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Antimicrobial Activity of Core–Sheath Surgical Sutures Modified with Poly-3-hydroxybutyrate

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Abstract—To impart antimicrobial activity to surgical sutures, weaved polyester fibers are coated with poly-3-hydroxybutyrate (PHB), containing the antimicrobial agent furazolidone (FZ). The prolonged FZ effect (7–14 days) is achieved by two-step application of a sheath, constituting 10% of the suture weight and containing 2–6% FZ. The sheath structure and antimicrobial activity of sutures can be modified by the introduction of other biocompatible and biodegradable polymers.

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To improve parameters of surgical sutures, in particular, biocompatibility and antimicrobial properties, a modifying sheath is applied to form a core–sheath structure [1–3]. The modifying sheath allows modification of flexibility, friction, knot tightness, and color. Coated sutures can be noninvasive and low capillary. Also, medicines or antimicrobial substances can be introduced into the sheath.

Use of poly-3-hydroxybutyrate (PHB) as a coating polymer is particularly promising. This polymer is highly affine to tissues, biodegradable, and sterilizable. It can form durable sheaths from solutions in chloroform, dichloroethane, and dichloromethane, where it can be dissolved with preservation of its molecular weight [4].

The solubility of PHB in the volatile solvent chloroform allows efficient application of the dry method of sheath formation on the surface of surgical sutures [5]. This method produces a uniform sheath along the suture without significant defects.

The objectives of this work are as follows: the modification of polyester or nylon sutures by application of a sheath based on the biocompatible and biodegradable polymer poly-3-hydroxybutyrate with an antimicrobial agent and the study of the effect of composition and sheath structure on the agent release and antimicrobial activity (AA) of the sutures.

MATERIALS AND METHODS

Experiments were performed with braided nylon and polyester fibers produced by Projectmashdetal' (Moscow) and PHB with molecular weights (MW) 200 kDa (PHB-200), 430 kDa (PHB-430), and 960 kDa (PHB-960) provided by the Bach Institute of Biochemistry. Molecular weights were viscosimetrically determined by using chloroform solutions of the polymer at the constant temperature 30°C and applying the equation $[\eta] = 7.7 \times 10^{-5} \times MM^{0.82}$. To obtain composite sheaths with controlled structure, porosity, and hydrophobicity, polyethylene glycol (PEG) with MW = 6 kDa (Fluka, Germany) or polycaprolactone (PCL) with MW = 65 kDa (Aldrich, Germany) were added to the PHB solution. In some experiments, a surfactant was added to the modifying solution. Antimicrobial activity of modified sutures was achieved by introduction of the antimicrobial agent furazolidone (FZ) of a broad range of activity. Formerly, it had been used for obtaining antimicrobial polytrifluoromonochloroethylene sutures [6]. The sheath was formed by application of the polymer (or polymer mixture) solution to polyamide and polyester fibers and evaporation of chloroform as in [5]. The fiber was supplied to the PHB solution in chloroform or dichloromethane (1–7%) at the speed 1.2 m/min, passed through an orifice of the exact diameter 0.7 mm precisely at the center to remove the excess solution, dried in a 90-cm high drying column either without blowing or in air flow at 20–25°C, and reeled. The structure and topography of the resulting samples were studied in microphotographs of the surfaces and sections obtained with a JSM-5300 LV electron microscope (Joel, Japan).

To study the kinetics of FZ release from the sheaths, a weight of suture was placed into physiological solution. Flasks with the sutures were stored in a thermostat at 36.6°C for 340 h. Furazolidone concentrations were estimated at 24-h intervals from the change of the optical density of the solution by using a calibration plot constructed before. Optical density was measured with a KFK-2-UKhL photocolorimeter (Russia) at λ 364 nm.

