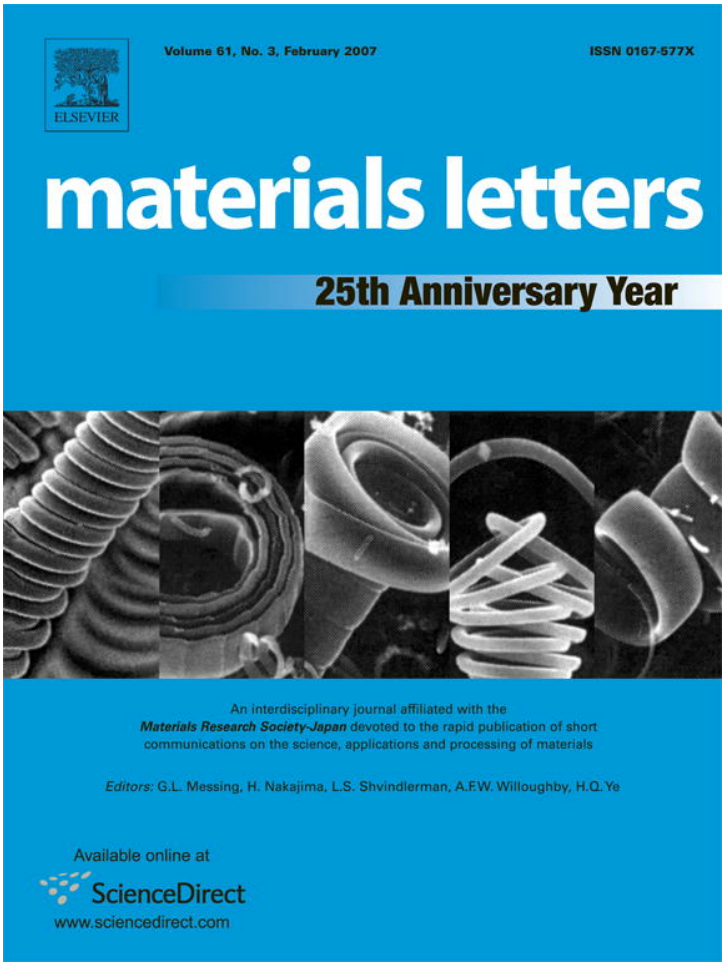

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Probing nanomechanical properties of nickel coated bacteria by nanoindentation

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Abstract

As a powerful method for the study of mechanical properties at micro-/nanoscale, nanoindentation was applied to measure the hardness and elastic modulus of bacteria-templated metallic nanomaterials for the first time. Based on the morphological characterization by Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM), nanoindentation testing results showed that after coating with nickel via electroless chemical plating, the elastic modulus and hardness of bacterial cells were increased about 17 times and 50 times, respectively, indicating a great improvement in mechanical properties. This study would lay a forceful mechanical foundation for a better and general understanding of this kind of biotemplated metallic nanomaterials, which showed potential applications in nanoelectronics, nanomagnetism and nanomechanics.

Keywords: Mechanical properties; Nanoindentation; Atomic Force Microscopy; Biotemplate; Metallization; Bacterial cell

1. Introduction

Over the past few years, metallic nanostructures or nanomaterials have attracted a great attention from scholars and scientists all over the world owing to their potential application in electronics, mechanics, optics and sensors[1–3]. Recently the synthesis and fabrication of metallic nanostructures or nanomaterials based on biological templates has become a novel and attractive trend. Among other biological templates such as DNA [4–6], microtubules [7,8], S-layer protein [9], protein cages [10] and natural pollen particles [11], bacterial cells have been attractive candidates for their inherent small size (about 1 μm), various standard geometrical shapes (such as spherical, bacilliform, tubular, gyroidal, corn-shaped or banana-shaped, etc) and abundant source. It is quite difficult to fabricate such small standard three-dimensional metallic nanostructures even with any available micro-machining methods. For example, gram-positive bacteria of *Citeromyces matritensis* and *Bacillus* [12] have been used as templates to construct nickel–phosphorus shell nanostructures by Li et al.

