Antithrombotic Suture Modification: Long-Term Storage Stability

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The aim of the study was to study thromboresistant properties of modified suture material after three-year storage.

Materials and Methods. We used polypropylene Serapren 3.0.-based suture. To modify suture material we applied 3% biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate), molecular weight 280 kDa in chloroform on suture surface. Unfractioned heparin was used as a pharmaceutical substance to produce an antithrombotic and antiproliferative effect. Suture material was modified in several stages using a multistep chemical reaction that enabled to rigidly attach the coating on suture surface.

Results. The assessment of uniformity and integrity of a modifying layer has revealed a modified suture surface after 3-year storage to remain evenly covered by a biodegradable layer. Spectroscopic study enabled to determine reliably the presence of a heparin layer in the coating, as evidenced by the presence of sulfo groups in spectrum.

Histology of biomaterial samples stitched by modified and unmodified suture showed the difference in tissue response to suture. The samples sutured by an unmodified suture material had marked inflammatory signs, significant lymphocyte accumulation being found around. However, the samples with modified sutures showed insignificant lympholeucocytic infiltration.

Conclusion. The suggested chemical technique of surgical suture modification is promising, since pronounced antithrombotic properties of the suture and high biocompatibility persist over a three-year period.

Key words: suture material; suture modification; antithrombotic coating; biopolymers; heparin.

Introduction

Suture material originated in 2000 BC, though suture improvement lasts until the present day [1, 2]. A promising trend today is suture material with biological activity [3–9]. Currently, in the market there is a great variety of modified suture materials with various therapeutic characteristics [5, 6, 10, 11]. Generally, there are suture materials with antibacterial activity [4, 7, 9, 11]. At the same time, there are no suture materials with an antithrombotic effect to be applied in reconstructive vascular surgery. In addition, the number of reconstructive operations on various vascular circulations is increasing annually [12, 13]. Chemical antithrombotic suture modification we have suggested [14] enables to reduce the number of early and delayed sequelae in vascular surgery.

A key feature of suture materials is the preservation of their sterility, biocompatibility and given properties within scheduled storage time [1, 2]. Shelf-life for unmodified suture materials averages 5 years depending on sterilization type applied. A storage period for

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modified sutures can differ, it depending on biologically active components composing the coating. It should be taken into consideration when developing a modified suture material. Previously, we showed [14] that the suggested technique of chemical modification using a biodegradable polymer of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and unfractioned heparin enables to increase bio- and hemocompatibility of sutures.

The aim of the investigation was to assess the efficiency of antithrombotic coating on the modified suture surface after three-year storage under standard conditions.

Materials and Methods

In the study we used polypropylene Serapren 3.0.-based suture. For further modification, we applied a biodegradable PHBV polymer layer, molecular weight 280 kDa, synthesized in Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino, Moscow region, Russia), on the suture surface. PHBV exhibits high biocompatibility, since a monomer comprising the polymer is a natural human and animal metabolic product, it being present in blood in the norm [15]. For modification we used 3% PHBV solution in chloroform. The solution concentration is optimal, since it enables to form an even coating, 6–7 nm wide, on the suture surface. Unfractioned heparin (Belmedpreparaty, Belarus) was used as a pharmaceutical substance to produce an antithrombotic and antiproliferative effect.

Suture material was modified by unfractioned heparin in three stages [14]: (i) initiation of grafting polymerization centers on the biopolymer layer surface by ozonizing; (ii) formation of an additional sublayer of poly(methacryloyl chloride) comprising active acid chloride groups, which can form stable covalent bonds with heparin; (iii) chemical grafting of heparin on a formed sublayer made of heparin solution in bicarbonate buffer at 2–5°C within 10 h, and then 14 h at room temperature, followed by air drying at room temperature. Modified suture samples were stored at room temperature in a shadowed place, single-packed. Shelf life expiration is three years after the date of modification.

Modifying coating evenness was assessed by scanning electron microscopy using the microscope Hitachi S-3400N (Hitachi, Japan). The samples under study were mounted on special tables and covered by a gold-palladium coating by means of ion sputtering using the exhaust cart Emitech SC7640 (Quorum Technologies, United Kingdom).

