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Features of morpho functional changes in periodontal structures in rats with the presence of a photo of a polymer or cement seal

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Abstract

High prevalence of caries is a great public health burden. To choose the optimal filling material for the restoration of hard tissues of teeth in caries patients is quite relevant. When choosing materials for dental restoration the doctor is guided by aesthetics quality, long-term guarantee of therapy, comfort at work and cost/quality ratio. **The objective:** under the conditions of an experiment to establish the relationship between the morphorfunctional state of periodontal components in the presence of either photopolymeric or cement filling and compare the data obtained with the results of intact animals. **Materials and methods.** The jaws of 41 white laboratory rats were used. 3 groups were formed: group 1 - intact; group 2 included animals with a photopolymer filling; group 3 consisted of rats with a cement filling. Histobacteriological, immunohistochemical, histochemical and statistical methods were used. **Results:** the animals with photopolymer filling material, in contrast to animals of the intact group, and those with cement filling, had a greater number of microbial cells in the biofilm during the whole experiment. The microbial cells exhibited enzymatic activity, against which there was a reduced number of glycosaminoglycans in the intercellular substance of the epidermis. **Conclusions:** Against the background of microorganisms increase in the biofilm of the epithelium, changes in carbohydrate-containing biopolymers of the biofilm, reducing the total number of glycosaminoglycans in animals with photopolymer filling material, the

number of lymphocytes and functional activity of antigen-presenting cells in the lamina propria of the gums took place. This indicates the activation of humoral nonspecific and specific links of immunity.

Key words: photopolymeric filling; cement filling; carbohydrate-containing biopolymer; morphorfunctional state of periodontium.

Introduction. The increase to 80-87% of caries prevalence of caries, which is closely connected with environmental problems and growing somatic diseases rate in the population is a burden of modern dentistry. To choose the optimal filling material for the restoration of hard tissues of teeth in patients with carious process, taking into account their compatibility is quite relevant. When choosing materials for dental restoration the doctor is guided by several criteria: aesthetics quality, long-term guarantee of therapy, comfort at work and cost/ quality ratio.

These requirements are fully met by plastic photopolymer X-ray contrast composite GC Gradia Direct Flo (Japan), which includes a traditional resin - dimethylacrylate - BisGMA [26].

BisGMA is derived from diphenylol propane, i. e. consists of Bisphenol A mixed with methacrylic acid, which is able to break down into components under the action of the aggressive environment of the oral cavity. Bisphenol A mimics the female hormone - estrogen. When this component leaks, it enters the circulation system. The second main component of BisGMA - methacrylate by 25% - 50% remains unpolymerized after photopolymerization, which poses a toxicological threat to the patient, including allergic contact stomatitis [22].

Of course, the drug BisGMA should affect the body's immune system, but to date this issue has not been highlighted in the scientific literature. In recent decades, there has been an increase in the incidence of pseudoallergies around the world, especially in dental diseases [7]. Therefore, there is a need for an experimental study of the reactivity of periodontal tissues and its lymphoid component under the influence of a photopolymer filling of the composite GC Gradia Direct Flo [28, 29].

Glass ionomer cement can be used as an alternative to photopolymer filling. But so far it has not been studied in a comparative aspect which filling materials - photopolymer or cement cause a more pronounced reactivity of the lymphoid component of periodontal tissues [2].

To date, it has not been studied how the extracellular matrix is affected by the system of "external barriers" of the oral mucosa, which is the body's first line of defense against various environmental pathogens and reproduces the internal environment state of stability [1, 21]. The resistance of anatomical formations and oral mucosa to damaging factors of microbial origin depends on the state of the protective systems, namely the lymphoid component of the gingival mucosa [3, 6, 10].

We share the opinion of leading scientists, and decided to confirm the biofilm attribution to the anatomical structure of the periodontium as its structure integral part [14]. To date, the carbohydrate composition of the epithelial biofilm has not been studied against the background of changes in the number of microorganisms in it, the composition of the glycosaminoglycon matrix of the epithelium against the background of photopolymer or cement filling, which may cause activation of various immune systems links and provoke an immune response by allergic type [5, 19].

