DOI 10.1007/s10517-016-3207-7

MORPHOLOGY AND PATHOMORPHOLOGY

The Use of Atomic Force Microscopy for Cytomorphological Analysis of Bacterial Infection Agents

I. S. Nemova, O. E. Falova*, and N. I. Potaturkina-Nesterova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 160, No. 10, pp. 509-512, October, 2015
Original article submitted October 31, 2014

Cytomorphological signs of bacterial infection agents were studied by atomic force microscopy. Analysis of the elastic mechanical characteristics of *Staphylococcus spp.* from the skin of patients with chronic dermatoses showed lower elasticity of *S. aureus* cell membrane in comparison with that of transitory flora representatives. Significant differences in characteristics of cell membrane relief and presence of *fimA* pathogenicity factor were detected in *E. coli* isolated from the reproductive tract mucosa of clinically healthy women and patients with inflammatory urogenital infections.

Key Words: *atomic force microscopy; genetic determinants; microflora; pathogenicity factors*

Atomic force microscopy (AFM) is a method of scanning probe microscopy widely used in microbiological studies [2]. This method allows computer-aided diagnosis in 3D mode and provides data on the biological variety of microorganisms [6]. The morphology and functions of microorganisms under conditions of various abiotic and biotic exposures can be studied by AFM. The method is used for evaluation of morphofunctional reaction of bacterial cells with various types of cell wall structure to antibiotics [3].

Studies of surface structure of infection agents by probe microscopy are now in the focus of interest. It was found that the surface formations of bacterial cells determine the type of their interactions with the host determining the development of pathological or immune processes [4].

Here we studied cytomorphological signs of agents of bacterial infections by AFM.

MATERIALS AND METHODS

The study was carried out on 45 *E. coli* strains isolated from the reproductive tract mucosa of clinically healthy women (group 1) and patients with urogenital infections – endocervicitis, adnexitis (group 2), and on 35 strains of staphylococci of various species collected from the skin of patients with chronic dermatoses: psoriasis, eczema, atopic dermatitis (group 3) and from healthy subjects (group 4). Ten of these strains (28.5%) were representatives of *S. aureus*, 8 (22.8%) were *S. epidermidis* strains, 12 (34.3%) were *S. haemolyticus*, and 5 (14.3%) strains were referred to *S. hominis*.

To detect microorganisms, the cervical discharge was analyzed by real-time PCR using Femoflor 16 test systems (DNA-Technologies). Cloned DNA specimens were analyzed by gel electrophoresis in 1.6% agarose after EtBr staining. Staphylococcal genome DNA was isolated using Politub kit (Litekh) at Research Institute of Physicochemical Medicine (TU-9398-410-172-53567-97) according to the instructions [1]. *E. coli* strains were cultured in solid nutrient media MPA (BioHold) and Endo (HiMedia Laboratories Pvt. Ltd) at 37°C.

Genetic determinants of pathogenicity factors of the isolated strains were studied. Primers and annealing temperature for *E. coli* were selected using Lasergene software. Primers for *E. coli*: *fimA* (adhesins) – 5'-TGG-CTG-CCG-CAC-TAT-TCG-CC-3'. Primer

Ulyanovsk State University; *Ulyanovsk Technological State University, Ulyanovsk, Russia. ***Address for correspondence:*** falova@rambler.ru. O. E. Falova

and probe oligonucleotides were synthesized by the amidophosphite method (Syntol). Primers for *Staphylococcus spp.*: *mecA* – 5'-TCC-CAG-ATT-ACA-ACT-TCA-CCA-GG-3'. Bacterial DNA was isolated by the standard method using a kit of reagents for DNA isolation from biological samples (Proba GS; kit No. 1).

Morphometric signs of isolated strains were studied by AFM in the tapping mode using Solver P47-PRO scanning probe microscope (NT-MDT). We used gold-coated cantilevers, lot NSG10 (NT-MDT), with the following parameters: 95×30 μ, 17 N/m rigidity, 10 nm needle curve radius, and 271 kHz resonance frequency. For more detailed studies of gram-positive and gram-negative microorganisms, two AFM modes: tapping and dissociation. The tapping mode was used to acquire 2D and 3D topographic images of bacteria and evaluate their linear parameters (length and width) of the cell. The resultant images were processed by Debug Nova 1.1.0.1.847 software (NT-MDT). The linear parameters of bacteria were measured in μ, roughness in nm. For more detailed visualization of bacterial ultrastructure, cantilever vibration amplitude was registered in the dissociation mode. The elastic viscous characteristics of the cell membrane were studied using Young's modulus (membrane isometric compression modulus), characterizing the cell deformability, higher values indicating lesser cell deformation [5].

The data were statistically processed by Statistica 6.0 software.

