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STUDY OF THE SPECTRUM OF DENTAL COMPOSITES BIOLOGICAL ACTIVITY IN MODEL EXPERIMENTS IN VITRO

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ИССЛЕДОВАНИЕ СПЕКТРА БИОЛОГИЧЕСКОЙ АКТИВНОСТИ СТОМАТОЛОГИЧЕСКИХ КОМПОЗИТОВ В МОДЕЛЬНЫХ ОПЫТАХ IN VITRO

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The nature of the effect of composite dental materials, based on methacrylate and oxirane, on the structural and functional properties of the blood cells of healthy subjects was investigated in model experiments in-vitro. There were revealed the changes of structural and functional characteristics of red blood cells, indicating the membrane damaging effect of the monomer composites. It is shown that incubation of blood with unpolymerized material causes expressed deformation of the membrane, erythrocyte aggregation. Methacrylate based composites have a more pronounced negative potential due to biodegradation that requires strict compliance with the polymerization conditions. Oxirane based materials are bioinert after polymerization, which allows recommending them for wider application. The study substantiated the feasibility of using of the developed experimental model for biological testing of dental filling materials.

Key words: monomers, dental composites, biological activity, blood, red blood cells

Проведено исследование характера влияния композитных стоматологических материалов на основе метакрилата и оксирана на структурно-функциональные свойства клеток крови клинически здоровых лиц в модельных опытах in-vitro. Выявлены изменения структурно-функциональных характеристик эритроцитов, свидетельствующие о мембраноповреждающем действии мономеров композитов.

Показано, что инкубация крови с неполимеризованными материалами вызывает выраженную деформацию мембран, агрегацию эритроцитов. Композиты на основе метакрилата вследствие биодеградации обладают более выраженным негативным потенциалом, что требует строгого соблюдения режимов полимеризации. Материалы на основе оксирана после полимеризации биоинертны, что позволяет рекомендовать их для более широкого применения. Проведенное исследование обосновало целесообразность использования разработанной экспериментальной модели для биотестирования стоматологических пломбировочных материалов.

Ключевые слова: мономеры, стоматологические композиты, биологическая активность, кровь, эритроциты

One of the important reasons for the rapid development of dental composites market is imperfection of classical materials, unsatisfying objective requirements for biocompatibility, durability, aesthetic perfection of restorations, as well as the ever-increasing patients' subjective demands. The complex of these conditions predetermined the development of new filling materials. Currently, composites form one of the most important groups of materials in dental practice. They are used for filling of all the groups of teeth, fissure sealing, fixing the dentures, as facing composites [5, 15]. The most widespread are the materials, organic matrix of which is a copolymer of acrylic and epoxy resins, one of the basic monomers of which is Bisphenol – glycidyl methacrylate (Bis-GMA), comonomers are triethyleneglycoldimethacrylate (TEGDMA) and urethanedimethacrylate (UDMA) [8, 12, 13]. The ratio and composition of monomers varies according to the specificity of use, as well as the polymer manufacturer [11, 14]. Fundamentally new by chemical structure are Tet-Sil composites based on siloranes and oxiranes; these are open rings monomers not containing methacrylic groups. These filling materials are designed to overcome and reduce the polymerization shrinkage. Obviously, different types of monomers in the composite determine the material properties, affect its polymerization degree, biodegradation, chemical reactivity [3, 6, 10]. To assess the potential risk of adverse reactions due to dental materials, physical, chemical and mechanical properties of the polymer are predominantly studied [4, 7]. In order to determine biocompatibility of materials at preclinical stage the cytotoxicity data are analyzed on murine fibroblast cell culture, the contact tests of polymers with human whole blood basophils for histamine release detection, the composite tissue implants in the oral cavity of laboratory animals [1, 2, 9]. Application of these methods makes it necessary to use special equipment to determine individual response of the organism on the filling material in a particular clinical situation. In this context, the creation of new in vitro tests to study the biocompatibility of polymer dental materials is important.

The aim of this study was to investigate the spectrum of the biological activity of dental composites based on methacrylate and oxirane in model experiments in vitro.

