HOW TO PREPARE BACTERIA WITH ADSORBED NANOPARTICLES FOR SEM AND TEM OBSERVATIONS

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Introduction

Growing applications of nanoparticles (NPs) lead to their accumulation in the environment. The majority of scientific reports reveal their negative impacts on aquatic organisms, invertebrates, mammals, and humans. There is a need to identify and quantify the parameters governing the NPs interaction with the biological surfaces such as bacterial cell walls. Most of the studies are focused on the interaction between NPs and bacteria, leading to an apoptotic disintegration of the bacterial cells (cells death), but there is a need to optimize a biological model of this interaction and propose reliable methods for electron microscopy observations. We believe that such knowledge may be used for further investigation of microorganism-based effective systems, not only for the capture of NPs from the environment but also for the development of new functionalities. The aim of this study was to optimize the procedures for preparing bacteria coated with NPs for SEM/TEM observations.

Materials and Methods

The investigated bacteria (*Pseudomonas putida* ATCC 31483) were cultured on a glass/ITO substrate and then prepared in two different protocols: (a) the bacteria cultures were directly prepared on glass/ITO substrate and after the 15 min contact with the AuNPs suspension and simply dried in air; (b) the bacteria cultures were fixed for the SEM observations according to the protocol [1,2]. Briefly, samples on a glass/ITO substrate were fixed in 3 % buffered glutaraldehyde for 24 h, and then carefully washed two times with Dulbecco's Phosphate Buffered Saline (DPBS). They were next dehydrated in the water-alcohol solutions with gradually increasing ethanol concentration (50, 60, 70, 80, 90, 96 and 100%) for 10 min each. Finally, the samples were dried using hexamethyldisilazane (HMDS). The prepared samples were mounted on a SEM holder by using adhesive carbon tape and carbon conductive paint. Prior to the observations, the specimens were coated with a thin layer of gold (approximately 15 nm) with the use of sputter-coater (Quorum Q150T S). The samples were characterized with the use of the field-emission scanning electron microscope (FE-SEM, Hitachi S-4700).

Transmission electron microscopy (TEM) observations were carried out using a Tecnai Osiris instrument (FEI) with the X-FEG Schottky field emitter operated at an accelerating voltage of 200 kV. Samples for TEM characterization were prepared by the standard procedure [3]. The investigated bacteria strain suspension was washed three times in DPBS, fixed in 3% buffered glutaraldehyde for 24 h. The pellets were washed three times in DPBS, rinsed with 1% osmium tetraoxide solution (DPBS) for 2 h and washed again with DPBS. Samples were dehydrated in the water-alcohol solutions with gradually increasing ethanol concentration (50, 60, 70, 80, 90, 96 and 100%) for 15 min each. The pellets were rinsed with propylene oxide (20 min) and incubated in 1:1 propylene oxide/resin ration overnight (Durcupan, Sigma-Aldrich). Following samples incubation in 100% resin at 37°C for 24 h and then 60°C for 48 h. The samples were sectioned using an ultramicrotome (Leica) equipped with the glass-edged knife (Diatome). The ultrathin lamellas were placed onto Cu TEM slots with the carbon-coated membrane and stained with lead citrate and uranyl citrate for contrast enhancement procedure or UranyLess contrast enhancement solution.

Results and Discussion

The SEM and TEM observations revealed significant differences in cell morphology of observed materials depending on the preparation procedure. The standard fixation for SEM observations (FIG. 1a and b) results in the reduction of cell volume, changes in cell morphology and removal of adsorbed NPs from the cell wall. The protocol for TEM observations, with the UranylLess contrast enhancement results in falsified cell morphology (FIG. 1c) in comparison with the standard procedure (FIG. 1d).

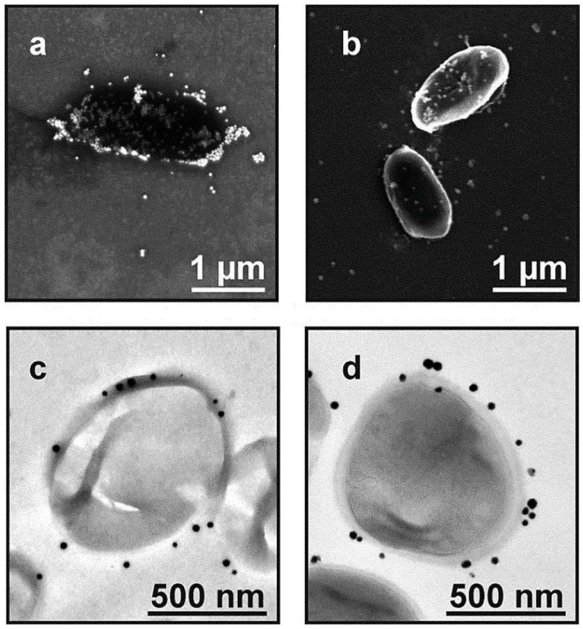

FIG. 1. Electron Microscopy microphotographs of *P. putida* coated with AuNPs. (a) bacteria cultured on the glass, simply dried in air (SEM), (b) bacteria cultured on ITO, fixed and dehydrated (SEM), (c) UranylLess contrast enhancement (TEM), (d) standard contrast enhancement procedure (TEM)

Conclusions

The most effective procedure for SEM observations of bacteria coated with NPs is a protocol, in which glass substrate is used and the probe is simply dried in air, without fixation and dehydration of material. The most appropriate procedure for TEM visualization is the standard one with contrast enhancement using uranyl citrate and lead citrate.

Acknowledgments

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