To better explore the possible applications in broad fields, it is of great significance to understand the mechanical properties of such bacteria-templated metal nanostructures. Their extremely small three-dimensional sizes impose a tremendous challenge to many existing testing and measuring techniques for experimental studies of their mechanical properties. A promising and appropriate method is the direct indentation of these nanostructures. As a viable technique for local mechanical assessment, nanoindentation has been conducted on many biological materials, such as bone, vascular tissues, tooth enamel and dentin, and mineralized matrix [13–16]. The hybrid structures of the microorganism with a metal shell provide a number of challenges in the field of nanoindentation. However, the ability of nanoindentation to probe local, nanoscale mechanical properties of heterogeneous materials makes it feasible to adopt it for application to these hybrid structures.

In this paper, a nanoindentor has been used to study the nanomechanical properties of bacteria-templated nickel nanostructures, which are fabricated via electroless chemical plating. For comparison, nanoindentation tests are conducted on bare cells

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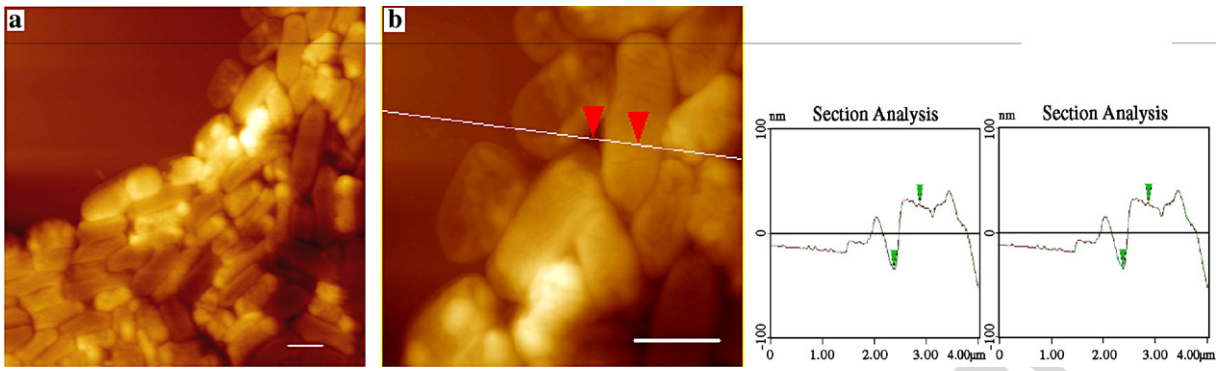

Fig. 1. AFM height images of dry *E. coli* cells on mica surface. (a) Due to dehydration, the cells aggregate to form biofilm. Scan size=10×10 μm; scan bar=1 μm; height scale=150 nm; (b) a section curve for one magnified bacterial cell, which shows a height of 62.681 nm. Scan size=4×4 μm; scan bar=1 μm; height scale=150 nm.

and the mica substrate at the same time. The aim of the study is to supply nanomechanical information of biotemplated metal nanostructures for their potential applications in the broad fields of nanoscience.

2. Experimental

2.1. Sample preparation

Escherichia coli (ATCC 25922) was obtained from Nanjing General Hospital of Nanjing Military Command (Nanjing, China). The pure culture of the bacteria was grown in Luria–Bertani (LB) medium for 5 h at 37 °C [17]. After cultivation, bacterial cells were collected by centrifugation and suspended in pure water. It is a common knowledge that the cell wall of gram-negative bacteria such as *E. coli* is mostly composed of proteins, lipopolysaccharide, polysaccharides and peptidoglycans. It is hypothesized that the negatively charged lipopolysaccharide protruding out of the cell wall supplies the possibility of binding with positively charged colloidal Pd ions via electrostatic interaction, which serves as further metallization nucleation sites. Here tin-free chloride-rich Pd(II)-based colloidal solutions were chosen as the activation initiator, nickel sulfate as the deposited metal source and hydrazine as the reducing agent.

2.2. Methods

For AFM characterization and nanoindentation testing, freshly cleaved mica was chosen as the substrate. A 40 μl sample solution was pipetted onto a mica disk for 10 min, rinsed in pure water and allowed to dry for imaging and testing. Except for special indication, all samples were freshly prepared from the same group and characterized within the drying period of 2 h.

A Nanoscope IIIa AFM (Digital Instruments, Santa Barbara, CA, USA) operating in contact mode in air was used. The relaztive humidity was 50–60% and no capillary forces were observed during operation. The nanoprobe cantilevers of silicon nitride (Si_3N_4) has a spring constant of K=0.06 N/m (DI). The Digital Nanoscope software (version 4.32) was used to analyze the topography of the cell surface. All AFM images were only treated with flatten command.

