Biosynthetic Wound Coatings as Susbtrates for Cell Growth

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Translated from *Kletochnye Tehnologii v Biologii i Meditsine*, No. 1, pp. 52-58, January, 2007
Original article submitted October 9, 2007

Experimental studies of composite materials formed on the basis of fluorine-containing latex and bioactive polysaccharides showed that physicochemical properties of composite materials and their adhesion characteristics can be modulated by variations of polysaccharide-latex ratio and the nature of polysaccharides. The ratio of components ensuring the formation of biosynthetic films that meet the standards for modern wound coating and maintain adhesion and growth of substrate-dependent mammalian cells was determined. These materials can considerably increase the efficiency of treatment of extensive and deep skin wounds in cases when application of cell cultures is indicated.

Key Words: *wound coating; skin; cell culture; transplantation; composite material*

Burns and traumas associated with burns are important medical and social problem. According to official data of the Federal Agency for Health Care and Social Development, the annual number of burned patients in Russia is about 700,000, or 4-5 per 1000 in population. Total burn-related mortality in Russia is 2.3-3.6%. Every year, of 180,000-200,000 victims hospitalized into medial institutions 8000-10,000 die, of them 85-90% are children and working-age people. According to different sources, 6-9% patients become disabled (more 80% disabled individuals are of working age) [7]. Similar mortality statistics was reported in other countries, but the degree of disability and the incidence of lethal outcomes depend on the quality of medial attendance. Introduction of new-generation polymer-based dressing materials ino clinical practice accelerated healing processes and reduced the mortality and disability rates. However, the existing wound coatings do not provide the possibility of treating deep and extensive burns and trophic and radiation ulcers. Cell transplantation is now an effective method for the treatment of these injuries. This method is sometimes low effective because of the absence of materials creating conditions for atraumatic transfer of cells on the wounded surface and for subsequent wound regeneration [1]. Unfortunately, wound coatings on the basis of synthetic [8, 10,11] and natural [4,5,16] polymers do not possess all necessary characteristics. Synthetic polymers ensure adequate protection of the wound, but in most cases are not cell-adherent and do not stimulate regeneration processes in the wound. Despite high biological activity, natural polymers are characterized by low mechanical endurance and high biodegradation rate.

Creation of composite materials combining advantages of synthetic and natural polymers faces the problem that biological activity of natural polymers disappears when routine methods of formation of polymeric structures from high-temperature melted mass or solutions in organic solvents are used. This problem can be solved by the formation of multilayer materials, where the structure and mechanical characteristics are determined by synthetic polymers, while biological activity is deter-

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0007-4888/08/1451-0158

mined by protein and polysaccharide macromolecules, antimicrobial preparations, and other active components. Another way for solving this problem is modification of the surface of synthetic constructions with natural macromolecules. This approach is realized in recently appeared sandwich-type composite materials, where natural polymer is applied on the synthetic matrix by the method of step-by-step modification [12,14]. Both ways have serious disadvantages, the main of them are low stability of multilayer sandwiches under conditions of wet wound, rapid decrease in the number of bioactive substances not covalently bound to the polymer surface, and considerable decrease in biological activity of biomacromolecules chemically bound to the polymer surface.

The aim of the present study was to create a new class of biosynthetic materials combining the properties of modern wound coatings and substrate for culturing and transport of cells to the wound. A new approach to the formation of biosynthetic polymers was proposed. It consists in the use of colloid nanosystems, aqueous dispersions of synthetic fluorine-containing latex and natural polymers [2,3,9], which preserves biological activity of natural polymers after their incorporation into synthetic matrix and makes it possible to form biomaterials combining advantages of synthetic and natural polymers. The main task was to determine the composition of the mixture for the formation of materials maintaining adhesion of substrate-dependent mammalian cells and meeting the standards for modern wound coating.

MATERIALS AND METHODS

Aqueous latex dispersion (SKF-26, Kirovo-Chepetsk Chemical Plant) representing a vinylidene fluoride and hexafluoropropylene co-polymer served as the basis polymer. Water-soluble polymers of plant origin sodium alginate and methylcellulose (both from Sigma) were used as bioactive additives. Aqueous dispersion of latex SKF-26 and a calculated volume of 1% aqueous solution of the corresponding polysaccharide were used for preparation of materials (the total amount of latex and polysaccharide in the mixture was constant).