Material and Methods. The study of influence of oxirane and me-methacrylate on the structural and functional characteristics of blood cells was conducted in model experiments in vitro. The study involved 60 somatic healthy volunteers with the sanitized oral cavity aged 18 to 21 years. As a material of laboratory testing venous blood taken with a closed vacuum system «BD Vacutainer» («Becton Dickinson», USA) was used. Total 85 blood samples were analyzed. The control group consisted of 17 venous blood samples taken from persons without somatic pathology. Effect of methacrylate and oxirane on the biomaterial was studied in two experimental groups of 34 samples each. The samples of the 1st experimental group were affected by methacrylate: one subgroup was acted by unpolymerized material, the second – by polymerized material. The samples of the 2nd experimental group consisting of two similar subgroups were affected by the oxirane based material.

Composite samples of 30 mg (5×5×5 mm) were incubated with venous blood for 30 minutes in a sterile Petri dish («SciLabware», UK) at room temperature. 17 composite material portions in each group were pre-polymerized with halogen curing lights «Demetron LC», company «KERR Hawe» (Germany). The light output was 600 mW/cm^2, wavelength – 500 nm, the exposure time – 40 seconds. Further study of the structural and functional characteristics of blood cells was carried out using an automated hematology analyzer «Sismex KH-21» before and after 30-minute incubation with composites. Also, after pre-staining by Leishman method, blood products ultramicroscopy was performed with the microscope Zeiss Axiostar (×1500). Statistical processing of the results was performed using «ANOVA» computer program.

As composite dental materials «Filtek™ Supreme XT» («3M-ESPE», USA) were used for bioindication of methacrylate and «Filtek® Silorane» («3M-ESPE», USA) – for bioindication of oxirane.

«Filtek™ Supreme XT» is nanocluster universal composite for restoration of front and lateral groups of teeth. The organic matrix comprises Bis-GMA, TEGDMA, UDMA and Bis-EMA acrylic and epoxy resins. Inorganic inclusions (72.5 % by weight; 57.7 % by volume) are a combination of aggregate silica cluster filler (average size of particles in the cluster: 0.6–1.4 microns; primary particle size – 75 nm) and non-agglomerated silicon filler with particle size of 75 nm.

«Filtek® Silorane» is low shrunk micro-hybrid composite material for the restoration of lateral teeth group. Chemical composition: the organic matrix is a combination of a new class of polymers – siloxanes and oxiranes (siloranes). Inorganic inclusions (81.8 % by weight; 64.2 % by volume) are a combination of silanized quartz with the addition of X-ray contrasting particles of yttrium trifluoride (average particle size 0.5–0.6 micrometers, primary particle size 150 nm).

Investigation of the spectrum of biological activity of monomers of filling materials «Filtek™ Supreme XT» and «Filtek® Silorane» was performed using «Prediction of Activity Spectra forsubstances: Complex & Training» computer system. Interpretation of the results is carried out using the program «Pharma Expert», proposing mechanisms for implementing of specific biological effect of the tested compound.

Results and Discussion. In the analysis of the results changes of hematological parameters concerning mainly structural and functional properties of erythrocytes are revealed. So, after the incubation of whole blood with the unpolymerized portions of the material based on methacrylate in the absence of quantitative changes there is a tendency to an increase in hemoglobin, hematocrit, which is likely due to the agglutination and aggregation of red blood cells, changes in cell shape, which affects the detection (Table 1). The data obtained are confirmed by ultramicroscoping, during which in the control blood preparations erythrocytes of a normal form with central clearing are revealed (Fig. 1a). In blood preparations after incubation with methacrylate based unpolymerized material, agglutination, aggregation of red blood cells, severe deformation of the membrane, acquiring an irregular shape (Fig. 1b) are marked.