For contrast, a Transmission Electron Microscopy (Hitachi, JEOL) was used to supply more exterior and composition information of samples under an accelerating voltage of 200 kV.

A Nanoindenter (SA2, MTS, USA) was used to determine the hardness and elastic modulus of bare and nickel coated bacterial cells. It has a displacement and load resolution of 0.0002 nm and 1 nN, respectively. A Berkovich tip with a curve radius of about 20 nm was used for the indenter. During indentation, a curve

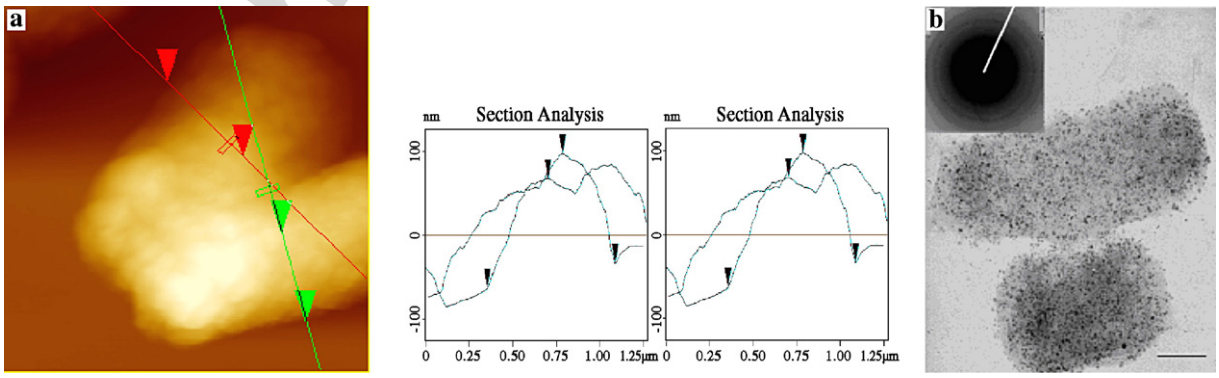

Fig. 2. Microscopic photos of metallized bacterial cells. (a) An AFM height image of two linking metallized bacterial cells. The section curves indicate that the height is ~ 130 nm. Scan size=1×1 μm; height scale=250 nm; (b) TEM image of two separate metallized bacterial cells. The insetting electron beam diffraction validates the presence of nickel particles at the surface. Scale bar=200 nm.

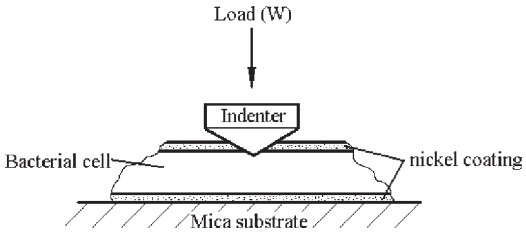

Fig. 3. A nanoindentation illustration of nickel coated bacterial cells on mica substrate.

describing the relationship between load W and displacement h is continuously monitored and recorded. The mechanical properties of the materials can be derived through analysis of the load–displacement data during the loading–unloading indentation cycle using the method of Oliver and Pharr [18]. At peak load, the load and displacement are W_{max} and h_{max}, respectively. The hardness is given by Eq. (1) as

$$H = \frac{W_{max}}{A} \quad (1)$$

where W_{max} is the maximum applied load and A is the area function.

Similarly, using the method of Oliver and Pharr, the elastic modulus of the sample, E, is given by Eq. (2) as

$$E = \frac{\sqrt{\pi}dW}{2\sqrt{A}dh} \quad (2)$$

where dW/dh is the slope of the unloading curve at maximum load or stiffness, S.

Just after AFM characterization, nanoindentation tests were performed for bare bacterial cells and those after metallization. A total of at least ten intends were made on separate cells for every sample. In order to reduce the testing errors, the same nanoindentation tests were repeated for three times for every sample and the average testing results were taken.

