Original paper

Effectiveness of methods for removing the *Candida albicans* biofilm from the dental acrylic surface

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Abstract

Background. Approximately half of the adult population in Europe have used some form of dental prosthesis. Much effort has been put into developing denture cleaning methods and the most recommended are brushing the prosthesis after meals and cleaning it with special liquids (sometimes prepared just before the procedure). However, these simple techniques are often omitted or insufficient due to, i.e., age-related mental or motor disabilities.

Objectives. The aim of the study was to compare a range of techniques that can be performed at home and do not require patient dexterity in order to find the most efficient method of reducing the viability of the *C. albicans* biofilm and removing it from acrylic surfaces.

Material and methods. The 20 mm × 25 mm × 1 mm unpolished acrylic plates were inoculated with *C. albicans* and incubated for 72 h. Plates with formed biofilms were divided into 6 equal groups: a control group and 5 groups for different cleaning procedures: a dental cleaner with liquid, a dental cleaner with phosphate-buffered saline (PBS), air drying, antiseptic liquid, and an ultrasonic cleaner. Biofilm viability was assessed by plating serial dilutions and counting the colonies of *C. albicans* on the Sabouraud dextrose agar (SDA) medium.

Results. The study found that both MultiClean fluid and Sonic-3 ultrasonic cleaner were effective against *Candida* cells. MultiClean fluid showed the strongest biocidal properties, both when used with the Sonic Denture Cleaner and independently.

Conclusions. Cleaning acrylic surfaces with a dental cleaner followed by antiseptic liquid is more effective than using these methods separately.

Keywords: *Candida albicans*, polymethyl methacrylate, denture cleaners

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Introduction

Approximately half of the adult population in Europe have used some form of dental prosthesis. Removable dental prostheses are used by 13–29% of adults. Among them, 3–13% of edentulous subjects wear complete dentures in both jaws.[1] In Poland, more than 66% of people aged >60 years wear dental prostheses, and the use of dentures increases with age.[2] Due to the material characteristics of dentures[3] and the favorable environmental conditions that they create for the growth of microorganisms, denture wearers often suffer from denture stomatitis.

Denture stomatitis is an inflammatory process that occurs due to the use of removable prosthetic restorations. Poor oral and denture hygiene is the cause of polymicrobial biofilm formation on the surfaces of prostheses. *Streptococcus* species and *Staphylococcus aureus* were the most common microorganisms isolated from the dental prosthesis. Prolonged usage of the prosthesis resulted in the growth of other bacteria, including *Escherichia coli*, coryneform bacteria and *Micrococcus*.[4] The dental prosthesis offers a reservoir for microorganisms associated with bacterial endocarditis, aspiration pneumonia, gastrointestinal infections, and chronic obstructive pulmonary disease. Gram-positive cocci such as *Streptococcus mutans* show synergistic interactions within biofilm formations in inter-kingdom biofilms.[5] These are often complicated by fungal infection.[6,7] The adhesion of *Candida* strains to the mucosa and dentures as well as the formation of biofilms are responsible for the development of *Candida* stomatitis.[8,9]

Candida albicans is the most common commensal of the digestive and reproductive tract mucosa and, under some conditions, becomes the most common fungal pathogen. It is present in the oral microbiome of 30–75% of the world population.[6,10] The immune system activity modulates its colonization of numerous niches in the human body. When immune reactions are suppressed to some extent, for example, as a result of corticosteroids, diabetes or elimination of the natural human microbiota, these yeasts can cause local infections of the mucous membranes and, in predisposed individuals, can lead to life-threatening invasive infections.[11] One of the key mechanisms of the pathogenicity of *C. albicans* is its ability to form biofilms on the surfaces of host tissues and inanimate surfaces (catheters, prostheses, etc.).[12,13] These structures are difficult to clean and often act as reservoirs for infection. Therefore, proper hygiene of dental prostheses, including effective removal of biofilms, is crucial to prevent denture stomatitis.

