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The analysis of nanomechanical properties of *Candida* spp. by atomic force microscopy (AFM) method

Analiza własności nanomechanicznych *Candida* spp. z zastosowaniem mikroskopu sił atomowych (AFM)

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Summary

Aim: The aim of the study was to analyze the selected nanomechanical properties of *Candida* spp: *Candida albicans* (standard strain ATCC 10231), *Candida albicans* (clinical strain, cultured from an oral swab), *Candida lipolytica* (clinical strain, cultured from a nosal swab) in atomic force microscopy (AFM).

Material/Methods: The culture *Candida* spp. was performed of Tryptone Soya Broth (BioMaxima). The topography and sample properties were analysed in AFM (Ntegra Spectra C from NT) and the results were carried out using NOVA 1.1.0.1824 software.

Results: *C. albicans* ATCC 10231 cells were significantly higher 1.81 μm (p = 0.001) from clinical strains: *C. albicans* (1.30 μm) and *C. lipolytica* (1.23 μm). *C. albicans* ATCC 10231 cells, and *C. albicans* cells of the clinical strain were softer, especially in the top parts of cells, than *C. lipolytica* cells. Adhesion force measured for *C. albicans* ATCC 10231 was 62.83 nN, and was significantly higher compared to the values obtained for *C. albicans* (41.93 nN, p = 0.0002) and *C. lipolytica* (41.78 nN, p = 0.0002). The stiffness of the *Candida* spp. cell surface was comparable and was in the range of 5–6 nA.

Conclusions: The differences in height may result from different conditions in which clinical strains grow. Adhesion force can be helpful in the analysis of the degree of destruction of the cell wall by various substances. The conducted analyses showed morphological differences and the differences in mechanical properties of the researched *Candida* spp. This data may be important in assessing their susceptibility to the effects of various substances of a lytic nature.

Keywords: ***Candida albicans*** • ***Candida lipolytica*** • **AFM**

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INTRODUCTION

The occurrence of diseases that go along with the reduction of the immunological potential and related therapies are not without the influence on the threat for patients of infections caused by various *Candida* species. They are responsible for about 15% of all hospital-acquired infections and they constitute not only a serious and growing clinical problem but also a significant issue in the field of public health [1, 4, 8]. The disorders of natural defense mechanisms and imbalance not only in the composition of the mucosa of the mucous membranes but also skin which appear in patients as a result of applied broad-spectrum antibiotics, anti-cancer treatment or immunosuppression, may contribute to *Candida* spp. infections, both on the aspect of surface and systemic infections [1, 6, 10, 15].

The research shows that among numerous species *C. albicans* is most often identified and is responsible for 32–58% of candidiasis. However, infections are also often caused by *C. parapsilosis* (13–30%), *C. glabrata* (8–24%), *C. tropicalis* (3–14%), and less frequently by *C. krusei* (8%) and *C. guilliermondii* (2 %). Other *Candida* spp. such as *C. dubliniensis, C. kefyr, C. lipolytica* [1, 6, 8]. *C. pelliculosa, C. lusitaniae, C. palmioleophila, C. curvata,* and *C. pulcherrima* [9], also participate in the generation of infections. As the research shows, the number of infections caused by non-*C. albicans* species increases [2].

Y. lipolytica (*C. lipolytica*) strains are commonly found in nature and are isolated from meat products, sewage, soil and marine environments with high salinity and used in food biotechnology and in environmental protection [2, 7]. *Candida lipolytica* is not a frequent infection factor, but there are documented infections caused by this species of *Candida* [2].

Among the virulence factors of *Candida* spp. enzymatic activity, signaling and quorum sensing are mentioned, as well as the participation in biofilm formation. The occurrence of various morphological forms within the same species is also significant. *C. albicans* cell types (yeasts, hyphae, pseudohyphae and chlamydospores) differ in their morphology and virulence potential. Yeasts ("white" cells) have a round cell morphology and form creamy white, shiny, domed colonies on solid media. *C. albicans* can occur in "opaque" form that exhibit different properties and interactions with the host. Opaque cells are elongated, the colonies are darker, matte and flattened in comparison to white a colonies. The white and opaque phenotypes show different sensitivity to the content in the environment of CO_2, pH, glucose and they react differently to mammalian body temperature. Moreover, differences in the expression of many genes and resistance to phagocytosis by host macrophages and neutrophils are observed [11].

