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THE HUMAN MUCOSAL MICROBIOCENOSIS: ADHESION ON PLASTICS

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ABSTRACT

Adhesion, growth and formation of biofilms of microorganisms are possible on the surface of polymers used for prosthetics. The development and spread of microorganisms cause an infectious process. The processes of biofilm formation on acrylic plates used in orthodontic practice were studied. The possibilities and features of biofilm formation by strains of conditionally pathogenic microorganisms of the genera *Enterobacteriaceae, Staphylococcus* and *Candida* are shown. It has been established that colonization by microbial flora is determined by the characteristics of the pathogen and the type of basis of the dental apparatus. Using the example of *Escherichia coli, Staphylococcus aureus* and *Candida albicans* strains, the features of biofilm formation on acrylic plastics of cold polymerization such as "Belacryl pink" and "Belacryl white" (Russia); "Redont pink" and "Redont white" (Ukraine), "Villacryl Ortho pink" and "Villacryl Ortho white" (Poland) are shown. The possibilities of pathogen adhesion on basic devices made of a new light-curing polymer "Nolatek pink" and "Nolatek white" (Russia) are considered. The mechanisms of the microbiocenosis biofilm formation and degradation in the oral mucous biotope are discussed.

KEYWORDS: prosthetics; basis acrylic plastics; biofilm forming; conditionally pathogens; cold-curing polymers, light-curing polymer.

ABBREVIATIONS

BF	biofilm formation
CB	communicative body
CFU	colony-forming units
h	hours
min	minutes
MPB	meat-peptone broth
OD	optical density
PL	probiotic lectins
PS	physiological solution
µl	microliters

1. INTRODUCTION

On the one hand, mucosal biotope microbiocenoses of the human open cavities play important multiple role in infectious processes in organism.

On the other hand, orthodontic diseases of the oral cavity are an important medical problem. Despite the annual medical examination of the population, including dental care, there is an increase in dental anomalies from year to year, which leads to the need to use orthodontic devices. However, in many cases, the devices injure the oral mucosa, which contributes to infection and the development of further inflammatory complications affecting the integrity of the dental alveolar complex.

The development of inflammatory conditions of the oral cavity is associated not only with violations in the operation of plate devices, but also with a whole range of factors, including colonization of the oral cavity by conditionally pathogenic microflora and others. In more than 30% of the population, conditionally pathogenic microflora is found in the oral cavity, and in 85% of cases, the chronic properties of the infectious process was observed. An important role in the biotopes of the human body is played by taxonomic and compositional (in terms of the level of contribution to the composition of microorganisms) the status of microbiocenosis, its conditionally pathogenic orientation. A number of factors plays an important role in regulation of mucosal biotope microbiocenosis of oral cavity. So, there is a risk of complications in the provision of orthodontic care to the population.

It is known that from 20 to 60% of oral diseases are associated with microorganisms capable to biofilm formation (BF). The ability to BF is considered as a trigger in the development of the infectious process. Taking into account the variety of the basic material of dental devices, the problem of not only choosing the material of orthodontic structures, but also its resistance to the adhesion of microorganisms remains urgent. In this regard, an important practical task is the control of BF on the surfaces of orthodontic polymers.

The aim of the work is to summarize our data on microbiocenosis communicative body (CB) and to study BF by microorganisms on widely used acrylic materials, which are the basis for the manufacture of orthodontic structures.

2. MATERIALS AND METHODS

Polymer samples were used: "Belacryl pink" /"Belakryl white" (Russia); "Redont pink"/"Redont white" (Ukraine), "Villacryl Ortho pink"/"Villacryl Ortho white" (Poland) and "Nolatek" (Russia). Four plastic models were studied: three samples of cold-cured acrylic plastics (Redont, Villacryl Ortho, Belacryl) and one light-curing (photosensitive) polymer (Nolatek). The studies were carried out on plates of two color shades, most often used in orthodontic practice: white and pink. The objects of the study were strains of the genera *Enterobacteriaceae*, *Staphylococcus* and *Candida*. The dimensions and shape of the test plates corresponded to the parameters of the well of the standard 24-well plate and were 10 x 10 x 2 mm (7 samples for each type of plastic).

Candida albicans ATCC 24433 culture was grown on a dense *Sabouraud* nutrient medium at a temperature of 37°C for 48 h.

