submitted April 9, 2009.

We studied stability of complexes of silver nanoparticles and silicon nitride before and after their interaction with liquid media and after passing through bacterial filters. According to scanning electron microscopy data, contact with fluids and passage through bacterial filters reduced the content of silver nanoparticles in complexes compared to that in initial complexes. It was shown that these differences depended on both initial characteristics of the examined samples and the used liquid media. The complex Ag/Si_3N_4 obtained after 5 silver reduction cycles and containing 15% α- and 85% β-modifications of silicon nitride (sample No. 2) was characterized by maximum stability. Culture medium produced more aggressive effect on stability of the studied complexes. We concluded that the effect of culture media on stability of silver nanoparticles–silicon nitride complexes depends on phase composition of the substrate and method of application of silver nanoparticles.

Key Words: *culture medium; silver nanoparticles; silicon nitride substrate*

Stabilization of nonequilibrium nanoparticles without considerable reduction of their high reaction capacity and, hence, targeted synthesis of stable and active metal nanoobjects are important problems of nanotechnology [7]. Physicochemical and functional properties of nanoparticles should be determined by both their size and the nature of carrier substrate. Silicon nitride is a promising material for this substrate [1]. This is a highly covalent compound existing in two thermodynamically stable modifications: low-temperature (<1200°C) α-modification and high-temperature (>1500°C) β-modification (phases). It was demonstrated that the main characteristics of silicon nitride such as specific surface (S_{sp}=1.5-66.0 m^2/g), mean size of particles (0.1-10.0 μ), phase composition (α/β ratio), and thermochemical properties depend on synthesis conditions [6]. For instance, increased relative content of silicon nitride α-modification and reduced content of amorphous fraction in the carrier considerably affect the catalytic properties of palladium microparticles applied onto this substrate [11].

The use of complexes on the basis of nanoparticles in biomedical studies attracts now much attention, especially in light of new achievements of cell technologies. Nanoparticles can be used not only for the formation of 3D engineering constructions, but also as carriers of differentiation factors for cell cultures [10].

Substances, including metals, change their characteristics and acquire new properties upon transition into the nanostate [7]. It cannot be excluded however that new properties will be harmful for cells [2,12]. Silver long ago showed itself to advantage in medicine. However, the properties of silver nanoparticles can also differ from the properties of traditional silver forms. In light of this, we studied stability of the

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0007-4888/10/1501-0160

complex silver nanoparticles–silicon nitride in liquid cell culture media.

MATERIALS AND METHODS

Silicon nitride samples obtained by self-propagating high-temperature synthesis allowing creation of materials with desired structure and chemical and phasic composition [3] were used as the carriers for silver nanoparticles. The technology of this process is described at length in papers of Russian scientists [8,9]. Phasic composition of the synthesized silicon nitride and its dispersibility were determined by temperature regimen of the synthesis, which was regulated by adding haliod ammonium salts into the initial alloy [8,9].

For obtaining complexes of silver nanoparticles with silicon nitride, the method of fractional chemical reduction of silver particles with sodium borane on the surface of the carrier from silver oxide solution followed by drying at 60-90°C and ignition at 600°C in oxygen flow was used [4,5]. Three types of samples differing by phase composition (α-phase/β-phase ratio) and by the number of cycles of silver particle reduction on the carrier surface were used. The main characteristics of the obtained samples are presented in Table 1.

Before testing stability of the silver–silicon nitride complexes, all samples (300 mg) were grinded in a porcelain mortar and placed in a fluid (30 ml) at constant stirring on a magnetic stirrer. The following fluids were used: distilled water, 0.9% NaCl solution, and culture medium containing 75% DMEM, 12.5% FCS, 12.5% equine serum, 280 mg/liter L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10^{-6} M dexamethasone, and 40 mg/liter HEPES buffer. The samples contacted with the test fluids for 10 and 60 min and 24 h. After that, the suspension of the test samples (10-ml aliquots) was passed through bacterial filters (0.22 μ, TPP) used for culture media sterilization.