RESULTS

AFM of gram-negative *E. coli* strains (n=25) isolated from the reproductive tract mucosa of group 2 patients with reproducible identification of the morphological characteristics showed that the length of bacterial cells was 2.53±0.32 μ and width 1.22±0.21 μ (Fig. 1). The parameters of *E. coli* strains (n=20) isolated from the urogenital tract mucosa of clinically healthy women were different: cell length was 1.4 times less and was 1.84±0.22 μ, width – 0.88±0.07 μ (p<0.05). Studies of the viscous-elastic characteristics of *E. coli* cell membrane showed that Young's modulus in group 2 was 2.4±0.3 MPa, adhesion strength of these bacteria was 20.6±3.1 nN; in group 1 strains these parameters were 1.4 times less (1.7±0.2 MPa and 13.9±1.7 nN, respectively; p<0.05).

Studies of the elastic mechanical characteristics of *E. coli* with genetic pathogenicity determinants were particularly interesting. Testing of *E. coli* strains isolated in groups 1 and 2 for *fimA* (adhesins) gene showed that the incidence of this fragment was 88% (22 strains) and 10% (2 strains), respectively. These data indicated that elastic characteristics of the bacteria differed: the mean quadratic roughness of the surface was 40.00±0.18 nm in strains with *fimA* gene and 20.00±0.09 nm in strains without it.

Cell diameters of the studied representatives of gram-positive *Staphylococcus spp.* (group 3) varied significantly; for *S. aureus* cells, this parameter was 2.03±0.08 μ (Fig. 2). In other staphylococci, representatives of opportunistic microflora, this parameter was lower – from 1.10±0.03 μ in *S. epidermidis* to 1.55±0.06 μ in *S. haemolyticus* (in group 4 – 0.95±0.03 μ for *S. haemolyticus*; p<0.05).

The elasticity of *S. aureus* membranes was characterized by Young's modulus of 377.26±133.20 MPa, with adhesion strength of 29.9±20.1 nN. No *S. aureus* was detected in group 4.

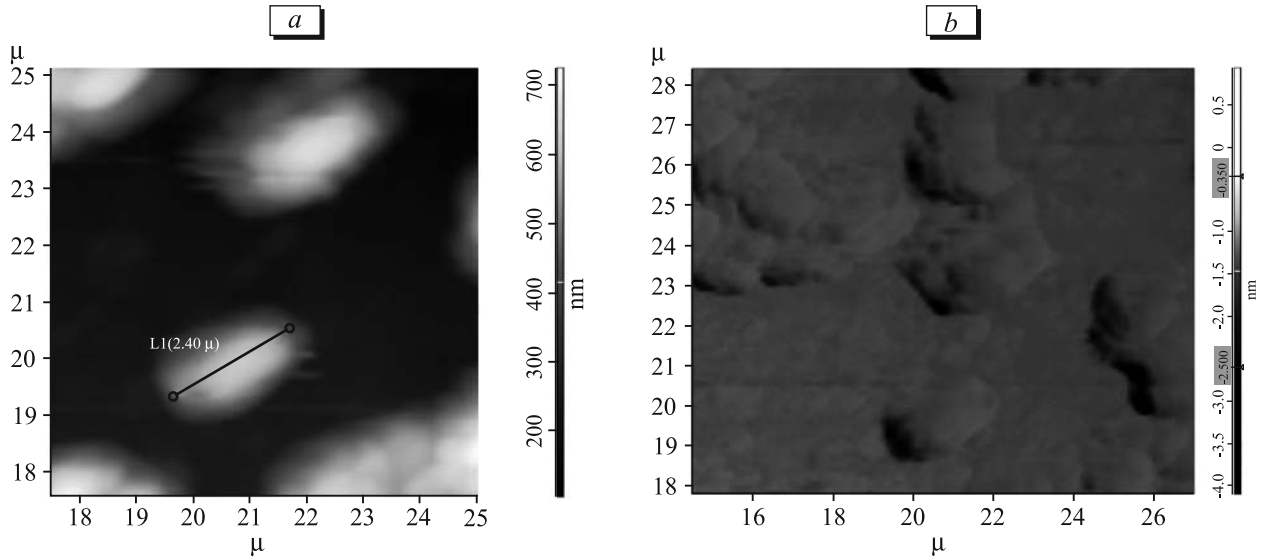

Fig. 1. AFM images of *E. coli* acquired by scanning in the tapping mode, scanning area 25×25 μ (*a*); by dissociation method, scanning area 26×28 μ (*b*).