The polymeric substrates were formed of a horizontal glass surface by the method of air drying of the latex-polysaccharide suspension. This method yields homogenous (by volume) composite films. The films were sterilized by γ-radiation on a GUBE experimental device (2.5 Mrad at room temperature).

The processes of swelling and polysaccharide release into the solution were studied by the method of gravimetry. To this end, the samples were bathed in distilled water for 24, 48, and 72 h at 37°C. The degree of swelling for polymeric substrates were determined by the standard method and calculated by the formula:

$$\alpha = \frac{W_1 - W_2}{W_1} \times 100\%,$$

where M_1 and M_2 are the weight of the polymer before and after swelling.

The release of polysaccharides into the solution d was determined as the change in specific weight of the polymer and calculated by the formula:

$$d = \frac{W_1 - W_2}{W_1} \times 100\%,$$

where W_1 is the initial weight of the polymer and W_2 is the weight of the polymer after swelling and drying.

The permeability of polymeric substrates for water vapors was determined gravimetrically by the standard method (GOST 22900-78). Glass vials containing 15 ml distilled water at 37°C were hermetically closed with film samples. The vials were weighed and placed in a thermostat at 37°C and relative humidity 48% for 1, 2, 3, or 4 days, and then weighed again. The permeability of polymeric substrates for water vapors (P) was determined as the amount of water crossing the area unit and calculated by the formula:

$$P = \frac{W_1 - W_2}{S \times t},$$

where W_1 and W_2 are the initial and final weights of the vial with film, S is area of the film, and t is the time of the test.

Wettability of the test materials was evaluated using special equipment: Sony XC77E video camera mounted on the ocular of horizontal MBS-2 binocular microscope and connected via DigitEye DE-15 interface (Candela) to IBM PC 486 PC computer. Measurements were performed at 20°C. A drop of bidistilled water (0.02 ml) was applied onto the substrate. The limiting wetting angle was measured in the point of three-phase contact using drop image analysis and Klimov 640 software. Free energy of the substrate surface was determined by an equation [13]:

$$\cos\theta = -1 + 2\sqrt{\frac{\gamma_{sv}}{\gamma_{lv}}}\, e^{-\beta(\gamma_{lv} - \gamma_{sv})^2},$$

where Q is contact angle of wetting, γ_{lv} is surface tension of the fluid, γ_{sv} is free energy of the surface, $\beta = 0.0001247$ is a parameter determined in experi-

ments with various fluids. The data were input into a special program for the solution of the equation by the Newton method.

Cultures of human fetal fibroblasts were isolated routinely from the musculocutaneous tissue of 8-10-week embryos. The cells were cultured in DMEM supplemented with 10% FCS (Sigma), 50 μg/ml penicillin, 50 μg/ml streptomycin, and 1% L-glutamine at 37°C in a humid atmosphere with 5% CO_2. The cells were harvested using 0.25% trypsin and 0.02% Versene (1:1). Seeding density was 60-80 thousands cells/cm^2. The cells were counted in a Goryaev chamber, viability was evaluated by trypan blue exclusion. Microphotographs were prepared using Peraval Interphako microscope by the method of differential interferention contrast.

RESULTS

When developing new type of wound coatings we presumed that these materials should combine the following functions:

1) protection from infection and mechanical damage to the wound;
2) adsorption of the exudate;
3) therapeutic activity (stimulation of wound healing and compatibility with drugs);
4) transport function, *i.e.* permeability for air and water vapors sufficient for preventing accumulation of the exudate under the coating on the one hand and drying of the wound on the other;
5) adhesion properties (maintenance of adhesion and flattening of fibroblasts and possibility of fixation on the wound).

The proposed approach for the formation of composite materials on the basis of aqueous latex and polysaccharide dispersions makes it possible to introduce bioactive polymers and water-soluble drugs into the hydrophobic polymer matrix without losing their biological activity. The structure of composite materials is modulated during drying of the dispersion due to interaction of latex particles with each other and with polysaccharides. Permeability for gases and vapors as well as sorption capacity and adhesion properties of the latex matrix can be modified by introduction of the hydrophilic component.