The objective: to establish the relationship between the morphorfunctional state of periodontal components (chemical state of biopolymer biofilm, the number of biofilm microorganisms, the composition of glycosaminoglycan matrix of its own plate, qualitative and quantitative composition of the immune component of the gums own plate) in animals with the presence of either photopolymeric or cement filling and compare the data obtained with the results of intact animals.

Materials and methods

The experiment was conducted on 41 white laboratory rats. The object of the study were the jaws of laboratory adult rats. The animals were divided into 3 groups: group 1 - intact; group 2 included animals with a photopolymer filling; group 3 consisted of rats with a cement filling (glass ionomer polymer with low solubility in the oral cavity). Intact animals of the appropriate age were a control group.

The animals were sacrified from 13:00 to 14:00 p.m. by decapitation. Within a few minutes after slaughter fragments of the jaws were taken for examination. The material was fixed in Buen's liquid and in 10% formalin solution, decalcified, dehydrated, filled with paraffin mixture and made histological sections 5 μm thick. The jaws of the animals were removed after two, four and six weeks of the experiment [24, 25].

When working with experimental animals, bioethical rules were observed in accordance with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes“ (Strasbourg, 1986), the provisions of the “Rules of Conduct for Experimental Animals” approved by the Ministry of

Health of Ukraine № 753 of August 12, 1997, as well as the "Common Ethical Principles of Animal Experiments", approved at the I National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine "On Protection of Animals from Cruelty" (Law of February 21, 2006, 3447-IV, version of December 9, 2015, grounds 766-19).

The filling location was chosen taking into account the anatomical structure of the rats' incisors. Since the enamel on the incisors is present only on the vestibular surface of the tooth, respectively, the artificial tooth cavity was made on its anterior surface, slightly laterally. Given the high growth rate of the incisors (2.2 mm per week), the place of preparation was done closer to the gingival sulcus. On the prosected surface of the enamel and dentin one-component adhesive system of the fifth generation "Prime & BondNT" (Dentsply) was applied. It remained on the surface for 15-30 s to penetrate the etched enamel, open dentinal tubules, demineralized dentin surface for binding to collagen fibers to form hybrid layer.

Further, the adhesive was polymerized with the light of the activating photopolymer LED lamp B with a blue light source and a wavelength of 420-480 nm. The next stage consisted of the introduction of a portion of plastic light-curing X-ray contrast composite GC Gradia Direct Flo (Japan) with a syringe, followed by plastic treatment of the composite. Mechanically, with the tool, oxygen was displaced from the composite material. The final treatment was carried out by polymerization of the composite material under the action of a photopolymer lamp.

Thus, the method of establishing the filling material on the incisors of rats for further study of the reactivity of the local immune system of the periodontium to foreign filling material was developed [11].

Histobacteriological method was used to fix biopsy material for biofilm preservation according to Morozov (1999) [3]. Histological specimens were stained according to Brown-Brenn. Gram-positive bacteria turn black, gram-negative bacteria turn red. The number of microorganisms in the biofilm was counted using the morphological grid of Glagolev. The results of the calculations were standardized - the number of microorganisms per 100 μm2.

To study carbohydrate biopolymers of biofilms lectingochemical method with the use of lectins panel (peanuts (βDGal), soybeans (αNAcDGal), conconovalin A (αDMan), grape snail (NAcαDGal) was used. Standard sets of lectins (RPA "Lectinotest", Lvov) was involved. The reaction results were evaluated by a semi-quantitative method. The intensity of the benzidine label deposition was evaluated as follows: +++ - strong reaction (brown color), ++ - moderate reaction (yellow-brown color), + - weak reaction (light brown color), 0 - no

reaction Intermediate shades of color were evaluated respectively: ++ / +++, + / ++, etc. Peanut lectin (PNA) was used to identify β-D-galactose carbohydrate residues that detect PNA^+ lymphocytes; soybean lectin - to determine B-lymphocytes, α-D-mannose residues that detect LCA^+ - dendritic cells, used lentils lectin (LCA). To detect B_1-lymphocytes double conjugation of soybeans lectin and concovalinal A lectin with horseradish peroxidase, i. e. double label was used. Receptors for the first lectin, conconovalin A, are detected on B_1 lymphocytes by a benzidine label, and receptors for the second receptor, the soybean receptor, are visualized by α-naphthol combined with methylene green. The sections were immersed in glycerol-gelatin [15].