The biofilm of *C. albicans* is difficult to remove from acrylic resin surfaces. The most common physical method of controlling plaque development is brushing with a denture brush, which can remove 90–96% of yeast cells from the acrylic surface.[14,15] However, the effectiveness of brushing depends on the skill of the person performing the task,[14] and can be reduced due to diseases and disorders common to older people. Alternative methods, including dedicated mechanical and chemical cleaners, may solve the problem of denture hygiene. Older people who develop dementia are at an increased risk of developing oral health problems due to diminished self-care and motor skills. Daily removal of denture plaque is essential in maintaining good oral health, especially in older age.[16]

Denture stomatitis can be prevented by controlling the formation of *C. albicans* biofilms on the acrylic.[8,9,17,18] This approach is critical in preventing the overgrowth of mycobiota and the development of oral mucosal infection. Significant effort has been put into developing effective cleaning methods. The most recommended is brushing with a denture brush after meals and cleaning with dedicated liquids (sometimes prepared just before the procedure). However, these simple measures are often neglected or insufficient due to age-related mental and motor disabilities.

A few independent studies on this topic have tested commercially available denture cleaning devices, but the results were contradictory. This study compared a range of methods that can be used at home and do not require patient dexterity, such as cleaning with sonification and denture liquid, to find the most efficient method of limiting the viability of *C. albicans* biofilms and removing them from acrylic surfaces for patients with limited motor skills. The study employed an in vitro single-species biofilm model.

Material and methods

Preparation of acrylic plates

A total of 180 acrylic plates made of routine denture material, Vertex Rapid Simplified (Vertex Dental, Soesterberg, The Netherlands), were prepared using the method described by Johnson et al.[19] First, molds were made for the casting of stone acrylic tiles (type III). Standard flasks were used.

While still warm, the stone was coated with sodium alginate solution and left to dry. Following the manufacturer's instructions, the acrylic resin was prepared by mixing 2.3 g of acrylic powder with 1 mL of water. The liquid was measured and poured into a clean and dry mixing vessel. The powder was added slowly, ensuring that each particle was wetted by the monomer.

After the powder was mixed with liquid, the mixture was stirred and left to settle in a closed container for 15 min. After this time, the acrylic mass had the consistency of dough; it was placed in the mold and pressed firmly until the halves of the mold joined. The flask was

placed in a spring clamp and tightened. Then, it was placed in a water curing bath at room temperature that was subsequently raised to 100°C. The boiling time was 2 h. Afterwards, the flask was removed from the curing bath, and the clamps were released to open the flask and remove the plates.

According to denture manufacturing procedures, it is recommended that denture surfaces in close proximity to mucosa are not polished. During the polishing treatment, acrylic material is lost, which may result in a poorer fit of the finished denture. The unpolished acrylic plates used in this study imitated the real conditions.

Strain and inoculum preparation

The effectiveness of the cleaning method was evaluated by using a *C. albicans* biofilm model. We adapted the technique described by Krom and Willems.[20] The biofilm-forming strain, *C. albicans* LIG.1.2. F.A. was isolated from the human oral cavity.[21] A loop of overnight yeasts cultured on Sabouraud dextrose agar (SDA; BioMaxima S.A., Lublin, Poland), was used to prepare the standardized suspension (10^7 CFU/mL). Subsequently, the yeast suspension was diluted to 1:25 in Roswell Park Memorial Institute (RPMI) 1640 medium (cat. No. R6504; Sigma-Aldrich), supplemented with 18 g of glucose and buffered with 3-(N-morpholino)propanesulfonic acid (MOPS; Fluorochem UK, Hadfield, UK) for further use.

Biofilm formation

The 180 unpolished acrylic plates measuring 20 mm × 25 mm × 1 mm were used in this experiment. Before use, all plates were sterilized with ethylene oxide. Next, they were placed in duplicate into Petri dishes (Φ 5 cm) and sunk in 10 mL of the previously inoculated RPMI 1640 medium. The incubation was carried out at 35 ±2°C for 4 h to ensure that the cells could adhere. The medium was then changed, and further incubation was carried out at 35 ±2°C for 72 h, with the medium changed daily.

