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REVIEW PAPERS PRACE POGLĄDOWE

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The influence of base resin of removable dentures on the planktonic growth of individual representatives of oral microflora

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Summary

Introduction. Microbiological researches of the role of denture base materials in violation of the oral cavity micro ecology were conducted.

Aim. Therefore, the purpose of research is to study the effect of different polymeric materials on denture bases, on the intensity of planktonic growth of resident and transient representatives of oral cavity microflora in a periodic culture on the basis of registration of its optical density.

Material and methods. 7 types of denture base materials and their influence on the planktonic growth of microorganisms have been studied.

Results. The planktonic growth of the *S. oralis* strain was suppressed by all base materials: SYNMA (by 35.5%), Bre.Flex (by 27.5%) and Protacryl-M (by 25.7%), in the smallest – Nylon (by 5.4%), Villacryl (by 9.5%).

Conclusions. Through experimental research, we have proved the direct involvement of basic resin materials in changes in the oral cavity micro ecology and the process of occurrence of prosthetic stomatitis.

INTRODUCTION

One of the main complications that arise during using full or partial dentures is prosthetic stomatitis (common recurrent disease with localization on a mucous membrane of both prosthetic bed and intact areas of gum and vestibular part of oral cavity). Despite the multi-factor etiology, the significant importance in the development of stomatitis belongs to the bacterial and fungal microflora of oral cavity (1, 2). Previously we demonstrated substantial changes of microbiocenosis of the mucous membrane of the prosthetic bed and periodontal area of patients with partial and complete dentures (3, 4), that are deepening due to poor oral hygiene (5), accompanying somatic pathology and adverse effects of environmental conditions.

The presence in the oral cavity of dental and orthodontic constructions prevent the free washing the mucous membrane of the saliva, which provides a constant clearance of microbial cells. In addition, numerous researches confirm the ability of oral microorganisms to adsorb on dental materials to form on their surface-resistant groups – multi-view microfilms. Their composition is quantitatively dominated by α-hemolytic streptococcus (in particular *Streptococcus oralis*, *S. sanguinius*, *S. gordonii*, representatives of the S. mutans-group), *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus*, *A. naeslundii*, and also yeast fungi *Candida albicans*, *C. tropicalis*, *C. dublinensis*. There are complex directional interrelations in both synergistic and antagonistic nature between separate species of microbiocenosis that form biofilm on the surface of the mucous membrane of oral cavity, prosthetic bed and dental materials. These include competition for certain nutrient deficiencies and growth factors, level of aero tolerance, sensitivity to compete types of hydrogen peroxide and lactic acid, the ability to withstand the effects of factors of immunity of the body (anti-lysozyme activity, inactivation of complement system factors and SIgA, phagocytosis resistance). Minimal extra external influence changes the relationship between different species, some of which receive additional preference for increased growth. In light of modern ideas about the mechanisms of communication of microbes and their ability to coordinate their behavior by secretion of molecular signals (quorum sensing) after achieving the specific critical cell number the conditionally pathogenic bacteria are becoming quite aggressive to cause the disease – in this case, the involvement of the mucous membrane of oral cavity. In addition, microbial colonization (along with mechanical damage) is an important factor in reducing the durability of dentures (6).

A significant impact on the micro ecology of oral cavity in the area of the prosthetic bed can have residual monomer (metylmetacrylat) and formaldehyde, which continue to be released from resin base (7, 8). They can also cause adverse reaction on the mucous membrane of the prosthetic bed, since are released at concentrations, potentially

high enough to detect cytotoxic effect and cause irritation, inflammatory and allergic response to mucous membrane tissues (8). Thus, in the water environment (including oral fluid) is washed off 5-10% of residual monomer, that equivalent ≈ 2% weight of acrylic resin of most types. Chemical methods have shown that the main part of the residual monomer is released from the base resin for the first 3 days, although this release is also on the 4-7th days (which was the limit of observation) (7). However, the mutagenic activity of the extract of acrylic dentures were also found in individuals with an average prosthesis use time of 14.3 years (9, 10).