Cultures of *Escherichia coli* ATCC 2592 and *Staphylococcus aureus* ATCC 6538-R were grown for 24 hours on *Muller-Hinton* agar. A suspension was prepared from each culture of microorganisms in sterile 0.9% saline solution with NaCl (as physiological solution [PS]) with a final turbidity of the microbial suspension of 3.0 x 10^8 CFU/ml (turbidity No. 1 according to *McFarland*). 1.8 ml of meat-peptone broth (MPB) and 20 µl of the suspension of the studied culture were added to the wells of the plate, the number of which corresponded to the number of test plates and the studied cultures, thus the working concentration of platonic microorganisms was 3 x 10^6 CFU/ml. The plates were incubated at 37°C for 24 h.

After incubation, the broth culture (plankton cells) was carefully removed from the by pipetting without damaging the plates, washed twice with PS and then fixed by adding 300 µl of 96% ethyl alcohol (C_2H_5OH) to each plate well. The fixation time was 20 min. The recorded samples were washed with distilled water, after which a 0.1% solution of *gentian violet* dye was added to each plate well (the volume of the dye corresponded to the capacity of the well) and kept for 30 min at room temperature (24^{o}C). For better extraction, a 24-well plate was placed on a Vortex machine for thorough mixing. After incubation, the dye solution was removed, and the plates were washed five times by PS, adding 300 µl to the wells, until the color disappeared. The remaining dye was extracted with 96% ethanol. The quantification of ethanol eluates in optical units (OD) was performed using an INFINITE F50 Tecan microplate spectrophotometer with Magellanfor F50 software at a wavelength of 620 nm. Wells to which a liquid nutrient medium without bacteria was added served as a control. In the calculations, a similar indicator was subtracted from the values of the test samples for the control sample, which made it possible to consider the intensity of BF as directly proportional to the dye concentration of the extracted eluates of the experimental samples. The measurement results were processed using a parametric criterion between groups and, when comparing related values within a group, using a *Student's* paired criterion. For all indicators, the reference values were calculated after discarding 5% of the extreme values that fall outside the range of the unimodal distribution. For convenience, the average values of thousandths are represented by integers. The level of significance between the compared values was assumed to be reliable at p 0.05. Statistical processing of the obtained data was carried out using the *Microsoft Excel* program.

3. RESULTS AND DISCUSSION

3.1. *Concept of the adhesive microbiocenosis as CB, factors regulating its BF in the human mucosal biotope*

We studied the features of sorption of microbial populations of the human mucosal biotopes of open cavities in respect of solid-phase hydrophilic and hydrophobic surfaces such as agar, porous membranes and plastic, including wells of micropanels and materials for prosthetics.[1-12]

The possibility of BF is associated with the etiological significance of microorganisms in the development of associated infections, which determines the choice and application of the

basis of dental devices, and is especially relevant in patients suffering from chronic or progressive inflammatory diseases of the oral cavity.

In this regard, it is important to understand the adhesive microbiocenosis as CB in the mucosal biotope.

The formation and functioning of microbiocenosis as CB was recorded already in the first 3 days of cultivation of microorganisms in the solid or liquid phase. On the first day, differences were observed between subpopulations of cells of a strain of planktonic microorganisms under the influence of microbial lectins (we can talk about the formation of liquid-phase CB). On the second and third days, the differences were leveled against the background of the manifestation and intensification of the processes of degradation and lysis of CB components. The presence of probiotic cells in case of liquid-phase CB helps in uncoupling the species status at the level of co-existing microbial niches of mixed microbiocenosis.

The following microbiocenosis CB adhesion properties were identified in the mucosal biotope:

*multicenter early (days) simultaneous assembly of biofilm components of CB microbiocenosis and late (weeks-months) degradation of solid-phase (interphase) CB (including on plastic) with the participation of microbial lectins and enzymes;

*effects on the opportunistic pathogenic compartment of CB through probiotic factors (including endogenous ones from CB), including those involving probiotic lectins (PL) that mimic the action of probiotics:

- PL initiates the synchronization of metabolic processes of planktonic microorganisms of microbiocenosis in preparation of CB for further lysis;
- PL, with prolonged action, contribute to the production and further conservation of the residual partially degraded multi-island landscape of solid-phase microbiocenosis CB, making it more accessible to antibiotics and other antimicrobials;
- Probiotic microorganisms within composition of the biofilms of conditionally pathogenic microbiocenoses can act as target-in-target with subsequent destruction of CB;
- With solid-phase CB assembly on PL ("incorrect" CB assembly), CB becomes unstable and quickly collapses under the influence of additional external factors.

