DOI: 10.56871/RBR.2023.97.69.004
UDC 616.98+578.8+616.314-008.1-036.1-08+579.61+691.175+678

# EVALUATION OF THE GENUS *CANDIDA* FUNGI ADHESIVE PROPERTIES ON THE MATERIALS USED IN DENTISTRY

© Anna V. Zachinyaeva[1], Yaroslav V. Zachinyaev[2], Dmitriy P. Gladin[1], Ilya A. Baranov[3], Anna S. Nabieva[1], Oleg G. Gorbunov[1], Anna N. Andreeva[1]

[1] Saint Petersburg State Pediatric Medical University. Lithuania 2, Saint Petersburg, Russian Federation, 194100
[2] Petrozavodsk State University. Lenin str. 33, Petrozavodsk, Russian Federation, Republic of Karelia, 185910
[3] North-Western State Medical University named after I.I. Mechnikov. Piskarevskiy pr. 47, 195067, Kirochnaya str., 41, 191015, Saint Petersburg, Russian Federation

**Contact information:** Anna V. Zachinyaeva — Doctor of Biological Sciences, Professor, Department of microbiology, virology and immunology. E-mail: anvz60314@gmail.com ORCID ID: 0000-0003-1521-3060 SPIN: 5208-2419

***For citation:*** Zachinyaeva AV, Zachinyaev YaV, Gladin DP, Baranov IA, Nabieva AS, Gorbunov OG, Andreeva AN. Evaluation of the genus *Candida* fungi adhesive properties on the materials used in dentistry // Russian biomedical research (St. Petersburg). 2023;8(4):27-31. DOI: https://doi.org/10.56871/RBR.2023.97.69.004

**Received: 15.09.2023** **Revised: 08.11.2023** **Accepted: 20.12.2023**

**Abstract.** ***Introduction.*** Acrylamide plastics are widely used in orthopedic dentistry. Studies of their susceptibility to microbial adhesion are relevant, since the restorative material made on the basis of these polymers can become a reservoir for microorganisms that can infect peri-implant tissues and cause inflammation. ***The purpose of the study*** was to test the adhesive ability of clinical strains of *Candida* to samples of acrylamide plastics. ***Materials and methods.*** 50 clinical strains of *Candida* fungi have been studied for the formation of biofilms during cultivation on acrylamide plastics. Plastic samples were treated with an inoculum of fungal cultures for 48 h at 37 °C. The values of their optical density (λ=560 nm) were a quantitative assessment of the biomass of the formed films. ***The results of the study.*** Quantitative analysis of the biofilm biomass showed that after 48 h all fungal strains formed a biofilm on the surface of the tested polymer discs. The highest quantitative values of the biofilm biomass were noted in the cultivation of *C. albicans.* ***Conclusion.*** It was noted that the type of material is not a key growth restriction factor for *C. albicans*. A set of measures is needed that combines optimal mechanical processing methods with the use of antimicrobial drugs to prevent the formation and accumulation of biofilms.

**Key words:** fungi of the genus *Candida*; *Candida albicans*; biofilms; acrylamide plastics.

# ОЦЕНКА АДГЕЗИВНЫХ СВОЙСТВ ГРИБОВ РОДА *CANDIDA* НА МАТЕРИАЛАХ, ИСПОЛЬЗУЕМЫХ В СТОМАТОЛОГИИ

© Анна Владимировна Зачиняева[1], Ярослав Васильевич Зачиняев[2], Дмитрий Павлович Гладин[1], Илья Андреевич Баранов[3], Анна Сергеевна Набиева[1], Олег Геннадьевич Горбунов[1], Анна Николаевна Андреева[1]

[1] Санкт-Петербургский государственный педиатрический медицинский университет.
194100, Российская Федерация, г. Санкт-Петербург, ул. Литовская, 2
[2] Петрозаводский государственный университет. 185910, Российская Федерация, Республика Карелия, г. Петрозаводск, пр. Ленина, 33
[3] Северо-Западный государственный медицинский университет им. И.И. Мечникова. 195067, Российская Федерация, г. Санкт-Петербург, Пискаревский пр., 47; 191015, ул. Кирочная, 41

**Контактная информация**: Анна Владимировна Зачиняева — д.б.н., профессор, доцент кафедры микробиологии, вирусологии и иммунологии. E-mail: anvz60314@gmail.com ORCID ID: 0000-0003-1521-3060 SPIN: 5208-2419