The adhesion ability allows *Candida* spp. to adapt to host cells and it is the stage preceding colonization, penetration of tissues and creation of biofilms [6, 14]. Among the *Candida* spp. Adhesives, the following can be enumerated: cell wall proteins (e.g., agglutinin-like sequence proteins, Als1-Als9; hyphae wall protein, Hwp1; epithelial adhesion protein) and proteins not associated with the cell wall (e.g., integrin-like protein; Int1) [6].

Determination of the physicochemical properties of pathogenic microbial cells is of key importance in the process of recognizing and understanding their life strategies, the ability to colonize tissues and biomaterials surfaces, the "host-pathogen" interactions and the process of infection. This is particularly important due to the growing number of candidiasis, including those caused by rare species of *Candida* spp. [9].

Atomic force microscopy (AFM) enables the analysis of the topography of pathogenic microorganisms and their biophysical properties in response to various active substances [13]. In order to use AFM to analyze and assess the degree of destruction of microbial cells under the influence of drugs or natural lithic factors, it is essential to know their physicochemical parameters [3]. AFM gives a wide range of measurement possibilities: height of cells, elasticity, physico-chemical parameters like charge density and turgor pressure [12], adhesion and mechanics of cell wall [3].

Not only adaptation to conditions prevailing in the host organism but also the rate of infection processes taking place as well as the sensitivity or resistance to drugs depend on physico-chemical properties of pathogenic microorganisms. The increasing number of *Candida* spp. infections, including rare species, and the increasing difficulties associated with the development of resistance to used drugs, encourage more research to be conducted into the characteristics of cells from different species of *Candida* spp. The aim of the study was to analyze the selected nanomechanical properties of *Candida* spp.: *Candida albicans* (standard strain ATCC 10231), *Candida albicans* (clinical strain), *Candida lipolytica* (clinical strain) in atomic force microscopy (AFM).

MATERIALS AND METHODS

Culture conditions and microscope imaging of Candida spp. cells

The culture *Candida albicans* standard strain ATCC 10231 (Microbiologics, distributor bioMerieux), *Candida albicans* (clinical strain, cultured from an oral swab from the patient after surgery within the abdominal cavity), *Candida lipolytica* (clinical strain, cultured from a nasal swab from the patient after partial resection of the large intestine due to familial polyposis) was performed in 5 ml of Tryptone Soya Broth (BioMaxima). After 48 hours incubation at 30°C, the culture was gently mixed and centrifuged per 8 min 2000 x g. *Candida* spp. samples were performed according to the procedure given by Janeczko [5]. Distilled water (5 ml) was added to the test tubes with the cells and centrifuged (8 min x 2000 g). The supernatant was removed from the precipitate. The cells were suspended in distilled water (3 ml) and applied to degreased glass slides. The slides were dried at room temperature [5].

The topography and sample properties were analyzed in atomic force microscopy (AFM; Ntegra Spectra C from NT) and the results were carried out using NOVA 1.1.0.1824 software. The semicontact topography meas-