Table 1

Effect of methacrylate based unpolymerized and polymerized material on haematological parameters, (M±m)

№	Indices	Control	Unpolymerized methacrylate	Polymerized methacrylate
1	Leukocytes (WBC,×10^9/l)	6.10±0.45	5.97±0.40	6.18±0.56
2	Erythrocytes (RBC,×10^{12}/l)	4.90±0.15	5.06±0.15	5.27±0.14
3	Hemoglobin (HGB,×10 g/l)	14.33±0.44	15.34±0.54	15.74±0.41
4	Hematocrit (HCT, %)	43.11±1.29	45.79±1.44	46.29±1.28
5	Mean corpuscular volume (MCV, vial)	88.01±0.57	88.40±0.58	87.86±0.70
6	Mean cell hemoglobin in the red blood cell (MCH; pg)	29.21±0.18	30.25±0.32	29.88±0.27
7	Mean corpuscular hemoglobin concentration (MCHC; g/l)	33.26±0.18	34.22±0.28	34.03±0.27
8	Platelets (PLT, × 10^9/l)	248.06±20.03	247.08±22.22	237.94±19.61
9	Lymphocytes (LYM, %)	34.56±2.04	33.36±1.93	33.03±2.29
10	Medium-sized cells (MXD, %)	10.79±1.13	11.21±2.35	8.78±1.82*
11	Neutrophils (NEUT, %)	55.48±3.89	56.24±4.68	60.88±4.33*
12	The absolute number of white blood cells in/ml of whole blood (LYM)	2.09±0.16	1.96±0.15	2.00±0.19
13	The absolute number of basophils, eosinophils and monocytes in ml of whole blood (MXD)	0.74±0.09	0.70±0.13	0.67±0.15
14	The absolute number of neutrophils in ml of whole blood (NEUT)	3.92±0.45	3.82±0.47	4.20±0.69
15	The weighted distribution of platelets (PDW, vial)	13.79±0.42	13.82±0.49	14.57±0.56
16	Mean platelet volume (MPV, vial)	10.89±0.23	10.86±0.22	11.28±0.24
17	The ratio (%) of large platelet volume to the entire volume of the platelet (P-LCR)	32.29±1.90	33.15±1.85	35.38±2.0

Investigation of data obtained during the hematology analysis of blood samples incubated with a polymerized methacrylate-based material, more significant changes are identified as by the number of modified indexes, and by the degree of difference from the control values. Under the influence of a polymerized methacrylate there occurs more pronounced increase in hemoglobin concentration (+10.0 %), the hematocrit value (+7.4 %), which is probably due to aggregation of erythrocytes that is clearly observed at microscopy of these samples (Fig. 1c).

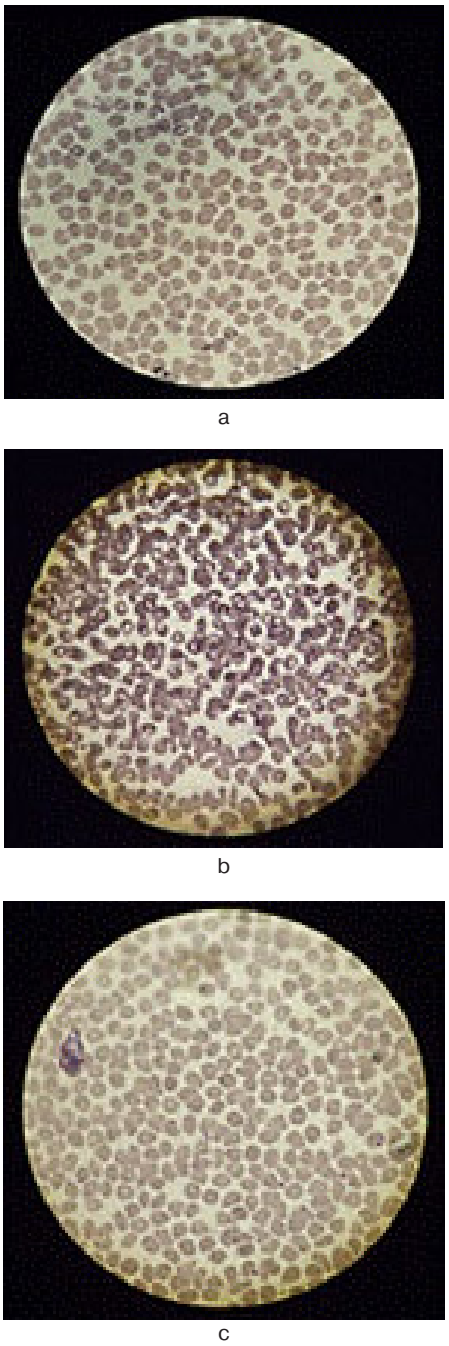

Fig. 1. Blood preparations incubated with methacrylate-based composites: a – control sample; b – a blood product incubated with unpolymerized material; c – blood preparation incubated with the polymerized material

