ORIGINAL ARTICLE

CONTENTS

Experimental study of the adhesive capacity of resident and transient representatives of the oral cavity microflora to different base materials

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ABSTRACT

Aim: To compare the adhesive ability of resident and transient representatives of the oral cavity microflora to various base materials used for the manufacture of removable dentures.

Materials and Methods: The presented report is an experimental study in vitro. 3 types of base materials, namely Vertex, Breflex and Villacryl were used in order to evaluate the adhesive ability of microorganisms. Strains of opportunistic pathogens representing the facultative anaerobic transient microflora of the oral cavity were used for the research. After the cultures cultivation, the number of isolated colonies grown from the microbial cells that adhered to the material sample was counted.

Results: *Staphylococci* adhered to all tested samples of base materials very weakly. Adhesion of methicillin-sensitive *S. aureus* (to Villacryl was at the level of the control material, i.e. glass (adhesion indices constituted 0.58 and 0.64). It was significantly weaker in relation to Breflex (adhesion index was 0.47) and, especially, to Vertex (adhesion index was 0.39).

All materials demonstrated adhesion indices lower than glass for α-hemolytic streptococci and staphylococci. Adhesion indices of Breflex and Villacryl resin for *β-hemolytic streptococci* differed little from the corresponding values of glass.

Conclusions: All tested samples of base materials showed significant biological inertness: the adhesion indices of the vast majority of the tested microorganisms were lower than those of the control material. Oral *streptococci* showed the weakest adhesive ability to Villacryl (average values of adhesion indices 0.51 and 0.68 appropriately). *Staphylococci* and *Candida* yeast-like fungi showed a weak adhesive ability to the samples of base materials, especially to Vertex.

KEY WORDS: oral cavity, removable dentures, base resin, microbial cultures, adhesive capacity

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INTRODUCTION

Microbial adhesion on the denture surface is a serious issue in the clinical findings of prosthetic dentistry. Physical and chemical features of the oral fluid and physiological conditions in the oral cavity as a whole provide an ideal environment for microbial colonization not only of the surface of the mucous membrane and teeth hard tissues, but dental prostheses as well. Despite the relative biological inertness of most modern prosthetic materials, the surfaces of prostheses can easily retain bacteria, fungi and other microorganisms. Adhesion of the oral microflora representatives is the initial stage of the multispecies biofilm formation. This process is facilitated by such factors as the surface roughness, chemical nature and free surface energy of the material of the dental prosthesis [1]. Colonization of the prostheses surfaces by microorganisms followed by the formation of a biofilm makes them a potential permanent source of oral pathogens [2]. This results in prerequisites for the development (or further progression) of tooth decay, periodontitis, and gingivitis. Epidemiological studies show that the use of removable dentures leads to prosthetic stomatitis in 70% of the examined individuals [3]. Dissemination of infectious microorganisms localized on the denture surface leads to the damage of the gastrointestinal tract, cardiovascular and pleuropulmonary systems [4-6].

The ability of microorganisms to adhere to the polished surface of the base of a removable denture plays a key role in the pathogenesis of prosthetic stomatitis. Such adhesion provides an opportunity to withstand mechanical clearance, which is provided by swallowing movements and the constant washing effect of saliva, and to act as a focus for further colonization [7].

Due to the seriousness of the problem of candidal prosthetic stomatitis The study of the adhesion of *Candida albicans* yeast-like fungi to polymer materials attracts considerable attention of researchers [8-10].

AIM

Therefore, the objective of the research was to compare the adhesive ability of resident and transient representatives of the oral cavity microflora to various base materials used for the manufacture of removable dentures.

MATERIALS AND METHODS

The presented report is an experimental study *in vitro*. 3 types of base materials samples, namely Vertex (Vertex-Dental B.V., Netherlands), Breflex (Bredent, Germany) and Villacryl (Zhermapol, Poland) commonly used in the prosthetic dentistry for the manufacture of bases for complete and partial removable dentures, were used in order to evaluate the adhesive ability of laboratory microbial strains of clinical origin.