During the analysis of literary sources we haven't found any researches related to the influence of base resin of dentures and classic acrylic resin methylmetacrylat monomer on growth of microorganisms (although other microbiological aspects, as adhesion of microorganisms and formation of biofilm on the surface of plastics are intensively studying). Okita et al. (11) did not find noticeable antimicrobial activity in 4 acrylic fabric conditioners by classical microbiological methods – disco-diffusion and diffusion in agar.

AIM

Therefore, the purpose of research is to study the effect of different polymeric materials on denture bases, on the intensity of planktonic growth of resident and transient representatives of oral cavity microflora in a periodic culture on the basis of registration of its optical density.

MATERIAL AND METHODS

To assess the impact on the growth of microorganisms, 7 types of denture base materials samples (for production of bases on complete and partial removable dentures) were used.

The table 1 presents the physicochemical compositions and their methods of polymerization.

The finished base materials samples for the experiment were like 2 mm thick plates and 1 cm^2 in area. As a control, we used similarly sized glass plates. The research and control samples were placed in sealed cellophane packaging and sterilized X-ray irradiation in a dose of 0.44 mGy during 1,540 s.

The research used strains of conditionally-pathogenic microorganisms that are represent optionally anaerobic transient oral cavity microflora (3-5), as well as α-hemolytic streptococci *Streptococcus oralis*, *S. gordonii*, *S. Sanguinis* as major representatives of resident microflora of this biotype. Microbial culture has been isolated from the oral mucosa (prosthetic bed, gingival pockets) of patients with removable dentures with prosthetic stomatitis and identified on the basis of morphological, cultural properties and biochemical microtests using "STAPHYtest 16", "STREPTOtest 16" (Lachema, Czech Republic) та VITEK 2 YST (bioMerieux, France).

The test sample was placed in the tube of 2.0 ml of Brain Heart Broth (HiMedia Laboratories Pvt. Ltd., India) with the addition of 1% glucose, pre-freshly seeded test-strains of microorganisms in the ultimate concentration of 1 10^4 KUO/ml. The crops where cultivated for 24 hours at a temperature of 37°C with constant agitation on the MR-1 shaker (SIA BIOSAN, Latvia) at a stirring frequency of 20 times/min. After cultivation, we selected 5 servings of 200 μl planktonic culture per well polystyrene tablet. The optical density of the culture (OD) was determined using a multi-mode photometer for the micro-tablet Synergy™ HTX S1LFTA (BioTek Instruments, Inc., USA) with wavelength 495 nm using Gen5™ Data Analysis Software. Control growth of crops was evaluated under similar culture conditions in test tubes with nutrient plastic samples. Based on the results of the experiment for each sample tested of the base material was calculated by the integral index of inhibition of growth of microorganisms:

$$IGI = \sum \left(100 - \frac{OD_{research}}{OD_{control}} \right)$$

The results were processed by method of variational statistics.

RESULTS

Conducted experimental researches have demonstrated that the tested samples of materials exhibit ability to inhibit the planktonic growth of microbial cultures. Growth intensity of most microbial crops in the presence of comparison material (glass) wasn't significantly different from the control values. A credible decline of planktonic growth in the glass presence has been registered only in *Staphylococcus epidermidis* (by 31.5%, $p < 0.01$), *Streptococcus oralis* (by 23.7%, $p < 0.05$).

Tab. 1. Characteristics of materials, which were used in the research

Brand name	Chemical composotion	Manufacturer (producer)	The type of polimerization
Polian IC	Polymethyl methacrylate	Bredent, Germany	Heat polimerization
Bre.Flex	polyamide/nylon	Bredent, Germany	Heat polimerization
Nylon	Polyamide		Heat polimerization
Protacryl-M	Fluorine-containing acrylic copolimer, methacrylate	AT "Стома", Ukraine	Cold polimarization
Villacryl	Polymethyl methacrylate		Heat polimerization
Biocryl	Polymethyl methacrylate	SHEU-DENTAL, Germany	Pressing Heat polimerization
SYNMA	Fluorine-containing copolimer, mixture of acrylic monomers and oligomers.	AT "Стома", Ukraine	Heat polimerization

The most practical interest is the study of the effect of polymer materials on growth properties of resident representatives of oral microflora – α-hemolytic streptococci (fig. 1).

