Introduction The technique of synthesis of new embolization materials based on 2-hydroxyethyl methacrylate with the use of dimethacrylate glycols with different quantity oxyethyl fragments between acrylic groups as cross-linking agents is offered. An accessible and reliable method of an estimating the biocompatibility of a polymeric material with blood cells is developed. It is shown that synthesized embolization materials have a regular superamolecular structure, have a strengthened hemostatic effect at biocompatibility preservation. Since the middle of the 70s yr of the last century, there has been steadily increasing interest in polymers for biomedical cross applications, which are widely and successfully used in medicine and pharmacology. Polymers for biomedical applications must satisfy such requirements as: occlusion to the body, resistance to a biological environment, etc. [1-4]. One of the representatives from of a large class of such polymers are three-dimensional (cross-linked) hydrogels based on 2-hydroxyethyl methacrylate (HEMA). The main advantages of such gels are large porosity, swelling in aqueous solutions (up to 65%), non-toxic properties, elasticity and resistance to external biological environment [5]. They are the properties, which determine the high biocompatibility of these materials with body tissues, enabling to apply them in the target embolization of vessels. [6]. Thus, the embolization material (EM) based on poly-HEMA has been successfully used for the treatment of certain tumor (ischemia of liver tumors, kidney, myomatous nodes, etc.). The high porosity of the hydrogels based on poly-HEMA has a positive effect on the material compatibility with the tissues body tissues and promotes a growth of connective fibrous tissue in the pores of the hydrogel, which leads to a stable fixation in the blood vessel [5]. Another obvious advantage of the porosity is a possibility of using EM for delivery and controlled release of drugs [7]. Traditionally, ethyleneglycol dimethacrylate (EGDMA) has been using as the cross-linking agent. A possibility of using cross-linking agents different in qualitative or quantitative structure of allows modifying physical and chemical properties of the resulting synthesized hydrogels, the functional characteristics being preserved. In order to expand the range of EM based on poly-HEMA for application in various pathologies and with a possibilities of speed regulation of the organization of blood clot, ingrowing of tissues into polymeric material, and also saturation by medications we have developed methods of syntheses of poly-HEMA with use tri-ethyleneglycol dimethacrylate (tri-EGDMA) and tetra-ethyleneglycol dimethacrylate (tetra-EGDMA) as cross-linking agents. The development and application of the EM based on poly-HEMA in clinical practice primarily requires the development of simple, available and reliable methods of assessing the biocompatibility of a polymeric material (PM) with blood cells, as well as studying the genesis of its haemostatic effect. Analysis of changes in parameters of donor blood during the interaction with the newly obtained materials, synthesized with different types of cross-linking agents should include a obligatory program of research: that is the definition of the number of leukocytes, erythrocytes, the levels of Hb, platelets and the assessment of the degree of hemolysis of the blood. The present

study has been aimed at investigating the possibility of using tri-EGDMA and tetra-EGDMA as cross-linking agents, as well as the research of the morphological structure of the polymers, their biocompatibility and hemostatic effect. Materials and methods Cylindrical emboli have been synthesized in aqueous solution under the action of the initiating system: ammonium persulfate - N, N, N', N'-tetramethylethylenediamine at 20ºC [8], with a constant ratio of monomer: cross-linker 46,7:1 mol/mol. As the cross-linking agents were used EGDMA, tri-EGDMA or tetra-EGDMA. The polymerization has been carried out in tubes of a length of 12-15 cm and a diameter of 1 mm. After polymerization the blanks of hydrogels have been cut into pieces 1 cm long and washed in water at 80ºC for several h with the replacement of one to remove unreacted monomer. Washed emboli have been placed in sterile containers with isotonic sodium chloride solution and stored at room temperature before further research. The surface morphology and pore