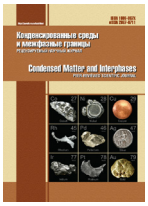

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X-ray photoelectron spectroscopy of hybrid 3T3 NIH cell structures with internalized porous silicon nanoparticles on substrates of various materials

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Abstract

The work is related to the study of a biohybrid material based on mammalian 3T3 NIH mouse fibroblast cells with immobilized porous silicon particles including nanocrystals about 10 nm in size by photoelectron spectroscopy. The influence of the surface material of the substrate on which the biohybrid material is grown on the possibility of conducting studies of the physico-chemical state of the developed surface is studied. Nickel as well as gold and titanium, known for their biocompatibility, were used as surface materials for cell growth and subsequent internalization of silicon particles. The method of optical microscopy in the reflected light mode was used to assess the distribution of cells on surfaces. It is shown that the nickel surface is not suitable for the synthesis and subsequent studies of biohybrid structures. At the same time, on the surface of gold and titanium, cellular material and structures based on it are available for measurements, including by photoelectron spectroscopy, a high-precision method for studying the atoms charge state and the physico-chemical state of the surface as a whole. The X-ray photoelectronic spectra show all the main components expected to be detected after drying and subsequent vacuuming of the studied objects: the surface material of the substrates and arrays of cell cultures grown on the substrates. No signal from silicon atoms was detected on the nickel surface. In the case of a gold surface, the proximity of the binding energies of the gold core levels (substrate) and silicon (internalized particles) leads to the fact that the signal of gold atoms, which is significant in its intensity, does not allow detecting a signal from silicon atoms, which is weaker in intensity. The signal of silicon atoms in biohybrid structures is reliably detected only when using titanium substrates, including for a control sample containing porous silicon nanoparticles without incubation in cells. Thus, it is shown that the surface of the titanium foil can be used for studies by photoelectron spectroscopy of a biohybrid material based on mammalian 3T3 NIH mouse fibroblast cells with immobilized porous silicon particles.The obtained result is important for high-precision diagnostics of the physico-chemical state of biohybrid materials and structures based on them with a low content of silicon atoms when solving problems of studying the compatibility and possibilities of using silicon nanomaterials for medical, including therapeutic and other applications.

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1. Introduction

Biohybrid structures, which are essentially a combination of biological objects and inorganic materials [1–4], are positioning at the subject of interest intersection of physics, chemistry and biology and stimulates special attention to the properties of such objects and is the subject of research by high-precision diagnostic methods. The physico-chemical processes under the internalization of inorganic particles into living cells, the associated changes in their physico-chemical state, composition, structure and other properties are insufficiently and sometimes fragmentary studied. On the other hand, information about the result of these processes is certainly important when studying the application of biohybrid structures. Structures in which silicon nanoparticles act as an embedded object are no exception [4–6]. Silicon nanoparticles, due to their special biological properties, such as biocompatibility [6–7], biodegradability [8-9], sensibilization of impacts [10–11] and low toxicity [6, 12], represent a promising material in the fields of therapy and diagnostics (theranostics) [4, 10, 13]. It is worth noting that nanoparticles created from crystalline silicon are inferior in efficiency to nanoparticles formed from porous silicon, primarily due to the extremely developed surface [14]. Therefore, the study of biohybrid structures, for which the embedded element is porous silicon nanoparticles, is relevant and in demand.

The method of X-ray photoelectron spectroscopy (XPS) has an extremely high sensitivity to the physico-chemical state of the developed surface [15–17]. An urgent issue is to establish the applicability of the XPS to the study of biohybrid structures, where one of the main tasks is to select the material on which a complex composition and structure of the sample will be applied. The need for an adequate choice of substrate is given by the issues of storage and transportation of the prepared sample, its stability and the effectiveness of choosing a research strategy generally. The substrate material should be inert to the biological processes taking place during the formation of the sample on given surface, on the other hand, the substrate should not make a significant contribution to the results of spectroscopic, microscopic or other studies. The question of the suitability of various substrate materials, on the surface of which a biohybrid material can be layered for investigation by the XPS is investigated in this paper.