Immunohistochemical method. To study the distribution of receptors to CD^{5+}, CD^{20+}, CD^{4+} lymphocytes, standard sets of monoclonal anti-mouse antibodies from ascitic fluid (Sigma-Aldrich, St. Louis, USA) were used.

Histochemical method. Histochemical detection and differentiation of carbohydrate compounds was performed according to the scheme (Avtsyn AP, Strukov AI, Fuchs BB, 1971). The whole complex of glycosaminoglycans was detected with alcyan blue at pH 2.6 with a critical concentration of magnesium chloride of 0.2 M, without and after pre-treatment of sections with testicular hyaluronidase. To differentiate sulfated glycosaminoglycans (chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan - sulfate) staining sections with a solution of alcyan blue with a concentration of MgCl2: 0.6M and 1M was used. Taking into account the results of staining, histochemical detection of glycosaminoglycans was performed by a semi-quantitative method: +++ - turquoise color, ++ - blue, + - pale blue, 0 - no staining.

Statistical method. Statistical processing of the numerical results obtained was performed on a personal computer with Windows 7 operating system using the licensed program "Statistica for Windows 13" (TIBCO Software Inc., №JPZ804I382130ARCN10J) using the methods of variation statistics. The data were presented as M ± m (arithmetic mean and standard error of the mean). Significance of differences between independent samples of values was evaluated with the use Student's t-test. Differences at $p < 0.05$ were considered significant.

Microphotography of the objects under study was performed with a Primo Star microscope with Axiocam 105 color video system (Carl Zeiss, Germany).

The results of own research. In the system of "external barriers" the oral cavity mucous membrane is the first line of defense of the body against various pathogenic environmental factors and reproduces the state of stability of the internal environment. In animals of the intact group, the biofilm is represented by a thin layer of biopolymer. The

biofilm layer has an inhomogeneous structure and is represented by transparent wavy structures that have a brown color due to the accumulation of benzidine, which joins the complex of carbohydrate residues in the structure of the biofilm and horseradish lectin peroxidase ligand. The intensity of accumulation of hydrocarbon βDGal residues is identified by the semi-quantitative method on ++, i. e. the strata are brown, αDMan also on ++ (brown) and αNAcDGal on + (light brown). In animals of the intact group, the number of microbial cells per relative unit (RU) of biofilm area i- 256.8 ± 13.65 cells.

Gram-positive coccal microflora predominates in the biofilm in animals with a photopolymer seal at the 2nd week of observation. The number of microbial cells per unit area is 290 ± 16.34. In the biofilm, microorganisms have clusters in the form of aggregates, in contrast to the intact group animals, in which they are evenly distributed throughout the thickness of the biofilm. The biofilm of the epithelium of the sulcus in animals with a photo polymer filling is 1/3 thicker than in animals of the first group and has a layered, fluffy appearance, which is a condition for creating a gingival pocket. However, in the biofilm of animals with photopolymer fillings increases the number of biopolymers having the following carbohydrate residues: βDGal, αNAcDGal and αDMan. The number of biopolymers with galactose (+++) and mannose (+++) receptors is particularly increasing. The number of αNAcDGal carbohydrate residues has the level of animals of the intact group.

For the 4th week of observation, the number of biopolymers with carbohydrate residues: βDGal, αNAcDGal and αDMan increases, so the biofilm is brown. The number of biopolymers with receptors for galactose (+++) and mannose (++ / +++) and sialic acid and galactose (+++) is increasing, thus increasing the adhesion of biopolymers of biofilm to microorganisms. The number of microbial cells on the 4th week of observations increases to 315.28 ± 24.41 per RU of area.