Heparin integrity and presence in a superficial layer of suture material were studied by Raman spectroscopy (RS) of high spatial resolution (micro-Raman spectroscopy) using the spectrometer Horiba LabRAM HR800 (HORIBA, France). Sample surfaces were locally scanned by exciting laser radiation with predetermined precise calibration of laser pumping power to perform a non-destructive molecular assay. Sequential spectral scanning was carried out along a longitudinal spatial coordinate Z (deeply in the coating, away from the surface) at a pitch of 1 μm. The research area in XY plane (i.e. along the suture) was randomly selected, with statistical average of measurement results.

Biocompatibility *in vivo* was estimated by subcutaneous implantation of xenopericardial samples (ChemPeriplas; NeoCor, Kemerovo) with modified and unmodified suture to male Wistar rats (weighing 55–70 g). ChemPeriplas fragments, 6×6 mm, were implanted as controls. The implantation period was 2 months, after that xenopericardial samples with a part of surrounding tissues were removed and put in buffered formalin. Tissue response to suture material was assessed by light microscopy (Axio Imager A1; Carl Zeiss, Germany). Tissue specimens were van Gieson's stained. All procedures with animals were carried out in accordance with the standards set out in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011); with the National standard of the Russian Federation 33044–2014 "Principles of Good Laboratory Practice"; with the ethic principles established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 2006). The research protocol was approved by the Ethics Committee of Research Institute for Complex Issues of Cardiovascular Diseases.

Antithrombotic properties of suture material were assessed relying on platelet aggregation parameters. Platelet aggregation activity after donor's blood came in contact with modified and unmodified suture material was studied using 4-channel platelet aggregation analyzer APACT 4004 (LABiTec, Germany). The contact of samples and blood lasted for 3 min. Fasting blood was taken in the morning by the ulnar artery puncture, in separate plastic collecting tubes with 3.8% sodium citrate, in 9:1 ratio. Platelet aggregation was induced by collagen solution, 2 mg/ml (Renam, Russia).

The findings were statistically processed using Statistica 6.0., and presented as a median and quartile deviation. The differences were considered significant if $p<0.05$.

Results and Discussion

The first stage enables to assess heparin safety on a modifying layer surface. When studying vibration spectra of heparin layer on a modifying suture surface, the emphasis was made on the analysis of RS line intensity typical for sulfo groups in its molecular structure. Micro-Raman spectroscopy used enables to measure vibration spectra at photo-excitation of substance of microscopic extent (in the order of units of cubic μm). In this case in laser 1D, 2D, or 3D scanning

of any sample, transparent in a selected frequency range of laser pumping and inelastic light scattering, one can obtain a spectral RS pattern with high resolution along any spatial coordinates. Micro-Raman spectra of suture samples were measured in infra red laser excitation, wave length being 785 nm. It enabled to entirely eliminate the effect of very strong spurious photoluminescence, which is observed in the materials under study under electromagnetic radiation in a visible wave length region.

Obtained micro-Raman spectra in the range of 100–1800 cm^{-1} (Figure 1) are situated from top to bottom, while the caustic of an exciting laser beam is lowered deep inside the surface. False coordinate of the caustic centre in depth in a sample is indicated in μm. Zero μm corresponds to the sample surface. In the selected hardware configuration, caustic has its own Gaussian length, about 2 μm, so the coordinate is –1 μm (the highest spectrum), it corresponding to the case when a caustic centre is elevated above the object surface under study to receive a signal from the submicrometer (in depth) part of a sample. Color code helps correlate the information with the diagrams themselves.

The spectral fragments enable to see a considerable number of peaks and spectral bands of inelastic light scattering, which refer to the excitement of a set of stretch and deformation vibrations for different functional groups. Due to high transparency of the system under study, a part of these spectral components at RS excitement length wave refer to polypropylene and PHBV, many of them being overlapped by bands in heparin spectrum. Reliable deconvoluting of such integral components in RS spectra is not always possible and effective. For this reason, we restricted the width of analytical spectral range by a narrow range of 1000–1100 cm^{-1}, in which there can be found RS bands typical for sulfo groups in sulfated polysaccharides. Figure 1 shows the gradual loss of signal from sulfo groups in the range 1085 cm^{-1} with growing excitement depth. Thus, spectral analysis enables to assess heparin layer thickness (about 2 μm) in modified suture material. All other peaks suggest the presence of polypropylene and biopolymer in the coating.