2. Experimental

The following materials were selected to study the suitability of the substrate when registering the XPS data: nickel foil (AlfaAesar, 99.7%), a gold film with a thickness of about 100 nm on a nickel foil formed by magnetron sputtering of a gold target (99.99%) and titanium foil (AlfaAesar, 99.5%). The biohybrid material was a cell culture of 3T3 NIH mouse fibroblasts with immobilized porous silicon particles including nanocrystals ~10 nm in size according to [18]. The cells were grown in Petri dishes on selected substrates, after which they were incubated with porous silicon nanoparticles (p-SiNPs) for 24–72 hours. p-SiNPs were obtained by mechanical grinding of porous silicon films in suspension mode in a planetary mill Fritsch Pulverisette 7 [14, 19]. Porous silicon films were produced by electrochemical etching of c-Si(100) crystalline

silicon wafers for an hour in $HF:C_2H_5OH$ = 1:1 (current density 50 mA/cm^2) [14, 19]. Suspensions of nanoparticles with a concentration of 0.5 mg/ml were used. After the time elapsed, the cells were fixed with formaldehyde, then washed and dried. The samples obtained by this method were named, in accordance with the substrate material, as BioHyb on Ni, BioHyb on Au, BioHyb on Ti. As a control sample a suspension of porous silicon nanoparticles were used applied to titanium foil and dried in natural conditions before vacuuming in a spectrometer chamber (p-SiNPs on Ti). The samples obtained were studied on a Bresser science MTL-201 optical microscope in the reflected light mode to observe the specifics in the cells distribution and collect statistics on the substrates coverage by cellular material.

XPS studies was carried out on the ESCA module of the ultrahigh vacuum "KISI-Kurchatov" synchrotron NANOPES experimental end-station of the National Research Center "Kurchatov Institute" (Moscow) equipped with the SPECS Phoibos 150 energy analyzer [20]. Monochromatized Al Ka radiation of an X-ray tube (1486.61 eV) was used with the analysis depth that was estimated as ~ 2–3 nm [21]. Survey spectra were recorded in the binding energy range 0–1200 eV. A standard approach to data calibration based on independent recording of the pure gold foil (Au 4f) signal was used since due to the presence of cellular material on the foils surface it was not possible to use standard C1s calibration of the hydrocarbon contamination line [21]. To compare and analyze the main features of the XPS spectra the well-known databases were used [21–23].

3. Results and discussion

The biohybrid structures formation modes, including the times and concentrations of components, were selected during incubation so that a one cell thickness layer was formed on the surface of the substrates, without significant agglomerations. The process was controlled microscopically. The results of optical microscopy (Fig. 1) showed noticeable differences in the surfaces coverage by cells containing p-SiNPs. In the case of BioHyb on Au and BioHyb on Ti samples (Fig. 1), in contrast to the BioHyb on Ni sample (Fig. 1, indicated by arrows) an integral and compacted structure of cellular material distributed over the surface is visible. In the case of nickel substrates, the coating with cells is smeared and their number is smaller. For the surface of the gold, the edges of cellular structures are sharper, clearer, which confirms the known biocompatibility of gold [24, 25]. However, significant areas of the gold surface are noticeable that are not covered with cellular material and can give a significant intensity of the XPS signal from the substrate that are not from hybrid structures. For titanium foil that is also known for its biocompatibility the number of cells on the surface is maximum, the coating is more uniform.

Statistical diagrams calculated with the use of ImageJ software package show that in the case of BioHyb on Ni and BioHyb on Au samples the total area of structural elements (in Fig. 1) is less for biohybrid material on the surface if compared to the BioHyb on Ti sample.

The percentage ratio of the biohybrid structures total areas to the ones of the substrate, for BioHyb on Ni sample was 17%. This value is less than for BioHyb on Au and BioHyb on Ti (23 and 24%, respectively). Thus, from the surface coating point of view nickel can be considered the least suitable. During incubation, nickel and its oxides can be toxic to cells (with silicon nanoparticles) [24]. Additionally, as the result of the cellular material weak adhesion to the nickel surface a significant part of the cells can be found destroyed and left the substrate after drying and vacuuming.

The X-ray photoelectron spectroscopy results are presented by the survey spectra in Fig. 2. It can be seen that in the spectrum of the biohybrid structure on a nickel substrate (Fig. 2, BioHyb on Ni) there are lines of core levels and Auger series of sodium, nickel, carbon, nitrogen and oxygen. The silicon lines absence may indicate an insufficient number of p-SiNPs combined with the cell culture for detection, or their complete absence in the sample. The presence of sodium and nitrogen lines may be due to the nutrient medium components used in cell growth or directly by elements of cell culture.

