

## ANTIADHESIVE ACTION OF DENTIFRICES\*

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*During the clinical studies of the toothpaste ROCS the investigators obtained data about the significant improvement of oral hygiene. The inhibition of dental plaque accumulation was noticed both by investigators and by study subjects. These data urged us to conduct additional research to assess the influence of the proteolytic enzyme bromeline (one of the key components of the patented formula) on the antiadhesive action of the toothpaste ROCS.*

The establishment of relation between the pathogen and the target cell as a result of bacterial adhesion is the constitutive element of the inflammatory process. Bacterial adhesion and following growth, with the creation of microcolonies and/or film, provide them with better conditions, connected, in particular, with the resistance to mechanical removal of bacteria from the host. It is proved that the adhesiveness of pathogenic bacteria often correlates with their pathogenicity and virulence. By the example of non-virulent strain of *Escherichia coli* it was shown, that moved plasmid, which controls synthesis of the antigen K88, is not only responsible of increase of microorganisms adhesion to the brush border of enterocytes, but also of the virulence of experimentally modified strain. Adhesion of *E.coli* to the epithelium of urinary tract, besides mechanical fixation of microorganism in the new ecological niche, leads to the metabolic changes adequate to new conditions. In addition to the metabolic changes, mechanical contact and adhesion of the P-fibers to epithelial cells lead to the changes in new fibers construction process (they become shorter) and stimulate the expression of some genes of *E.coli* virulence (pap-complex and hemolysine).

The molecular mechanism of bacterial adhesion is non-specific for pathogenic and commensal species, which is confirmed by the example of microbial flora of upper respiratory tract, lower digestive and genitourinary tracts. The basis of interaction of any biological systems and intercellular communications is the ligand-receptor recognition. Ligand is the smaller (by size and molecular mass) participant, for example, superficial structures of the bacterial cell. Receptor is the bigger complementary partner, for example, sites of adhesion on the cytolemma of eukaryotic cell.

Ligands and receptors are represented by polymers of glycolipidic or glycoproteinal nature, consisted of multiple copies of unique subunits, and defining the tropism of different pathogens to their target cells. The latter contributes to bacterial colonization in host tissues, there the concentration of receptors is higher. In vivo the process of adhesion is significantly influenced by dissolved components of biological liquids and secrets, which have the same chemical structure as cell receptors. Pathogens contact with these components earlier then with target cells. Orksov and Birch-Anderson (1980) demonstrated that *E.coli* adheres to the mucine of saliva before the oral epithelium. Oral streptococci (*Streptococcus sanguis*, *S.mitis*, *S.salivarius*) are able to absorb protein components of saliva. It was shown in the study, that this adhesion can be destroyed by the proteolytic enzyme tripsine.

Characteristics of adhesion as a multifactor process depend on different properties of bacteria as well as host. It is known that kind of species significantly influences adhesive characteristics of the microorganism. For example, *Streptococcus mutans* virtually cannot fix to epitheliocytes of tongue and cheeks, but it irreversibly adheres to dental surface. Arbuthnott and Smith (1979) note, that adhesiveness of *Sp.pyogenes* to oral epithelium is 6 times higher then that of *E.coli*. The direct relation between hydrophobicity of the cell surface and its adhesiveness is shown for the series of bacteria. For example, *St.aureus* from suppurative sites is more hydrophobic then *St.aureus* from environment, nasal cavity, skin surface.

Among factors influencing adhesive properties of host's cells and tissues there is an individual parameter of the patient: high level of colonization by *Str.pyogenes* in subjects suffering different inflammatory diseases, low level in carriers, and almost absence in healthy individuals. The adhesion of microorganisms to different sites in one host organism in different. In consideration of *Str.salivarius* and *Str.aureus*, the lower surface of tongue has the biggest amount of receptors and is the most favorable niche for invasion. The variability of the receptor apparatus of epitheliocytes may change under the influence of heterogeneity of cell pull. This heterogeneity is determined by physiological changes of superficial

cell structures during differentiation or aging. Pathological changes of host's tissues create additional conditions which stimulate the adhesion of bacteria.

Study of the molecular nature of ligand-receptor complexes generated during the interaction of bacteria with appropriate target cells, as well as study of factors influencing adhesive process in vivo and in vitro, permit us develop preventive measures aimed to earlier stages of infective process.

The search for antiadhesive agents is based on creation of effective blocks or obstacles (with different mechanisms of action) which would act upon the ligand-receptor interaction. One of the best known mechanisms, used for the search of adhesion inhibitors, is the insertion of soluble substances into the system bacteria-eukaryotic cell; these substances can compete with ligands of receptors for the adhesion sites on cell surfaces. Considering this, all soluble substances can be divided into two groups: able to interact with bacterial or with eukaryotic cells. The selective adhesion to bacterial ligands is more preferable because of less influence on receptors of target cells and, consequently, on different processes in host tissues.