The protocols of experiments performed in the laboratory of microbiological research of the Department of Microbiology, Virology and Immunology of the Ivano-Frankivsk National Medical University were developed.

Ready resin samples for the experiment had the form of plates with a thickness of 2 mm and an area of 1 cm^2. Glass plates of similar size were used as controls. The study and control samples were placed in a sealed cellophane package and sterilized by X-ray irradiation at a dose of 0.44 mGy for 1.540 s.

Strains of opportunistic pathogens representing the facultative anaerobic transient microflora of the oral cavity were used for the research: *Streptococcus pyogenes* (Group A β-hemolytic streptococcus), *Streptococcus dysgalactiae ssp. equisimilis* (Group G β-hemolytic streptococcus), methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) with associated resistance to fluoroquinolones, macrolides, tetracyclines and aminoglycosides, methicillin-susceptible *Staphylococcus epidermidis*; yeast-like fungi *Candida albicans* and *Candida tropicalis* as well as the mitis group α-hemolytic streptococci (*Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus gordonii*) as the main representatives of the resident microflora of this biotope. Microbial cultures were isolated from the oral mucosa (prosthetic bed, gingival pockets) of the patients with removable dentures with manifestations of prosthetic stomatitis and identified on the basis of morphological, cultural properties and biochemical microtests "STAPHYtest 16", "STREPTOtest 16" (Lachema, Czech Republic) and VITEK 2 GP and VITEK 2 YST test systems (biomerieux, France) using the VITEK 2 Compact analyzer.

The adhesive ability of microorganisms was evaluated according to the method, where suspensions in an isotonic NaCl solution with a concentration of 1 10^5 CFU/ml were made from daily bacterial cultures grown in blood agar and from 48-hour cultures of *C. albicans* grown in Sabouraud agar according to the optic standard. The studied material sample was cultivated in bacteriological test tubes in a microbial suspension for 1 hour at a temperature of 37°C with constant stirring by means of MR-1 shaker (SIA BIOSAN, Latvia) with a stirring frequency of 20 times/min. Then, the sample was transferred to a new sterile test tube and washed three times in a sterile isotonic solution in order to remove non-adherent microbial cells. The washed samples were placed in a sterile ultrasonic tub-washer for dentures TSM 226 289 (50W, 42KHz) with the ultraviolet irradiator turned off for 5 minutes in order to separate the adhered bacteria from the resin surface. Adhered microorganisms were washed off in 1.0 ml of sterile nutrient broth. Then, the number of viable microbial cells was determined in it by the method of tenfold serial dilutions. The cultivation of staphylococci and streptococci was conducted in blood agar, Escherichia coli cultivation was performed in Endo agar, *Candida* fungi were cultivated in Sabouraud agar.

After the cultures cultivation, the number of isolated colonies grown from the microbial cells that adhered to the material sample was counted. Then, recalculation was performed per 1 $см^2$ of the sample and the value of the decimal logarithm of the number of viable microbial cells was determined. The percentage of adhered microorganisms was determined for each of the test cultures and the adhesion index was calculated according to the formula (1):

$$IA = lgA/lgN, \quad (1)$$

where IA is the index of adhesion;
A is the number of adhered microbes;
N is the number of microbes in the initial suspension.

The experiments to check the adhesion of each test culture to each material sample were repeated three times. The percentages of adhered microbial cells and adhesion indices were calculated for each experiment separately based on the counting of microbial colonies. The quantitative data obtained in the experiments by the type of dispersion corresponded to the normal Gaussian law (based on the Shapiro-Wilk's W test). To describe the central tendency of the quantitative data, the interval (M±m) was used: arithmetic mean (Mean) ± standard error (Standard error), and to assess the reliability of the differences in the results obtained in comparison with the control, a parametric t-test (Student's test) for independent samples.