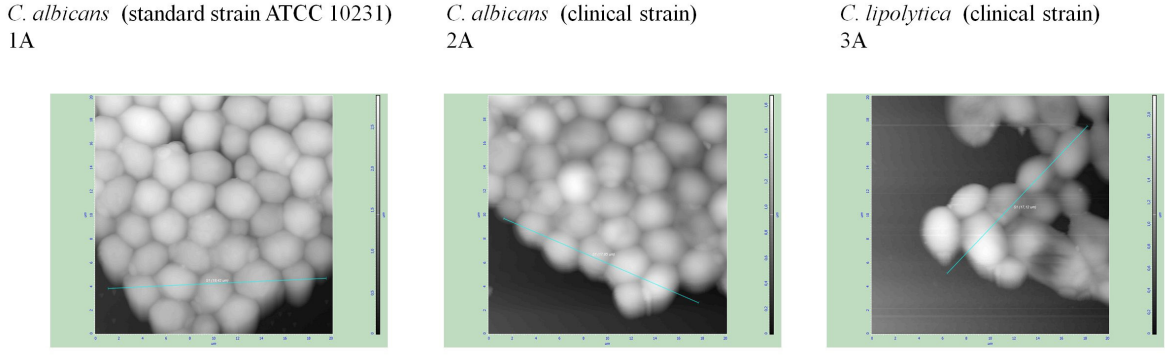

Fig. 1. The height of *Candida* spp. cells in AFM

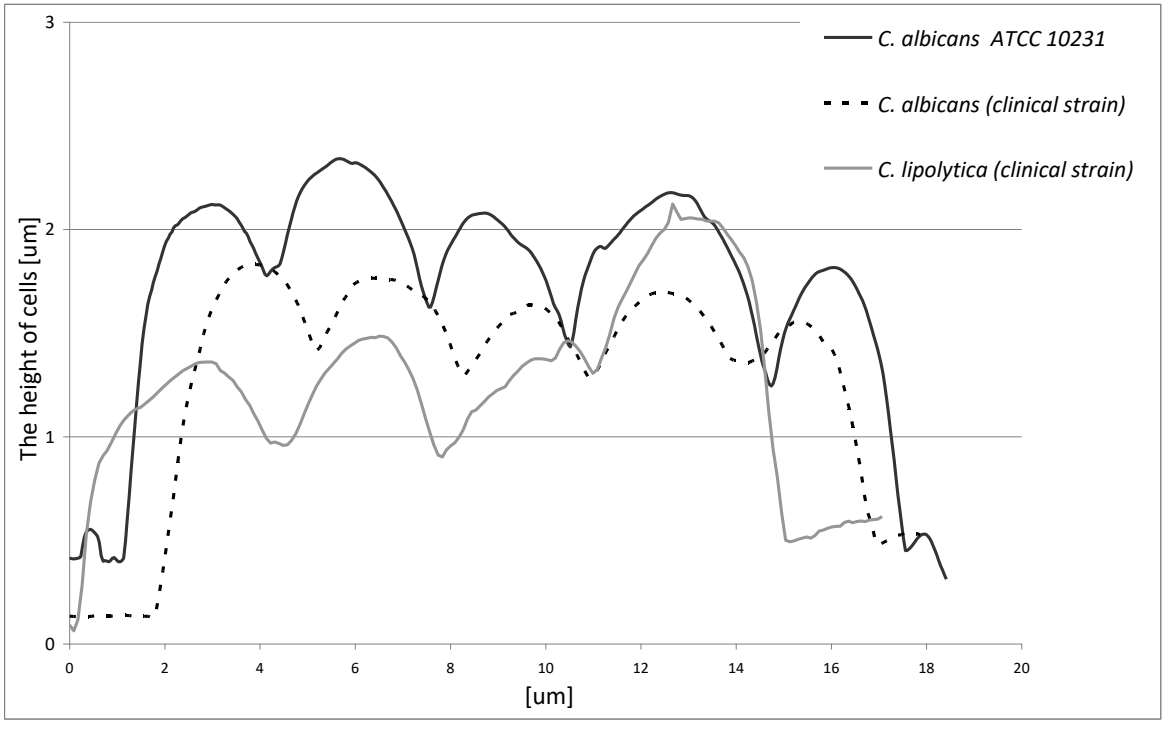

Fig. 2. The difference in height between *C. albicans* ATCC 10231, *C. albicans*, and *C. lipolytica*