In the survey spectrum for the BioHyb on Au sample (Fig. 2) there are lines of carbon, nitrogen, oxygen, sodium, chlorine, nickel and

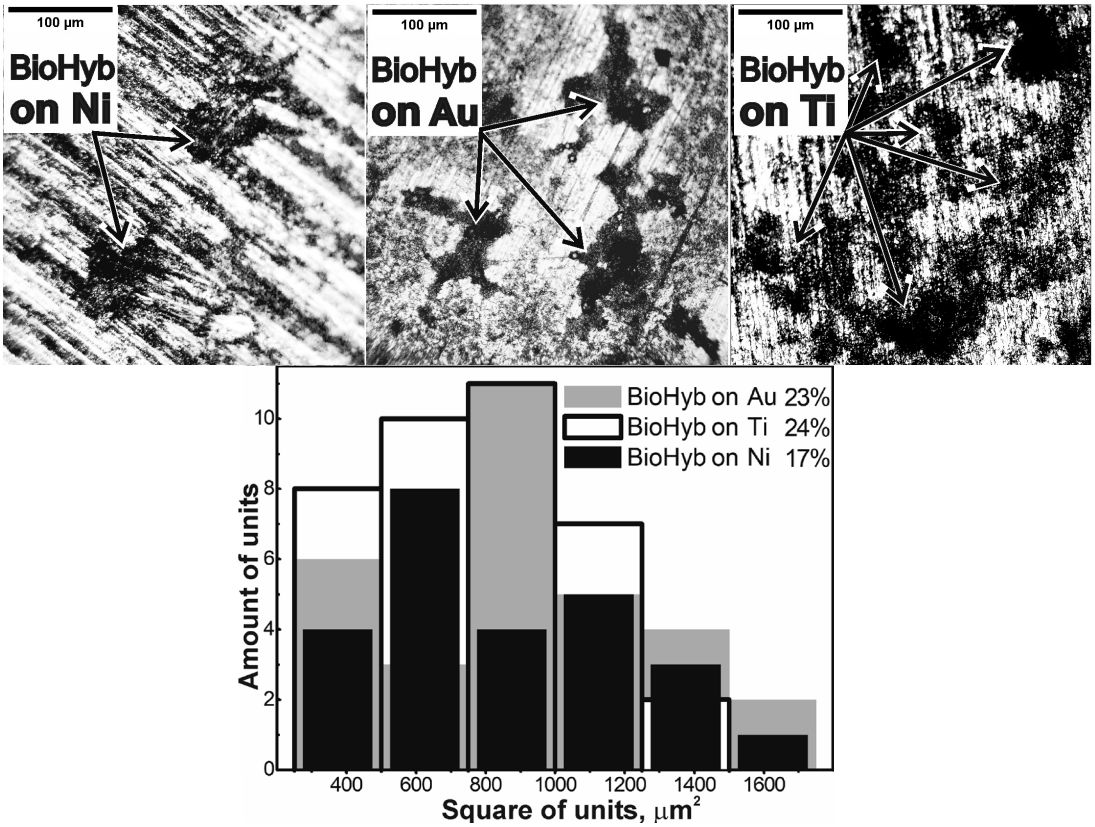

Fig. 1. Optical microscopy and statistical diagrams for biohybrid structures grown on nickel (BioHyb on Ni), gold (BioHyb on Au) and titanium (BioHyb on Ti)