Evaluation of antibiofilm properties

Acrylic plates with a formed biofilm were divided into 6 groups of 30 pieces each: one positive control group (1) and 5 groups for different cleaning procedures: (2) Sonic Denture Cleaner, consisting of a vibration-generating part and a removable denture container (referred to as DC or dental cleaner; Roko Dental Systems, Częstochowa, Poland), used simultaneously with MultiClean antiseptic fluid (30–40% of potassium peroxymonosulfate and <5% of potassium persulfate, referred to as liquid and prepared according to the manufacturer's instruction: adding 5 g of MultiClean powder to 200 mL of water, stirring and leaving for 3 min before use; Rokodent); (3) dental cleaner with phosphate buffered saline (PBS); (4) air drying; (5) MultiClean antiseptic fluid; and (6) ultrasonic cleaner (Sonic-3; Polsonic Palczyński Sp. J., Warsaw, Poland) corresponding to the test procedure. Cleaning the samples with an ultrasonic cleaner was used as a negative control because, according to the literature,[15] no growth was expected when using this method. Similar to the positive control (no intervention), the negative control provides a benchmark for the results obtained with other experimental methods.

All cleaning procedures were carried out by the same person on a single day to ensure maximum repeatability.

The plates were washed with PBS and air-dried for 15 min. The following test procedures were then carried out in the plate groups: (2) 2 cycles of 7 min in DC using 200 mL of antiseptic liquid; (3) 2 cycles of 7 min at 25°C using DC with PBS; (4) air drying for 24 h; (5) immersion in 200 mL of MultiClean antiseptic fluid for 20 min at 25°C, following the manufacturer's recommended procedure; (6) ultrasound cleaning in 2 L of distilled water for 7 min using a Sonic-3 ultrasonic cleaner at 25°C with a 40 kHz frequency/160 W. The positive control group (1) was processed for further actions.

After the cleaning procedures, the plates were washed again with PBS and divided into 2 groups of 90 pieces each. In the first group of plates, 2 biofilm cell samples of 1 cm^2 each were collected with a swab from the same surface. The swab was vortexed for 20 s with 1 mL of saline, and a series of 10-fold dilutions was prepared. The dilutions were inoculated on SDA and incubated at 25 ±2°C for 24 h, after which the yeast colonies were counted.

The second half of the plates, from which the biofilm was not collected, were stained with 0.1% crystal violet solution for 30 min. The stained biofilm was washed with cold water, air-dried for 24 h, and 2 biofilm cell samples of 1 cm^2 each were collected from the plates with a scalpel. Each sample was then placed in a well of a 96-well microtiter plate and discolored with ethyl alcohol for 2 h. The alcohol with the dissolved stain was transferred to fresh microtiter plates. The amount of the biofilm was estimated by measuring the absorbance of the stained alcohol at a wavelength of 560 nm.

Statistical analysis

Statistical calculations were conducted using the R 4.1.1 statistical package.[22] The Kruskal–Wallis χ^2 test for non-parametric variables was used to test the differences between the groups. Pairwise comparisons were evaluated by using the Tukey and Kramer (Nemenyi) test for post hoc analysis with a Tukey lambda distribution approximation for independent samples. A p-value <0.05 was considered significant.

Results

Evaluation of *C. albicans* viability

The first step of this research involved determining the effect of cleaning procedures on biofilm viability by comparing the recovery of colony forming units in samples treated with the tested methods to positive and negative control groups (samples untreated and cleaned with an ultrasonic cleaner, respectively). This allowed us to assess the cell-damaging or cell-killing potential of the cleaning methods.