***Для цитирования:*** Зачиняева А.В., Зачиняев Я.В., Гладин Д.П., Баранов И.А., Набиева А.С., Горбунов О.Г., Андреева А.Н. Оценка адгезивных свойств грибов рода *Candida* на материалах, используемых в стоматологии // Российские биомедицинские исследования. 2023. Т. 8. № 4. С. 27–31. DOI: https://doi.org/10.56871/RBR.2023.97.69.004

**Поступила: 15.09.2023** **Одобрена: 08.11.2023** **Принята к печати: 20.12.2023**

**Резюме.** ***Введение.*** Акриламидные пластмассы широко используются в ортопедической стоматологии. Исследования их восприимчивости к микробной адгезии актуальны, поскольку изготовленный на основе этих

полимеров реставрационный материал может стать резервуаром для микроорганизмов, которые могут поражать периимплантные ткани и вызывать воспаление. ***Целью исследования*** было проверить адгезионную способность клинических штаммов грибов рода *Candida* к образцам акриламидных пластмасс. ***Материалы и методы.*** *И*сследовано 50 клинических штаммов грибов рода *Candida* на предмет образования биопленок при культивировании на акриламидных пластмассах. Образцы пластмасс обрабатывали инокулятом культур грибов в течение 48 ч при 37 °С. Количественной оценкой биомассы сформировавшихся пленок были значения их оптической плотности (λ=560 нм). ***Результаты исследования.*** Количественный анализ биомассы биопленки показал, что через 48 ч все штаммы грибов образовали биопленку на поверхности тестируемых полимерных дисков. Самые высокие количественные значения биомассы биопленок были отмечены при культивировании *C. albicans*. ***Заключение.*** Было отмечено, что тип материала не является ключевым фактором ограничения роста для *C. albicans*. Необходим комплекс мероприятий, сочетающий оптимальные методы механической обработки с использованием антимикробных препаратов для предотвращения образования и накопления биопленок.

**Ключевые слова:** грибы рода *Candida*; *Candida albicans*; биопленки; акриламидные пластмассы.

## INTRODUCTION

The oral cavity is the most important biotope of the human body which is inhabited by various microflora represented by more than 700 kinds of the microorganisms and which plays an important role in cooperation of the human body with its environment [6]. A specific microbiota inhabits the oral cavity and tends to colonize the surfaces of the teeth, tongue and mucous membranes of the mouth, soft tissues, tooth implants and restorative materials.

The microorganisms colonizing the oral cavity mainly remain in the biotopes where the biofilm formation takes place. As a part of biofilms the microbes become more resistant to the immune factors, antibiotics etc. instead of those solely inhabiting ones, this factor promotes and aggravates such oral conditions as caries, periodiodontal disorders, infections associated with the implants and candidosis of oropharynx. Polymicrobial biofilm infection on the teeth implants is considered to be the main cause of periimplant diseases. Oral streptococci such as *Streptococcus sanguinis, Streptococcus mutans, Streptococcus oralis* and *Streptococcus mitis* are considered to be the colonization "pioneers" prior to plague formation [11], the process involves both the bacteria and the fungi. Thus the oral cavity is being colonized by various genus *Candida* fungi which are commonly associated with the disorders of the mucous membranes of the oral cavity. *Candida albicans*, *Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei* and *Candida dubliniensis* are most common types among them antibiotic-resistant strains are present [1, 4]. It is revealed that genus *Candida* fungi are characterized by expressive adhesive properties and they can be absorbed in biotic and abiotic surfaces including acrylic dentures. Adhesion and colonization seem the first and the most important stage in infectious process underlying and preceding the biofilm formation which involves not only the mucous membranes but also the surfaces of the medical devices [3], thus, resulting in infection and the following medical assistance. Nowadays the genus *Candida* fungi ability to produce biofilms is considered as the most important factor of virulence [5], resulting in grave clinical conditions. Genus *Candida* fungi present in biofilms is highly resistant to antifungal therapy and resists to some immune factors of the host.

## OBJECTIVE OF THE STUDY

The purpose of the study was to test the adhesive ability of clinical isolates of *Candida* strains to samples of acrylamide plastics which is used in prosthetics.