In addition, we identified the phenomena of agglutination, aggregation of red blood cells, so-called «coin columns», concentration and an increase in the number of red blood cells in the field of view are defined, and the phenomenon of poikilocytosis (elliptocytosis) is observed. Also the influence of the material on the performance of leukocyte number is revealed. It is noted marked reduction of cell number of MXD-group –

monocytes, eosinophils, basophils by 18.6 % compared to the control values, increased neutrophil count by 9.7 %, reflecting changes in the leukocyte formula. A tendency to a decrease in the number of platelets (–8.2 %) is revealed.

At evaluation of the cellular composition of blood, incubated with oxirane-based material, an increase of hemoglobin content (+13.0 %) is revealed, there is a tendency to increase of the hematocrit (+9.0 %) (Table 2).

Table 2

Influence of oxirane-based unpolymerized and polymerized material on the haematological parameters, (M±m)

№	Indices	Control	Unpolymerized oxirane	Polymerized oxirane
1	Leukocytes (WBC, $\times 10^9$/l)	6.10±0.45	5.96±0.46	6.12±0.53
2	Erythrocytes (RBC, $\times 10^{12}$/l)	4.90±0.15	5.31±0.14	5.30±0.14
3	Hemoglobin (HGB, ×10 g/l)	14.33±0.44	16.20±0.52	16.09±0.52
4	Hematocrit (HCT, %)	43.11±1.29	46.98±1.30	46.65±1.29
5	Mean corpuscular volume (MCV, vial)	88.01±0.57	88.54±0.58	87.98±0.69
6	Mean cell hemoglobin in the red blood cell (MCH; pg)	29.21±0.18	30.49±0.34	30.33±0.36
7	Mean corpuscular hemoglobin concentration (MCHC; g/l)	33.26±0.18	34.44±0.28	34.46±0.30
8	Platelets (PLT, × 10^9/l)	248.06±20.03	243.42±23.65	226.35±19.71
9	Lymphocytes (LYM, %)	35.15±2.04	33.90±1.61	33.31±2.21
10	Medium-sized cells (MXD, %)	12.12±1.41	12.74±1.75	10.27±2.65
11	Neutrophils (NEUT, %)	50.75±4.79	52.30±4.28	56.15±4.57
12	The absolute number of white blood cells in/ml of whole blood (LYM)	2.09±0.16	1.99±0.15	2.03±0.18
13	The absolute number of basophils, eosinophils and monocytes in ml of whole blood (MXD)	0.71±0.12	0.79±0.11	0.75±0.20
14	The absolute number of neutrophils in ml of whole blood (NEUT)	3.90±0.57	3.73±0.59	3.63±0.55
15	The weighted distribution of platelets (PDW, vial)	13.79±0.42	13.85±0.46	14.48±0.53
16	Mean platelet volume (MPV, vial)	10.89±0.23	11.07±0.23	11.26±0.25
17	The ratio (%) of large platelet volume to the entire volume of the platelet (P-LCR)	32.29±1.90	33.44±1.88	35.24±2.03