Of all the methods tested, air drying (Tukey and Kramer (Nemenyi) test, p = 0.41) and DC with PBS (p = 0.99) did not result in a significant reduction in recovered colonies of *C. albicans* compared to the control samples. MultiClean fluid and Sonic-3 ultrasonic cleaner (p = 0.00) showed effective activity against *Candida* cells. MultiClean fluid showed the strongest biocidal properties when used with the Sonic Denture Cleaner (p = 0.00) and independently (p = 0.00). Detailed data is presented in Fig. 1 and Table 1.

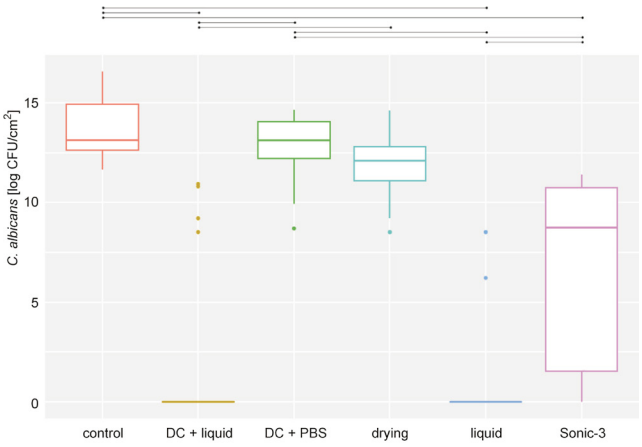

Fig. 1. Impact of different cleaning procedures on the recovery of *Candida albicans* cells

The Kruskal–Wallis test revealed significant differences between the cleaning methods (χ^2 = 82.302, df = 5, p = 2.768e-16). The line represents the median, the box shows the interquartile range, the whiskers stand for the minimum and maximum ranges, and the colored circles represent the outliers. The horizontal lines indicate statistically significant differences between variables.
Cleaning methods: control – untreated samples; DC + liquid – Sonic Denture Cleaner and MultiClean fluid; DC + PBS – Sonic Denture Cleaner and phosphate-buffered saline; drying – air-dried samples; liquid – MultiClean fluid; Sonic-3 – Sonic-3 ultrasonic cleaner.

Assessment of biofilm biomass: Crystal violet staining

Crystal violet staining shows the effectiveness of the cleaning method on the biofilm removal without assessing its viability. The Tukey and Kramer (Nemenyi) test showed that the ultrasonic cleaner (p = 0.00) and the Sonic Denture Cleaner with PBS (p = 0.00) almost completely removed the biofilm from the surface of the acrylic plates. Air drying (p = 1.00), MultiClean fluid (p = 0.21), and the Sonic Denture Cleaner with MultiClean fluid (p = 1.00) were insufficient to remove *C. albicans* cells from the acrylic plates compared to the control group. Detailed data is presented in Fig. 2.

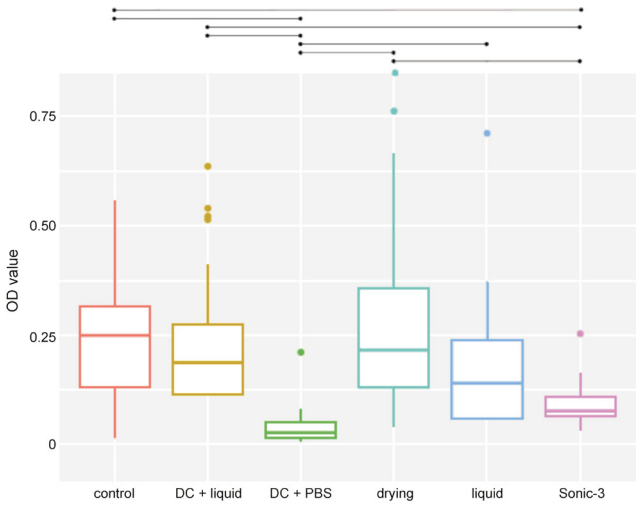

Fig. 2. Effectiveness of different cleaning procedures on biofilm removal as measured by crystal violet staining

The Kruskal–Wallis test revealed significant differences between the cleaning methods (χ^2 = 65.473, df = 5, p = 0.000); OD – optical density.