## MATERIALS AND METHODS

Methyl methacrylate plastics Belacryl-M HO (Russia), ethyl methylmethacrylate plastics Belacryl-E HO (Russia) and Protacryl-M material (Ukraine) samples were investigated. 10 samples from each material with the diameter of 20 mm and height of 8 mm were involved. 50 strains of Candida fungi were studied: *C. albicans* — 23, *C. tropicalis* — 14, *C. krusei* — 8, *C. glabrata* — 5. All the strains were cultivated in 5% blood agar (24 h at 37 °C) to obtain separate colonies. To prepare inoculum the cultures were suspended in fluid Biomedia (Saburo) environment until they reached the density of $D_{600}$ 0,025±0,005 (NanoPhotometer N60-Touch, Germany). The tested materials were placed in the plates (Fudau Biotechnology, Russia) were 2 ml of inoculum were introduced. Cultivation lasted 48 hours at 37 ° C. After incubation the samples were thoroughly washed twice in phosphate buffered saline (PBS), pH 5.0, to remove planktonic cells. The biofilm biomass was assessed by the method [2] according to the research modi-

*Table 1*

**Quantitative characteristics of biofilm biomass based on absorption values depending on the type of restoration material**

*Таблица 1*

**Количественные характеристики биомассы биопленки на основе значений поглощения в зависимости от типа реставрационного материала**

| Материал / Material | *C. albicans* | *C. glabrata* | *C. tropicalis* | *C. krusei* |
|---|---|---|---|---|
| Белакрил-М ХО / Belacril-M HO | 2,62 | 1,29 | 1,54 | 1,9 |
| Белакрил-Э ХО / Belacril-E HO | 2,07 | 1,43 | 1,8 | 1,54 |
| Протакрил-М / Protacril-M | 2,19 | 2,0 | 2,04 | 1,62 |

*Table 2*

**Number of viable microorganisms in biofilms, expressed in colony forming units (CFU/ml)**

*Таблица 2*

**Количество жизнеспособных микроорганизмов в биопленках, выраженное в колониеобразующих единицах (КОЕ/мл)**

| Материал / Material | *C. albicans* | *C. glabrata* | *C. tropicalis* | *C. krusei* |
|---|---|---|---|---|
| Белакрил-М ХО / Belacril-M HO | $5,87\times10^6$ | $1,63\times10^6$ | $2,28\times10^6$ | $2,86\times10^6$ |
| Белакрил-Э ХО / Belacril-E HO | $5,14\times10^6$ | $1,80\times10^6$ | $2,62\times10^6$ | $2,24\times10^6$ |
| Протакрил-М / Protacril-M | $5,99\times10^6$ | $3,05\times10^6$ | $3,14\times10^6$ | $2,24\times10^6$ |

fication: the biofilm stained by gentian violet was extracted with ethanol, decanted, and separated 20 times to assess the optical density by spectrophotometer (PE-5400 UV) at a wavelength of 560 nm. Sterile disks were used as a negative control.

The amount of viable microorganisms in the biofilm was detected by counting colony forming units (CFU). After biofilm formation the samples were thoroughly washed three times in 1 ml of PBS to remove the cells which were not detached. Then they were placed in centrifuge tubes filled with 1 ml of PBS, they were vigorously shaked for 2 min in LAUDA Varioshake VS 15 R (Germany) to disperse the cells which sticked to the disk surface. The cellular suspension of each sample was three times consequently dissolved in PBS ten times and applied Saburo agar. They were incubated for 48 h at 37° C. The amount of the viable cells which formed the colonies was indicated in CFU/mL. All the results are represented statistically according to STATISTICA 12.0.

## RESULTS AND DISCUSSION

The quantitative analysis of the biomass formed on the biofilm revealed that all strains of the fungi formed the biofilm on the surface of the tested polymer disks in 48 h. The quantitative analysis noted the highest number of biofilm biomass represented by of *C. albicans* (Table 1).

High values of biofilm biomass in cultivation of *C. albicans* to a great extent are associated with the adhesive activity of these fungi. In comparison with the other *Candida* genus, *C. albicans* forms complicated biofilms which are composed of a merging basal layer of blastospores and covered with thick layer of matrix and containing extracellular material and hypha. The other isolates form only blastospore basal layer [8;10]. Morphological transition of *C. albicans* from yeast to micelial cells is one of the main factors of virulence as it contributes not only to significant spread of the culture on the polymer surface but also impairment of the mucous membrane of the oral cavity caused by acid proteases.