The quality of the applied coating after three-year storage was studied by scanning electron microscopy. We estimated the evenness and integrity of a modifying layer (Figure 2). The modified suture surface after three-year storage was found to be covered uniformly by a biodegradable layer (Figure 2 (c)). Furthermore, there are some insignificant fragmentary areas with broken integrity of a modifying layer that is inessential and has no effect on antithrombotic properties of sutures, as demonstrated by the findings presented hereafter.

In contrast we presented a modified suture, which

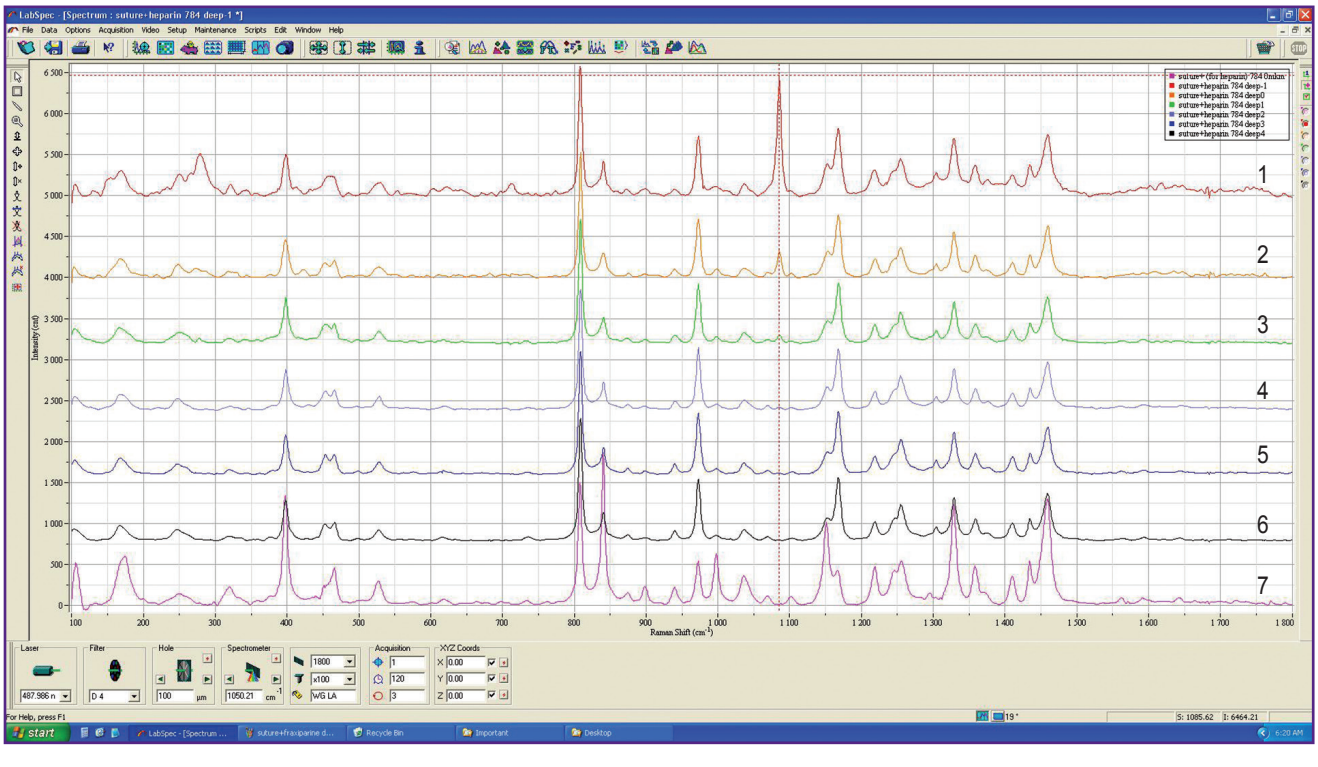

Figure 1. Micro-Raman spectra when deepening the caustic center of a laser beam inside a sample (top to bottom): (*1*) suture material + heparin, 1 μm deep; (*2*): suture material + heparin, 0 μm deep; (*3*): suture material + heparin, 1 μm deep; (*4*): suture material + heparin, 2 μm deep; (*5*): suture material + heparin, 3 μm deep; (*6*): suture material + heparin, 4 μm deep; (*7*): reference spectrum of Raman scattering of suture material (polypropylene + poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) without heparin