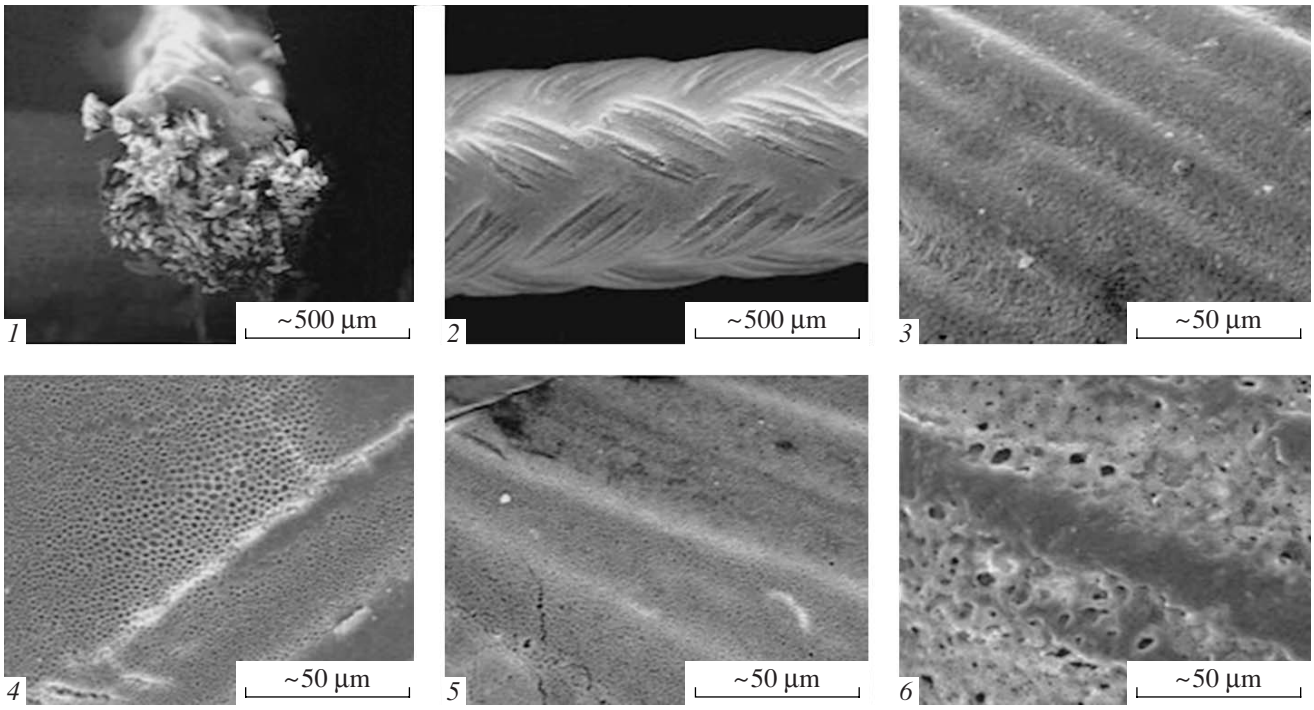


Fig. 1. Microphotographs of sections and surfaces of sutures modified by coating from polymer solutions (PHB, PCL, PEG) of various compositions. *1, 2,* section and surface of the suture coated with PHB sheath; sutures modified by coating from 4–6% solutions: *3*, PHB-430; *4*, PHB-200; *5*, mixture PHB-430 + PEG; *6*, mixture PHB-430 + PCL.

The optical paths of the cells with the reference solution (physiological solution) and the assayed solution were 5.055 and 5.057 mm, respectively. The results are expressed in terms of percentage of released FZ with respect of the amount introduced into the sheath (**R1**, %) per 1 cm of the suture (**R2**, μg/cm) and the time of maximum release (τ_{max}). Antimicrobial activity was measured with agar infected with *Staphylococcus aureus* (microbial load 10^6 cells/ml).

RESULTS AND DISCUSSION

Suture structure. An important parameter of surgical sutures is sheath porosity, which depends on composition, PHB molecular weight, and the fastness of solvent evaporation. Elevated porosity should increase capillarity and simultaneously allow diffusion of medicines from the suture to the medium, thereby ensuring the antimicrobial activity of the suture. For this reason, evaluation and control of sheath porosity are important tasks in the development of surgical suture modification. Porosity, achieved by solvent evaporation from the polymer sheath, can be controlled by varying the fastness of this process, polymer molecular weight, and addition of a dope to the coating PHB solution in chloroform, which would favor phase separation. Microphotographs in Fig. 1 show (*1*) sections and (*2*) surfaces of sutures modified by application of a sheath from solutions of (*3*) 4–6% PHB-430, (*4*) PHB-200, (*5*) PHB-430–PEG mixture, and (*6*) PHB-430–PCL mixture. As illustrated, porous sheath forms in experiments with relatively low-molecular-weight PHB-200 (Fig. 1, *3*) or mixtures of high-molecular-weight PHB-430 with other low-molecular-weight polymers (Fig. 1, *4* and *5*). Pore sizes differ significantly. The smallest (1–3 μm) pores form from the mixture of PHB-430 with a small PEG amount, and the largest (up to 8 μm), from the PHB–PCL mixture 1 : 1. The porous structure pattern is related to specific features of phase separation processes occurring during solvent evaporation from solutions of various polymers with various molecular weights. Thus, controlled change of the structure of sheath in sutures for indirect controlling of their capillarity, rate of medicine release, and AA can be achieved by varying PHB molecular weight and mixing it with other polymers.