For the 6th week of observations the number of biopolymers with carbohydrate residues βDGal, αNAcDGal and αDMan increases compared to the previous observation period. Especially increases the number of biopolymers with receptors for galactose (+++) and mannose (+++) and for sialic acid and galactose (+++) and increases the number of microbial cells to 332 ± 17.57 per RU of area. With cement filling the morphology of the biofilm corresponds to the morphology of animals of the intact group on the 2nd week of observations. In animals with a cement filling, the number of microbial cells per RU of biofilm area is 269.32 ± 14.43 cells. The intensity of accumulation of carbohydrate residues in animals with cement filling βDGal is identified by the semi-quantitative method on ++, as in

animals of the intact group, i. e. the layers are brown, αDMan also on ++ (brown) and αNAcDGal on + (light brown), as in the 1st group animals.

For the 4th and 6th week of observations, the number of microbial cells per RU of area increases slightly compared to the previous observation period, and is 278.64 ± 13.92 and 290.32 ± 24.58. Similarly, the amount of carbohydrate residues in the biofilm does not change compared to the previous observation period.

The qualitative and quantitative composition of the immune component of the gums is related to the peculiarities of the biofilm structure and the number of microbial cells. In animals with photopolymer filling, the number of antigen-presenting cells in the epithelium by the 2nd week from the beginning of the experiment does not differ from that in animals of the intact group (3.4 ± 0.08) and is 3.35 ± 0.12 per RU of area. The cells are also located in the basal layer of the epidermis, have an irregular polygonal body shape and an irregularly shaped nucleus.

The body sizes are insignificant, but larger and are 16-18 microns, rather thickening of the epidermis, compared with animals of the intact group. The number of outputs of the shoots is 3-4. The intensity of accumulation of the benzidine label is the same as in the intact group animals, i. e. Langerhans cells are in the same morphofunctional state. At the 4th and 6th week of observation, the number of antigen-presenting cells does not change significantly compared with the 2nd week of observation and is, respectively, 3.6 ± 0.17 and 3.5 ± 0.14 per RU of area. In cement filling animals for the 2nd week of observations, the topography of Langerhans cells - antigen-presenting cells corresponds to the topography of cells in all above mentioned groups in the basal layer of the epidermis. The cells also have a typical shape - star-shaped with 3-4 outgrowths of processes. Their number is, approximately, as in animals of the intact group - 3.8 ± 0.06 on the 2nd week of observations per RU area; 3.4 ± 0.65 - for the 4th week of observations and 3.1 ± 0.15 for the 6th week.

Immunologically immature PNA lymphocytes having, mainly, average diameter are found in own plate. They are located diffusely in the reticular and papillary layers. Their number increases in the presence of a photopolymer filling by 20% at the 6th week of observations compared with animals of the intact group and is 6.05 ± 0.48 per RU of area.

In the presence of a cement filling, the number of immunologically immature PNA lymphocytes also increases to 6.98 ± 0.36 lymphocytes per RU of area at the 6th week of observations.

In animals of the intact group SBA^+ -B-lymphocytes having receptors for soy lectin have a diameter of 11-12 μm. On the surface of the cytoplasmic membrane is a layer of brown

benzidine particles and such lymphocytes are identified as B-lymphocytes. SBA^+ -B-lymphocytes, which form humoral immunity, are located under the epithelium of the free layer, under the epithelium of the gingival sulcus, around the vessels. Lymphocytes are located singly, without forming clusters. The number of SBA^+ -B-lymphocytes is 4.07 ± 0.25 per RU of area. In animals in the presence of a photopolymer filling, the number of SBA^+ -B-lymphocytes increases by 2-2.3 times during all observation periods. In the presence of a cement filling, the number of SBA^+ -B-lymphocytes remains at the level of indicators of animals of the intact group.

