Analysis of nanomechanical properties of *Borrelia burgdorferi* spirochetes under the influence of lytic factors in an *in vitro* model using atomic force microscopy

**Summary**

Atomic force microscopy (AFM) is an experimental technique which recently has been used in biology, microbiology, and medicine to investigate the topography of surfaces and in the evaluation of mechanical properties of cells. The aim of this study was to evaluate the influence of the complement system and specific anti-*Borrelia* antibodies in *in vitro* conditions on the modification of nanomechanical features of *B. burgdorferi* B31 cells.

In order to assess the influence of the complement system and anti-*Borrelia* antibodies on *B. burgdorferi* s.s. B31 spirochetes, the bacteria were incubated together with plasma of identified status. The samples were applied on the surface of mica disks. Young’s modulus and adhesive forces were analyzed with a NanoScope V, MultiMode 8 AFM microscope (Bruker) by the PeakForce QNM technique in air using NanoScope Analysis 1.40 software (Bruker).

The average value of flexibility of spirochetes’ surface expressed by Young’s modulus was 10185.32 MPa, whereas the adhesion force was 3.68 nN. AFM is a modern tool with a broad spectrum of observational and measurement abilities. Young’s modulus and the adhesion force can be treated as parameters in the evaluation of intensity and changes which take place in pathogenic microbiorgansisms under the influence of various lytic factors. The visualization of the changes in association with nanomechanical features provides a realistic portrayal of the lytic abilities of the elements of the innate and adaptive human immune system.

**Keywords:** *Borrelia burgdorferi* - AFM - Young’s modulus - adhesion force
Bacterial strains and culture conditions

A reference strain of *B. burgdorferi* s.s. B31 (ATCC 35210) was used in the study. To cultivate spirochetes, 0.1 ml of strain was inoculated in 5 ml of BSK-H Complete medium. The strain was incubated in a 5% CO₂ atmosphere at 35°C for 7 days, to a cell density of 10⁷/ml [9].

Acquisition of plasma of identified status

In order to assess the influence of the complement system and anti-*Borrelia* antibodies on *B. burgdorferi* s.s. B31 spirochetes, the bacteria were incubated together with plasma of identified status. A blood sample taken from a person who did not reveal the presence of IgM/IgG anti-*Borrelia* was the source of plasma with the active complement system. A blood sample taken from a person with clinical symptoms of Lyme disease and IgM/IgG anti-*Borrelia* was the source of plasma with the active complement system and specific antibodies. Whole blood (2.5 ml) was collected in polypropylene tubes containing r-hirudin (SARSTEDT). Recombinant hirudin is a highly specific thrombin inhibitor that minimally influences complement activation [3,8,13]. The complement system activity was blocked as a result of taking a blood sample (2.5 ml) from a person with clinical symptoms of Lyme disease and IgM/IgG anti-*Borrelia* using syrin-
The blood samples were centrifuged for 10 min at 1000 g. The plasma was separated. The bacteria were suspended in specific status plasma (200 μl), and the samples were incubated for 3 h at 37°C [18].

Microscopic imaging of bacterial cells

*B. burgdorferi* s.s. B31 in BSK-H medium and in specific status plasma was centrifuged for 10 min at 2500 rpm. The supernatant was removed from the precipitate of bacteria. Bacteria samples were prepared according to the procedure given by Zdybicka-Barabas [21]. The samples were applied on the surface of mica disks and allowed to dry at 28°C before imaging. Young’s modulus and adhesive forces were analyzed with a NanoScope V, MultiMode 8 AFM microscope (Bruker) by the PeakForce QNM technique in air using NanoScope Analysis 1.40 software (Bruker).

Results

Analysis of nanomechanical properties of *B. burgdorferi* s.s. B31 spirochetes expressed by Young’s modulus and adhesion force

The image record of bacteria in DMT Modulus format (Fig. 1) enables one to perform a series of measurements of Young’s modulus on the precisely determined operative fields of the analyzed spirochete’s surface. The image record of bacteria in the Adhesion format (Fig. 2) enables one to take measurements of the adhesion factor, i.e., intermolecular forces on the surface of a junction between the AFM probe tip and a bacterial cell. The average value of flexibility of spirochete’s surface expressed by Young’s modulus is 10 185.32 MPa (min. 5812.00 MPa – max. 15 181.0 MPa; SD 2590.34), whereas the adhesion force is 3.68 nN (min. 3.05 nN – max. 4.37 nN, SD 0.45). Young’s modulus and the adhesion force were designated for *Borrelia* spirochetes which were not subjected to the action of destructive factors. They were compared to results achieved for spirochetes incubated with plasma of identified status.

Young’s modulus determined for *Borrelia* spirochetes incubated with plasma of different status

The value of Young’s modulus measured for incubated bacteria with plasma containing active complement was significantly lower (4511.70 MPa, \(p \leq 0.0000001\)) in comparison with the control (10 185.33 MPa). Young’s modulus determined for spirochetes incubated with plasma containing active complement and specific anti-*Borrelia* antibodies was significantly lower (3075.82 MPa, \(p \leq 0.0000001\)) in comparison with a control and significantly lower (\(p \leq 0.0000001\)) in comparison with the value measured for spirochetes exposed to plasma with active complement. Spirochetes incubated with plasma containing specific anti-*Borrelia* antibodies and blocked complement obtained lower values of Young’s modulus (3752.72 MPa, \(p \leq 0.0000001\)) in comparison with control cells. The obtained values were also significantly lower (\(p \leq 0.000008\)) in comparison with Young’s modulus measured for spirochetes incubated with plasma with active complement (4511.70 MPa) and significantly higher (\(p \leq 0.0000001\)) in comparison with the value measured for bacteria incubated with plasma with active complement and specific anti-*Borrelia* antibodies (3075.82 MPa) (Fig. 3).