TABLE 1. Characteristics of Silver-Containing Silicon Nitride Complexes

Ag/Si_3N_4 sample	Content of α- and β-modifications of Si_3N_4, %		Number of precipitations during Ag/Si_3N_4 complex preparation
	α	β	
No. 1	5	95	5
No. 2	15	85	5
No. 3	15	85	6 (with argon treatment)

Phase composition, structural parameters, and silver content were determined using a complex of physicochemical methods. Phase composition and structural parameters of pure carrier and its complexes with silver nanoparticles were evaluated by X-ray phase analysis (XPA) on a XRD-6000 diffractometer using CuKα radiation. The relative content of α- and β-modifications in silicon nitride was determined by band intensity ratio on diffractograms [11]. Structural peculiarities of the studied samples before and after their interaction with biological medium were evaluated by scanning electron microscopy (SEM) on SEM 515 Philips and Tescan LMU II and by transmission electron microscopy (TEM) on Philips CM 30 electron microscope. Structural and phasic states and morphological peculiarities of the studied samples were

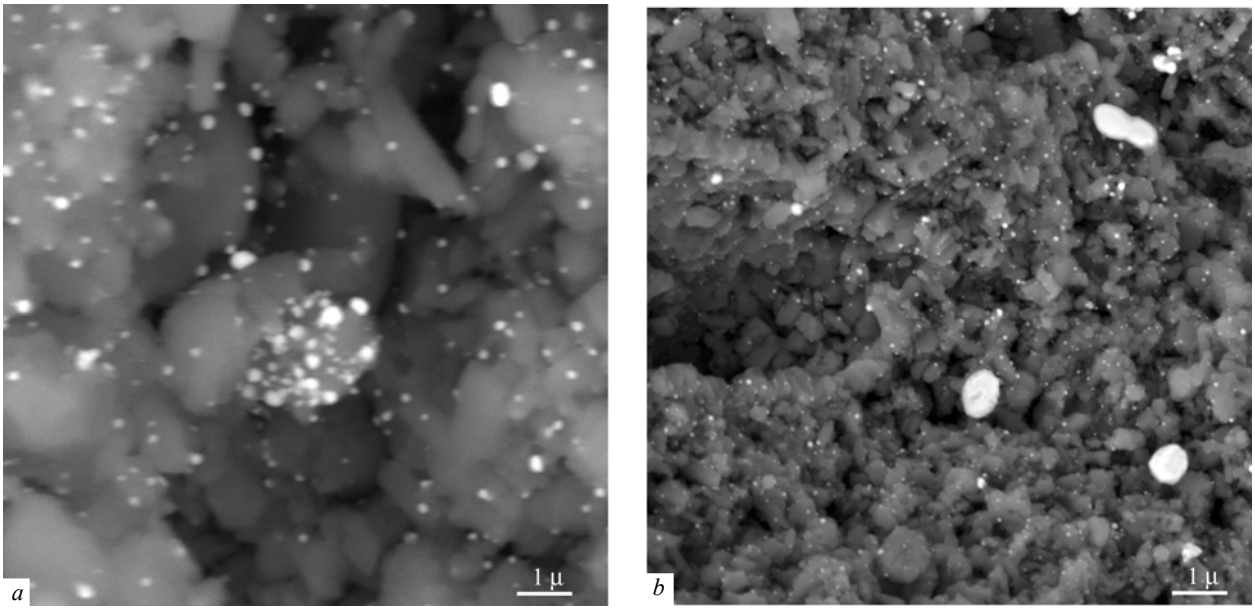

Fig. 1. SEM of the surface of silver containing Ag/Si_3N_4 complex before (*a*) and after (*b*) interaction with liquid medium.

TABLE 2. Silver Content in Ag/Si_3N_4 Complex after Interaction with Liquid Medium

Time of exposure	Ag/Si_3N_4 sample								
	No. 1			No. 2			No. 3		
	DW	PS	CM	DW	PS	CM	DW	PS	CM
10 min	2.2±0.1	2.2±0.1	2.0±0.1	3.1±0.2	2.9±0.2	3.1±0.2	3.8±0.3	3.5±0.2	3.6±0.2
60 min	2.0±0.1	2.1±0.1	1.8±0.1	3.0±0.2	2.8±0.2	2.8±0.2	3.3±0.2	2.5±0.2	3.2±0.2
24 h				3.0±0.2	2.8±0.2	2.7±0.2	3.0±0.2	1.8±0.1	2.1±0.1
Initial Ag content in Ag/Si_3N_4 complex	3.5±0.2	3.0±0.2	4.0±0.3						

Note. DW: distilled water; PS: physiological saline; CM: culture medium.

studied at Common Laboratory Center, Tomsk State University. The content of silver nanoparticles on the carrier surface was controlled using a Quant'X X-ray-fluorescent spectrometer (FLS). The spectra were recorded and silver content was measured 3 times for each sample, the samples was dispersed and agitated before each measurement. Relative error of silver measurement in the sample was calculated for metal content ranging within 2-4% and was equal to 7%.