Table 1. The percentage of microorganisms adhered to different types of base resin materials

Microorganisms	Vertex	Breflex	Villacryl	Glass
S. aureus ATCC 25923	0.16±0.008*	0.38±0.02*	1.13±0.06*	2.13±0.11
S. aureus MRSA	0.01±0.0004*	0 *	0.06±0.003*	0.17±0.09
S. epidermidis	0.03±0.002*	0.14±0.007*	0.07±0.004*	0,80±0,04
β- hemolytic *Str. pyogenes* (group A)	35.29±1.76*	17.65±0.88*	6.18±0.31*	10.88±0.54
β- hemolytic Str. dysgalactiae *ssp. equisimilis* (group G)	22.50±1.13*	2.00±0.10*	3.25±0.16*	7.00±0.35
α- hemolytic *Str. gordonii*	0.71±0.036*	0.48±0.024*	0.48±0.024*	1.75±0.09
α- hemolytic *Str. sanguinis*	5.00±0.025*	3.75±0.19*	1.67±0.08*	12.50±0.63
α- hemolytic *Str. Oralis*	2.89±0.14*	2.11±0.11	0.45±0.022*	2.11±0.11
C. albicans	0.20±0.01*	0.60±0.03*	0.30±0.015	0.09±0.005
C. tropicalis	0 *	0.08±0.004	0.75±0.038*	0.17±0.008

Note. * – $p<0.05$ when compared to the control (glass)

Table 2. Adhesion index of microorganisms to various types of base resins

Microorganisms	Microbial load of the initial suspension, CFU/ml	Index of adhesion			
		Vertex	Breflex	Villacryl	Glass
S. aureus ATCC 25923	4.60±0.14	0.39**	0.47*	0.58	0.64
S. aureus MRSA	4.78±0.15	0.15**	0**	0.32*	0.42
S. epidermidis	4.70±0.14	0.25**	0.39*	0.33*	0.55
β- hemolytic *Str. pyogenes* (group A)	4.23±0.13	0.89*	0.82	0.71	0.77
β- hemolytic *Str. dysgalactiae ssp. equisimilis* (group G)	4.30±0.12	0.85*	0.60*	0.65*	0.73
α- hemolytic *Str. Gordonii*	4.80±0.15	0.55*	0.52*	0.52*	0.63
α- hemolytic *Str. Sanguinis*	4.08±0.11	0.68*	0.65*	0.56*	0.78
α- hemolytic *Str. oralis*	4.28±0.12	0.64	0.61	0.45*	0.61
C. albicans	4.70±0.14	0.43*	0.53**	0.46*	0.35
C. tropicalis	4.78±0.15	0.06**	0.36	0.56*	0.42

Note. * – $p<0.05$, ** – $p<0.01$ when compared to the control (glass)

RESULTS

The adhesion of microorganisms to the control material (glass) was very weak (Table 1). Only β-hemolytic streptococci strains differed in relatively high adhesiveness both to glass and to the tested materials of base resin. Thus, 10.88±0.54% of the cells from the suspension of β-hemolytic streptococcus of group A *Streptococcus pyogenes* adhered to glass, 35.29±1.76% and 17.65±0.88 adhered to Vertex and Breflex resin, respectively ($p<0.05$). A high percentage of adhered cells of β-hemolytic streptococcus of *Streptococcus dysgalactiae ssp. equisimilis* group G was observed when studying glass (7.00±0.35%) and Vertex resin (22.50±1.13%) ($p<0.05$). Among oral cavity-resident α-hemolytic streptococci, only the *Streptococcus sanguinis* strain showed a high percentage of cells adhered to glass. The studied strains of staphylococci and candida showed extremely low percentages of cells adherent to both glass and all base materials.