$ConA^+$, SBA^+ -B_1 lymphocytes are located mainly in their own plate under the attachment epithelium, closer to the basement membrane and closer to the attachment epithelium. In animals of the intact group, B - lymphocytes having receptors for the lectin conconovalin A and soy have a diameter of 11-12 μm. On the surface of the cytoplasmic membrane is a layer of brown benzidine particles and green particles. Their number per RU of area is 2.02 ± 0.08.

In the presence of stopping material - photopolymer filling, the number of $ConA^+$, SBA^+ - B_1 lymphocytes increases, especially for the 6th week of observations and is 6.04 ± 0.24 per RU of area. In the presence of cement filling, the number of $ConA^+$, SBA^+ -B_1 lymphocytes increase 1.5 and 2 times compared with animals of the intact group.

Immunohistochemical examination revealed that in animals of the intact group CD^{5+} lymphocytes have a medium diameter and are located in the thickness of its own plate, under the basement membrane of the gum epithelium and the attachment epithelium. Lymphocytes are located singly or forming contacts with connective tissue cells. CD^{5+} -B_1 lymphocytes produce the main class of antibodies of the mucosal immune system - IgA antibodies, which are immunoglobulin receptors of B_1 lymphocytes. In animals of the intact group, the number of CD^{5+} -B_1-lymphocytes in its own plate is 5.5 ± 0.71 cells per RU of area. In the presence of a photopolymer filling, there is an increase in the number of CD^{5+} lymphocytes than in a norm and is 6.0 ± 0.4; 6.3 ± 0.42; 6.9 ± 0.36 lymphocytes per RU of area on the 2nd, 4th and 6th week of observation. In the presence of a cement filling the number of CD^{5+}-lymphocytes statistically do not differ from the indicators of intact group animals and their quantity is equal to 5. 7 ± 0.42; 6.6 ± 0.68; 6.6 ± 0.68, respectively, on the 2nd, 4th and 6th week of observation.

A study of the distribution of CD^{20+} lymphocytes showed that they are located in their own gum plate, under the epithelium, around the epithelium of the sulcus, near the vessels. Lymphocytes have a diameter of 14-16 μm. Benzidine layers are deposited on the surface of

the cytoplasmic membrane. In animals of the intact group, their number is 6.08 ± 0.64 lymphocytes per RU of area. In the presence of a photopolymer filling, the number of CD^{20+} lymphocytes on the 2nd week of observation is – 7.5 ± 0.5 per RU of area.

In animals of the intact group, their number is 6.08 ± 0.64 lymphocytes per RU of area.

In the presence of a photopolymer filling, the number of CD^{20+} lymphocytes on the 2nd week of observation is - 7.5 ± 0.5 per RU of area, on the 4th week, it practically does not change and is 7.5 ± 0.6 and on the 6th week of observations it is 8.0 ± 0.2. Topography observed is similar to that of the intact group animals.

The presence of a cement filling is characterized by the presence of CD^{20+} lymphocytes in its own plate in the same amount as in the animals of the second experimental group. Their number by the 2nd week of observations is 6.8 ± 0.64 cells per RU of area. On the 4th week of observations, their number increases slightly to 7.7 ± 0.48 and on the 6th week of observations it rises to 8.4 ± 0.48 cells per RU of area.

In animals of the intact group CD^{4+} lymphocytes are found singly in the thickness of their own plate and their number is 3.7 ± 0.56 cells per RU of area. The presence of a photopolymer filling is combined with an increase in the number of CD^{4+} lymphocytes, compared with the control group. Lymphocytes are located in their own plate in small clusters of 3-5 lymphocytes. The presence of a photopolymer filling leads to an increase in the number of CD^{4+} lymphocytes during all observation periods to 4.8 ± 0.48; 5.4 ± 0.6; 5.2 ± 0.8 lymphocytes per RU of area, compared with animals of the intact group. Cement filling shows no statistically significant differences in the number of CD^{4+} lymphocytes compared with animals of the intact group. The number of lymphocytes per RU of area is 3.5 ± 0.5; 4.1 ± 0.36; 4.8 ± 0.48, respectively, on the 2nd, 4th, 6th week of observations.