RESULTS

Comparative analysis of silver nanoparticles applied onto silicon nitride before and after interaction with liquid media revealed significant differences. According to scanning electron microscopy data, contact with fluids and passage through bacterial filters reduced the content of silver nanoparticles in complexes compared to that in initial complexes (Fig. 1). However, these differences depended on both initial characteristics of the examined samples and the used liquid media. This effect was confirmed by the results of X-ray-fluorescent spectrometry of silver-containing complexes (Table 2). Samples No. 2 and No. 3 did not practically loose the applied silver particles after their 10-min interaction with liquid media, which can be explained by stronger interaction between the carrier and silver nanoparticles compared to sample No. 1. After longer exposure (especially, 24 h) in saline solutions (physiological saline and culture medium), a decrease in silver content in sample No. 3 was observed. The maximum decrease was observed in samples contacting with saline solutions (physiological saline and culture medium).

The results obtained for sample No. 1 differed significantly from the results obtained during testing of other samples. Analysis of complexes passed through filters showed that they lost up to 13% nanoparticles after 10-min contact with liquid medium irrespective of its composition. The losses of silver nanoparticles tended to increase with increasing the time of exposure of silver-containing complexes with liquid me-

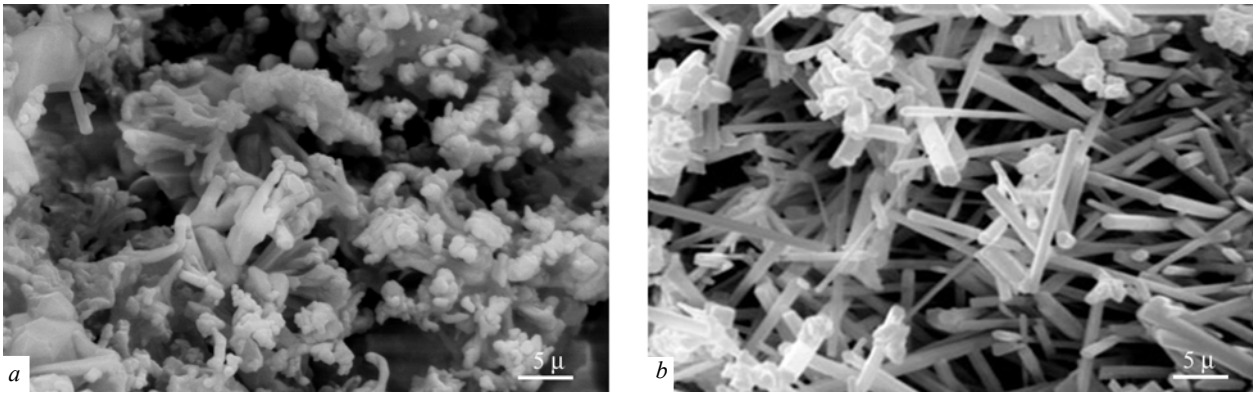

Fig. 2. SEM of surface of silicon nitride synthesized by different methods. *a*) silicon nitride obtained by ferrosilicon burning in nitrogen in the presence of ammonium chloride and used for preparing sample No. 1; *b*) silicon nitride obtained using ammonium fluoride and used for preparing samples No. 2 and No. 3.

