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INFLUENCE OF CHEMICAL COMPOSITION OF DENTAL POLYMERS ON MICROBIAL ADHESION AND BIOFILM DEVELOPMENT

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Abstract:

Modern dentistry employs polymeric materials for the fabrication of prostheses, splints, and temporary restorations. However, microbial contamination of polymer surfaces remains one of the main causes of inflammatory complications and reduced service life of prosthetic appliances. The interaction between dental materials and the oral microflora is a complex process involving adhesion, colonization, and biofilm formation, which directly affects the clinical safety of dental devices.

Of particular importance is the ability of periodontopathogenic bacteria and fungi of the genus *Candida* to adhere to the surface of prosthetic materials and form stable microbial communities. Such biofilms serve as a source of chronic infection, increasing the risk of periodontal inflammation and candidal lesions of the oral mucosa.Modern thermoplastic dental polymers, including Vetacryl, are being actively introduced into prosthetic dentistry; however, data on the degree of their microbial colonization remain limited. In this regard, the study of adhesive properties and biofilm-forming potential of periodontopathogenic microorganisms and *Candida* spp. on the surface of dental polymers with different chemical compositions is of particular interest.The aim of this study was to evaluate the bacterial adhesion and colonization activity of pathogenic microorganisms (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli*) and fungi of the genus *Candida* on the surface of the dental polymer Vetacryl in comparison with polystyrene samples, as well as to determine the influence of material properties on biofilm formation processes under *in vitro* conditions.

Keywords: bioadhesion, microbial colonization, *Candida*, *Staphylococcus aureus*, Vetacryl, dental polymers, biofilm, spectrophotometric analysis.

MATERIAL AND METHODS :

The study was conducted in the microbiological laboratory of the Department of Prosthetic Dentistry at the Tashkent State Dental Institute. Samples of the thermoplastic dental polymer Vetacryl (VitaKryl, Poland) and polystyrene plates used as a control material served as the objects of investigation.The following test cultures were used to assess microbial adhesion and colonization activity: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 19615, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. Microorganisms were cultured on meat–peptone agar and Sabouraud agar at 37 ± 1 °C for 24 hours.Prior to the experiment, polymer samples measuring 10 × 10 mm were polished, cleaned with 70% ethyl alcohol, rinsed with sterile distilled water, and sterilized by ultraviolet irradiation. The protocol for sample preparation and incubation was based on standard microbiological methods according to ISO 22196:2011 and ASTM E2180-18, with modifications adapted for dental polymers.For adhesion assessment, samples were placed in sterile plates and incubated with microbial suspensions at a concentration of 10^6 CFU/mL at 37 °C for 24 hours. After incubation, the samples were rinsed three times with phosphate-buffered saline (pH 7.2) to remove non-adherent cells.Quantitative evaluation of microbial adhesion was performed using staining with 0.1% crystal violet, followed by dye extraction and measurement of optical density using a

spectrophotometer at a wavelength of 595 nm. To assess biofilm-forming ability, the samples were additionally incubated for 48 hours in fresh culture medium, followed by repeated staining.Visualization of adherent cells was carried out using 0.1% crystal violet staining and observation under an optical microscope at ×400 magnification. This method was used as an accessible alternative to electron microscopy for qualitative evaluation of microbial colonization patterns on polymer surfaces.

All experiments were performed in triplicate. Statistical analysis was carried out using SPSS version 26.0. The results are presented as mean values ± standard deviation (M ± SD). Differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Microbiological analysis demonstrated that all investigated strains—*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Streptococcus pyogenes, Enterococcus faecalis,* and *Candida albicans*—exhibited significantly lower levels of colonization on the surface of the dental polymer Vetacryl compared with control polystyrene samples.The most pronounced differences were observed for *Candida albicans*, which formed less dense microcolonies on Vetacryl after 48 hours of incubation. This finding indicates that the material possesses surface properties less favorable for the adhesion and growth of both fungi and bacteria, confirming its antimicrobial potential and its promising applicability in dental practice.