structure of cylindrical emboli has been investigated with the use of a scanning electron microscope Jeol YSM 5300 LV (Japan). Contrast samples have been coated with gold on the installation Jeol YFC-1100 E "Ion sputtering device." The thickness of the sputtering was about 40 Å. To assess the preservation of blood cells 0.1 ml of the solution in which EM was stored (0.08 g of PM in 10 ml) has been added to 0.1 ml of blood. In stained blood smears for Romanowsky-Giemsa have been studied the morphology of leukocytes, erythrocyte and platelets, which characterize the degree of integrity and intactness of the blood during its contact with the PM. Increased hemolysis (20%), leukopenia, anemia and sharp thrombocytopenia, as well as the morphological features of damaged blood cells (leukocytes, erythrocytes and platelets) is an indicator of biological incompatibility of the PM with blood, precluding a possibility of its application in clinical practice [5]. Results and discussions The process of three-dimensional polymerization is of microheterogeneous nature [9]. Primary gel structures appear at the earliest stages of polymerization, and the concentration of such microgels, ceassing to increase since some moment. The reactionary system consists of a set of local microreactors in which the polymerization reaction proceeds though is at different stages. Simultaneously with chemical processes of polymerization and formation of cross-link occurs in microreactors. The physical processes of interlacing of both formed and growing chains also takes place. The polymerization occurs on the surface of individual microreactors (globule) building-up the new macromolecules chains. As a result of increasing globules their contact and aggregation is occurring. Further polymerization takes place in zones of contact and in the space out of these zones. The processes described above define structural and morphological features of EM being formed. The resulting polymer has a complex heterogeneous structure and contains both dense globular formation and flexible weak links between them. For EM, formed by poly-HEMA-EGDMA, the size of the globules varies in the range of 2-10 microns, the packaging is friable, and the linear-chain-like aggregates of globules organize multi-strands, leading to the organization of macro-cell structure. The latter, in turn, create

macropores up to several tens of microns (Fig. 1a). The material based on poly-HEMA-tri-EGDMA virtually do not exhibit communication between globules, packing is more dense, the size of the globules varies in the range of 2-10 microns (Fig. 1b). The structure of poly-HEMA-tetra-EGDMA is more regular, the bond between the globules is absent, and there appear “unloaded” chain-like aggregates. The size of the globules varies in the range of 1-2 microns (Fig. 1c). Similar to poly-HEMA-EGDMA, this polymer tends to create a macro-cell structure, but without voluminous voids. a b c Fig. 1 - The structure of: a) poly-HEMA-EGDMA; b) - poly-HEMA-tri-EGDMA; c) poly-HEMA-tetra-EGDMA Previously we have shown the following [2, 10]: - under the contact of the donor blood with poly-HEMA-EGDMA used after several removals of residual low molecular weight substances by water washing out, have been observed minimal damage of blood cells and for these indicators this material is suitable for application in endovascular surgery; - Hb levels varied in the range 11.5 ÷ 12 g/L; - hemolysis of the blood did not exceed 11 mg%; - the number of erythrocytes was not less than 3.5 × 10^{12} L-1, at the average, this reduction amounted to 10÷15% relative to the level of erythrocyte of the donor blood, with no observed morphological changes of erythrocytes being observed; - number of leukocytes decreased in the range the 15÷18%, i.e., from (6.2 ± 0.4)×109 to (5.1 ± 0.3)×109 L-1, p 0.05; - morphological preservation of white blood cells (mean, 4 of damaged cells to 200 cells), at the average. Figure 2-3 shows the micrographs of the original donor blood (2) and the blood after the interaction with the medium in which EM has been stored (3, a-c). The photomicrographs present the unchanged blood cells, mainly erythrocytes, leukocytes, and platelets. The interaction of the donor blood with a liquid medium of poly-HEMA-EGDMA exhibit no signs of hemolysis. The morphology of blood is good, there are small aggregates of platelets, some neutrophilic leukocytes, erythrocytes and their aggregates (Fig. 