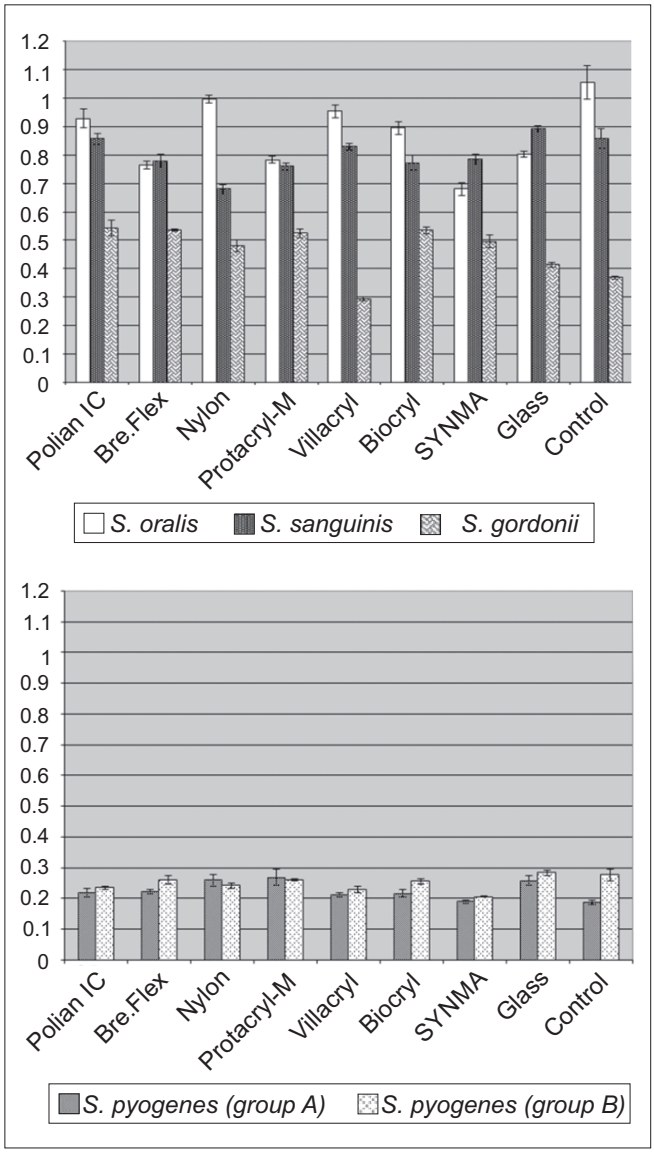

Fig. 1. The impact of base resin materials on the planktonic growth of oral streptococci cultures

The planktonic growth of the *S. oralis* strain was suppressed by all base materials, to the greatest extent SYNMA (by 35.5%, $p < 0.01$), Bre.Flex (by 27.5%, $p < 0.05$) and Protacryl (by 25.7%, $p < 0.05$), in the smallest – Nylon (by 5.4%, $p > 0.05$) and Villacryl (by 9.5%, $p > 0.05$). The test-strain *S. sanguinis* showed significantly weaker sensitivity to all tested base materials. Its growth reliably suppressed only by Nylon (by 20.7%, $p < 0.05$). Inhibition of growth of α-hemolytic *S. gordonii* was found only in a presence of Villacryl (by 20.5%, $p < 0.05$).