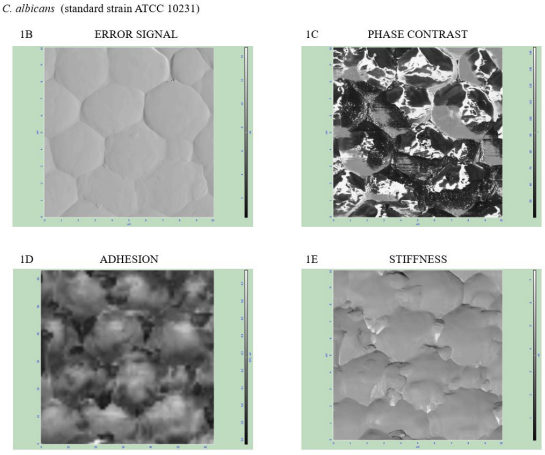

Fig. 3. AFM images of *C. albicans* ATCC 10231

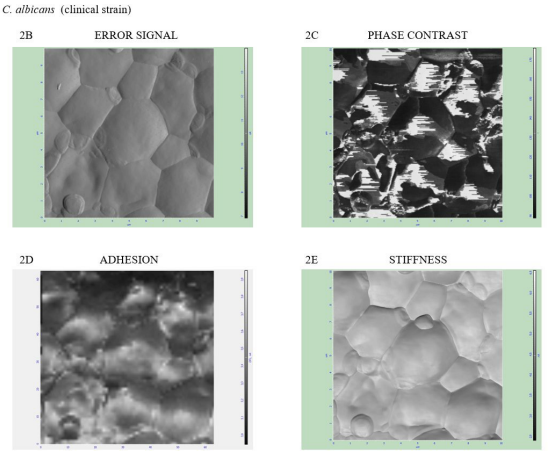

Fig. 4. AFM images of clinical strains of *C. albicans*

urements (Error Signal, Phase Contrast) were performed with a 135 µm NT-MDT NSG03 Cantilever, Resonant frequency 90 kHz and Force constant 1.74 N/m. The contact topography measurements (Error Signal, Lateral Force) were performed with a NT-MDT CSG30 tip (190 µm), Force constant 0.6 N/m. The results were analyzed statistically (Statistica v.10 software). The maximum and minimum values, arithmetic average and standard deviations (SD) were calculated. The Mann-Whitney U test and the Kruskala-Wallisa test were used. The assumptive level of significance was p = 0.05.

RESULTS

The turbidity of the liquid microbiological medium indicated the growth of *Candida*. Only yeast forms were present in the culture, no forms of hyphae, pseudohyphae or chlamydospores were observed. The yeast cells were round or oval. The height of *Candida* spp. cells: *C. albicans* ATCC 10231, *C. albicans* (clinical strain), and *C. lipolytica* (clinical strain) are shown on images 1A, 2A and 3A, respectively (Fig. 1). The line on the height images represents the location of the height measurement. The difference in height between *C. albicans* ATCC 10231, *C. albicans* (clinical strain), and *C. lipolytica* (clinical strain) illustrated in the Fig. 2 *C. albicans* ATCC 10231 cells were significantly higher 1.81 µm (SD 0.44, min. 0.31 µm - max. 2.34 µm, p = 0.001) from clinical strains: *C. albicans* – 1.30 µm (SD 0.52, min. 0.13 µm – max. 1.83 µm) and *C. lipolytica* – 1.23 µm (SD 0.44, min. 0.06 µm – max. 2.34 µm). Differences in height of *C. albicans* and *C. lipolytica* were also important (p = 0.000021).

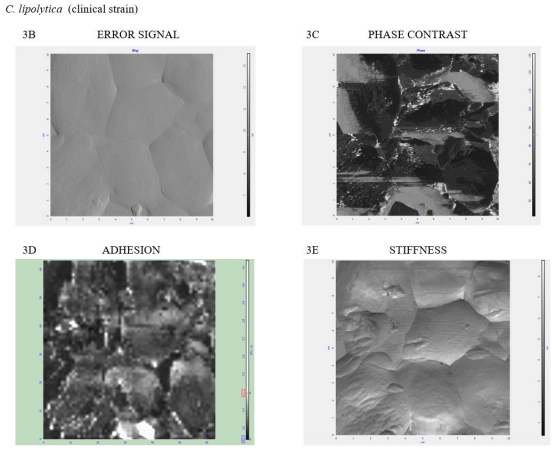

Fig. 5. AFM images of clinical strains of *C. lipolytica*

Fig. 3, 4 and 5 show AFM images of *C. albicans* ATCC 10231, and clinical strains of *C. albicans*, and *C. lipolytica*, respectively. Cells have a smooth surface, a regular shape (Error signal 1B, 2B and 3B). Phase contrast imaging (1C, 2C and 3C) allows the visualization of areas in a surface of cells that have a different softness (scale shows force values ranging from 40 to 170 degrees). *C. albicans* ATCC 10231 cells (1C) and *C. albicans* cells of the clinical strain (2C) were softer, especially in the top parts of cells, than *C. lipolytica* cells (3C).

Adhesion force measured for *C. albicans* ATCC 10231 was 62.83 nN, (SD 4.47, min. 56.19 nN – max. 69.49 nN) and was significantly higher compared to the values obtained for *C. albicans* (41.93 nN, SD 3.67, min. 36.46 nN – max. 147.40 nN, p = 0.0002) and *C. lipolytica* (41.78 nN, SD 3.46, min. 36.63 nN – max. 46.93 nN, p = 0.0002). Figure 1D, 2D, 3D show AFM adhesion images of *Candida* spp. cells.

