Magnetron sputtering deposition of ultra-thin metal coatings for the visualization of protein-containing objects of nanometer size by electron microscopy

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Abstract. Electron microscopy is widely used in biological and medical research, enabling to obtain images of the investigated objects with nanometer resolution. Exposure of biological objects under electron beam during the visualization can, however, cause their damage. Destructive effect of the electron beam can be significantly decreased by formation of metal coating on the surface of the objects to be studied. In the present study, an approach for the formation of ultra-thin metal coatings on the surface of protein-containing structures is proposed. Magnetron sputtering in argon plasma in DC mode was employed to form 8 Å-thick tungsten films on the surface of model protein-containing objects—tobacco mosaic virus particles—for their subsequent visualization by electron microscopy.

1. Introduction

Electron microscopy (EM) is widely used in biomedical research [1], enabling to obtain images of single protein-containing particles (viruses [2, 3] and proteins [4]) with nanometer resolution. Upon visualization by EM, the biological objects under study can be damaged by the electron beam, what causes significant loss of quality of the obtained images [3, 5, 6]. Due to this circumstance, to enhance clearness of the EM images obtained during visualization of biological objects, techniques employing preliminary treatment of the studied sample with heavy metals (Os, Ta, Au, W, Pt, Pd) or their compounds (osmium tetraoxide [1], phosphotungstic acid [2], potassium silicotungstate [4] etc.) are employed. Advantages and disadvantages of these techniques are discussed in more detail in our previous study [3]. Destructive effect of the electron beam can be significantly decreased by formation of metal coating on the surface of the sample to be studied [3]. In the present study, we have experimentally demonstrated that magnetron sputtering of ultra-thin metal coatings (whose thickness is less than 1 nm) for the visualization of nanometer-sized protein containing objects by EM.

Magnetron sputtering allows one to obtain coatings with good adhesion to the treated surface and low porosity, even at low thickness (<10 nm [7]). The advantage of magnetron sputtering consists in that it allows one to vary the deposition rate in a broad range by changing a number of parameters (power applied to the magnetron, sputtering mode — DC or RF, working gas pressure etc.). This provides flexibility of the method and enables one to obtain coatings with different characteristics, including ultra-thin ones with ~1 nm thickness [3, 7, 8].
In the present study, magnetron sputtering has been employed to obtain ultra-thin (of 8 Å thickness) layers of tungsten on the surface of biological protein-containing structures. As model object, tobacco mosaic virus (TMV) particles, which were extensively studied and characterized in much detail in the literature — have been used. In comparison with our previous report [3], the thickness of the formed coating has been significantly (from 13 Å to 8 Å) decreased. The thermal effect on the treated sample during the coating deposition with regard to the coating thickness is discussed.

2. Methods
The techniques used in this study were similar to those described in our previous paper [3]. The tungsten deposition procedure was modified to provide thinner coating.

2.1. Sample preparation
Tobacco mosaic virus sample (21.1 mg/mL in standard Dulbecco modified phosphate buffered saline (PBS-D) buffer) used in the study was kindly provided by Dr. V. Makarov (Belozerskii Institute Physicochemical B., Moscow State University). The samples were prepared as follows. An aliquot of the initial TMV suspension was diluted with PBS-D buffer (in the ratio of 1:99) and then incubated in a shaker (Eppendorf Thermomixer Comfort (Germany), 600 rpm, 20°C) for 30 min. After that, 5 µL of the resulting diluted suspension was carefully dispensed onto an amorphous carbon film adhered to copper grid (Ted Pella, Inc., USA; Prod # 01843-F), and incubated for 6 min, and then rinsed off with 1 mL of ultrapure water. The samples prepared in such a way were finally dried in air at room temperature.

2.2. Ultra-thin tungsten film deposition
To form ultra-thin metal coatings on the surface of the samples, Orion-3 magnetron sputtering system (AJA Inc., USA) with installed tungsten target (99.95%, thickness 0.25'', diameter 2'', Girmet, Russia) was used. Base pressure in the system was not higher than 6×10⁻⁷ Torr. The sputtering was carried out using argon plasma in DC mode at constant power 70 W and sputtering gas pressure 50 mTorr during 16 s. The distance between the target and the specimen was 15 cm. During sputtering, rotation of the substrate to be coated with constant angle velocity 40 rpm was provided. At that, the sample plane was tilted relative to the plane of the disc by 45° (analogous to [3, 9]). The target was pre-sputtered for six minutes with the closed shutter prior to the deposition onto the substrate. The thickness of the tungsten layer was determined by QCM (according to the mass of tungsten deposited onto the quartz sensor surface during fixed time period) using MCM-160 thickness monitor (McVac Manufacturing, USA), installed into Orion-3 system. Under the specified conditions, the deposition rate was 0.5 Å/s; accordingly, the thickness of the coating deposited in 16 s was 8 Å.