The research samples had showed less expressed impact on the growth of β-hemolytic streptococci cultures. The suppression of intensity of planktonic growth of β-hemolytic streptococcus G group was observed in the presence of SYNMA materials (by 25.7%, $p < 0.05$), Villacryl (by 17.1%, $p > 0.05$), Polian (by 15.0%, $p > 0.05$). Growth of the β-hemolytic streptococcus A group was kept at the control level only in the presence of SYNMA material, and in the presence of all other materials, on the contrary, it intensified – maximum for Protacryl (by 43.5%, $p < 0.01$) and Nylon (by 38.2%, $p < 0.05$).

Test-strains of staphylococci also found different sensitivity to the presence of different base materials samples in the cultural environment. All tested materials were essential suppressed the planktonic growth of staphylococcus epidermidis (usually by 24.6-35.8%, $p < 0.01$). The minimal impact on the growth of *S. epidermidis* was shown by Bre.Flex and Protacryl materials (growth inhabitation indexes are 17.0 and 19.7% respectively, $p < 0.05$). The antibiotic-sensitive strain of *S. aureus* (MSSA) showed sufficiently high sensitivity to Villacryl and SYNMA materials (growth inhabitation indexes are 24.2 and 22.0%, respectively, $p < 0.05$). Protacryl, Biocryl and Polyan materials have proved itself indifferent, and in the presence of Nylon planktonic growth MMSA increased by 55.1% ($p < 0.01$). Planktonic growth of the polyantibiotic-resistant strain of *S. aureus* (MRSA) was sensitive to the presence of Protacryl, Bre.Flex and Nylon (growth inhabitation indexes are 32.4, 31.2 and 25.0%, $p < 0.01$) in the cultural environment. Villacryl has stimulated MRSA growth culture (by 24.8%, $p < 0.01$).

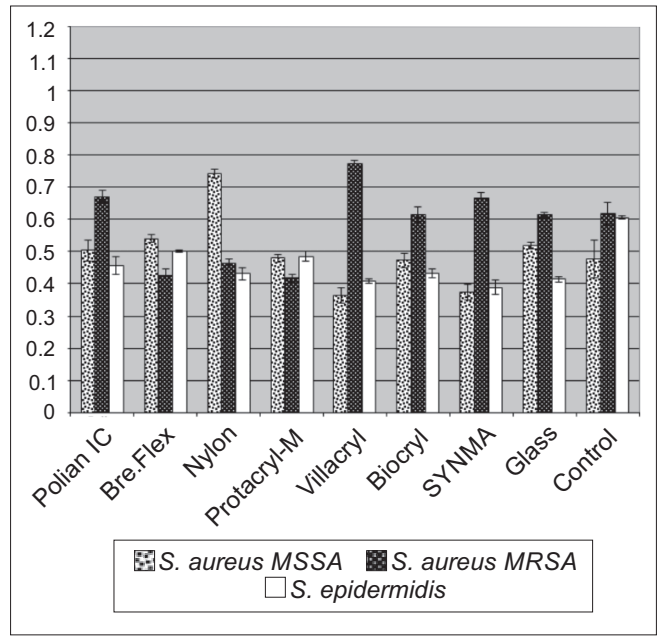

Fig. 2. Effect of basic plastics materials on the growth of planktonic cultures of oral staphylococci

Staphylococcus test strains also showed different sensitivity to the presence of different samples of basic materials in the culture medium. All tested materials were essential suppressed the planktonic growth of staphylococcus epidermidis (usually by 24.6-35.8%, $p < 0.01$). The minimal effect on the growth of *S. epidermidis* was shown by the materials Breflex and Protacryl (Breflex and Nylon indices (growth inhibition indices 32.4, 31.2 and 25.0%, $p < 0.01$)). Villacryl has stimulated MRSA growth culture (by 24.8%, $p < 0.01$).