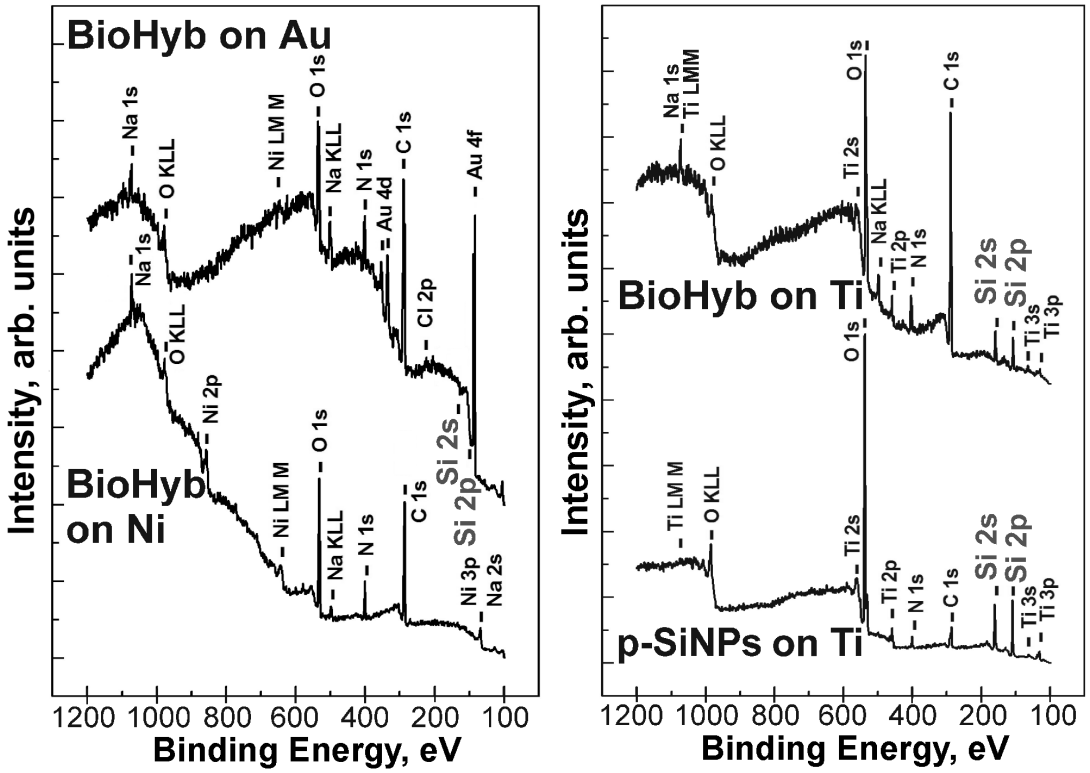

Fig. 2. XPS surveys for biohybrid material grown on gold (BioHyb on Au), nickel (BioHyb on Ni), titanium (BioHyb on Ti), and reference p-SiNPs suspension sample on titanium substrate dried in normal conditions

possibly silicon. Although the Au 4*f* gold line is particularly intense. The presence of a nickel atoms signal in the survey spectrum is most likely due to a part of the nickel substrate that is not covered with gold ingress under the X-ray beam when recording spectra. The sodium and nitrogen lines are associated with the nutrient medium components used in cell cultivation or directly with the elements of cell culture. It is worth noting that the extremely high intensity of the gold $4f_{5/2,7/2}$ spin-doublet line at energies of 84 eV and 87 eV practically leveled the observing and registering possibility of the Si 2*p* silicon line at binding energies of ~ 100 eV (as well as for Si 2*s*, ~ 150 eV). In these areas of observation, the silicon core levels lines signal is probably present, but due to the insignificant amount of the p-SiNPs substance (possibly screened by the cells bio-shells) its intensity is significantly small. Including at the level of XPS survey spectrum background intensity formed by inelastic scattered electrons of substrate gold atoms which are much more quantitatively than silicon atoms. This makes gold unsuitable material for the study of biohybrid structures with silicon nanoparticles, despite good biocompatibility. Thus, the intense gold $4f_{7/2,5/2}$ lines presence hinders the identification and further study of the studied biohybrid materials.

In turn, in the spectrum of the BioHyb on Ti sample (Fig. 2), lines of titanium, carbon, nitrogen, oxygen, sodium and silicon can be observed. The sodium and nitrogen lines, as before, are due to the presence of these elements in the composition of the cellular material. Here, the silicon lines are significantly intense and clearly visible. As a result, it can be emphasized that the sample contains a sufficient number of porous silicon nanoparticles for their clear detection by the XPS method. The lines from the titanium substrate are far from the silicon lines and do not interfere with their identification.

A survey spectrum of the initial porous silicon nanoparticles dried from their suspension on a titanium substrate under natural conditions before vacuuming is given as a comparison (Fig. 2, p-SiNPs on Ti). All the lines characteristic of a biohybrid sample grown on the titanium surface are observed in the spectrum (Fig. 2 BioHyb on Ti), with the exception of the sodium line which is apparently a component of organic compounds or a nutrient medium used in cell growth. The low-intensity nitrogen line observation can be related to the specifics of titanium foil face cleaning.

4. Conclusions

The paper shows that according to optical microscopy data, gold and titanium are the best substrate materials for growing biohybrid structures with subsequent internalization of silicon nanomaterial and studying such objects. The biohybrid structure has good adhesion to the materials of such substrates and is stable in its distribution on the surface. The use of X-ray photoelectron spectroscopy to study the charge state of surface atoms, their physico-chemical state, including the substrate, cells and silicon particles, excludes the use of gold surfaces. It is shown that the surface of titanium foil can be used for XPS studies of biohybrid material based on mammalian 3T3 NIH mouse fibroblast cells with immobilized porous silicon particles. The obtained result can be used for high-precision diagnostics of the biohybrid materials physico-chemical state and structures based on them with a low content of silicon atoms, which is necessary to study the compatibility and possibilities of using silicon nanomaterials for medical, including therapeutic and other applications.

Author contributions

All authors made an equivalent contribution to the preparation of the publication.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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