Sorption properties of materials can be related to their capacity to swell in aqueous media. Study of swelling of composite materials in water revealed an increase in sorption capacity of latex films after introduction of polysaccharides into their composition (Fig. 1). Sorption capacity of materials almost linearly increased with increasing the content of methylcellulose; sodium alginate also increases to a certain extent sorption capacity of materials.

Introduction of methylcellulose and sodium alginate into the composition of latex films considerably increased vapor permeability of composite materials compared to latex film (Fig. 2). Vapor permeability of films containing sodium alginate was somewhat lower than that of films containing methylcellulose.

Swelling of materials and their permeability for vapors are determined by their capacity to bind water molecules and by the rate of diffusion processes. Differences in the degree of steady-state swelling and vapor permeability for materials with similar methylcellulose content can be determined by differences in the microstructure of these materials. Non-ionogenic and capable of crystallization methylcellulose probably forms larger pores, whereas the formation of hydratation lattice by sodium alginate more strictly depends on its interaction with the latex. Sorption capacity of the polymer coating depends on the free volume of pores, while the rate of water adsorption depends on the nature of the polymer. Polysaccha-

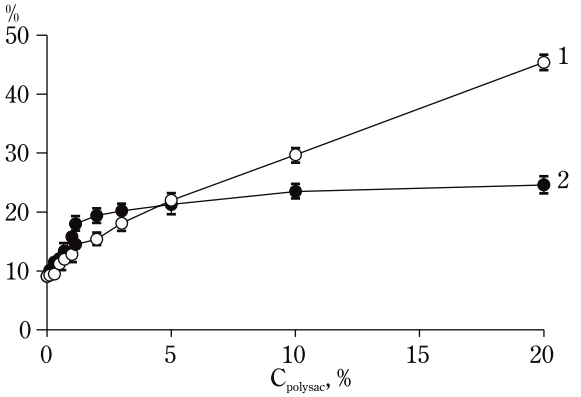

Fig. 1. Steady-state swelling of latex films modified by polysaccharides methylcellulose (*1*) and sodium alginate (*2*) in water at 37°C for 48 h. Ordinate: degree of swelling.

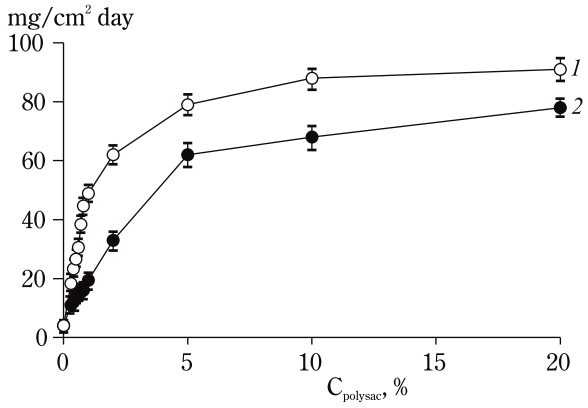

Fig. 2. Vapor permeability of latex films modified by methylcellulose (*1*) and sodium alginate (*2*).

rides act as water sorbents: they form hydrophilic channels for water and hydratation lattices of various topologies depending on the supramolecular structure of the latex-polysaccharide complex. Differences in the degree of steady-state swelling and vapor permeability for materials with similar methylcellulose content can be determined by differences in the microstructure of these materials.

When developing new biomaterials we compared them with modern wound coatings Omiderm (ITG Laboratories Inc.) and Op-Site (T.Y.Smith & Nephew Ltd.) used for the treatment of exudative wounds. For these wound coatings, the degree of swelling in water was 42% and 170%, respectively, and vapor permeability 96 and 48 mg/cm^2×day, respectively.

For composite materials based on fluorine-containing latex containing 20% sodium alginate and methylcellulose, the degree of swelling in water was 23% and 46%, respectively, and vapor permeability 78 and 91 mg/cm^2×day, respectively. Thus, composite materials developed by us by their vapor permeability and sorption capacity are close to modern wound coatings intended for the treatment of exudative wounds [6].