Today there is multiple proven evidences, that usage of natural or synthetic cell receptors can significantly decrease or even prevent the adhesion of microorganisms to host cells. The interaction of microbial ligands with proteins and glycoproteins of blood (immunoglobulines A and G, p2-microglobuline, fibrinogene, fibronectine, albumine, transferrine and others), urine (TH-protein), saliva (mucine, agglutinins) was proved. This fact permitted the usage of most of listed compounds as inhibitors of bacterial adhesion in experiments, including clinical. Now we have data about antiadhesive action of exogenous proteolytic enzymes. The effect of enzymes is not limited to changes of the adhesion character between microorganism and target cell, but also lead to destruction of already formed colonies. Different enzymes demonstrate different efficacy levels. The results of analytical studies show the specificity of this action.

The goal of this study is to assess the effect of the toothpaste containing bromeline on the adhesion of human oral bacteria.

### **Materials and methods**

Materials. The experimental product was the dentifrice ROCS containing bromeline.

Control: the dentifrice of the identical formula without bromeline.

The study was conducted blind. The investigational products were marked with conventional numbers: 56 (ROCS) and 57 (control).

Test-cultures of microorganisms. Clinical strains of microorganisms isolated from the oral cavities of volunteers: *Staphylococcus aureus* 20, *Streptococcus salivarius* 67, *Streptococcus sanguis* 12, *Streptococcus sobrinus* 83.

Cell culture of musculocutaneous fibroblasts of human embryo.

Equipment. Bacteriological analyzers: IEMS-photometer (LabSystems, Finland), BBL Crystal (Becton Dickenson, USA); imaging system Video-TEST-Morphology (Germany).

Methods: microbiological, morphological.

All measurements were repeated three times.

#### ***Phase 1.***

The pure cultures of *Staphylococcus aureus* 20, *Streptococcus salivarius* 67, *Streptococcus sanguis* 12, *Streptococcus sobrinus* 83 were extracted by direct inoculation from volunteers' oral cavities to 5% blood agar.

The extracted strains were identified using listed bacteriological analyzers.

#### ***Phase 2.***

The antiadhesive effect of test toothpastes was studied on the cell culture (CC) of musculocutaneous fibroblasts of human embryo. Fibroblasts had been grown in Leiton vials on glass strips (Igl nutrient medium, 24 hours, 37°C) using method of Grabovskaya&Totolyan (1977), to the formation of confluent monolayer.

After that the nutrient medium was removed and there was added 1,8 ml of test toothpastes and 0,2 ml of 24-hour culture of appropriate test-strain ( $10^8$  CFU/ml). The mixtures were incubated 2 hours (37°C).

After the incubation monolayer cells were cleared of non-adhered bacteria by the multiple change of nutrient medium. They were fixed by 96% ethanol, dyed by Giemsa staining and studied under the microscope.

The assessment of inhibition of adhesion of test-strains was conducted after 1:20000 dilution of each paste in the presence of human serum.

The intensity of adhesive process was assessed by the following parameters: 1) index of adhesion (IA) counted as an average number of bacterial cells on one eukaryotic cell; 2) percentage of damaged monolayer cells (PC%); 3) semination of 100 monolayer cells, or bacterial load (BL), counted as  $BL=IA \times PC\%$ .

The degree of adhesion of each species is defined from the bacterial load in reference to control, accepted as 100%.

In the experiment 2 expositions were used: 2 hours and 3 minutes, with the toothpaste concentration 1:20000, which virtually doesn't damage the monolayer of cells (figures 1 – 4).

The table 1 shows, that dentifrices 56 and 57 didn't inhibited adhesion of test-strains strongly enough, then exposed for 2 hours. The percentage of the inhibition of adhesion was the following:

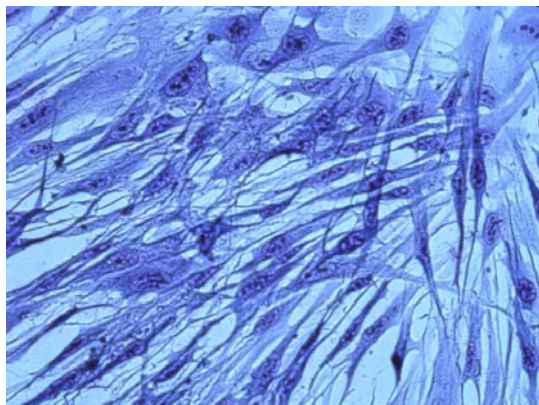
- S.aureus – 28% and 16%.
- Str.salivarius – 30% and 17%.
- Str.sanguis – 26% and 13%.
- Str.sobrinius – 31% and 17%.

The table 2 shows, that then the exposition time was reduced to 3 minutes the efficacy of toothpastes 56 and 57 increased sharply, and the percentage of inhibition of adhesion was the following:

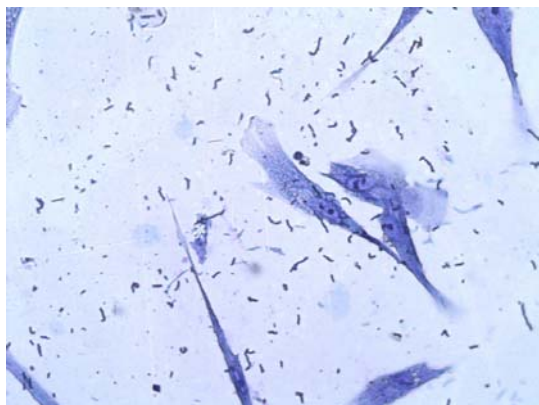
- S.aureus – 80% and 70%.
- Str.salivarius – 80% and 70%.
- Str.sanguis – 83% and 72%.
- Str.sobrinius – 79% and 67%.