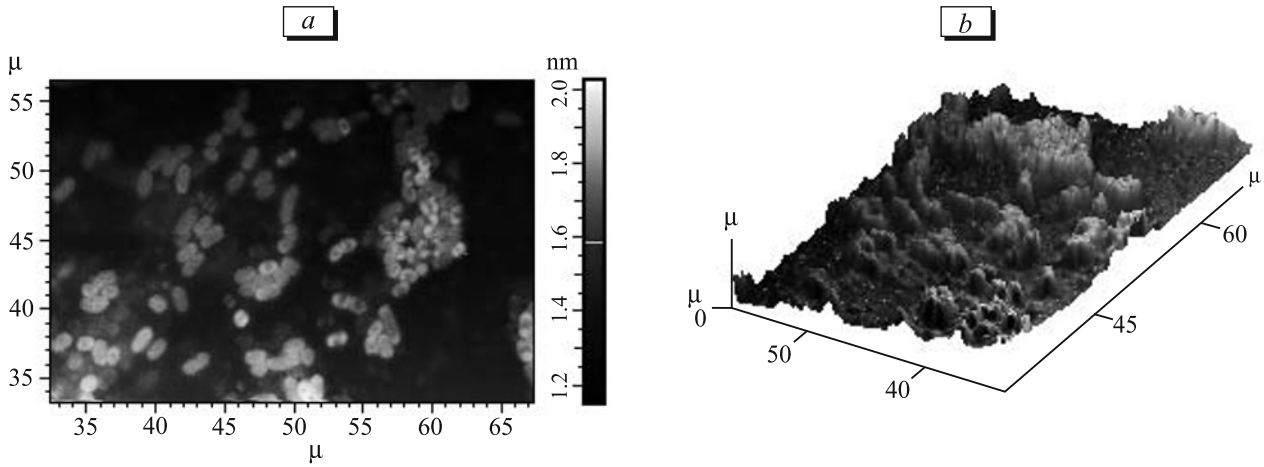

Fig. 2. AFM images of *Staphylococcus spp*. acquired by scanning in the tapping mode, scanning area 55×65 μ (*a*), in 3D mode, scanning area 55×65 μ (*b*).

Representatives of opportunistic bacteria, for example, *S. epidermidis*, exhibited cell membrane elasticity of 94.8±23.5 MPa, with 73.93±59.8 nN adhesion strength. Young's modulus of *S. haemolyticus* strains was higher than that of *S. epidermidis* and was 211.0±171.7 MPa ($p<0.05$). Adhesion strength of *S. haemolyticus* and *S. epidermidis* virtually did not differ (160.13±2.7 nN; $p>0.05$). In group 4, *S. haemolyticus* strains exhibited elastic properties (Young's modulus 115±58 MPa; $p<0.05$).

Hence, *S. haemolyticus* and *S. epidermidis* exhibited the highest adhesive characteristics, while *S. aureus* – the lowest ones.

The elastic mechanical characteristics were also studied in *Staphylococcus spp.* strains, for which the genetic pathogenicity determinants were studied. Of the coagulate-positive staphylococci in group 3, only 4 *S. epidermidis* strains and 4 *S. haemolyticus* strains had *mecA* gene, coding for methycillin resistance – a fact significant for analysis of hospital staphylococcal population structure. *S. epidermidis* strains carrying *mecA* gene were less elastic (Young's modulus 216.7±64.0 MPa). *S. haemolyticus* cells were more elastic (Young's modulus 333.0±67.4 MPa; $p>0.05$).

AFM demonstrated heterogeneity of morphological signs of bacterial infection agents. Analysis of elastic mechanical characteristics of *Staphylococcus spp.* representatives isolated from patients with chronic dermatoses showed lower elasticity of *S. aureus* cell membranes in comparison with the transitory flora representatives. *S. epidermidis* cells exhibited the highest elasticity. Differences in cell membrane relief and in the presence of *fimA* pathogenicity factor in *E. coli* isolated from the reproductive tract mucosa of clinically healthy women and patients with inflammatory urogenital infections were detected.

REFERENCES

1. M. V. Afanas'ev, S. V. Karakashev, E. N. Il'ina, *et al.*, *Mol. Gen., Mikrobiol. Virusol.*, No. 2, 20-24 (2010).
2. A. S. Vasil'chenko, *Vestn. Ural. Med. Akad. Nauki*, No. 4/1, 24 (2011).
3. D. G. Deryabin, A. S. Vasil'chenko, E. S. Aleshina, *et al.*, *Ross. Nanotekhnol.*, **5**, Nos. 11-12, 135-141 (2010).
4. N. P. Konnov, Yu. P. Volkov, M. N. Kireev, and O. S. Kuznetsov, *Probl. Osobo Opasnykh Infektsii*, No. 3, 60-63 (2008).
5. D. V. Lebedev, A. P. Chuklanov, A. A. Bukharaev, and O. S. Druzhinina, *Pis'ma Zh. Tekhnich. Fiziki*, **35**, No. 8, 54-61 (2009).
6. F. J. Verbeek and P. J. Boon, *Three-Dimensional and Multidimensional Microscopy: Image Aquisition and Processing IX. Proceedings SPIE*, San-Jose, **4621**, doi: 10.1117|12.467833(2002).