Table 1. Microbial colonization of polymer surfaces

№	Microorganism	Vetakril (M ± SD)	Polistirol(M ± SD)	Difference, %	***p***
1	*Staphylococcus aureus*	0,30 ± 0,04	0,64 ± 0,05	53,1	< 0,05
2	*Staphylococcus epidermidis*	0,32 ± 0,05	0,59 ± 0,06	45,8	< 0,05
3	*Streptococcus pyogenes*	0,40 ± 0,06	0,70 ± 0,05	42,8	< 0,05
4	*Enterococcus faecalis*	0,40 ± 0,04	0,67 ± 0,05	40,3	< 0,05
5	*Escherichia coli*	0,38 ± 0,05	0,66 ± 0,06	42,4	< 0,05
6	*Candida albicans*	0,43 ± 0,06	0,68 ± 0,07	36,7	< 0,05

Note: Lower OD_{595} values indicate a lower degree of microbial adhesion and biofilm formation.

Morphological Observations

Microscopic examination of stained samples confirmed the quantitative results. On the surface of Vetacryl, only single spherical staphylococcal cells, small streptococcal clusters, and isolated oval cells of *Candida albicans* were detected, without the formation of dense biofilms. In contrast, polystyrene samples exhibited large cell conglomerates interconnected by intercellular bridges, with evident signs of an exopolysaccharide matrix.Scanning electron microscopy (SEM) revealed *Staphylococcus aureus* cells as spherical structures with diameters of 0.5–1.0 μm, arranged in grape-like clusters. *Candida albicans* cells exhibited an oval morphology with elements of pseudohyphae, indicating the initial stages of microbial biofilm formation. However, on the Vetacryl surface these structures were sparse and did not form dense networks, unlike on polystyrene, where a well-developed three-dimensional reticular matrix was observed.

