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Biocomposites Based on Degradable Materials as Biotechnological Dermal Equivalents

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*Hybrid tissue-engineered biosystems based on biodegradable polymers of microbiological origin – bacterial cellulose (BC) and a copolymer of 3-hydroxybutyric and 4-hydroxybutyric acid P(3HB/4HB) – were constructed using various methods: 1) the dried BC pellicles were soaked with a 2% P(3HB/4HB) solution in chloroform, kept for 24 h, and dried in a dust-free cabinet until the solvent had completely evaporated; 2) nonwoven membranes were placed into the *K. xylinus* B-12068 culture, and bacterial cellulose synthesized in it grew under static conditions; 3) powdered cellulose (particle size of 120 μm) was added to a 3% P(3HB/4HB) solution in chloroform and mixed ultrasonically to homogeneity; then, the films were produced by the solvent evaporation technique, at polymer to cellulose ratios of 2:1 and 1:1. Investigation of surface properties of the samples showed an increase in surface hydrophilicity of the composites produced by mixing a solution of P(3HB/4HB) with BC at ratios of 2:1 and 1:1 (the contact angle was $43.9 \pm 17.55^\circ$ and $36.1 \pm 0.66^\circ$) relative to the starting materials. The physical/mechanical properties of the composites based on P(3HB/4HB) and BC were superior to the properties of the neat materials, and they were largely determined by the methods of production of the composite and the properties of the materials used. Based on the study of surface and physical/mechanical properties of the hybrids, films prepared by casting the polymer solution with BC powder (2:1 and 1:1) followed by solvent evaporation were chosen for studying cell adhesion. These scaffolds were loaded with drugs promoting wound healing (actovegin, solcoseryl) and tested in the culture of fibroblasts derived from the adipose tissue MSCs. The MTT assay showed that the most effective hybrid systems were polymer:bacterial cellulose powder (1:1) samples loaded with actovegin or solcoseryl at a concentration of 5%. Those systems produced a stimulating effect on fibroblasts, and, thus, they can be regarded as promising wound dressings to repair skin defects.*

Keywords: bacterial cellulose, polyhydroxyalkanoates, hybrids, drugs, biocompatibility.

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Биокompозиты на основе разрушаемых биоматериалов в качестве биотехнологических дермальных эквивалентов

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*С применением различных методов конструирования матриц получена и исследована серия гибридных тканеинженерных биосистем на основе биоразрушаемых полимеров микробиологического происхождения – бактериальной целлюлозы (БЦ) и сополимера 3-гидроксимасляной и 4-гидроксимасляной кислот П(ЗГБ/4ГБ): 1) высушенные пленки БЦ погружали в 2%-й раствор сополимера П(ЗГБ/4ГБ) в хлороформе на 24 ч и затем высушивали в беспылевом боксе до полного испарения растворителя; 2) нетканые мембраны П(ЗГБ/4ГБ), полученные методом электроспиннинга, помещали в культуру *K. xylinus* В-12068, растущую и синтезирующую БЦ в условиях периодической культуры; 3) к 3%-му раствору П(ЗГБ/4ГБ) в хлороформе добавляли измельченную целлюлозу (размер частиц 120 мкм, в соотношении П(ЗГБ/4ГБ):БЦ 2:1 и 1:1), гомогенизировали ультразвуком, затем отливали пленки и сушили до полного испарения растворителя. Исследование свойств поверхности этих образцов показало повышение гидрофильности поверхности относительно исходных материалов для композитов, полученных путем смешивания раствора П(ЗГБ/4ГБ) с крошкой БЦ в соотношении 2:1 и 1:1 (краевой угол смачивания водой составил $43,9 \pm 17,55^\circ$ и $36,1 \pm 0,66^\circ$). Установлено, что физико-механические характеристики композитных матриц во многом зависят от способа получения самого композита и свойств используемых материалов (П(ЗГБ/4ГБ), БЦ) и превосходят последние. По результатам исследования поверхностных и физико-механических характеристик полученных гибридов для исследования адгезии клеток были отобраны пленочные системы, полученные технологией полива раствора полимера с целлюлозной крошкой в соотношении 2:1 и 1:1 с последующим испарением растворителя. Эти типы матриц были нагружены лекарственными препаратами, стимулирующими ранозаживление (актовегин, солкосерил) и протестированы в культуре фибробластов, полученных из ММСК жировой ткани. По результатам МТТ-теста наиболее привлекательными в качестве гибридных систем являются образцы «полимер:порошок бактериальной целлюлозы (1:1)», нагруженные актовегином или солкосерилом в концентрации 5 %, оказывающие стимулирующее действие*

на фибробласты, что дает возможность использовать их в качестве перспективных раневых повязок для восстановления дефектов кожных покровов.

Ключевые слова: бактериальная целлюлоза, полигидроксиалканоаты, гибриды, лекарственные препараты, биосовместимость.

