Research of Properties of Polyfunctional Polymeric Films

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SUMMARY: At present the problem of creating materials for medical application, which possess surface thromboresistant and antiseptic properties is of the great importance. The method of creating hydrogel films, containing anticoagulants, biologically active and antiseptic substances on the surface of well-known polymers of medical purity allows to give their surface special functions and properties and to retain the good mechanical properties [1,2]. In the majority of cases, the methods which are used for the creation of such films have a limitations which impede their wide application as they do not allow to obtain surface layers, possessing different medico-biological and physic-chemical properties.
The aim of our research was the electroformation of biocompatible, polyfunctional poly(vinyl alcohol) (PVA) films with immobilizied anticoagulant, enzyme and antibiotic on the surface of a composite material on the base of polysiloxane of medical purity [3,4].

Materials and methods

The composite material polydimethylsiloxane (PDMS) modified by graphite was used as a plate (25 cm^2) for the electroformation of the polyfunctional polymer films. Medical PVA (TU 605-05-26-75) with a high content of hydro groups; anticoagulant-heparin, «RIHTER» Hungary; the enzyme trypsin «LENMYSOKOMBINAT»; the antibiotic sodium salt of benzylpenicilline, factory «KRASNOYRSKMEDPREPATAT» were used.

The samples of composite material were soaked in 1 N NaOH aqueous solutions at 70°C for various periods, washed with distilled water and dried at room temperature for 24 h. The creation of the PVA layers on the surface of the composite material was carried out in electric field of infralower frequency in the regime of signal «meander». The electrodes have been fixed parallely in glass cell, containing the composition consisted of the PVA, boracic acid or glutaric-dialdehyde as a cross-linked agents, glycerine. The anticoagulant, enzyme and antibiotic were added to the composition immediately before process of electroformation. The density of voltage of electrical field was 20-80 V/cm. The period of electroformation was 10-120 sec.

The adhesion of the PVA films was determined by the method of lattice notches[5]. Amounts of heparin and trypsin immobilized on the surface of the composite material was determined by the methods [6]and [7]. The activity of trypsin was determined by the method based on the hydrolysis of kazein protein by the proteolityc enzyme [8]. The antimicrobial

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properties of the materials were studied by the method[9].The thromboresistance of the samples was investigated by the method of activated partial thromboplastin time (APTT) [10].

Results and discussions

It was stated that such characteristics of the electroformation's regime as density of the voltage 30 V/cm, period of process 30-120 sec. and composition (PVA 7%, H_3BO_3 0,1%) are optimal for the creation of PVA films in electric field. Previous treatment of the samples of the material's surface in 1 N NaOH aqueous solution allows to improve the adhesion of PVA films to its surface. The thermostating of samples with PVA layers at 50-60 C° allows to increase their mechanical properties and to improve the adhesion and water resistant of the films. The addition of glycerin (2%) provides the higher plasticity of the obtained films. It has been indicated that the electroformation allows to regulate the concentration of the substances in the PVA film on the composite's surface (Table 1).

Tab. 1. The characteristics of the PVA films containing active substances

Composition, mass %	Period of the electro-formation, sec.	Density of voltage of electric field, V/cm	Quantity of heparin in the film, mg/cm^2	Quantity of trypsin in the film, mg/cm^2	Activity of enzyme, % of initial	Adhesion of the film to the surface, points
PVA - 7% H_3BO_3 - 0,1%	30	30	0,10	0,18	86	1
Glycerin - 3%	60	30	0,17	0,30	86	1
Heparin - 0,3%	90	30	0,21	0,38	80	1
Trypsin - 0,3%	120	30	0,23	0,43	72	1-2

The character of the desorption of heparin and trypsin from PVA films given in Fig.1 shows that the quantity of retained heparin coupled onto crosslinked PVA hydrogel in the presence of glutaric dialdehyde is higher than the one in the presence of boracic acid (curves 1 and 2). As illustrated in Fig.1, the immobilized substances are preserved in the films the prolonged period. It is important for using them in medicine.