Release of the antimicrobial agent. The method of modification with a polymer film containing a biologically active substance used in this study allows concentration of the substance on the suture surface. This ensures disinfection of the area near the suture and prolongs the release of the active substance.

Furazolidone (N-(5-nitro-2-furfurylidene)-3-aminooxazolidone-2) was chosen in this study as the bio-

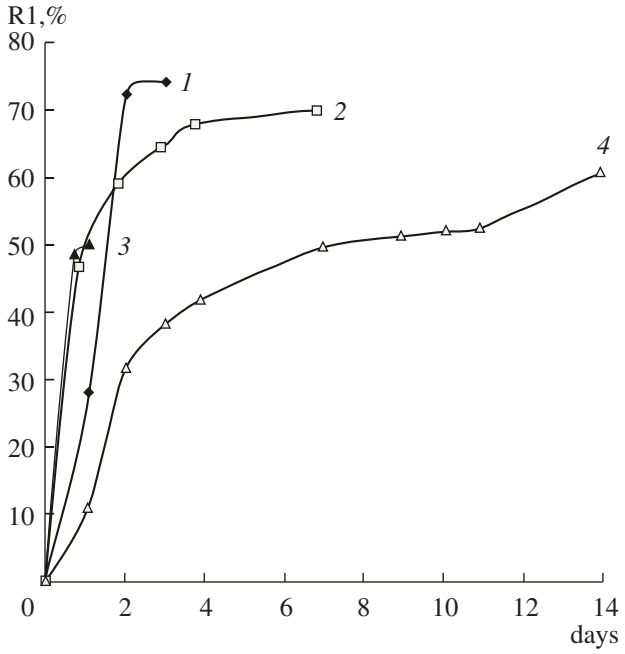
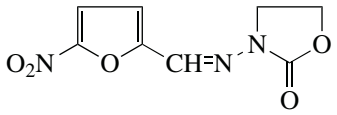


Fig. 2. Release of FZ (R1, %) from modified sutures coated with pure PHB with exposure in physiological solution. Storage time of modified sutures after production is 4–6 days. *1*, *C* = 9.5%, FZ = 20%; *2*, *C* = 10.9%, FZ = 6.3%; *3*, *C* = 1.1%, FZ = 47.4%; *4*, *C* = 23.0%, FZ = 20.0%. *C*, percentage of the sheath in the suture, w/w; FZ, percentage of FZ in the sheath, w/w.

logically active substance used to impart bactericidal properties to surgical sutures.



Furazolidone controls gram-positive and gram-negative microorganisms, lamblias, agents of dysentery and enteric fever, and trichomonads. Its therapeutic daily intake dose is 0.4–0.6 g/day. It should be mentioned that wound healing takes 7 to 14 days from the occurrence of the suture in the wound on average. The release of the antimicrobial agent should last all of this time.

As the AA of sutures depends on the amount of released FZ and duration of the release, we studied the dependence of the rate and amount of FZ released from the sheath on suture sheath composition and sheath percentage in the suture.

Our data indicate that the amount of released FZ (R1) increases with the time of suture exposure in the model medium (Fig. 2, curves *1–4*) and FZ content in the sheath (curves *1–3*). The amount of released FZ increased most rapidly in the first 1–3 days at the stage of the rapid release of the loading doze of the antimicrobial agent. Prolonged FZ release was observed in both the suture with 23.0% sheath and 20.0% FZ in the

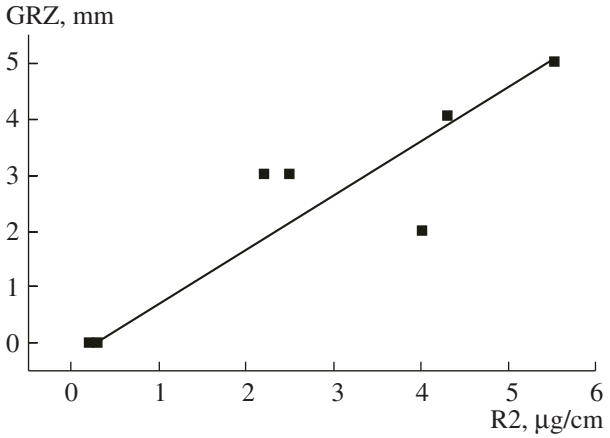


Fig. 3. Correlation between GRZ and released FZ, μg/cm suture.