The results of calculation of the viable cells derived from the biofilms referred to the dynamic of biofilm biomass formation by different genera of *Candidae* (Table 2).

It was noted that the type of the material was not a limiting key factor for *C. albicans* growth. Though there was decrease of *C. glabrata* and *C. tropical* growth on the surface of Belacryl-M HO.

Survival rate of *C. albicans* on polymer materials is associated with extracellular polymer matrix formation ability, which overlaps the cells and pseudohyphae of the fungi, thus, it prevents the inhibiting factors [12].

Acrylamyd plastics are the most common type of polymer materials in dentistry. Nevertheless there are no extended research works concerning biofilm formation on these materials. Microorganisms which adhere to the dental implants and dentures can cause different infectious processes, aggravating pulp pathology in particular [7, 9]. Intensive biofilm formation on the restorative materials in the oral cavity

is revealed in a day, even a temporary restorative material can aggravate the oral health condition, which requires measures to prevent accumulation of the microorganisms and formation of the biofilm.

## CONCLUSION

All the investigated genus *Candida* types have shown high capability in biofilm production in all mentioned above acrylamide plastic samples. It was revealed that *Candida albicans* was characterized by the highest quantitative value prevalence. Intensive biofilm formation by the fungi on these materials during the restorative work in the oral cavity is a risk factor for infectious complications. Complex of measures including optimum mechanical debridement in combination with antimicrobial medications are required for effective oral hygiene.

## ADDITIONAL INFORMATION

**Author contribution.** Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

**Competing interests.** The authors declare that they have no competing interests.

**Funding source.** This study was not supported by any external sources of funding.

## ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

**Вклад авторов.** Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

**Источник финансирования.** Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

## REFERENCES

1. Antonova Ye.A., Kosyakova K.G. Griby roda Candida — vozbuditeli infektsionnykh sostoyaniy. Zdorov'ye — osnova chelovecheskogo potentsiala. [Fungi of the genus Candida are causative agents of infectious conditions. Health is the foundation of human potential]. 2019; 14(1): 455–9. (in Russian).
2. Zachinyayeva A.V., Zachinyayev Ya.V. Deystviye poligeksametilenguanidina na aktivnost' katalazy griba Pyricularia oryzae. [Effect of polyhexamethylene guanidine on the activity of catalase in the fungus Pyricularia oryzae]. Uspekhi meditsinskoy mikologii. 2022; 23: 245–6. (in Russian).
3. Kozlova N.S., Barantsevich N.Ye., Kosyakova K.G. i dr. Chuvstvitel'nost' k antibiotikam enterobakteriy, vydelennykh v dvukh statsionarakh dvukh rayonov Sankt-Peterburga. [Antibiotic sensitivity of enterobacteria isolated in two hospitals in two districts of St. Petersburg]. Problemy meditsinskoy mikologii. 2017; 19(1): 34–42. (in Russian).
4. Kozlova N.S., Nesterova Ye.V., Vasiliyev O.D., Selezneva A.A. Spektry ustoychivosti k antifungal'nym preparatam shtammov Candida spp., vydelennykh v kozhno-venerologicheskom dispansere. [Spectra of resistance to antifungal drugs of Candida spp. strains isolated in the dermatovenerological dispensary]. Uspekhi meditsinskoy mikologii. 2023; 24: 221–5. (in Russian).
5. Kozlova N.S., Nesterova Ye.V., Trofimova N.N. i dr. Antibiotikorezistentnost' shtammov Sandida spp., vydelennykh u bol'nykh dermatozami. Zdorov'ye — osnova chelovecheskogo potentsiala: problemy i puti ikh resheniya. [Antibiotic resistance of Candida spp. strains isolated from patients with dermatoses. Health is the basis of human potential: problems and ways to solve them]. 2021; 16(2): 429–35. (in Russian).
6. Kuz'mina D.A., Novikova V.P., Shabashova N.V., Orishak Ye.A. Candida spp. i mikrobotsenoz polosti rta u detey s dekompensirovannoy formoy kariyesa. [Candida spp. and microbiocenosis of the oral cavity in children with decompensated forms of caries]. Problemy meditsinskoy mikologii. 2011; 13(1): 23–7. (in Russian).
7. Bertolini M., Costa R.C., Barão VAR. Oral Microorganisms and Biofilms: New Insights to Defeat the Main Etiologic Factor of Oral Diseases. Microorganisms. 2022; 10(12): 2413.
8. Cássia M. de Souza, Murilo M. dos Santos, Luciana Furlaneto-Maia. Adhesion and biofilm formation by the opportunistic pathogen Candida tropicalis: what do we know? Canadian Journal of Microbiology. 2023; 69(6): 207–18.
9. Engel A.S., Kranz H.T., Schneider M. et al. Biofilm formation on different dental restorative materials in the oral cavity. BMC Oral Health. 2020; 20 (1): 162.
10. Kuhn D.M., Chandra J., Mukherjee P.K. Comparison of Biofilms Formed by Candida albicans and Candida parapsilosis on Bioprosthetic Surfaces. Infect Immun. 2002; 70(2): 878–88.
11. Mazurek-Popczyk J., Nowicki A., Arkusz K. et al. Valuation of biofilm for-mation on acrylic resins used to fabricate dental temporary restorations with the use of 3D printing technology. BMC Oral Health. 2022; 22(1): 442.
12. Paulone S., Malavasi G., Ardizzoni A. Candida albicans survival, growth and biofilm formation are differently affected by mouthwashes: an in vitro study. New Microbiologica. 2017; 40(1): 45–52.