It is known that immobilization can influence on the enzyme activity [11]. The electroformation at the density of voltage 30 V/cm (Fig.2, curve 1) and time 60 sec.allows to creat films with higher activity of enzyme (table 1). The enzyme activity is reduced at temperature to 60 °C.

It has been reported that the activity of the enzyme can be changed by the co-immobilization the last with antibiotic [12]. Therefore the co-immobilization of sodium salt of benzylpenicilline with trypsin was studied. As illustrated in Fig.2 (curve3) the activity of the trypsin in the presence of antibiotic is reduced by 10 %. From the point of view of the total resistance of the material to microorganisms the application of trypsin with antibiotic is more effective.

Kinetics of the desorption of the active substances

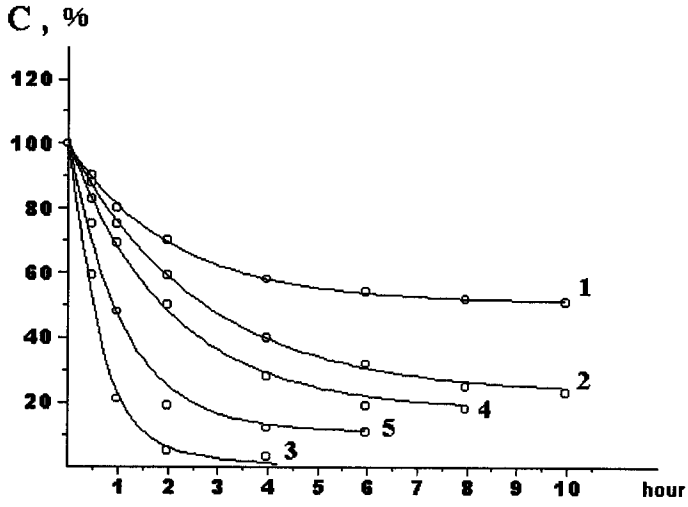

Fig. 1. **1,2**-heparin immobilized in electrical field (cross-linked agent glutaric dialdehyde -1; H_3BO_3 -2) for 60 sec.; **3**- heparin immobilized by the adsorption for 1 h.; **4**- trypsin immobilised in electrical field (cross-linked agent H_3BO_3) for 60 sec.; **5**- trypsin immobilized by the adsorption for 1 h.

Influence of the electroformation regime on the enzyme activity

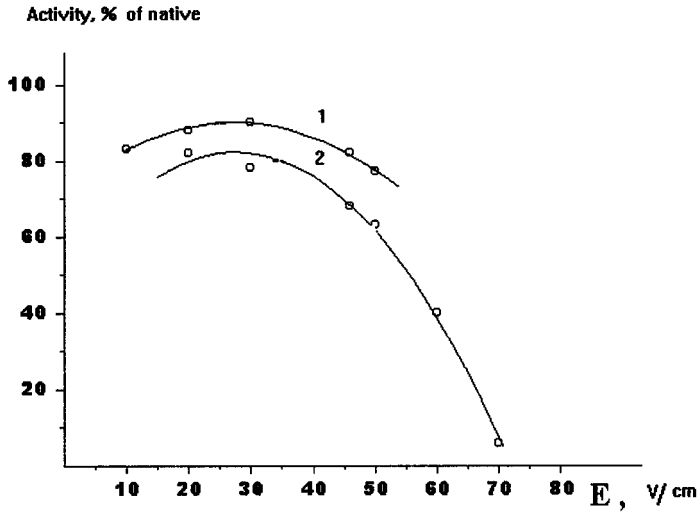

Fig. 2. **1**- trypsin immobilized onto PVA film; **2**- trypsin co-immobilized with antibiotic

The resistance to γ-sterilization of immobilized biologically active substances is one of the important properties for medical materials. The samples of the material with immobilizied trypsin and salt of benzylpenicilline were studied after sterilization by the X-rays. The reduction of the activity of trypsin immobilized in PVA polymer film caused by the remaining moisture in the samples after drying and radio-binding of PVA [13]. The conditions of drying at 40-50^0 C doesn't allow to remove the moisture from material completely. However the quite high level of the activity of trypsin (52 %) is preserved after γ-sterilization at 5 kGr (Table 2).