Table 1. *Candida albicans* count in the evaluated study groups

Group	Variable [CFU/cm²]					
	mean	median	min	max	Q1	Q3
Control	2,319,588	505,000	116,000	15,945,000	260,000	3,082,500
DC + liquid	6,694	0	0	56,000	0	2,500
DC + PBS	764,222	510,000	6,000	2,295,000	209,750	1,477,500
Drying	308,778	179,750	5,000	2,240,000	62,250	337,500
Liquid	583	0	0	5,000	0	0
Sonic-3	24,278	6,250	0	90,000	0	58,750

Cleaning methods: control – untreated samples; DC + liquid – Sonic Denture Cleaner and MultiClean fluid; DC + PBS – Sonic Denture Cleaner and phosphate-buffered saline; drying – air-dried samples; liquid – MultiClean fluid; Sonic-3 – Sonic-3 ultrasonic cleaner.

Discussion

The surfaces of removable dentures used by patients are often covered with plaque, which can promote infections in the denture-bearing area. In the case of partial dentures, inflammation can be observed on the gingival surfaces that are in contact with the denture plate. Plaque can be removed from dentures by using several different methods, such as brushing with a denture brush or soaking the plaque in chemical solutions (e.g., Corega tabs), as well as using devices such as microwave ovens or ultrasonic apparatus.[23] If patient dexterity has decreased with age, effective cleaning of the dentures becomes more difficult, which can significantly affect the health of the oral tissues.

This study aimed to compare denture cleaning methods that do not require dexterity and are available for home use.

Some patients believe that drying the prosthesis overnight dehydrates and kills microbes present on the denture. It can hardly be called a hygienic procedure, but it does not require skilled hand movements or the help of third parties in its application. The results of this study showed that this method was not effective in killing *C. albicans* biofilm cells. Drying the dentures does not involve any actions that would remove the biofilm from its surface or even lead to its fixation (Fig. 3). This method should never be recommended for patients with limited dexterity.

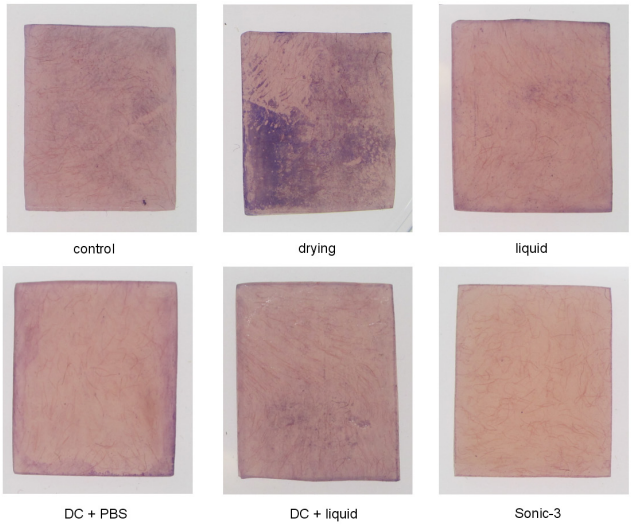

Fig. 3. Acrylic plates with *Candida* biofilm stained with crystal violet

The second method often used by patients is placing the dentures in the denture cleaning fluid. This method does not require a lot of dexterity but may require a little help in preparing the liquid, especially for people with diseases that affect cognitive abilities. The results show a significant antifungal activity of the liquid used in this study, but, like drying, it does not remove the biofilm from the surface of the prosthesis.

Other studies have shown that peroxide-based cleansers are the most effective cleaning method.[12,14,15,24] However, de Andrade et al.[25] and Nishi et al.[26] did not observe any antifungal activity resulting from the use of denture cleaning tablets alone, which is in contradiction with the results of the present study. The explanation for this situation may be the use of the single-species rather than the multi-species biofilm model in the aforementioned studies, which included the *Streptococcus* species. The *Streptococcus–Candida* biofilm tends to be more difficult to break due to synergistic actions between these microorganisms.[5] Peroxide-based cleansing solutions were found to be highly effective in killing *C. albicans* cells, but they tended to fix the biofilm structure,[25] which could then act as a base and a medium for microorganisms to rebuild the biofilm. These results are consistent with the outcomes of our study.