Table 2 shows the calculated values of the adhesion index of each of the used test strains of microorganisms to the samples of the base materials. Cultures of pathogenic β-hemolytic streptococci of *Streptococcus pyogenes* group A and *Streptococcus dysgalactiae ssp. equisimilis* group G demonstrated the highest adhesion indices to basic materials and glass (the values were within 0.73-0.89). The highest adhesive ability of β-hemolytic streptococci was manifested to Vertex resin. The α-hemolytic streptococcus *Streptococcus sanguinis* as a representative of the resident microflora of the oral cavity showed a high adhesive ability to the control material, namely glass (adhesion index constituted 0.78). However, it adhered to all tested samples of base resin significantly weaker ($p<0.05$), especially to Villacryl resin (adhesion index was 0.56). Weak adhesion to this resin was also demonstrated by α-hemolytic *Streptococcus oralis* (adhesion index was 0.45). α-hemolytic *Streptococcus gordonii* as another representative of the normal oral cavity microflora showed significantly less adhesion to all three samples of the tested base resins compared to glass as the control material (adhesion

Table 3. Comparative analysis of the adhesive ability of base resins (according to the average adhesion index) in relation to different groups of tested microorganisms

Microorganisms	Vertex	Breflex	Villacryl	Glass
Oral α-hemolytic streptococci	0.62±0.07	0.59±0.07	0.51±0.06	0.67±0.09
Oral β-hemolytic streptococci	0.87±0.03*	0.71±0.15	0.68±0.04	0.75±0.03
Oral staphylococci	0.26±0.12*	0.29±0.25	0.41±0.14	0.54±0.11
Candida yeast-like fungi	0.24±0.26	0.44±0.12	0.51±0.07*	0.39±0.05
For all tested microorganisms	0.49±0.17	0.49±0.15	0.51±0.11	0.59±0.12

Note: * – $p<0.05$ when compared to the control (glass)

indices constituted 0.52-0.55 vs. 0.63, $p<0.05$) (Table 2).

The lowest adhesion indices to the control material (glass) were demonstrated by the strains of *C. albicans* and *C. tropicalis* yeast-like fungi constituting 0.35 and 0.42, respectively. *C. tropicalis* strain practically did not adhere to Vertex resin (adhesion index constituted 0.06, $p<0.01$). However, its adhesion to Villacryl resin was even significantly higher than to glass (adhesion index was 0.56, $p<0.05$). The strain of *C. albicans* also showed weak adhesive ability to all tested materials (adhesion index values amounted 0.43-0.53). However, the intensity of adhesion in all cases was higher compared to the control material (glass) ($p<0.05$).

We used glass as a control material. The adhesive properties of the tested base materials (in percent) relative to glass were presented in Fig. 1 for illustration purposes (Fig. 1: 1.1.-1.4.). As it was already mentioned, a very small percentage of *Candida* yeast-like fungi adhered to all tested materials (no more than 0.60-0.75% of those present in the suspension). Moreover, a regularity could be noticed that the adhesive capacity of *C. albicans* (as the most common causative agent of candidal prosthetic stomatitis) was higher than that of *C. tropicalis*. The adhesion index of *C. albicans* to all three samples of base resins was significantly higher than to glass: Vertex – by 20.3%, Breflex – by 49.8% and Villacryl – by 31.6% ($p<0.05$). The test strain of *C. tropicalis* adhered to Breflex resin and, especially, to Vertex resin weaker than to glass (by 15.1% and 84.9%, respectively, $p<0.05$). The adhesion index of *C. tropicalis* to Villacryl resin was 32.7% higher compared to glass ($p<0.05$) (Fig.1).

Considering the fact that the test strains used in the study represented a rather wide range of microorganisms, the adhesive ability to base materials was summarized in Table 3 taking into account their taxonomic and ecological affiliation. According to the average values of adhesion indices of all tested microorganisms, the tested samples of base materials were inferior to the control material (glass) indicating their significant biological inertness. In general, oral streptococci (both α- and-hemolytic) adhered less strongly to Villacryl resin. Staphylococci and *Candida* yeast-like fungi adhered weakly to Vertex resin (Table 3).