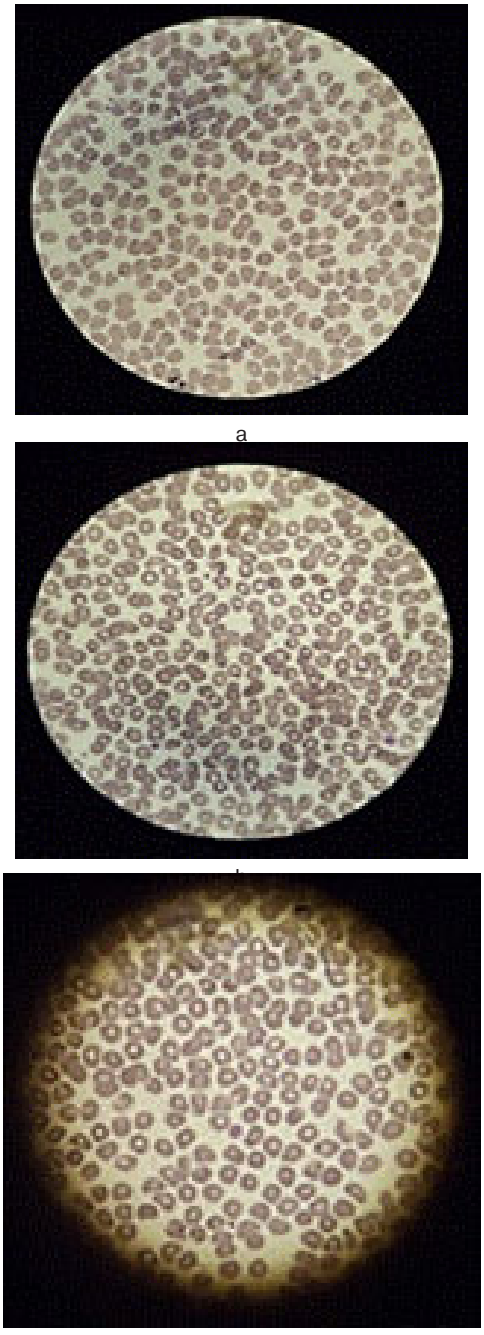

Fig. 2. Blood preparations incubated with oxirane-based composites: a – control sample; b – a blood preparation incubated with unpolymerized material; c – blood preparation incubated with the polymerized material

At blood microscopy after incubation with unpolymerized material based on oxirane samples demonstrate changes in shape and increased erythrocyte aggregation with many aggregates (++++) (Fig. 2b). When compared hematological parameters after incubation with polymerized portions of the material from the control group, increase of the hematocrit (+8.6 %) is determined, which is probably due to the tendency to increase of the number of red blood cells and a higher content of hemoglobin (+8.6 % and 12.6 %, respectively) (Table 2). The changes in leukogram are also identified, they are manifested in the reduction of the amount of MXD-cells by 15.3 %, increase in the number of neutrophils by 10.6 %, the downward trend in the number of lymphocytes (–5.2 %), platelets

(–9.6 %). These changes are probably due to fixation of the cells in the protein monolayer, which is formed on the polymer surface by its interaction with blood. Notable is the fact that by microscopy of blood samples after incubation in polymerized oxirane-based material the picture is close to normal. Erythrocyte monolayer without severe aggregation is determined (Fig. 2c).

Thus, these data indicate membrane damaging effect of methacrylate and oxirane on blood cells, resulting in a change in the quality characteristics of red blood cells. And methacrylate has a more pronounced damaging potential compared with oxirane, which is clearly visualized by microscopy of the samples. It should be also noted that the blood incubation with polymerized portions of the studied dental filling materials causes multidirectional changes of hematological parameters. After 30 minutes of action of polymerized methacrylate we found changes in the qualitative and quantitative characteristics of red blood cells, confirmed by microscopy, caused, probably, by continuing membrane damaging action of monomer because of its biodegradation even with standard polymerization mode. Another pattern was observed by us after a similar 30-minute incubation of blood samples with polymerized portions of oxirane. A minor deviation from the norms of the studied parameters is revealed, there are no phenomena of aggregation of red blood cells in blood smears that indicates the final polymerization and bioinertion of filling material. The identified changes in the leucocyte count and a decrease in the number of platelets by incubation of blood samples with polymerized portions of methacrylate and oxirane are consistent with the available scientific data of domestic and foreign researchers about the interaction of non-biological materials with the blood and are probably conditioned by fixation of the cells in a protein monolayer formed on polymer surface. Removing of the last of the tested samples leads to a decrease in the number of leukocytes and platelets from baseline values [2,10,11,14,15].

Conclusions. Destructive effect of monomers of composite filling materials (methacrylate, oxirane) on the structural and functional characteristics of blood cells is revealed. Methacrylate has a more pronounced membrane damaging capacity compared with oxirane. Biodegradation observed after the polymerization of the composite determines the importance of adhering to the finishing modes of fillings. Consistently stable indices of bioinertia of oxirane-based polymerized composites are an important advantage among selection criteria for composite materials in dental practice. The expediency of using of these in vitro tests for determining the biocompatibility and personalization in the choice of a composite dental material is substantiated.

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