3. Results and discussion

3.1. Morphological and microstructure characterization by AFM and TEM

The morphological images of bare *E. coli* cells and those after metallization with nickel coating were shown in Figs. 1 and 2, respectively. Bare *E. coli* cells are observed to aggregate into biofilm in Fig. 1a. They have relatively smooth surface and the roughness of Ra is measured to be 1.1 nm within the box size of 300×300 nm (roughness data not shown here). Due to the attachment of the bacterial cell with mica surface, its section curve doesn't show either rounded or semi-rounded, but arciform with an unproportionable ratio of height to width, indicating an evident outspreading effect. Fig. 2a presents one AFM image of linking metallized bacterial cells, whose height is measured to be ~ 130 nm by section analysis. A lot of particles with the diameter of about 15 nm were observed to uniformly distribute at the metallized bacterial surface, which had been approved to be nickel with TEM characterization (see Fig. 2b). During AFM repetitive scanning cycle, no position displacement of the bacterial cells was observed, which illuminates the strong adhesion interaction between the bacteria and substrate.

3.2. Nanoindentation testing

After AFM characterization, the samples were indented by a nano-indentor as illustrated in Fig. 3. The applied load was recorded as a function of penetration. It is generally accepted that the depth of indentation should never exceed 30% of the film thickness [19,20]. If this condition is satisfied, the substrate effect on the measurement of the hardness and elastic modulus of the film can be ignored. Herein, the height of bare and metallized bacterial cells adsorbed on mica obtained from AFM section analysis is to be in the range of 60–70 nm and 100–130 nm, respectively. So the depth of indentation is chosen to be 20 nm and 40 nm, respectively. Considering the effect of surface roughness on indentation results, the choice of indentation depth is reasonable.

Fig. 4 presents a typical load–unload curve for metallized bacterial cells. For contrast, nanoindentation tests were performed for those bare cells and the mica substrate. A comparison of their elastic modulus is shown in Fig. 5. It can be seen that the lower the penetration of nanoindentation, the higher the elastic modulus of the samples, which is attributed to the strain gradient effect (size effect) during nanoindentation for most materials [21,22]. After a short-distance descending (within the penetration of 5 nm), the values of elastic modulus reaches a relatively flat and stable stage, which is particularly distinct for bare and metallized cell. The nano-indentation elastic modulus and hardness of the metallized bacterial cells were measured to be 71.484±1.804 GPa and 10.353±0.606 GPa, respectively. Compared with bare bacterial cells with an elastic modulus value of 4.382±1.74 GPa and a hardness value of 0.203±0.049 GPa, there appears a great improvement in the mechanical properties of those cells after metallization, in which an increase in elastic modulus was almost 17 times and hardness was 50 times.

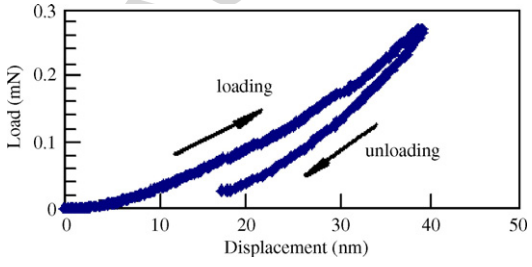

Fig. 4. Load vs. depth curve for bacterial cells after metallization obtained by nanoindentation.

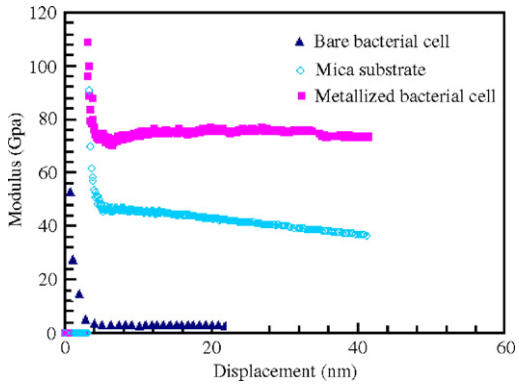

Fig. 5. Elastic modulus comparison among mica substrate, bare and metallized bacterial cells.

4. Conclusions

In summary, we have used nanoindentation to study the mechanical properties of bacteria-templated nickel nanomaterials, which are fabricated via electroless chemical plating. It is found that these metallized bacterial cells exhibit elastic modulus of 71.484±1.804 GPa and hardness of 10.353±0.606 GPa. Compared with bare bacterial cells, their elastic modulus is increased 17 times and hardness is increased almost 50 times, which will supply a powerful mechanical theoretic foundation for their potential application in nanoelectronics, nanomagnetism and nanomechanics in the future.

Acknowledgements

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