The particular importance in the etiology of prosthetic stomatitis play yeast fungi (12). The results of studying the influence of different types of denture base materials on planktonic growth of yeast fungi isolated from patients with candida stomatitis are presented in figure 3. Growth of the most widespread opportunistic fungal pathogen *Candida albicans* was suppressed by such materials as Protacryl, Polian, Villacryl – by 31.1, 30.0 and 28.8% respectively ($p < 0.01$). There was a slight increase in the growth of *C. albicans* (by 12.0%, $p < 0.05$) in the presence of Bre.Flex. Suppression of planktonic growth poliresistant to natural and synthetic antifungals of clinical strain *C. tropical* in the presence of all tested base resin material samples was not observed at all. In the case of Protacryl and Villacryl research its intensity did not differ from the control indicator, but under the influence of Bre.Flex, Polian and SYNMA it even increased (by 77.1, 56.8 and 53.6% respectively, $p < 0.01$).

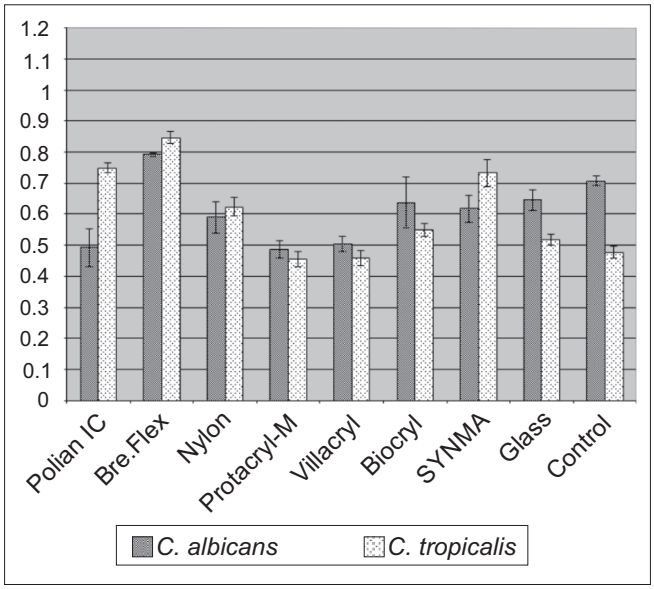

Fig. 3. The influence of resin base materials on the growth of planktonic cultures of Candida yeast

To summarize the results of the experiment, the characteristics and comparisons of antimicrobials properties of different samples of resin base materials we have applied integral growth of inhibition index for each of them, which is the mathematical sum of the corresponding growth inhibition indices of all used test cultures (fig. 4). Based on this, we can conclude that the most pronounced ability to suppress planktonic growth of oral microorganisms is possessed by Villacryl >> Protacryl ≈ SYNMA. Biocryl and the glass (material of comparison) are indifferent to the effect on oral microflora. Bre.Flex > Polian ≥ Nylon enhances planktonic growth of oral microflora representatives.

At the same time, particular attention is paid to the results of the study of resin base material impact on planktonic growth of oral microflora resident representatives – α-hemolytic streptococci *S. oralis*, *S. gordonii* and *S. sanguinis*. Thus, *S. gordonii* has a high affinity for the pellicle on the surface of the tooth enamel and

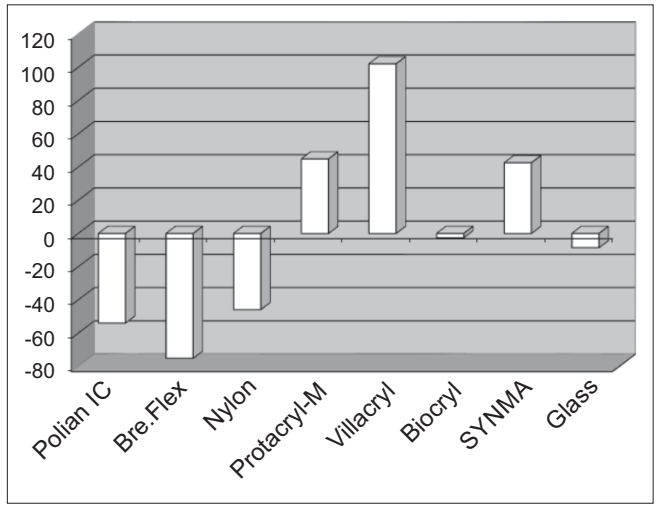

Fig. 4. Comparison of researched resin base materials: integral index of microorganism's suppression growth

acts as indicator of adhesion of other types of oral microorganisms.