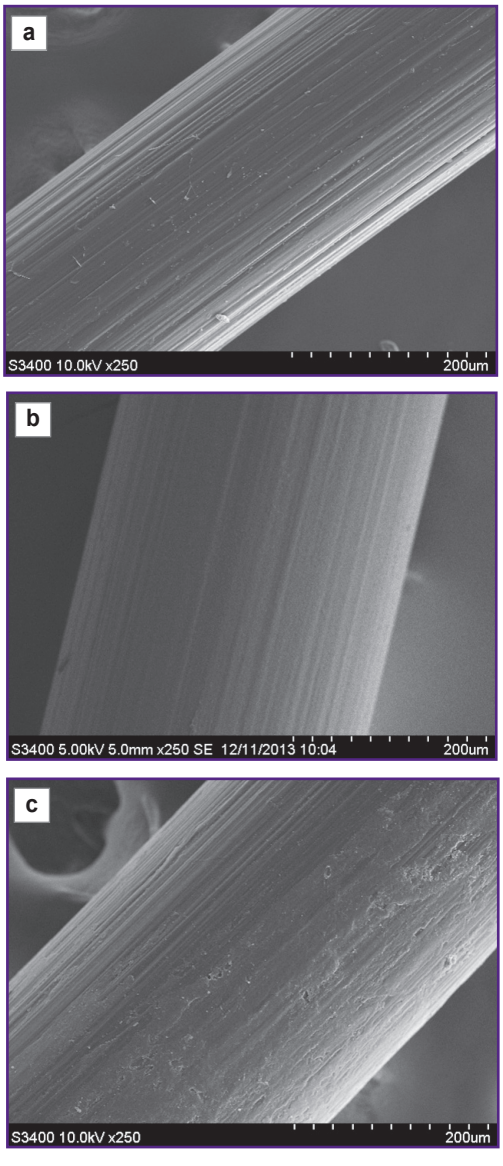

Figure 2. Scanning electron microscopy of suture material surface:
(a) non-modified suture; (b) suture immediately after modification; (c) modified suture after 3-year storage; ×250

surface was studied immediately after the modification (Figure 2 (b)). On the surface one can see a uniform coating throughout the suture with no exfoliation sings and integrity damage.

Since one of the key characteristics of suture material is biocompatibility, the factor variation was assessed in subcutaneous suture implantation in laboratory animals. The histological examination of the removed xenopericardial samples with a part of surrounding tissues sutured with modified and unmodified suture material revealed the difference in tissue response to suture material (Figure 3).

The examination of the samples with an unmodified suture showed the signs of marked inflammation of granulomatous inflammation type (Figure 3 (b)). Collagen fibers in the area adjacent to the suture were loosely arranged, in some places they being fragmented.