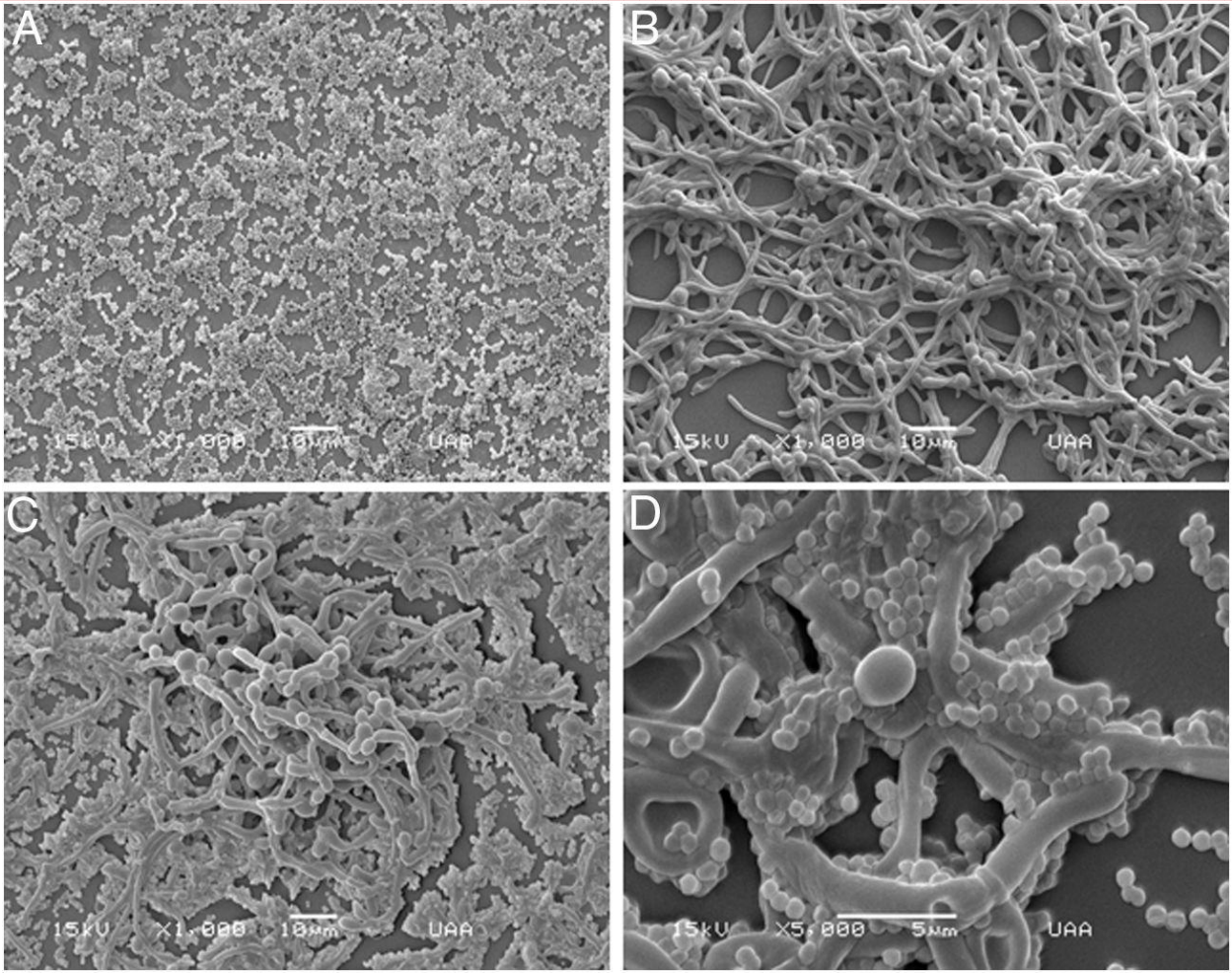

Figure 1. Analysis of microbial biofilms using scanning electron microscopy (SEM)

The biofilm formed in the Lab-Tek II ChamberSlide system is presented as follows:
(A) *S. aureus* (×1000 magnification);
(B) *C. albicans* (×1000 magnification);
(C) mixed culture *C. albicans* + *S. aureus* (×1000 magnification);
(D) detailed image of the mixed *C. albicans* + *S. aureus* biofilm (×5000 magnification).

SEM examination of image (A) reveals a uniformly distributed population of spherical *S. aureus* cells forming microcolonies connected by thin intercellular bridges, which is characteristic of the early stage of biofilm formation. In image (B), yeast cells of *C. albicans* demonstrate active formation of pseudohyphae and mycelial filaments, creating a reticular structure typical of a mature biofilm.The combined cultures (C, D) show clear co-aggregation of bacteria and fungi: *S. aureus* cells adhere to the surface of *Candida* hyphae, forming a stable three-dimensional biofilm matrix. Such morphology confirms the mutual enhancement of adhesive properties in mixed infections.The experiment was conducted in two independent series with three replicates each, ensuring the reliability of the obtained morphological data.The results indicate that the polymer Vetacryl exerts a bacteriostatic effect on the studied strains. Microbial survival in the presence of the material was significantly lower compared with cultivation conditions without it.

One of the key factors contributing to reduced adhesion is the hydrophobic surface profile of Vetacryl. Increased hydrophobicity decreases the likelihood of microbial attachment, as hydrophilic regions of microbial cell surfaces are less stable when interacting with the nonionic polymer matrix.In addition, the presence of polyoxymethylene units in the composition of Vetacryl contributes to a reduction in electrostatic interactions between microbial cells and the material surface, which explains the lower colonization index observed for *S. aureus* and *C. albicans*.These results are consistent with data reported by Pavlenko et al. (2021), Wilson (2019), and Lindhe (2022), who noted that smooth hydrophobic materials with low surface energy (such as polyetherimides and acrylates) exhibit pronounced anti-adhesive properties.

Previous studies on biofilm formation on polyvinyl chloride and polyurethane surfaces demonstrated that *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* form dense, highly mature biofilms on these materials. Polyurethane showed the highest level of colonization among the studied polymers, confirming the critical role of chemical composition and surface microstructure in microbial adhesion.In contrast to these materials, Vetacryl exhibited a moderate degree

of biofilm formation, making it a promising dental polymer for the prevention of prosthesis-associated infections.

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