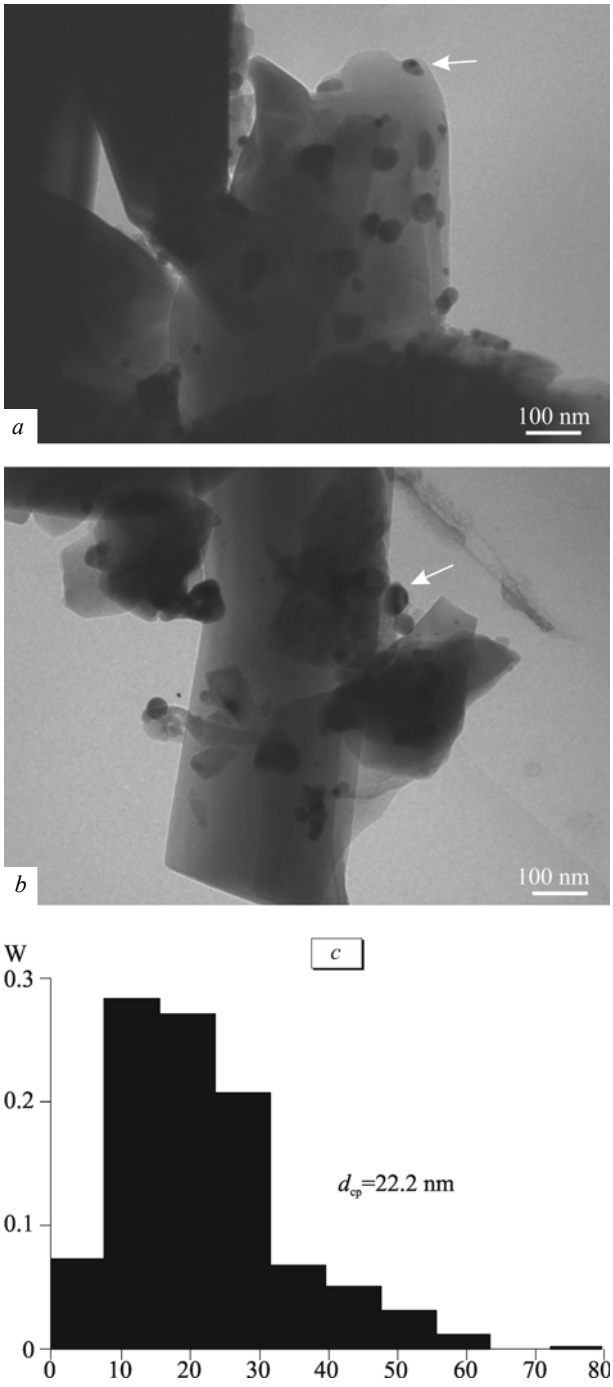

Fig. 3. TEM (*a*, *b*) and size distribution histogram (*c*) for silver-containing systems (sample No. 1) applied onto silicon nitride. Arrows show silver particles applied onto silicon nitride carrier.

dia. Samples with carrier No. 1 poorly passed through bacterial filters irrespective of the liquid medium used and time of contact. Therefore the use of this silver-containing complex is unpromising for preparing liquid media containing silver nanoparticles.

The differences between the samples detected in our study are most likely determined by the peculiarities of silicon nitride substrates used. Apart from α- and β-modifications with hexagonal crystalline lattice, polymorphous modifications of silicon nitride with tetragonal and orthorhomboid lattice are present in the sample [1,9], which probably affects the strength of Si_3N_4 interaction with silver nanoparticles and the possibility of obtaining (through crushing in the mortar) silver-containing complex capable of passing through bacterial filters. This assumption is confirmed by the redults of SEM (Fig. 2). The structure of silicon nitride in sample No. 1 drastically differs from that in samples No. 2 and No. 3. This difference is explained by peculiarities of sample synthesis protocol, which determines different possibility of obtaining silver complexes passing through bacterial filters.

Silver particles are localized on prominent sides of the carriers and sites of contact of conjoined particles in the carrier matter, *i.e.* in energetically uncompensated centers, which leads to the formation of stable complexes of silver nanoparticles with silicon nitride substrate. For instance, silver particles in sample No. 1 (predominantly α-phase) are characterized by wider size distribution (Fig. 3), have elongated shape, and are equally localized on projections and notches of silicon nitride crystal surface (Fig. 3, *a*). If the carrier contains cylindrical structures, silver particles are distributed unequally and are primarily located not on the silicon nitride surface, but in sites of contact between carrier particles (Fig. 3, *b*). This pattern of localization of metal particles is formed under conditions of weak chemical interaction between the carrier and the applied component [3].

Thus, the strength of interaction between silver nanoparticles and silicon nitride depends on phasic state of the substrate and the method of application of silver nanoparticles. In the series of the test samples, sample No. 3 most easily release silver nanoparticles in liquid media, and especially, in saline solutions. Silicon nitride substrate used in sample No. 1 does not stably binds silver nanoparticles. However, it does not form complexes passing through bacterial filters. It can be concluded that sample No. 3 can be used for introduction of free silver nanoparticles into culture media, while sample No. 2 is more suitable for obtaining stable spatial conglomerates containing silver nanoparticles.

The study was supported by the Russian Foundation for Basic Research (grant No. 09-03-00604_a).

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