The stiffness of the *Candida* spp. cell surface was comparable and was in the range of 5-6 nA (z-scale from 0 to 9 nA). Figure 1E, 2E and 3E show AFM stiffness images of *C. albicans* ATCC 10231 cells, *C. albicans* cells and *C. lipolytica*, respectively.

DISCUSSION

Chemical substances, both of natural and synthetic origin which are present in the growth environment of *C. albicans*, affect their cellular properties. Under the

influence of active substances, the *C. albicans* cell wall can be deformed, which is connected with changes in its mechanical strength. The tendency of cells to perforate may also increase [3]. According to the research conducted by Tyagi et al., the height of *C. albicans* cells (2000 nm.) can be changed under the influence of lemon grass oil (LGO) and LGO vapor up to 700 nm and 100 nm, respectively [15].

The height of *C. albicans* varies depending on the cell growth phase (from 1.5 µm to 3.5 µm), as was evidenced by Formosa et al. [4]. In the measurements conducted on the strain model cells of the *C. albicans* ATCC 10231, an approximate result (1.81 µm) was obtained.

It was also shown that the examined clinical strain cells of *C. albicans* and *C. lipolytica* have a lower height (1.30 µm and 1.23 µm, respectively) and a lower adhesion force (41.93 nN, 41.78 nN, respectively) than standard strain cells of *C. albicans* ATCC 10231 (62.83 nN).

It seems that the described nanomechanical properties depend on the strain. It is necessary to conduct further studies on more strains of one species and grown under different conditions to determine whether they are a permanent feature and whether they can be useful as a parameter of the pathogenic potential of yeasts.

Adhesion force can be helpful in the analysis of the degree of destruction of the cell wall by various substances (e.g., drugs). Janeczko et al. observed that *C. albicans* cells, being under the influence of DMSO, showed a lower value of the adhesion force (31.75 nN) compared to cells treated with Amphotericin B (48.32 nN) [5]. As it was showed by studies carried out in AFM by Formosa et al., surface proteins of the *C. albicans* cell wall form nanodomains with an area of 0.09 µm2, which show specific adhesive properties and different stiffness (13.4 nN/µm, 12.4 nN/µm). Mapping them is possible with the use of high resolution imagery. The presence of nanodomes and their properties may have a direct effect on the ability of cells to adhere to the surface [4]. The species of *Candida* spp. which were used in the research were not subjected to degradation factors, and the stiffness of their cells was similar and was in the range of 5–6 nA. This parameter may affect the ability to create biofilms on natural surfaces (skin, mucous membrane) and artificial surfaces (drains, catheters). Cell stiffness may change under the influence of chemical substances. On the surface of *C. albicans* treated with AMP-B, regions with reduced stiffness were observed compared to control cells [5]. Treatment with caspofungin alters the morphology of *C. albicans* cells and the topography of their surface, as was demonstrated by the analysis in AFM carried out by Quilès [13]. Cytomechanical changes were observed on the surface of *C. albicans* under the influence of *S. sanguinis* bacteriocin. Young's modulus (7.33 Mpa) and adhesion force (7.35 nN) registered in the AFM for *C. albicans* cells were changed under the influence of *S. sanguinis* bacteriocin to 5.03 Mpa and 5.69 nN, respectively. These changes increase the elasticity of the *C. albicans* cell wall, which also increases the possibility of cell deformation and reduction of adhesion capacity [10].

Candidemia associated with *C. lipolytica* are relatively rare but cause severe blood infections, particularly in patients with severely reduced immunity. The mechanism of these infections has not been thoroughly understood [8]. However, *C. albicans* infections are most commonly found, and the properties of their cells may change under the influence of various chemical compounds present in the growth environment during treatment. For this reason, in striving to constantly broaden the knowledge about the pathomechanisms of *Candida* spp. infections, it is significant to conduct analyses in the field of cell biology and cytomechanics. Research conducted with the use of AFM gives this opportunity. The conducted analyses showed morphological differences and the differences in mechanical properties of the researched *Candida* spp. This data may be important in assessing their susceptibility to the effects of various substances of a lytic nature.

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