Introduction

An essential requirement for effective treatment of skin injuries is the availability of biotechnological tissue-engineered skin equivalents. Materials used to fabricate such constructs should have a number of special properties and impart characteristics of living tissues to the engineered constructs (grafts). These characteristics include 1) the ability to regenerate themselves, 2) the ability to maintain the blood supply, and 3) the ability to alter their structure and properties in response to effects of the external factors, including mechanical loads (Zhang et al., 2016; Deng & Kuipe, 2018; Han et al., 2014).

The main tasks of tissue engineering are to optimize isolation, proliferation, and differentiation of cells and to construct scaffolds or delivery systems for supporting and coordinating the growth of three-dimensional tissues *in vitro*. Scaffolds must serve as templates and inducers of cell proliferation and differentiation into specialized cells, generating new tissue. The tissue can be grown on the scaffolds that, being implanted into the host organism, will be gradually resorbed and replaced by the new tissue (Stratton et al., 2016; Shelke et al., 2014). Another option is to implant a biocomposite, which comprises a scaffold and a partly formed tissue. Having been implanted into the defect site, the engineered construct must retain its structure and functions over the time period necessary for the normally functioning tissue to be regenerated and integrated with the surrounding tissues (Shishatskaya & Volova, 2016).

The purpose of the present study was to construct hybrid tissue-engineered biosystems for regeneration of injured skin based on materials of biotechnological origin (biocompatible and resorbable polyesters polyhydroxyalkanoates and bacterial cellulose).

Materials and methods

Bacterial cellulose (BC) pellicles were produced in the culture of acetic acid bacterium *Komagataeibacter xylinus* B-12068 (Prudnikova & Shidlovsky, 2017); biodegradable polymer, poly-3-hydroxybutyrate-co-4-hydroxybutyrate (P(3HB/4HB)), was synthesized in the culture of chemolithoautotrophic bacterium *Cupriavidus eutrophus* B10646 (Volova et al., 2014b).

Hybrids based on BC and P(3HB/4HB) were prepared using different methods: 1) the dried BC pellicles were soaked with a 2% P(3HB/4HB) solution in chloroform, kept for 24 h, and dried in a dust-free cabinet until the solvent had completely evaporated; 2) nonwoven membranes were placed into the *K. xylinus* B-12068 culture, and bacterial cellulose synthesized in it grew under static conditions; 3) powdered cellulose (particle size of 120 µm) was added to a 3% P(3HB/4HB) solution in chloroform and mixed ultrasonically to homogeneity; then, the films were produced by the solvent evaporation technique, at polymer to cellulose ratios of 2:1 and 1:1. Solutions of actovegin (OOO Takeda Pharmaceuticals, RF) and solcoseryl (MEDA PHARMA, Switzerland) were used for the loading of BC/P(3HB/4HB)-hybrids by immersion in solutions of drugs.

Samples of the BC/P(3HB/4HB) hybrids were analyzed in a number of studies, to estimate their physical/mechanical and surface properties, their adhesive properties towards fibroblasts, and their ability to facilitate cell proliferation. The methods were described in detail elsewhere (Volova et al., 2014a).

Statistical analysis of the results was performed using the standard software package of Microsoft Excel. Arithmetic means for three replicates and standard deviations were found.

Results and discussion

The measurement of the mechanical properties produced the following results: composites of nonwoven membranes and BC had the highest values of Young's modulus (272.3 ± 27.2 MPa), most probably due to the high mechanical strength of the P(3HB/4HB) membranes (113.54 ± 9.85 MPa) (Table 1). The values of Young's modulus of the composite of the dried BC pellicles soaked in a 2% P(3HB/4HB) solution was 110 MPa, while fracture strain increased to 6.25%. Although Young's modulus of the composites prepared by mixing the polymer solution and BC powder was low (65.08 ± 7.1 and 63.41 ± 5.6 MPa), it was higher than the corresponding parameter of pure BC (47.60 ± 6.32 MPa).

Measurements of water contact angles showed that BC film was rather hydrophilic. Its water contact angle was $45.5 \pm 17.6^\circ$, while the water contact angle of the nonwoven P(3HB/4HB) membrane was $66.5 \pm 4.4^\circ$ (Table 1). Soaking of the BC film in a 2% P(3HB/4HB) solution produced an insignificant effect on the surface properties of BC. Composites prepared by mixing the P(3HB/4HB) solution and BC powder at ratios of 2:1 and 1:1 had water contact angles of $43.9 \pm 17.55^\circ$ and $36.1 \pm 0.66^\circ$, respectively; the dispersive and polar components were larger than those of pure BC, suggesting higher hydrophilicity of the surface

of composites. The reason for the high values of water contact angle of the BC + nonwoven membrane composites, $65.3 \pm 2.72^\circ$, was that the nonwoven membranes used to grow cellulose were initially dense, with the water contact angle of about 60° .