In general, various options for therapeutic and preventive effects on CB may include broader tactics of direct influence on opportunistic pathogenic consortia of CB microbiocenosis, giving CB microbiocenosis a general (resulting) harmless orientation.

3.2. *Adhesion of conditionally pathogenic microorganisms to polymeric materials for orthodontic structures*

In the pathogenesis of microbial infections, processes such as physical contact of planktonic microorganisms with polymeric materials used in orthopedic and orthodontic structures are of no small importance. The process of BF and subsequent contact with the oral mucosa can serve as the initial stage of the infectious process and contribute to the development of an inflammatory disease. We have conducted a study of the possibility of BF by microorganisms to widely used acrylic materials, which are the basis for the manufacture of orthodontic structures. The intensity of BF was studied by three strains from the ATCC international collection: *C. albicans* ATCC 24433, *E. coli* ATCC 25922, and *S. aureus* ATCC 6538-R on samples of polymers from domestic and foreign manufacturers: "Belakryl pink" /"Belakryl white" (Russia); "Redont pink"/"Redont white" (Ukraine), "Villacryl Ortho pink"/"Villacryl Ortho white" (Poland) and "Nolatek" (Russia).

In Table 1, the results of research of BF of the test strains on polymer plastics used in the manufacture of orthodontic structures are presented.

The data obtained indicate that the adhesive properties of microorganisms vary greatly. A comparative assessment of the microbial contamination of various samples made it possible to identify differences in BF, depending on the strain of the microorganism and the features of the manufacturing technology of the polymer material. Taking into account the fundamental difference in the biological properties of *Gram*-positive and *Gram*-negative microorganisms, the data were analyzed separately for each group of bacteria. When studying the number of adhered microorganisms, it was revealed that the highest BF values were recorded for *E. coli* in relation to all samples different in color: the average group BF values were 71.3 to 23.3 OD; while for *S. aureus*, statistically significantly lower values were noted, not exceeding the maximum value of 10.4 OD for "Nolatek pink". It is established that the type of *S. aureus* has the same OD of BF to orthodontic structures made on the basis of "Redont" and "Villacryl Ortho" acrylates, regardless of the color spectrum.

It should be noted that when evaluating BF in *C. albicans*, there was a destabilization of the recorded indicators for various types of basis materials. Depending on the severity of BF in *C. albicans* to various polymers, the values ranged from 61.1 to 10.2 OD.

Table 1: The optical density (OD) of eluates obtained during the cultivation of microorganisms for 24 h on various plastics.

Microorganisms sorbed on Plastic	*Staphylococcus aureus***		*Escherichia coli**		*Candida albicans****	
	Pink	white	pink	white	pink	white
Redont	21,9±3,8	18,0±2,5	47,9±12,3	41,3±8,6	28,5±5,4	25,3±4,4
Villacryl Ortho	23,3±4,3	20,3±3,4	52,1±8,6	40,4±5,4	42,3±11,2	61,1±8,6
Belacryl	24,3±3,96	25,3±0,8	71,3±11,2	46,2±9,5	23,3±4,5	18,6±6,7
Nolatek **	10,4±0,72	14,4±0,27	23,3±0,78	25,3±0,75	10,2±0,88	11,3±1,74

Comments

*statistically significant differences in the colonization ability of *E. coli* when comparing Nolatek white/pink with respect to similar-colored samples of Redont, Villacryl Ortho and Belacryl ($p < 0.0001$); **statistically significant differences of Nolatek in comparison with samples of Redont, Villacryl Ortho and Belacryl: for *S. aureus* ($p < 0.001$ for pink/white polymer); for *C. albicans* ($p < 0.001$ in the case of a white polymer and $p < 0.05$ in the case of a pink polymer); ***statistically significant differences in the color of the comparison samples of Redont, Villacryl Ortho and Belacryl with respect to the tested cultures of microorganisms were not revealed.

It should be noted that the choice of the above polymers was based on the frequency of use and availability of their manufacture in the laboratory. However, one of the new plastics for dental structures is the domestic light-cured polymer "Nolatek", which compares favorably with previous acrylates in the speed of manufacturing the bases of orthodontic devices. The absence of "individual intolerance", the ease of polymerization makes it possible to distinguish it as a "standard" or "control" relative to any polymers currently existing among a variety of acrylic plastics. According to the data obtained (Table 1), Nolatek has significantly lower adhesive activity against all studied cultures when compared with cold-cured plastics. OD of BF was minimal in 93.7% of the studied samples from Nolatek (when compared with other materials) and was in the range of 10.2–25.3 OD. Thus, the Nolatek basis material exhibits a significantly lower adhesive colonizing ability and can be considered low-intact to pathogens.