DISCUSSION

Implant-supported prostheses improve patients` satisfaction with treatment and quality of life. Improvements in the implant`s surface and in attachment elements have made this type of treatment method very successful. However, some biological and mechanical complications remain [11, 12].

Conventional removable partial dentures are still common option for patient with distal-extension tooth loss [13]. Replacement of missing natural teeth is important to improve function, aesthetics and quality of life for patients, who is treated with partial dentures [14].

In the literature there are many studies devoted to the study effects of partial dentures on the oral health of soft tissues and remaining teeth, comparing commonly used materials with emergency materials for the fabrication of partial dentures [15-17].

In our study, we used 3 types of base material samples to evaluate the adhesive ability of microorganisms: Vertex (Vertex-Dental B.V., the Netherlands), Breflex (Bredent, Germany) and Villacryl (Zhermapol, Poland). The selection of these materials was based on prevalence of their use in the prosthetic dentistry clinic for the manufacture of bases for complete and partial removable dentures.

Analyzing previous studies, scientists studied the level of adhesion of microorganisms of the oral cavity to the soft linings of the bases of removable prostheses. As result, temporary soft lining materials are not resistant to adhesion and possible surface damage caused by oral bacteria, and therefore their use should be limited to short-term periods [18].

In our study, we performed microbiological analysis on equally well-polished prostheses. In this way, we rejected the factor of different roughness of the surfaces of the bases of dentures.

According to the results of the conducted experiment, all studied material samples were microbiologically quite inert. They sorbed and retained a small percent-