The adhesive factor for spirochetes *Borrelia* incubated with plasma of different status

The value of the adhesion force for *Borrelia* cells incubated with plasma containing active complement was significantly lower (3.11 nN; \(p \leq 0.0000001\)) in comparison with the value achieved for control cells (3.68 nN). The measures taken for spirochetes incubated with plasma containing active complement and specific anti-*Borrelia* antibodies revealed a significant decrease of the adhe-

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**Fig. 1.** The image record of bacteria *B. burgdorferi* s.s. B31 in the DMTM Modulus format (NanoScope Analysis 1.40; Bruker)

**Fig. 2.** The image record of bacteria *B. burgdorferi* s.s. B31 in the Adhesion format (NanoScope Analysis 1.40; Bruker)
sion force (2.07 nN; p≤0.0000001) in comparison with the control. The obtained adhesion force value was also significantly lower (p≤0.0000001) in comparison with the value measured for spirochetes which were exposed to plasma with active complement (3.11 nN). The adhesion force was significantly lower (2.96 nN) (p≤0.0000001) for B. burgdorferi spirochetes incubated with plasma containing specific anti-Borrelia antibodies and blocked complement, in comparison with the control. At the same time, the obtained adhesion force value was significantly higher (p≤0.0000002) in comparison with the measured value for spirochetes incubated with plasma containing active complement and specific anti-Borrelia antibodies (2.07 nN), and significantly lower (p≤0.000005) in comparison with the value measured for bacteria incubated with plasma and active complement (3.11 nN) (Fig. 4).

**DISCUSSION**

AFM is one of the most modern tools with a very broad spectrum of observational and measurement capabilities, and its significance in the study of microorganisms is immense [7,12]. The cell wall of gram-negative bacteria is one of the most complex structures in prokaryotic cells. The foundation consists of a thin layer of peptidoglycan and a double external layer with a rich protein-lipid fraction. If the external layer of a cell is damaged as a result of the activity of lytic factors, e.g. the complement system, the pressure present inside the cell radically decreases, which has an effect on the flexibility parameters. In conclusion, the shape of a cell is a resultant of all factors: turgor pressure, thickness of the peptidoglycan and the external membrane [20]. The mutual reaction between a pathogen and tissues of an infected organism is described as a decisive stage of colonisation. During this process, the mechanical parameters of external structures of a bacterial cell are very important [1,2,4,14].

Specific antibodies enable one to eliminate spirochetes when acting as described and favor their destruction via the complement system, whereas antibodies attached to the surface of a bacterial cell cannot lead to its lysis. Non-specific factors such as complement and immunologically competent cells with the participation of immunoglobulin can lead to the effective lysis of a pathogen. The presence of specific antibodies or non-specific mechanisms solely is insufficient in order to effectively eliminate an infection from the organism [6,15,16]. In the case of cooperation of specific and non-specific mechanisms, the killing effect is immensely greater. It was confirmed that the specimen of patients with erythema migrans (EM) is less effective in killing B. burgdorferi than the specimen of patients with later symptoms of the disease, who had high levels of specific antibodies against spirochete proteins. The specimen of patients with EM, Lyme arthritis and acrodermatitis chronica atropicans confirmed the activity of the killing effect as respectively 29.63 and 86% [5]. According to other data, B. burgdorferi has an influence on the activation of a complement without anti-Borrelia antibodies, although the killing effect was distinctly visible only after the addition of specific antibodies to the specimen [6]. AFM was used to observe how the surface of B. burgdorferi and its mechanical parameters change as a result of a reaction of complement and specific anti-Borrelia antibodies. The changes of flexibility of bacterial cells and their prospective abilities to adhere were determined by measuring Young’s modulus and the adhesion force. The values of Young’s modulus and the adhesion force were decreased due to B. burgdorferi spirochetes being subjected to the effect of plasma with active com-

![Fig. 3. Young's modulus determined for spirochetes Borrelia incubated with plasma of different status](image-url)
The knowledge of mechanisms responsible for the ability of bacteria to adhere to surfaces is crucial in relation not only to pathogenic bacteria but to probiotic ones as well. Tripathi et al., using AFM, analyzed the issues connected with adherence of *L. rhamnosus GG* [17].

The notion of adhesion is also crucial in terms of biology of pathogenic microorganisms including *B. burgdorferi* as regards their participation in pathogenicity of diseases. The destruction of outer structures as a result of the activity of complement and specific antibodies can lead to a decrease of adhesive abilities of bacteria. Spirochetes subjected to the reaction of a complement and antibodies demonstrate the lowest adhesion force. Thus, it is possible that these two factors can have the greatest influence on a decrease of abilities of *B. burgdorferi* to adhere to colonized surfaces.

**Conclusions**

Young’s modulus and the adhesion force can be treated as parameters in the evaluation of intensity and changes which take place in pathogenic microorganisms under the influence of various lytic factors. The visualization of the changes in association with mechanical features provides a realistic portrayal of the lytic abilities of the elements of the innate and adaptive human immune system.
Reference


The authors have no potential conflicts of interest to declare.