3a). Fig. 2 - The morphology of cells donor blood The contact of the liquid medium of poly-HEMA-EGDMA with the donor blood continued to cause thrombocytopenia due to increased formation of platelet aggregates, which reflects a specific property of poly- HEMA associated with the release of residual monomer at a concentration of 10-5 g/g, thus accelerating the process of platelet aggregation [2, 10]. The number of platelet decreased, by 3 times from (22.1 ± 0.5)×109 to (7.5 ± 0.3)×109 L-1 at the average. Thus, the emboli from the poly-HEMA-EGDMA are hemostatically active material, which primarily activates the adhesive-aggregative properties of platelets via desorption of residual monomer in this concentration. Activation of plasma hemostatic factors is secondary character and develops in 1-3 days and stored post embolization period of 10-15 days, depending on the speed and volume of injected EM in the blood vessel when the X-ray endovascular occlusion. In testing the newly synthesized EM with cross-linking agents, triethyleneglycol dimethacrylate or tetra-ethyleneglycol dimethacrylate on the proposed scheme the following results has been obtained. a b c Fig. 3 - The morphology of cells after interaction with isotonic sodium chloride solution, in which was kept embolization

material: a) - poly-HEMA-EGDMA; b) - poly-HEMA-tri-EGDMA; c) - poly-HEMA-tetra-EGDMA The contact of the donor blood with a liquid medium in which the emboli from poly-HEMA-tri-EGDMA have been stored also causes a minimal damage to blood cells, the preservation of blood remaining good (Fig. 3b). Leukocytes (neutrophils), stab neutrophil, platelet macroform are observed in the visible region. The level of Hb in this test varied in the range of 11.6÷12 L-1. Hemolysis of blood did not exceed 10 mg%. The number of erythrocytes did not decrease below $(3.35\pm0.6)\times10^{12}$ L-1 and the average was 10÷14% relative to the level of erythrocytes of the donor blood. There were no signs of morphological damage to cells. The number of leukocytes decreased in the range of 15÷20%, i.e., of (6.2 ± 0.6)×109 to (5 ± 0.4)×109 L-1, p 0.05. Morphological preservation of the white blood was good. The increased formation of platelet aggregates results in accelerated thrombocytopenia, the number of platelet decreased by 1.5÷2.5 times from (21.5 ± 0.7)×109 to (8.6 ± 0.2)×109 L-1, p 0.01, respectively. However, unlike the poly-HEMA-EGDMA the aggregation properties of erythrocytes increased and instantly organized into micro- and macroaggregates capable of adhesion to leukocytes and platelets. Under the contact the donor blood with the liquid medium in which the emboli from the poly-HEMA-tetra-EGDMA have been stored there was a slight damage of blood cells (damaged cells have not been identified), the preservation of the formed elements was satisfactory (Fig. 3c). In the visible region is observed densely organized aggregations of erythrocytes, neutrophils, leukocyte with the platelet adjacent to its surface. A characteristic feature of such contact is contiguity of platelets or their groups on the aggregations of erythrocytes. The level of Hb varied within 11÷12 L-1, hemolysis of blood did not exceed 12÷13 mg%. The number of erythrocytes did not decrease below (3.1±0.4)×1012 L-1, and the average was 15÷16% relative to the level of erythrocytes of the donor blood. In addition, an increasing erythrocytes aggregation activity has been observed, which is reflected in the organization of macroaggregates in the form of a erythrocytic clot. The number of leukocytes is reduced in the range of 20%, i.e., from (6.1±0.5)×109 to (5.05±0.3)×109 L-1, p 0.05. Table 1 presents the data on changes in of the cellular elements of the donor blood under contact with EM, which was synthesized with different cross-linking agents. Morphological preservation of white blood cells was also satisfactory. Apparently, an increased formation of platelet aggregates causes the activation of thrombocytopenia with a decreasing number of platelet by 3 times, from (21.5±0.7)×109 to (7.1±0.3)×109 L-1. It should be noted that the genesis of hyper-coagulative activity under the interaction of both poly-HEMA-tri-EGDMA and poly-HEMA-tetra-EGDMA can be specified as an increase in the aggregative properties of erythrocytes with the formation of macroaggregates and erythrocytic clots with participation of platelets, which instantly increases the area of obturation in the vessel.