Sonic cleaning methods involve the mechanical removal of the biofilm from the surface coupled with biocidal activity by using sonic or ultrasonic vibrations to disintegrate the biofilm and release cells; however, the use of only ultrasonic waves with specific parameters can damage yeast cells.[27]

The tools for sonic cleaning of dentures used in this study included a professional ultrasonic cleaner and devices that generate vibrations to mimic ultrasound (Sonic Denture Cleaner with MultiClean fluid or with PBS). The Sonic Denture Cleaner, due to its size and ease of use, was considered an alternative method of denture cleaning for individuals with limited dexterity. Vibrations generated by this device effectively removed the biofilm from the surface of the plates, but no reduction in the cell viability was observed. The results obtained with the Sonic Denture Cleaner and PBS regarding the biofilm removal were comparable to those of the ultrasonic cleaner. Although the technical specifications were not provided by the manufacturer, due to its structure, method of operation and power source, it can be concluded that the Sonic Denture Cleaner generates a mechanical wave at a much lower frequency and less power than an ultrasonic cleaner, which resulted in a much lower efficiency of the Sonic Denture Cleaner in damaging the structure of the biofilm. However, the combination of the Sonic Denture Cleaner with MultiClean fluid had the strongest anticandidal properties.

Ultrasonic devices without peroxide-based cleaning agents were not effective in reducing the number of *C. albicans* cells in complete dentures.[25,26] However, according to Ghazal et al.,[15] the use of ultrasonic cleaners resulted in a significant reduction in the number of *C. albicans* cells, and their findings are relatively consistent with our results. Despite the efficiency with which ultrasonic cleaning disintegrates biofilms, its main disadvantage is the lack of access to these cleaners. Ultrasonic devices capable of generating frequencies that reduce the viability and adhesion of biofilms are used primarily by professionals.[25]

Pellizarro et al.[28] suggested that the best results could be obtained by combining chemical and mechanical methods. They reported that sodium carbonate peroxide solutions can remove *C. albicans* from denture bases; however, soaking dentures in antiseptic liquid should be combined with brushing to control fungal growth more effectively. A similar effect can be achieved by cleaning the denture with the Sonic Denture Cleaner, followed by soaking in a disinfectant. Combining chemical disinfectants with dental cleaners might help avoid the difficulties associated with decreased mobility. However, simultaneous use of peroxide-based cleaners and ultrasonic or sonic dental cleaners may not produce the desired results due to the biofilm-fixing properties of the antiseptic fluid.

The main limitation of this study is the use of an in vitro model to assess the removal of *C. albicans* biofilms from acrylic surfaces. The results may not be applicable to the cleaning of multi-species biofilms from more complex denture surfaces. Most studies[12,15,26,29] evaluated the effectiveness of denture plaque cleaning methods on removable dentures that have been used for some time. This research model has obvious advantages in that it can confirm the observed ex vivo efficacy. On the other hand, we assessed the ability of different cleaning methods to kill and remove *C. albicans* from a porous material such as prosthetic acrylic. This study is also easily reproducible and adaptable to other biofilm models.

Our approach does not take into account the changes that occur in the physical properties of acrylic during denture use. The introduction of a multi-species biofilm model and coating of the acrylic with saliva would certainly help to obtain a better picture of the effectiveness of these methods.

Conclusions

The Sonic Denture Cleaner is a reasonably efficient tool for removing *C. albicans* biofilms from acrylic surfaces. The combination of cleaning with a dental cleaner and subsequently soaking the acrylic tiles in antiseptic liquid gave better results than using either method separately. Further studies evaluating the effectiveness of these tools for denture cleaning and taking into account multi-species complex biofilm models as well as other clinical conditions associated with denture wearers are required.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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