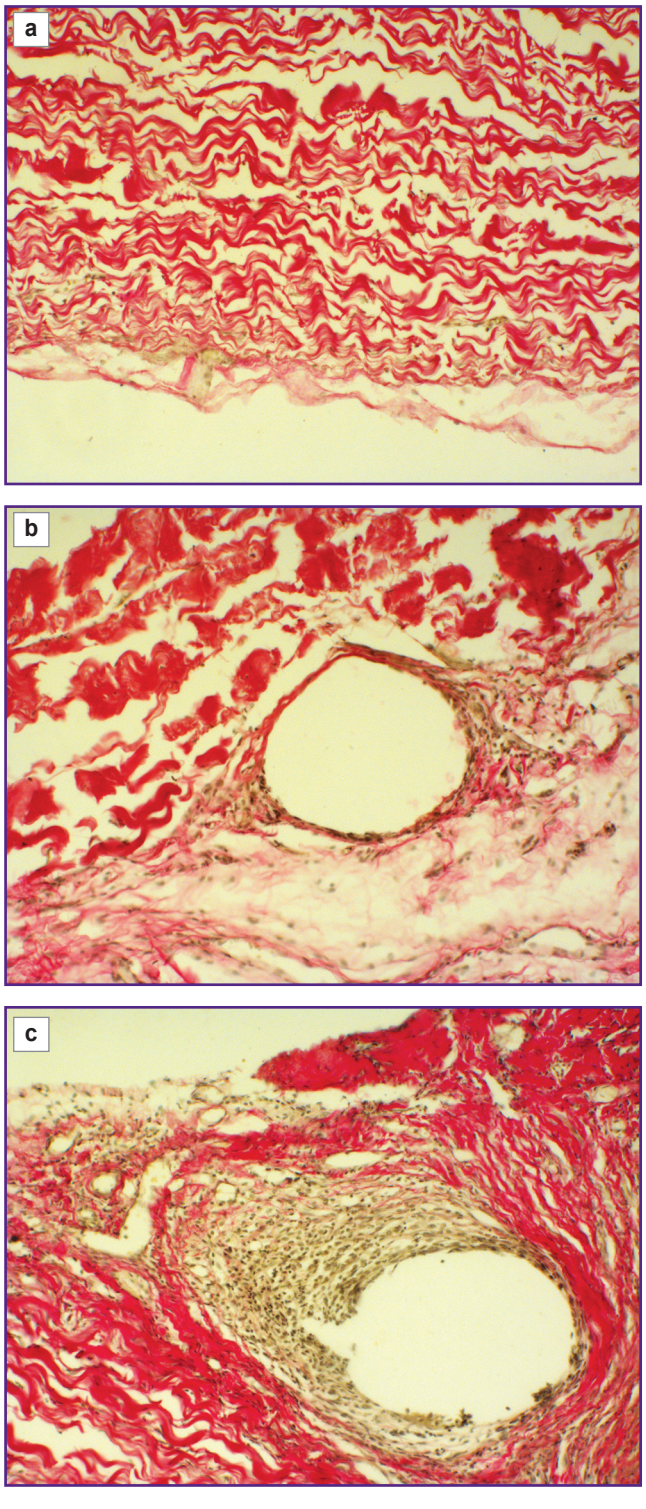

Figure 3. Light microscopy of histological specimens:
(a) pericardium, control; (b) pericardium + non-modified suture material; (c) pericardium + heparin modified suture material; van Gieson's staining; ×200

Around the suture there were significant accumulations of lymphocytes suggesting an inflammatory reaction to suture material. In addition, the samples with modified suture material were characterized by insignificant lympholeucocytic infiltration located along the suture (Figure 3 (c)); collagen fibers were tortuous, their structure preserved.

It should be mentioned that xenopericardial samples without sutures, which were implanted in rats as controls, caused no inflammatory reaction, collagen fibers being crinkled and densely arranged (Figure 3 (a)). The findings suggest suture material to be able to cause a significant inflammatory response. The inflammation reaction can result in integrity damage of surrounding tissues including those implanted that can be followed by adverse effects when cardiovascular biological grafts are implanted. Suture material modified by PHBV and heparin enables to reduce a negative effect on surrounding tissues, a protective effect being maintained after three-year storage.

In addition to the fact that suture biocompatibility increases, the developed modification improves hemocompatibility of polypropylene suture. Platelets were found to be among the first responding to foreign material when an implant comes in contact with blood: there occur their activation, adhesion, and further aggregation, they releasing biologically active substances potentiating cell aggregation and blood protein clotting [16]. Thus, thromboresistant properties of suture materials can be judged by their platelet aggregation degree.

The study findings of the effect suture material has on platelet aggregation over again confirmed that suture material is able to provoke platelet aggregation increase [14]. Then, platelet aggregation maximum in blood, which has not come in contact with suture material, does not exceed the normal level amounting to 51.06 [51.02; 51.08]%. Moreover, the samples with unmodified suture material enhanced platelet aggregation up to 55.05 [55.04; 55.09]%. Despite the fact that the findings had no statistical significance (p=0.08), we can suggest a significant increase of platelet aggregation properties. Surgical suture modified by heparin and PHBV solution enabled to reduce (p=0.04) platelet aggregation up to 44.53 [40.23; 48.38]% indicating the possibility to improve bio- and hemocompatibility of modified suture material.

Thus, the developed modification technique based on a multistep chemical reaction enables to firmly attach the coating on suture surface, the covering being preserved for three years.

Conclusion

The findings have demonstrated the potential of the suggested technique to improve surgical suture characteristics. After three-year storage, suture material modified by poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and heparin appear to have preserved high biocompatibility and marked antithrombotic activity. The modification technique of suture material can be applied to produce suture with antithrombotic characteristics for its further usage in cardiovascular surgery.