Wettability is a physical consequence of specific chemical structure of the surface and determines adhesion of the material to the wound surface. Free surface energy is a quantitative parameter characterizing wettability of the material. Free surface energy of a dense substrate determines cell interaction with the surface especially at the stage of initial attachment. We previously showed that irrespective of the presence of serum proteins in the medium cells poorly adhere to substrates with low surface energy (Y_s<50 Erg/cm^2), whereas on substrates with higher surface energy we observed good attachment and flattening of cells [15].

It was demonstrated that hydrophilic-hydrophobic properties of the surface of composite materials changed with time (Fig. 3).

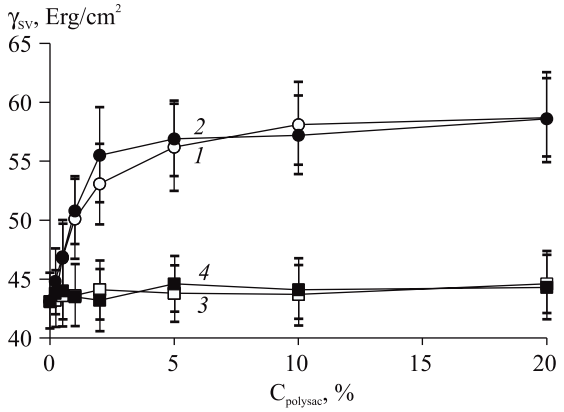

Fig. 3. Free surface energy of latex films modified by polysaccharides. *1*) sodium alginate after 24 h; *2*) methylcellulose after 24 h; *3*) sodium alginate after 72 h; *4*) methylcellulose after 72 h.

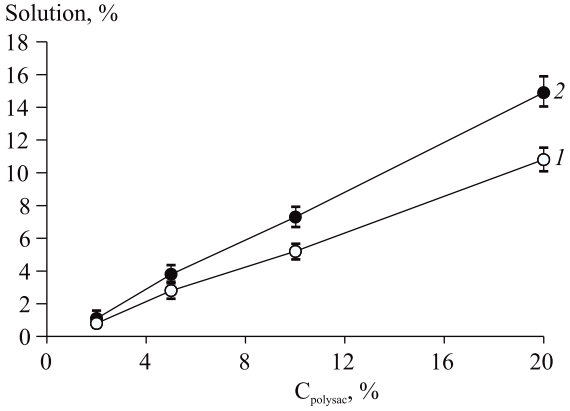

Fig. 4. Vapor permeability of latex films modified by methylcellulose (*1*) and sodium alginate (*2*).

During the first 24 h the material has moderately hydrophobic surface promoting adhesion and flattening of cells (Y_s≥50 Erg/cm^2). However, by the end of day 3 the material becomes highly hydrophobic and non-adherent for cells (Y_S<50 Erg/cm^2). These changes in surface properties are related to the release of unbound polysaccharides from the

TABLE 1. Comparison of Proposed Composite Materials with Modern Wound Coatings

Parameter	Composite A	Composite M	Latex	Omiderm	Op-Site
Chemical composition	Fluorine-containing latex 80%, sodium alginate 20%	Fluorine-containing latex 80%, methylcellulose 20%	Fluorine-containing latex	Polyurethane with acrylamide	Polyurethane
Thickness, μ	100	100	100	20	200
Vapor permeability, mg/cm^2×day	78	91	4	96	48
Degree of swelling, %	23	46	5	42	170

Note. Composite A: latex films modified by sodium alginate; composite M: latex films modified by methylcellulose.