The obtained results in the experimental research may serve as an indirect explanation mechanism of intensive microbial colonization of the surface of acrylic dentures from most types base resins *in vivo*. The vast majority of tested base resin materials do not suppress the planktonic growth of *S. gordonii* and *S. sanguinis*, and it can receive preferences for development during the denture usage. The mentioned types of oral streptococci, like *S. oralis* (which also exhibits a low sensitivity to the presence of most base resin materials), are intensive producers of hydrogen peroxide (13, 14). Along with the effects on other representatives of the oral microflora, hydrogen peroxide can also protrude into the role of the initiator of impaired tissue homeostasis in the mucous membranes, which leads to the development of inflammation and inflammatory tissue alteration. It is experimentally proved that due to production H_2O_2 *S. oralis* and *S. sanguinis* cause the death of macrophages (6, 15), which develops as a result of destabilization of lysosomal membranes (16) and also inhibit their protective function by lowering the expression of genes of pro-inflammatory cytokines TNF-α, IL-6 (10). We also demonstrated cytotoxic activity of the thought-out cytotoxic H_2O_2 of *S. sanguinis* strain for neutrophils (17). *S. oralis* and *S. sanguinis* cause death of epithelial cells of various origin (18). This action is also mediated by, as it does not manifest itself when adding in the pilot system of katylase.

DISCUSSION

The obtained results demonstrate that the base resin samples show the ability to inhibit planktonic growth of microbial cultures. This effect is not uncommon and is manifested in varying degrees relative to the individual test strains that represent the oral microflora. It can be assumed that in this case has matter the high biofilm ability of mentioned cultures, which causes the redistribution of microbial cells between planktonic phase and biofilm.

Summarizing the results of the research through the integral index of the planktonic growth suppression, we assume that most pronounced ability to inhibit the planktonic growth of oral microorganisms possess Villacryl → Protacryl = SYNMA.

Biocryl and the glass (material of comparison) are indifferent to the influence of oral microflora. Such sample as Bre.Flex, Polian, Nylon enhances planktonic growth of oral microflora representatives.

It can be assumed that the similar changes in the intensity of microorganisms' reproduction also occurs in the oral fluid of patients, that use appropriate type of dentures. Residual monomer is leached form resin base materials into the oral fluid, which can affect differently individual representatives of oral microflora. Increasing of the intensity of the planktonic growth contributes to accumulate a greater number of microbial cells in the oral fluid, which subsequently may increase the intensity of their adhesion on the mucous membrane and surface of prosthesis, contribute to the formation of dental plaque (especially with insufficient attention of the patient to the hygiene of the oral cavity and denture in particular).

It is established that even minor changes of micro ecology of oral cavity that arise under the influence of base resin materials should be consider as one of the main starting mechanisms for the development of prosthetic stomatitis.

Resin base materials for prosthodontic treatment should, at least, not cause strengthening of planktonic growth of microorganisms in the oral fluid, and ideally – possess antimicrobial properties that extend to the entire species spectrum of oral microflora. Therefore, attempts to create new resin materials, which consist of monomers with antimicrobial properties (metacrylic acid, methacryloxy-undecylpyridinium bromide, 12-methacryloxy-dodecylpyridinium bromide, 2-tert-butylaminomethyl-methacrylate), zeolite, nanoparticles of metals (silver, platinum) and their nanoxydes (silver, zirconium, titanium, vanadium) are relevant (19).

CONCLUSIONS

Through experimental research, we have proved the direct involvement of basic resin materials in changes in the oral cavity micro ecology and the process of occurrence of prosthetic stomatitis.

PROSPECTS FOR FURTHER RESEARCH

It is further planned to study the properties of basic materials by their influence on microbiological condition of the oral cavity to prevent complications from the use of removable dentures.

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