sheath (curve *4*) and the suture with lower sheath content (10.9%) and much lower FZ content in the sheath (6.3%) (curve *2*). However, it was absent from the suture with higher FZ content (47.4%) at a low percentage of the sheath (1.1%), and this suture demonstrated the fastest FZ release (curve *3*). Thus, the prolonged effect is determined by a thicker nonporous multilayer structure, which is formed by multiple treatment of the fiber. Multilayer structures impart barrier properties to the sheath [7] and slow down FZ release to physiological solution.

The large amounts of released FZ ($R1_{max}$, %) and release prolongation indicate that FZ forms covalent bonds with neither PHB nor polyethylene terephthalate, and the polymer sheath serves as microdispenser of the antimicrobial agent. Generally, FZ release illustrates the potential efficiency of the antimicrobial agent. In our sutures, it reaches as high values as 75%, but the amount of released FZ, measured after 2 days and expressed in μg/cm suture (R2), is in better agreement with AA, because these long suture samples and time interval are used for determination of the microbial growth retardation zone (GRZ). This conclusion is confirmed by data presented in Figs. 3 and 5, which are adequately described by a linear equation (correlation coefficient $r = 0.90$). It is apparent from Table 1 that the amount of released FZ decreases with the increase in the percentage of the sheath in the suture but increases with the time of exposure in the liquid medium. Also, AA correlates with the amount of released FZ in 2 days expressed in μg/cm.

The effect of suture storage on the amount of released FZ is of special interest. Storage of sutures with PHB-430 sheath decreased the amount of released FZ. As the temperature of PHB crystallization is within 0–50°C and PHB-430 samples are stored at –10°C, this loss can be related to crystallization during storage at room temperature. Crystallization rate is determined by many properties of the polymer, including isotacticity,

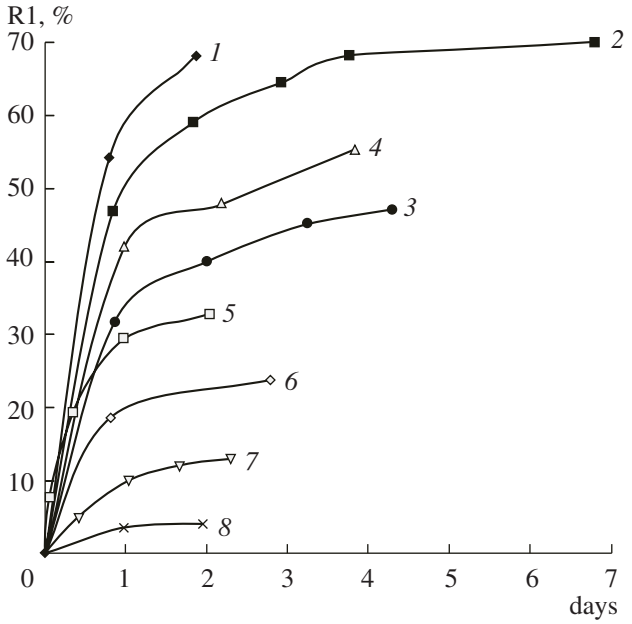


Fig. 4. Dependence of FZ release on the storage time (days) of sutures modified with pure PHB: *1*, 6; *2*, 20; *3*, 85, *4*, 3; *5*, 7; *6*, 8; *7*, 40; *8*, 71. *1–3*, PHB-960 kDa; *4*–8, PHB-430 kDa.

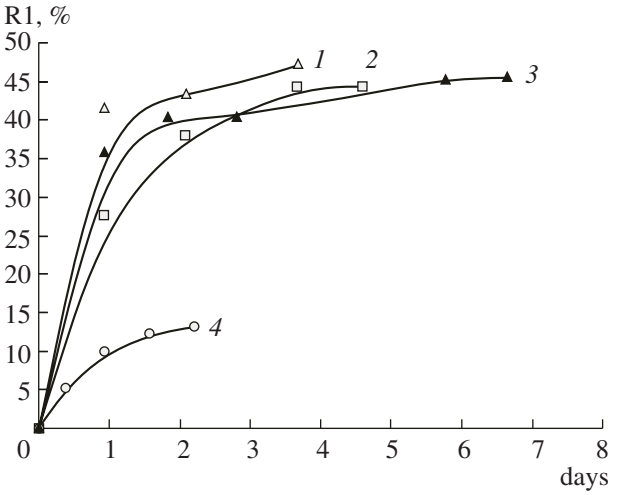


Fig. 5. Dependence of FZ release from modified braided polyester (MD = 1.5) sutures with various sheath compositions on the time of suture exposure in physiological solution; PHB with MW = 430 kDa and PEG with MW = 6 kDa. Sutures with various sheath compositions were stored for different times before the test:

1, PHB 90.0%, PEG 9.3%, FZ 0.7%, 40 days; *2*, PHB 90.0%, PEG 9.4%, FZ 0.6%, 112 days; *3*, PHB 49.3%, PCL 50.0%, FZ 0.7%, 40 days; *4*, PHB 99.3%, FZ 0.7%, 40 days.

purity, and molecular weight. Crystallization can be suppressed by the presence of other components in the polymer coating. For these reasons, the decrease in FZ release from the suture with easily crystallizable thick uniform sheath (30.5%) applied in one step from a laboratory PHB-430 sample with low FZ content (0.65%) (Fig. 4, curves *4–8*) was to be expected. This effect was not observed in sutures with less uniform sheaths, formed by two-step coating (10.9%) from higher molecular weight industrial PHB-960 with ten times as high FZ content (Fig. 4, curves *1–3*).

Smaller FZ release was observed in sutures with less uniform composite sheath (Fig. 5, curves *1–3*). The rate of FZ release from sutures with the composite PHB–PCL sheath (Fig. 5, curve *3*) was much higher than from the sheath of pure PHB (Fig. 5, curve *4*). Indeed, as expected, the application of a porous composition increased both the rate of FZ release from the sheath to the medium and its amount. The highest FZ release rate was observed in sutures with the sheath of mixed PHB–PEG (Fig. 5, curve *1*), apparently, owing to their higher hydrophilicity.

Thus, AA is determined by FZ release, which, in turn, depends on sheath thickness, composition, structure (porosity), and FZ amount. Therefore, to obtain a wide GRZ and prolonged effect, it is necessary to produce sutures with 10–12% sheath, which should be applied in two steps. The sheath should contain 5–7% FZ. Sheath composition and PHB brand should be chosen with regard to PHB purity and toxicity and to fiber strength.

Strength indices of modified polyester sutures are shown in Table 2. If PHB-960 with the highest molecular weight has no toxicity, it is appropriate for production of very strong sutures with metric dimension (MD) > 3, to stitch abdominal sutures. As seen from Table 2, the addition of 10% PEG to PHB has virtually no effect on suture strength, and the addition of PCL in the same amount as PHB reduces the strength significantly. However, even in this case the suture meets the requirements of European Pharmacopoeia.

Table 1. Correlation between antimicrobial activity and amount of FZ released from polyester sutures modified with PHB-960 kDa

Sheath percentage in the suture	FZ percentage in the sheath	Released FZ, percentage of its content in the sheat		FZ release, μg/cm 2 days	GRZ, mm/cm sutur 2 days
		2 days	14 days		
10.5	20	72	74	4.3	4
30.0	20	32	61	5.5	5

Table 2. Strength of modified polyester sutures*

Sheath composition	*T*, tex	*MD*	Weight increment, %	S_{knot}, N	S_{knot}, N, according to EPh no less tha	Compliance S_{knot}, N, according to EP
PHB + FZ	32.6	1.5	10	7.8 ± 0.9	5	+
	97.0	3.5	12	24 ± 0.2	22	+
	246.0	5	12	47.9 ± 0.8	35	+
PHB + PEG + FZ	249.2	5	14	47 ± 0.8	35	+
PHB + PCL + FZ	245.0	5	12	41.6 ± 0.6	35	+

* *T*, suture linear density, tex; *MD*, metric dimension; S_{knot}, suture strength in a knot, N; EPh, European Pharmacopoeia.

Thus, the optimal antimicrobial activity and prolonged effect of the antimicrobial agent (7–14 days) are achieved by two-step application of the sheath, constituting 10% of the suture weight and containing 2–6% FZ. The release of FZ from sutures with sheaths of different compositions and antimicrobial activity depend on suture storage conditions and time. Formation of a composite sheath suppresses PHB crystallization, thereby extending the range of storage conditions of sutures modified by application of PHB coating.

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