An important parameter of wound dressings is water vapor transmission rate – a measure of the passage of water vapor through the material. The experimental composites showed different water vapor transmission rates: for the P(3HB/4HB) + BC powder (2:1), it was the highest (5014 ± 20 g/m²·d), while for the P(3HB/4HB) film, it was the lowest (404 ± 9.1 g/m²·d). It is important to increase this parameter to control the build up of fluid underneath the dressing, make it more breathable, and prevent the establishment of conditions favorable for the development of pathogenic microorganisms.

The study of surface, physical/mechanical and biological properties of the experimental hybrids showed that the films prepared by casting the solutions of the polymer P(3HB/4HB) with cellulose powder followed by solvent evaporation were the most attractive candidates to be used as scaffolds for growing fibroblasts (Fig. 1). These types of hybrids were loaded with drugs promoting wound healing (actovegin, solcoseryl) and fibroblasts derived from the adipose tissue MSC (Fig. 1). Hybrids prepared by casting the solutions of the polymer: cellulose powder mixture (1:1) and loaded with 5.0% actovegin or solcoseryl were the most favorable for fibroblast growth.

Conclusion

Bacterial cellulose/resorbable polymer P(3HB/4HB) hybrid elastomeric systems were constructed as scaffolds for cell culture and as biotechnological dermal equivalents. The studies of the surface properties and physical/mechanical

Table 1. Physical/mechanical and surface properties of hybrids based on BC and P(3HB/4HB)

Sample	Young's modulus, MPa	Fracture strength, MPa	Fracture strain, %	Water contact angle, °	Dispersive component, mN/m	Polar component, mN/m	Water vapor transmission rate, g/m ² ·d
Initial BC	47.60±6.32	0.11±0.13	4.35±0.82	45.5±17.60	28.0±9.24	17.5±8.37	2655±21.0
P(3HB/4HB) membrane	113.54±9.85	9.30±1.24	180.33±20.71	66.5±4.40	22.9±17.60	42.2±17.60	404±9.1
BC + nonwoven membranes	272.30±27.20	8.67±0.86	3.65±0.40	65.3±2.72	45.6±1.29	19.7±1.42	2005±15.0
P(3HB/4HB) + BC powder (2:1)	65.08±7.10	0.88±0.08	3.45±0.34	43.9±17.55	22.0±9.39	21.9±8.17	5014±20.0
P(3HB/4HB) + BC powder (1:1)	63.41±5.60	0.83±0.07	3.47±0.57	36.1±0.66	35.5±0.58	29.6±0.08	1732±9.0
BC + 2% P(3HB/4HB)	110.99±10.90	6.65±1.20	6.25±0.94	46.2±13.77	45.2±11.74	21.0±2.03	4473±10.0

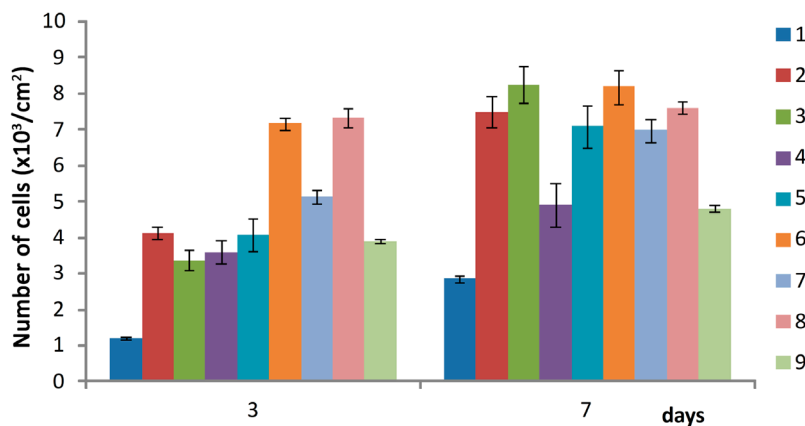


Fig. 1. Viable fibroblasts cultivated on BC/P(3HB/4HB)/drug hybrid scaffolds: 1 – control (polystyrene); 2 – BC + nonwoven membrane; 3 – BC + 2% P(3HB/4HB) solution; 4 – P(3HB/4HB) + BC powder (2:1); 5 – P(3HB/4HB) + BC powder (1:1); 6 – P(3HB/4HB) + BC powder (1:1) + actovegin (5.0%); 7 – P(3HB/4HB) + BC powder (1:1) + actovegin (1.0%); 8 – P(3HB/4HB) + BC powder (1:1) + solcoseryl (5.0%); 9 – P(3HB/4HB) + BC powder (1:1) + solcoseryl (1.0%) (MTT assay)

properties of the hybrids proved that the best scaffolds, which facilitated fibroblast growth, could be produced by combining the cellulose/polymer systems and drugs. They were found to be promising materials to fabricate wound dressings for healing skin defects.

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