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Conflicts of interest. The authors declare neither financial nor other conflicts of interest related to the present study.

References

1. Bontsevich D.N. *Khirurgicheskiy shovnyy material* [Surgical suture material]. Moscow: Integratsiya; 2005.

2. Buyanov V.M., Egiev V.N., Udotov O.A. *Khirurgicheskiy shov* [Surgical suture]. Moscow: Rapid-Print; 1993.

3. Kabeshev B.O., Zinovkin D.A., Bontsevich D.N., Nadyrov E.A. The effect of surgical suture material modified with silver nano-particles on the course of inflammatory wound process in vivo in microbial contamination. *Problemy zdorov’ya i ekologii* 2014; 2(40): 109–115.

4. Kniaziuk A.S., Lyzikov A.N., Zinovkin D.A., Nadyrov E.A., Bontsevich D.N. The influence of new antibacterial sutural material on the traumatory process in experiment. *Problemy zdorov’ya i ekologii* 2015; 1(43): 48–53.

5. Mokhov E.M., Sergeev A.N., Serov E.V. The development of new biologically active suture materials and using them in the abdominal surgery. *Novosti khirurgii* 2013; 21(3): 23–32, https://doi.org/10.18484/2305-0047.2013.3.23.

6. Patahov G.M., Ahmadudinov M.G. Bioactive of suture materials for gepatorafii. *Fundamental’nye issledovaniya* 2011; 7: 124–126.

7. Obermeier A., Schneider J., Föhr P., Wehner S., Kühn K.-D., Stemberger A., Schieker M., Burgkart R. In vitro evaluation of novel antimicrobial coatings for surgical sutures using octenidine. *BMC Microbiol* 2015; 15(1): 186, https://doi.org/10.1186/s12866-015-0523-4.

8. Saxena S., Ray A.R., Kapil A., Pavon-Djavid G., Letourneur D., Gupta B., Meddahi-Pellé A. Development of a new polypropylene-based suture: plasma grafting, surface treatment, characterization, and biocompatibility studies. *Macromol Biosci* 2011; 11(3): 373–382, https://doi.org/10.1002/mabi.201000298.

9. Li Y., Kumar K.N., Dabkowski J.M., Corrigan M., Scott R.W., Nüsslein K., Tew G.N. New bactericidal surgical suture coating. *Langmuir* 2012; 28(33): 12134–12139, https://doi.org/10.1021/la302732w.

10. Shkurenko S.I., Idiatulina T.S. Nikant biologically active surgical sutures. *Fibre Chemistry* 2002; 34(5): 346–349, https://doi.org/10.1023/a:1022159018362.

11. Wu X., Kubilay N.Z., Ren J., Allegranzi B., Bischoff P.,

Zayed B., Pittet D., Li J. Antimicrobial-coated sutures to decrease surgical site infections: a systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis* 2017; 36(1): 19–32, https://doi.org/10.1007/s10096-016-2765-y.

12. Bokeriya L.A., Gudkova R.G. *Serdechnososudistaya khirurgiya — 2015. Bolezni i vrozhdennye anomalii sistemy krovoobrashcheniya* [Cardiovascular surgery — 2015. Diseases and congenital anomalies of the circulatory system]. Moscow: NTsSSKh im. A.N. Bakuleva; 2016.

13. Pokrovskiy A.V., Gontarenko V.N. *Sostoyanie sosudistoy khirurgii v Rossii v 2014 godu* [Status of vascular surgery in Russia in 2014]. Moscow; 2015.

14. Akentyeva T.N., Borisov V.V., Kudryavtseva Yu.A., Doronina N.V., Ezhov V.A. Effect of polyoxyalkanoate-based cover on properties of suture material. *Angiologiia i sosudistaia khirurgiia* 2014; 4(2): 42–48.

15. Antonova L.V., Nasonova M.V., Kudryavtseva Yu.A., Golovkin A.S. Potential for polyhydroxyalkanoates and policaprolactone copolymer use as tissue-engineered scaffolds in cardiovascular surgery. *Bulletin of Siberian Medicine* 2012; 1(11): 128–134.

16. Zubairov D.M. *Molekulyarnye osnovy svertyvaniya krovi i tromboobrazovaniya* [Molecular basis of blood clotting and thrombogenesis]. Kazan: Fen; 2000.