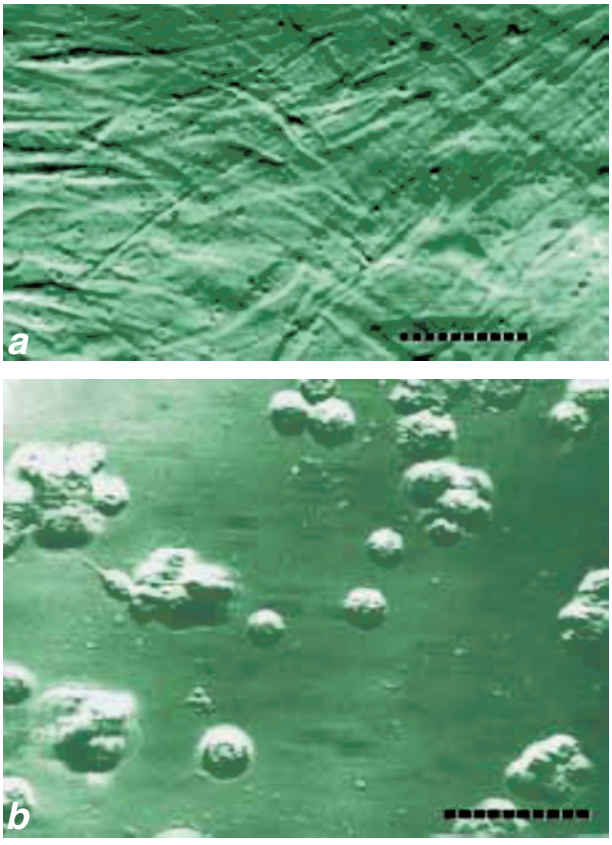

Fig. 5. External appearance of cells on the surface of substrates containing 80% latex and 20% methylcellulose (*a*) or 100% latex (*b*). Scale: 100 μ.

material, which shifts the ratio of hydrophilic and hydrophobic groups on its surface. The results of our study suggest that about 70% sodium alginate and 50% methylcellulose are released during the first 2 days (Fig. 4). The release of these polysaccharides into the wound provides conditions for cell migration and stimulates regeneration processes.

Study of the interaction of human fibroblasts with the surface of the test materials showed good cell adhesion and flattening on polysaccharide-modified latex films within 1-2 days. Adhesion properties of materials little depended on the concentration and nature of polysaccharide and in case of moderately hydrophobic substrates containing 5-20% polysaccharides these properties were similar to those of polystyrene specially treated for cell culturing. On the other hand, only 20-25% cells adhered to unmodified latex and flattening was poor in this case (Fig. 5).

Cells adhesion to the films with 20% sodium alginate and methylcellulose was 92 and 75% of the control, respectively, cell adhesion to Omiderm and Op-Site materials was 17 and 38%, respectively. In contrast to polystyrene treated for cell culturing, no cell growth was observed on the surface of composite latex-polysaccharide materials. On day 3 of culturing the cells detached from the surface of composite material, which was probably due to increasing hydrophobicity of their surface (Fig. 6).

This helps to solve the problem arising when hydrophilic wound coatings are used. The most serious disadvantage of these materials are their high adhesion to the wound, which makes redressing painful for the patient and traumatizing for the wound. To solve this problem the dressing surface facing the wound are made of hydrophobic synthetic polymer. These coatings do not tightly adhere to the wound and are characterized by low adsorption rate, which leads to accumulation of the exudate. We showed that modification of synthetic fluorine-containing latex films by polysaccharides