2.3. Electron microscopy
EM images of TMV particles were obtained with Hitachi S5500 electron microscope in both SE and STEM modes at 25 kV accelerating voltage.

3. Results obtained
Figure 1 displays EM images of TMV particles coated with 8 Å tungsten film.
Figure 1. EM images of TMV particle adsorbed onto amorphous carbon film and coated with 8 Å tungsten film by magnetron sputtering. The images were obtained in SE (a) and STEM (b, c) modes. Magnification 400 000× (a, b), 200 000× (c). Panels (a) and (b) display the images of a single TMV particle obtained in different modes. Panel (c) displays «end-to-end» dimeric TMV aggregate.

The images presented in Figure 1 (a, b) indicate that the length of single TMV particles makes up 295-300 nm. This is in a good agreement with the data obtained by AFM [10]. Dimers of these particles (in the form of «end-to-end» aggregates) have a characteristic length of 600 nm (Figure 1 (c)). The data obtained confirm our previously obtained results [3].

It is known that the image quality depends on its resolution, i.e. on the relative number of small details distinguishable in this image [11]. In this connection, it has to be noted that, with regard to the study of biological objects by electron microscopy, decreasing the thickness of the formed metal coatings down to sub-nanometer values allows one to improve quality and resolution of images of the surface of the studied objects in comparison with thicker coatings.

When employing magnetron sputtering for coating biological objects it is necessary to take into account thermal effect on the treated sample, i.e., its heating during the deposition process. This heating is caused by the influence of a number of factors: the heat, released during the condensation of a sputtered metal on the sample surface; kinetic energy of the sputtered metal atoms and of sputtering gas ions; thermal radiation from the target etc. [12]. In our case, short sputtering time (16 s) and low thickness (8 Å) of the formed coating have allowed us to minimize the impact of the above-listed factors on the objects to be studied.

4. Conclusions
The use of DC magnetron sputtering deposition of ultra-thin (8 Å) tungsten coatings onto biological protein-containing structures of nanometer size has been demonstrated with the example of tobacco mosaic virus particles. These coatings allow one to minimize the destructive impact of the electron beam on these structures during their imaging by electron microscopy. Short sputtering time (16 s) and low thickness (8 Å) of the formed coatings have allowed us to minimize the impact of thermal effect on the treated sample during the deposition process.

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References
[1] Kuo, J. 2014 Methods in Molecular Biology. Electron microscopy. N.Y.: Humana Press.
[2] Brenner S, Horne R W. 1959 Biochim. Biophys. Acta. 34 103-10
[3] Shumov I D, Kanashenko S L, Archakov A I, Ivanov Yu D, Pleshakova T O. 2017 Prikladnaya Fizika 4 10-5
[4] Tsuprun V L, Myasoedova K N, Berndt P, Sograf O N, Orlova E V, Chernyak V Ya, Archakov A I, Skulachev V P. 1986 FEBS Lett 205 35-40
[5] Ogura T. 2012 PLOS ONE 7 e46904
[6] Adrian M, Dubochet J, Lepault J, McDowall A W. 1984 Nature 308 32-6
[7] Petroff P, Sheng T T, Sinha A K, Rozgonyi G A, Alexander F B. 1973 J. Appl. Phys. 44 2545-54
[8] Salamon K, Milat O, Radic N, Dubcek P, Jercinovic M, Bernstorff S. 2013 J. Phys. D: Appl. Phys. 46 095304
[9] Hart R G. 1961 J. Mol. Biol. 3 701-2
[10] Dubrovin E V, Yaminsky I V, Kirikova M N, Novikov V K, Drygin Y F. 2004 Colloid J. 66(6) 673-8
[11] Sai S V, Sorokin N Yu. 2012 Dokl. TUSUR 2 (1) 78-82
[12] Andritscky M, Guimaraes F, Teixeira V. 1993 Vacuum 44(8) 809-13