## ЛИТЕРАТУРА


1. Антонова Е.А., Косякова К.Г. Грибы рода Candida — возбудители инфекционных состояний. Здоровье — основа человеческого потенциала. 2019; 14(1): 455–9.
2. Зачиняева А.В., Зачиняев Я.В. Действие полигексаметиленгуанидина на активность каталазы гриба Pyricularia oryzae. Успехи медицинской микологии. 2022; 23: 245–6.
3. Козлова Н.С., Баранцевич Н.Е., Косякова К.Г. и др. Чувствительность к антибиотикам энтеробактерий, выделенных в двух стационарах двух районов Санкт-Петербурга. Проблемы медицинской микологии. 2017; 19(1): 34–42.
4. Козлова Н.С., Нестерова Е.В., Васильев О.Д., Селезнева А.А. Спектры устойчивости к антифунгальным препаратам штаммов *Candida spp*., выделенных в кожно-венерологическом диспансере. Успехи медицинской микологии. 2023; 24: 221–5.
5. Козлова Н.С., Нестерова Е.В., Трофимова Н.Н. и др. Антибиотикорезистентность штаммов *Candida spp*., выделенных у больных дерматозами. Здоровье — основа человеческого потенциала: проблемы и пути их решения. 2021; 16(2): 429–35.
6. Кузьмина Д.А., Новикова В.П., Шабашова Н.В., Оришак Е.А. Candida spp. и микробоценоз полости рта у детей с декомпенсированной формой кариеса. Проблемы медицинской микологии. 2011; 13(1): 23–7.
7. Bertolini M., Costa R.C., Barão VAR. Oral Microorganisms and Biofilms: New Insights to Defeat the Main Etiologic Factor of Oral Diseases. Microorganisms. 2022; 10(12): 2413.
8. Cássia M. de Souza, Murilo M. dos Santos, Luciana Furlaneto-Maia. Adhesion and biofilm formation by the opportunistic pathogen Candida tropicalis: what do we know? Canadian Journal of Microbiology. 2023; 69(6): 207–18.
9. Engel A.S., Kranz H.T., Schneider M. et al. Biofilm formation on different dental restorative materials in the oral cavity. BMC Oral Health. 2020; 20 (1): 162.
10. Kuhn D.M., Chandra J., Mukherjee P.K. Comparison of Biofilms Formed by Candida albicans and Candida parapsilosis on Bioprosthetic Surfaces. Infect Immun. 2002; 70(2): 878–88.
11. Mazurek-Popczyk J., Nowicki A., Arkusz K. et al. Valuation of biofilm for-mation on acrylic resins used to fabricate dental temporary restorations with the use of 3D printing technology. BMC Oral Health. 2022; 22(1): 442.
12. Paulone S., Malavasi G., Ardizzoni A. Candida albicans survival, growth and biofilm formation are differently affected by mouthwashes: an in vitro study. New Microbiologica. 2017; 40(1): 45–52.