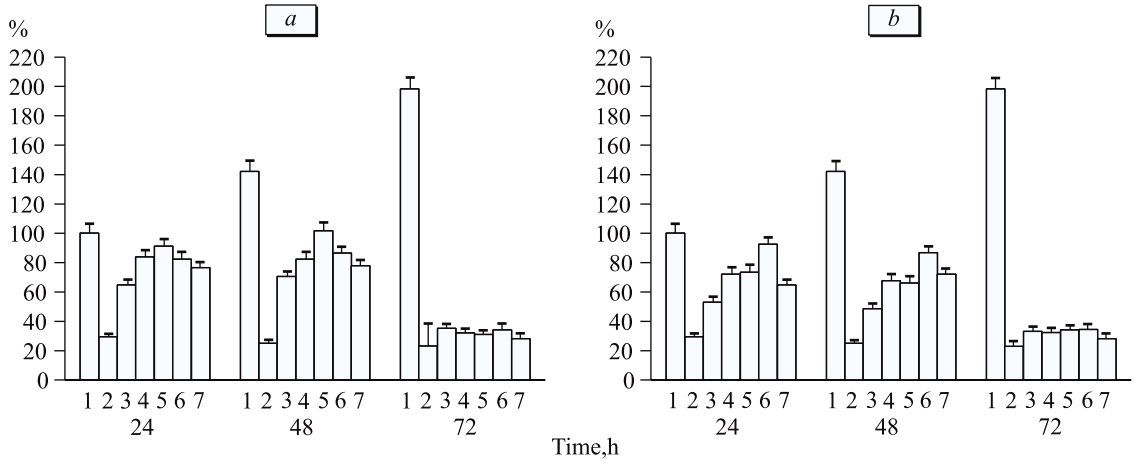

Fig. 6. Cell adhesion to latex films modified by methylcellulose (*a*) and sodium alginate (*b*). *1*) control, *2*) latex, *3*) 0.5%, *4*) 1.0%, *5*) 5.0%, *6*) 10%, *7*) 20.0%.

are more preferable, because they ensure good sorption properties and vapor permeability of the composite materials and effective transfer of cells to the wound during the first 2 days. Later, this coating can be atraumatically removed, if necessary, and replaced with a new one.

Thus, the proposed approach to the formation of biosynthetic polymer materials consisting in the use of aqueous dispersions of synthetic fluorine-containing latex and natural polymers allowed us to prepare composite materials with appropriate physicochemical characteristics and adhesion properties. The ratio of components ensuring the formation of biosynthetic films that by their physicochemical characteristics meet the standards for modern wound coating and maintaining adhesion and growth of substrate-dependent mammalian cells was determined. The main advantages of the developed biosynthetic wound coatings are their atraumaticity and combination of therapeutic and protective functions. These materials can considerably increase the efficiency of treatment of extensive and deep skin wounds in cases when application of cell cultures is indicated.

The study was supported by the program "Fundamental Sciences for Medical Practice" of the Russian Academy of Sciences and Federal Agency for Science and Innovations (Contract No. 02.513. 11.3090)

REFERENCES

1. A. A. Alekseev, *TsEMPINFORM*, **62**, No. 2, 15-18 (2004).
2. G. A. Davydova, I. I. Selezneva, and B. K. Gavrilyuk, *Biofizika*, **49**, No. 5, 809-814 (2004).
3. G. A. Davydova, I. I. Selezneva, and B. K. Gavrilyuk, *Vopr. Biol. Med. Farm. Khimii*, No. 2, 11-14 (2004).
4. V. I. Sevastyanov, V. A. Egorova, E. A. Nemets, *et al., Vestn. Transplantol. Iskusstven.Organ.,* No. 2, 47-52 (2004).
5. Yu. S. Khotimenko, V. V. Kovalev, O. V. Savchenko, and O. A. Ziganshina, *Marine Biology* [in Russian], **22**, No. 3, 151-162 (2001).
6. S. G. Shapovalov, *FARMindex-Praktic*, No. 8, 38-46 (2005).
7. V. N. Yuskov, *Surgery in Questions and Answers* [in Russian], Moscow (2000).
8. M. Ducharme-Desjarlais, C.J. Celeste, E. Lepault, and C. L. Theoret, *Am. J. Vet. Res*., **66**, No. 7, 1133-1139 (2005).
9. J. Ebbehoj, B. Gavrilyuk, V. Menzul, *et al*., *Burns,* **22**, No. 7, 557-559 (1996).
10. E. Hann, S. Reinartz, S. E. Clare, *et al.*, *Hybridoma (Larchmt).,* **24**, No. 3, 133-140 (2005).
11. D. Imran, E. Sassoon, and D. Lewis, *Plast Reconstr Surg.*, **113**, No. 3, 1093-1094 (2004).
12. U. Klinge, K. Junge, B. Spellerberg, *et al.*, *J. Biomed. Mater. Res*., **63**, 765-771 (2002).
13. D. Y. Kwok, C. N. C. Lam, A. Li, *et al., Colloids and Surfaces. A.* **142**, 219-235 (1998).
14. C. Mary, Y. Marois, M. W. King, *et al., ASAIO J.,* **44**, No. 3, 199-206 (1998).
15. J. M. Schakenraad, H. J. Busscher, CHR Wildevuur, and J. Arends, *J. Biomed. Mater. Res*., **20**, 773-778 (1988).
16. L. Wang, E. Khor, and L. Y. Lim, *Pharm Sci*., **90**, No. 8, 1134-1142 (2001).