The number of cytotoxic CD^{8+} lymphocytes in all groups under observation practically corresponds to the index of animals of the intact group, and their number is 7.60 ± 1.26 per RU of area. CD^{8+} lymphocytes are located throughout the thickness of its own plate.

Discussion. Thus, the presence of photopolymer filling material leads to quantitative changes in the structure of the biofilm. There is an increase in the accumulation of biopolymers with carbohydrate residues - galactose, which has adhesive properties against microorganisms. Their number also increasing, which is consistent with the data of other scientists [4]. In the cell wall of gram-positive cocci there are ligands that bind to galactose residues of biopolymers of the biofilm. At the same time, the level of carbohydrate residues to mannose increases in the biofilm. Carbohydrate residues of mannose are found on

macrophages, leukocytes, lymphocytes and thus in the biofilm grow adhesive properties for macrophages and leukocytes, lymphocytes that produce cytokines and chemokines, which can affect the morpho-functional state of the gingival epithelium. In the presence of photopolymer filling material, the microbial load in the biofilm of the epithelium of the gingival sulcus increases in comparison with the norm and in the composition of the biofilm increases the number of carbohydrates with terminal residues to mannose and galactose, as a response to changes in the composition of the microbial biocenosis of the biofilm.

In the presence of a cement filling changes in structure of a periodontium are not expressed, if compared with animals of intact group.

The animals with photopolymer filling material, in contrast to animals of the intact group, and those with cement filling, had a greater number of microbial cells in the biofilm during the whole experiment. The microbial cells exhibited enzymatic activity, against which there was a reduced number of glycosaminoglycans in the intercellular substance of the epidermis and therefore the intercellular space had [8].

It is known that a significant role in regulating the permeability of capillary-connective tissue structures is played by the system hyaluronic acid - hyaluronidase. Hyaluronidase (probably of bacterial origin) causes depolymerization of glycosaminoglycans, destroys the bond of hyaluronic acid with protein, thereby sharply increases the permeability of connective tissue, which leads to partial loss of barrier function [18, 23]. Thus, the protection of periodontal tissues from bacterial and toxic agents with glycosaminoglycans decreases. The number of toxic agents is greater in the biofilm of the gingival epithelium against the background of carbohydrate residues βDGal, αDMan, αNAcDGal increase in biopolymers of gum biofilm [16].

According to the morphofunctional state of antigen-presenting cells, we concluded that in animals with photopolymer fillings the functional activity of these cells increases, which can lead to the activation of the immune response [13]. In animals with a cement filling, the indicators of the morphofunctional state of antigen-presenting cells are almost the same as those in the intact group animals.

The increase of SBA^+-B-lymphocytes in groups of photopolymer fillings animals emphasizes the activation of a specific link of humoral immunity, which does not contradict the data of [17].

For the first time in periodontal tissues, $ConA^+$, SBA^+-B_1 lymphocytes were described using the lecting histochemical method. Lectin concovalinalin A and soy lectin with a dual imaging system that describes a nonspecific link of immunity were used. $ConA^+$, SBA

+ -B_1 increases in the group of animals with photopolymer filling and indicates the activation of nonspecific humoral immunity [9, 12].

To understand the pathogenetic mechanism of periodontal tissue rearrangement in the presence of photopolymer fillings, a scheme of morphological changes is proposed (Fig. 1). This may be the basis for prevention of complications after photopolymer filling to insert.