In all cases the toothpaste 56 (ROCS with bromeline) was more effective then the toothpaste 57.

**Figure 1.** Cell control.



**Figure 2.** Cells + Str.salivarius 67.



**Figure 3.** Cells + Str.salivarius 67 + dentifrice 56 (1:20000), exposition of 2 hours.



**Figure 4.** Cells + Str.salivarius 67+ dentifrice 56 (1:20000), exposition of 3 minutes.



## Conclusion

The effect of agents for the inhibition of adhesion of normal oral microflora depends on exposition time. If the time of exposition is 2 hours, the efficacy of toothpastes is low, but if the exposition time is reduced to 3 minutes, the effect increase sharply and the inhibition reach 70-80% of microorganisms. At the same time, 3 minutes is normal toothbrushing time. The result can be related to reversibility of adhesion soon after insertion of strains into the model system, because after 1 or 2 hours the adhesion usually becomes irreversible.

The obtained results confirm the prospects for development of these formulas, especially the dentifrice 56 (ROCS) containing bromeline, as agents for prevention of formation of oral bacterial biofilm.

**Table 1.** Adhesive action of test-strains in the presence of test dentifrices 56 (ROCS) and 57 (control); exposition time 2 hours.

| The intensity of adhesion of bacterial test-strains |                   |            |           |           |                             |            |           |           |                |            |           |           |  |            |           |           |
|---|-------------------|------------|-----------|-----------|-----------------------------|------------|-----------|-----------|----------------|------------|-----------|-----------|--|------------|-----------|-----------|
| Tested object                                       | Index of adhesion |            |           |           | Percentage of damaged cells |            |           |           | Microbial load |            |           |           | Percentage of adhesion comparing control |            |           |           |
|   | S.aureus          | Str.saliv. | Str.sang. | Str.sobr. | S.aureus                    | Str.saliv. | Str.sang. | Str.sobr. | S.aureus       | Str.saliv. | Str.sang. | Str.sobr. | S.aureus                                 | Str.saliv. | Str.sang. | Str.sobr. |
| Control   |                   |            |           |           |                             |            |           |           |                |            |           |           |  |            |           |           |
| Cell culture (CC) + bacteria                        | 8                 | 9          | 10        | 7         | 25                          | 30         | 29        | 27        | 200            | 270        | 290       | 189       | 100                                      | 100        | 100       | 100       |
| Experiment  |                   |            |           |           |                             |            |           |           |                |            |           |           |  |            |           |           |
| CC + bacteria + dentifrice 56                       | 6                 | 7          | 8         | 5         | 24                          | 26         | 27        | 26        | 144            | 182        | 216       | 130       | 72                                       | 70         | 74        | 69        |
| CC + bacteria + dentifrice 57                       | 7                 | 8          | 9         | 6         | 24                          | 28         | 28        | 26        | 168            | 224        | 252       | 156       | 84                                       | 83         | 87        | 83        |

**Table 2.** Adhesive action of test-strains in the presence of test dentifrices 56 (ROCS) and 57 (control); exposition time 3 minutes.

| The intensity of adhesion of bacterial test-strains |                   |            |           |           |                             |            |           |           |                |            |           |           |  |            |           |           |
|---|-------------------|------------|-----------|-----------|-----------------------------|------------|-----------|-----------|----------------|------------|-----------|-----------|--|------------|-----------|-----------|
| Tested object                                       | Index of adhesion |            |           |           | Percentage of damaged cells |            |           |           | Microbial load |            |           |           | Percentage of adhesion comparing control |            |           |           |
|   | S.aureus          | Str.saliv. | Str.sang. | Str.sobr. | S.aureus                    | Str.saliv. | Str.sang. | Str.sobr. | S.aureus       | Str.saliv. | Str.sang. | Str.sobr. | S.aureus                                 | Str.saliv. | Str.sang. | Str.sobr. |
| Control   |                   |            |           |           |                             |            |           |           |                |            |           |           |  |            |           |           |
| Cell culture (CC) + bacteria                        | 8                 | 9          | 10        | 7         | 25                          | 30         | 29        | 27        | 200            | 270        | 290       | 189       | 100                                      | 100        | 100       | 100       |
| Experiment  |                   |            |           |           |                             |            |           |           |                |            |           |           |  |            |           |           |
| CC + bacteria + dentifrice 56                       | 4                 | 3          | 2         | 2         | 10                          | 18         | 25        | 20        | 40             | 54         | 50        | 40        | 20                                       | 20         | 17        | 17        |
| CC + bacteria + dentifrice 57                       | 5                 | 4          | 3         | 3         | 12                          | 20         | 27        | 21        | 60             | 80         | 81        | 63        | 30                                       | 30         | 28        | 33        |

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