Tab. 2. Influence of γ-sterilization on the activity of the enzyme

Absorbed dose of irradiation, kGr	Activity of native trypsin, %	Activity of native trypsin in solution, %	Activity of immobilized trypsin, %
0	100	100	100
5	100	4	52
10	96	0	28
25	85	0	9
100	74	0	0

Table 3 shows the data of antiseptic activity of materials with immobilized sodium salt of benzylpenicilline. The γ-sterilization reduces the activity of immobilized antibiotic by 3-5% (Fig.3).It can be explained by disorientation or partial radio binding of the functional groups of antibiotic[13].

The electroformation of the PVA films allows to obtain the materials with higher content of antibiotic in the shorter period (0,5 min) in comparison with the method of dipping (120 min.). As illustrated in table 3 the presence of glycerin did not influence on the antiseptic activity of immobilized antibiotic. PVA films possess own antiseptic activity(the zone of suppression of reproduction of microorganisms 10 mm).

Influence of γ-sterilization on the antiseptic activity

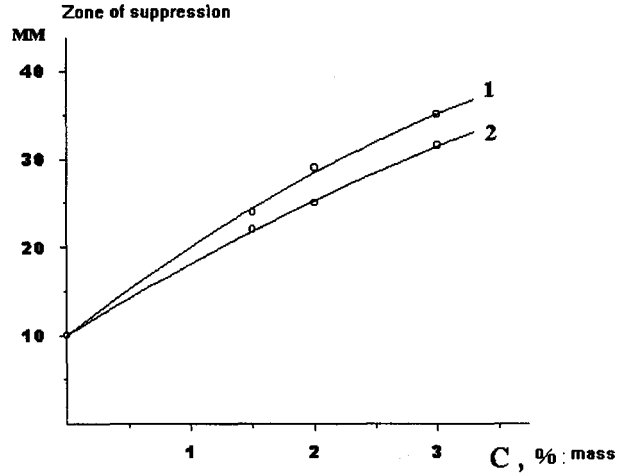

Fig. 3. 1- samples without γ-sterilization; 2-samples after γ-sterilization.

Tab. 3. The antiseptic activity of the material

Composition	Method of formation	The content of antibiotic in film, %mass	Zone of suppression of reproduction microorganisms, mm	
			without γ-sterilization	after γ-sterilization
PVA, H_3BO_3, glycerin	Electrical field,τ=0,5 min	0	10	10
PVA, antibiotic, H_3BO_3	Electrical field, τ=0,5 min	3	36,3	34,6
PVA, glycerin, antibiotic, H_3BO_3	Electrical field, τ=0,5 min	3	34,7	32
PVA, glycerin, antibiotic, H_3BO_3	Electrical field, τ=0,5 min	2	30,6	26,3
PVA, glycerin, antibiotic, H_3BO_3	Dipping, τ=120 min	1,5	25	22,5
Antibiotic-benzylpenicilline		100	> 50	> 50

Tab. 4. Activated partial thromboplastin time (APTT) on the surface of composite with films

Substrate	APTT, sec	
	Before incubation with material	After incubation with material
Initial PDMS	45	35
50 mass.p. of graphite	45	44
Composite with PVA layer, containing:		
trypsin	45	55
heparin	45	600
heparin and trypsin	45	600

In vitro thromboresistance of the immobilized heparin, trypsin, the APTT, which exhibits the thromboresistance of intrinsic blood coagulation factors[10], was studied, and results are given in Table 4. The APTT of the composite is higher than the one of the PDMS. The APTT of the composite with PVA films, containing trypsin and heparin was significantly prolonged compared to that of initial PDMS and composite.

The results of the investigation of some properties of obtained PVA films showed the effectivity of the electroimmobilization of the different biologicaly active substances coupled onto hydro gel films. The obtained materials possess of significantly biocompatibility, thromboresistance and antiseptic properties which allowed to use them in medicine practice.

Various types of the obtained materials as an artificial phalange of upper limbs without biologicaly active substances and with them were examined of in clinical condition on the base of St. Petersburg Research Institute of Traumatology and showed the good biocompatibility and antiseptic activity.

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