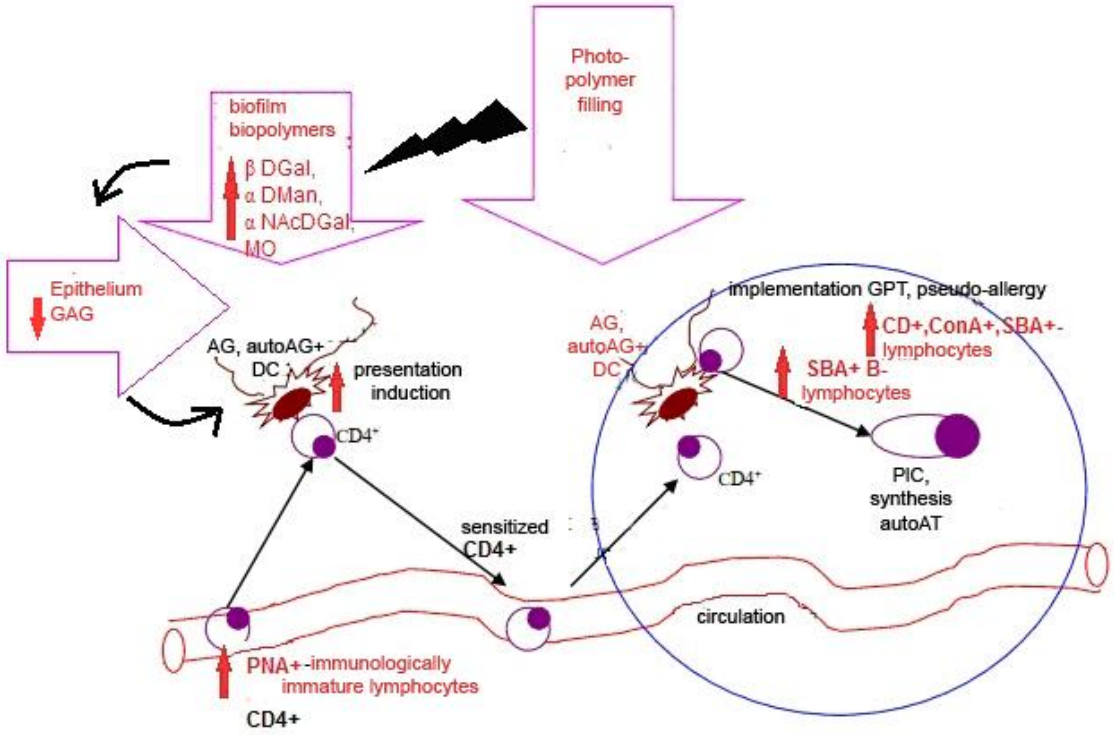

Fig. 1. Scheme of morphofunctional changes in paradont in the presence of photopolymer filling.

Note: the results of own researches are marked red: AG-antigen, DC-dendritic cell, GAG- glycosaminoglycan

↑ - *Increase of the indexes* ↓ - *Decrease of the indexes*

As in Fig. 1, the presence of CD^{4+} lymphocytes, the number of which exceeds the norm in animals with photopolymer fillings, and can probably be sensitized by periodontal autoantigens against the background of increasing microbial load in the biofilm, and their presence in periodontal tissues compatible with DC and B lymphocites. This involves three-cell cooperation and autoantibodies production. So, CD^{4+} lymphocytes have a central immunopathogenetic role in the periodontium destruction [20, 27].

Conclusions. 1. In the presence of filling material of photopolymer origin, the microbial load in the biofilm of the epithelium of the gingival sulcus increases, in comparison with the norm and in the presence of a cement filling.

2. In the presence of photopolymer filling material in the biofilm of the gingival epithelium, the amount of carbohydrates with terminal residues to mannose and galactose increases as a response to changes in the composition of the microbial biocenosis of the biofilm. This is in contrast to animals of intact group and group of animals with cement filling.

3. In animals with photopolymer filling, the accumulation of low- and high-sulfated glucosaminoglycans decreases in comparison with animals with cement filling and norm.

4. Against the background of microorganisms increase in the biofilm of the epithelium, changes in carbohydrate-containing biopolymers of the biofilm, reducing the total number of glycosaminoglycans in animals with photopolymer filling material, the number of CD^{4+} -, CD^{5+} -, SBA^{+}-B-, SBA^{+}, ConA-B_1 – lymphocytes and functional activity of LCA^{+}-antigen-presenting cells in the lamina propria of the gums, in comparison with the indicators of the intact group animals and those with cement filling increases. This indicates the activation of humoral nonspecific and specific links of immunity.

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