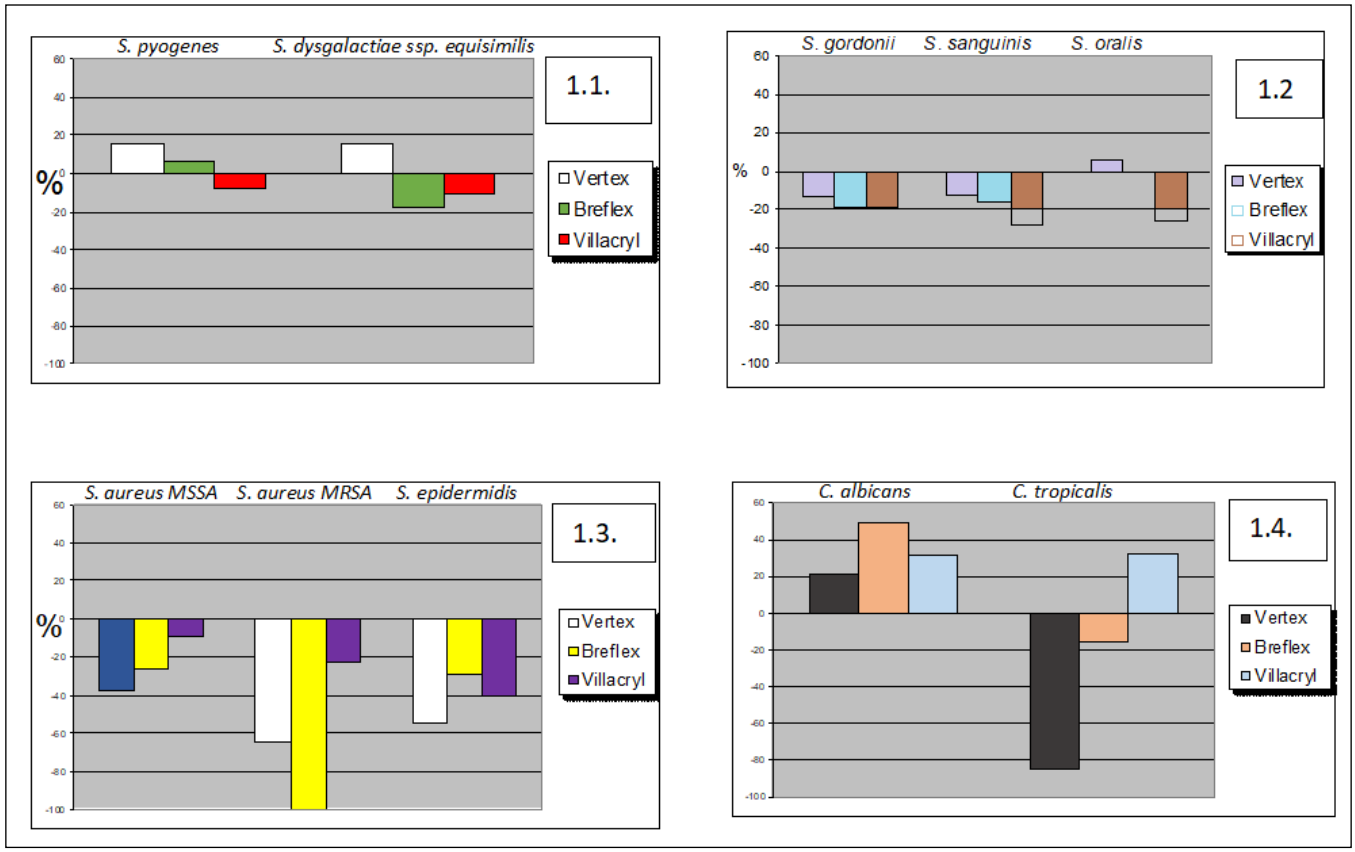

Fig. 1. Adhesive properties of the base materials in relation to the control material (glass). The adhesion index is presented as a percentage: 1). The adhesion index of *S. pyogenes* and *S. dysgalactiae ssp. Equisimilis*; 2) The adhesion index of *S. gordonii*, *S. sanguinis* and *S. oralis*; 3). The adhesion index of *S. aureus* MSSA, S. aureus MRSA and *S. epidermidis*. 4) The adhesion index of *C. albicans* and *C. tropicalis*

age of microbial cells from the suspension under the conditions of the performed experiment.

Staphylococci, especially *S. epidermidis* and methicillin-resistant *S. aureus* (MRSA), adhered to all tested samples of base materials very weakly. Adhesion of methicillin-sensitive *S. aureus* (MSSA) to Villacryl resin was at the level of the control material, i.e. glass (adhesion indices constituted 0.58 and 0.64, respectively, $p<0.05$). It was significantly weaker in relation to Breflex resin (adhesion index was 0.47, $p<0.05$) and, especially, to Vertex resin (adhesion index was 0.39, $p<0.01$).

The results indicate that all materials exhibited significant biological inertness, with microbial adhesion indices generally lower than those of the control material (glass). Among the microorganisms tested, *Streptococcus pyogenes, Streptococcus dysgalactiae*, and *Candida albicans* demonstrated varying degrees of adhesion to the base materials, with *Candida albicans* showing the weakest adhesion to the resins, especially Vertex.

Several previous studies have investigated microbial adhesion to denture materials, and many of their findings complement the results of this study. For example, Dantas et al. (2016) studied the effect of surface finishing and polishing on the adhesion of *Streptococcus sanguinis* to polymethyl methacrylate (PMMA). Their findings indicated that smoother surfaces reduced bacterial adhesion, confirming the importance of surface characteristics in microbial colonization [19].

Similar to the findings of this study, Dantas et al. noted that PMMA-based materials had lower microbial adhesion when compared to rougher surfacesr study by Nikhath Sultana et al. (2023) also found a direct correlation between surface roughness and microbial adhesion. Increased roughness led to higher adhesion rates of bacteria such as *Streptococcus mutans*, a key pathogen in dental caries. While this study controlled for surface roughness by using equally polished materials, it still aligns with the finding that surface properties—such as chemical composition and free surface energy—play a critical role in microbial adhesion [20].