Such stability of the polymer, apparently, can be explained by a technologically more advanced polymerization method, leading to a decrease in "porosity", as well as the presence of protective and destructive properties (including relief features) of the surface in relation to these microorganisms. Taking into account the significantly lower degree of biofouling of Nolatek in comparison with other samples, an additional study was conducted based on the study of the BF activity of the periodontopathogenic species *S. aureus*, as one of the most frequently isolated pathogens in the study of the oral microflora. To assess the adhesive ability of *S. aureus*, dynamic indicators of four-day (96 h) BF monitoring with respect to Nolatek were studied (Table 2). To substantiate the prognostic trends of colonization of the sample with the *S. aureus* strain, a pairwise comparison of the sample was performed based on the color-dependent division of the Nolatek basis. The adhesion study revealed that the *S. aureus* microorganisms have a high tropism to BF in the first 24 h with respect to pink plastic. The studied strain of *S. aureus* adhered equally to all seven pink plastic samples, while significantly lower BF values were observed on the surface of the colorless base ($p < 0.05$). It should be noted that the BF index after 48 h was already characterized by a biofouling of 3.8% with respect to white plastic, while the colonization of pink-tinged materials was characterized by a lower percentage of colonization. Further observation showed that after 72 h, in all cases, there was a gradual stabilization of values for both types of bases, and after 96 h, the results of quantitative assessment of biofouling of denture samples were practically compared – the total increase in biofilm for "Nolatek" pink was 11.2% and "Nolatek" white was 17.5%, respectively.

Despite the fact that the identified "anti-adhesive effect" is different only on the first day for the bases of the Nolatek material that are different in color, this fact indicates that this type of light-curing polymer molding initially provides lower adhesive properties to this material sample, and in cases of micro-traumation of the epithelium of the oral mucosa, the polymer will have the least risk of developing the pathogen(s)-associated infection.

Table 2: Dynamics of the biofouling ability of the dental basis of the plastic "Nolatek" in the presence of *S. aureus*.

S. aureus	**“Nolatek”**	
	white	**pink**
OD of BF		
24 h	14.4 ± 0.27	10.4 ± 0.72
Biofouling of plastics (%) in comparison with the daily indicator of BF		
48 h	3.8	2.1

72 h	9.2	8.9
96 h	6.8	4.2
Total biofilm growth (%)	19.5	11.2

Thus, the analysis of colony-forming adhesion of etiologically significant microorganisms objectively and reliably indicates the connection of BF of pathogens, depending on two types of manufacturing of the base material: cold or light-curing type of polymerization.

The data obtained indicate a high adhesive activity of microorganisms to cold-cured plastics, in many cases having equivalent values in the BF reaction. At the same time, BF differed significantly in the case of the basis of orthodontic devices made of light-curable Nolatek material.

As before, the relevance of choosing the optimal dental material remains, characterized by the lowest colonization index by opportunistic microorganisms, including its own microbial flora (with the prospect of its adaptive correction) of the oral cavity.

Considering the above, it is important to further study the plastic "Nolatek", as its manufacture is the most promising and affordable. Given the wide variety of orthodontic materials, the study of the above BF factor will help in choosing the material and assessing the feasibility of its use for providing dental care to patients.

4. CONCLUSION

The proposed concept of adhesive mucosal microbiocenosis as CB allows new approaches in consideration of the treatments of BF on solid phases. The results also allow to conclude that the BF of microbiocenosis is influenced not only by the species composition of the microbiocenosis of the oral cavity, but also by the technological factor of manufacturing the composite material. Microbiological studies of the BF process on samples of cold-cured plastic and photo sensitive polymer material have shown a potentially low possibility of adhesion of infectious pathogens to the light-curing polymer material. The latter indicates in favor of the fact that this type of light-curing base will have lower biofouling rates and can be recommended for the manufacture of wide-profile dental prostheses, especially immediate prostheses (dental orthopedic structures that are installed immediately after tooth extraction).

Disclosure of conflict of interest

The authors declare no conflict of interest.

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