The adhesion of *Candida albicans* to denture materials has been extensively studied, particularly because of its association with prosthetic stomatitis, a common condition affecting denture wearers. In studies like those by Klotz et al. (2005), *Candida albicans* was shown to exhibit higher adhesion to acrylic resins than other Candida species [21]. Our study similarly demonstrated that *Candida albicans* adhered more strongly to the resin materials than *Candida tropicalis*.

However, the adhesion of both yeast species was weak compared to bacterial pathogens, suggesting that these resins may help prevent fungal colonization. This observation is consistent with Klotz et al.'s finding that acrylic resins, particularly those with low surface energy, tend to limit the adhesion of *Candida* species. It was found that adhesion of *Candida albicans* to polymethylmethacrylate depend on morphilogical forms of fungi: yeast cells adhere less comparing with hyphe tubes. Moreover the virulent clinical strains (with capacity to survive in blood and resistance to complement opsonization) adhered to acrylate surfaces more active than collection strain [22].

In contrast, the this study regarding the adhesion of *Staphylococcus aureus* and *Staphylococcus epidermidis* are in line with the findings of a study by Espinel-Ingroff et al. (2007), which suggested that *Staphylococcus species* generally exhibit low adhesion to prosthetic materials, especially when compared to oral streptococci. This study demonstrated that methicillin-resistant *S. aureus* (MRSA) had minimal adhesion to the resins tested, particularly Vertex [23]. In a similar study by Weng et al. (2015), it was found that the adhesion of *S. aureus* to dental materials like acrylic resin was significantly lower compared to *Streptococcus* species [24].

The adhesive ability of orocci, particularly *Streptococcus pyogenes* and *Streptococcus dysgalactiae*, observed in this study contrasts with the general trend reported in other studies. A study by Morin et al. (2014) showed that *Streptococcus mutans*, a prominent oral pathogen, adhered more readily to dental materials compared to other microorganisms like *Streptococcus sanguinis*. The high adhesion observed for β-hemolytic streptococci (*S. pyogenes* and *S. dysgalactiae*) to Vertex resin, in particular, suggests that certain resins might be more prone to bacterial adhesion due to their surface chemistry, although the overall levels of adhesion were still relatively low [25].

The weak most microorganisms to the base materials in this study suggests that these materials could help minimize the risk of biofilm formation and associated infections, such as prosthetic stomatitis, a significant concern in removable denture wearers. Given that *S. aureus*, *Candida albicans*, and Streptococcus species are often implicated in oral infections, the results of this study are promising in terms of reducing the microbial load on denture surfaces, particularly for individuals with compromised immune systems or those prone to infection.

However, while the resins tested showed low microbial adhesion, further research into other factors, such as saliva composition, oral hygiene practices, and patient diet, is essential. Studies by Motallebzadeh et al. (2016) [26] and Gorseta et al. (2019) [27] emphasized that clinical factors like saliva pH, bacterial load, and the oral hygiene routine of denture users significantly influence the microbial colonization of denture surfaces . Therefore, while laboratory results are valuabre essential to confirm these findings in real-world settings.

The obtained results will be the basis for further experimental studies of the base materials influence on the oral microflora.

CONCLUSIONS

1. All tested samples of base materials showed significant biological inertness: the adhesion indices of the vast majority of the tested microorganisms were lower than those of the control material (glass).
2. Oral streptococci (both α- and β-hemolytic) showed the weakest adhesive ability to Villacryl resin (average values of adhesion indices 0.51 and 0.68 appropriately).
3. Staphylococci and *Candida* yeast-like fungi showed a weak adhesive ability to the samples of base materials, especially to Vertex resin (average values of adhesion indices 0.26 and 0.24 appropriately).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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