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The interaction of polyhydroxyalkanoate polymer group with cells and tissues

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In the recent literature devoted to the results of the study, formulation and application of polyhydroxyalkanoates (PHA), contains a wealth of experimental and clinical data on efficacy and safety of using these polymers with biomedical goals. However, the application of PHA, both independently and in combination with other drugs and substances that must surely have its advantages and disadvantages. It should be noted the complete absence, information on contraindications and complications of the PHA, and insufficient data on the patterns of degradation of PHA in vivo, and morphological processes connected with it of scientific literature.

Key words: polyhydroxyalkanoates, reconstructive medicine, biodegradation, cellular technologies.

Introduction. Successful introduction of the methods of long-term cultivation of cells, including the progenitor cells of specialized tissues, into the practice of experimental biology and medicine became the prerequisite of the elaboration of novel technologies and approaches of the reconstructive medicine [1]. Some recent works have reported the application of mesenchymal stem cells (MSC) for the purposes of regenerative medicine and tissue engineering, which enables the reconstruction of three-dimensional structure of the lost or damaged tissues and even whole organs [3–5] rather than just restoration of some tissue, for instance, after skin injuries [2]. The interdisciplinary research in tissue engineering is directed, first of all, at the elaboration of novel biocomposite materials with improved properties [6–9]. The main principle is the creation of biodegradable carriers and their application in combination with the donor MSC and/or bioactive substances for implantation into the damaged organ or tissue [10].

Therefore, in the last decade an enhanced attention of scientists and technologists has been paid to the new class of polymers – polyhydroxyalkanoates (PHA).

PHA characteristics and production PHA are aliphatic polyesters, the polymers of hydroxy derivatives of natural fatty acids – β-hydroxybutyric and β-hydroxyvaleric. There are over 150 different monomers in this family which can give rise to the materials with various properties [11].

The melting temperature of PHA is up to 180 °C, the decomposition point is over 200 °C, and the molecular mass is 100–800 kDa. The most important representatives of this family are polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV).

The industrial types of PHA are copolymers of PHB and PHV. All the PHA homologues are the products of bacterial activity [12], and for practical purposes they are manufactured by the biotechnological method of bacterial fermentation from vegetative sugars, for instance, glucose.

In the past, PHA were too expensive for widespread implementation. The current efforts are aimed at decreasing the cost of polymers by producing them from fermentable sugars, obtained from relatively inexpensive sources, for instance, from the sugar industry waste, from renewable plant raw materials [13]. At present PHA, PHB in particular, are produced on the industrial scale

in Germany (Biomer©), the USA (Metabolix©), in Great Britain (Biopol©).

PHA properties and spheres of application. Recently in China, Southern Korea, Japan, India, Brazil and Russia sharply increased the number of scientific publications devoted to extremely interesting and useful properties of PHA. The most active and successful Russian scientists from the Siberian federal university (Krasnoyarsk) elaborated the technology of PHA production, and in 2005 designed and launched the first domestic pilot manufacture of biocompatible polymers of different structure, resorbable in biological media, as well as fabrication of experimental goods for biomedical purposes. The developed PHA retention sutures, tubular endoprostheses and membranes are approved for clinical trials.

To date there has been accumulated a considerable experimental database, demonstrating valuable PHA properties, such as thermoplasticity, biocompatibility and, above all, biological degradability [12, 14, 15].

PHA are known for their non-susceptibility to hydrolytic degradation in aqueous media, therefore they are characterized by slow (months and years) kinetics of bioresorption, and their destruction in biological media is not accompanied with the change in the active reaction of the medium [14], which allows their application as a carrier-substrate for functioning cells [16]. PHA may be used as matrices for deposition, delivery and long-term controlled release of preparations (drugs, pesticides) [17–19], in particular, rubomycinum [20]. This class of polymers is widely used as a matrix or scaffold for the MSC delivery to tissues [21–24].

The interrelationship of PHA and the cellular environment *in vitro*. Shishatskaya *et al*. [10] applied PHA in different phase states (solutions, emulsions, powders) to obtain and to study the structure and properties of two- and three-dimensional matrices in the form of flexible transparent films, membranes, superfine fibers, microparticles, sponges, voluminous solid and porous constructions. Glass and polystyrene were used as controls. The suitability of PHA-matrices for the cultivation of cells *in vitro* was confirmed. The biocompatibility and cytotoxicity of matrices from PHA were estimated on the animal cells of different origin – mesenchymal (fibroblasts and cells of endothelium) and endodermal (hepatocytes) ones as well as on the primary culture of osteoblasts, isolated from the cells of red bone marrow. The investigation was performed using the microscopy and intravital staining of cells with trypan blue. The synthesis of proteins and DNA by the cultivated cells as well as the cellular and medicinal cytotoxicity were investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is based on the ability of the mitochondrial dehydrogenases to convert the soluble MTT into formazan, crystallized inside the cell. It was demonstrated that the morphology of cells, cultivated at direct contact with the matrices surface, did not differ from that of the control cells, cultivated on glass or polystyrene. The direct contact of the cells with the surface of PHA matrices neither reduced the cell viability, nor led to the inhibition of the DNA synthesis and the proliferative activity.

Therefore, the absence of cytotoxicity of the PHA matrices was proved as well as their high biocompatibility regarding the investigated cultivated cells. Besides, the osteogenetic potential of PHA-constructs was demonstrated *in vitro* [21]. These works did not reveal any differences between the biological activity of PHB and copolymer samples of PHB/PHV.

Previously Deng *et al*. [25] used the cultivation of chondrocytes to demonstrate that, compared to pure PHB, the mixtures of polymers (polyhydroxybutyrate-Co-hydroxyhexanoate/polyhydroxybutyrate) are the most suitable for the cell cultivation. Zhao *et al*. [26] showed a better biocompatibility of the PHA mixture (polyhydroxybutyrate-Co-hydroxyhexanoate/polyhydroxybutyrate) in comparison with the pure polyhydroxybutyrate-Co-hydroxyhexanoate.

The recent studies have shown that PHA may promote the growth and differentiation of stem cells, in particular, into neurons after CNS injury [27], and that PHA stimulate the growth of fibroblasts *in vitro* [28]. However, such publications are not numerous and the last ones are dated 2011, but the probability of the impact and change in the cell differentiation during the interaction with PHA cannot be rejected and is to be considered while using these polymers as scaffolds.

Some differences are revealed between the surface properties of the films, made of PHA of various kinds, in particular, of poly-3-hydroxybutyrate and poly-3-hydroxybutyrate-Co-3-hydroxyvalerate, which, in turn,

may impact the level of cell adhesion on the surface of these films. Additionally, the conclusion was made that the biomaterials for tissue engineering are specific for certain type of cells. For instance, poly-3-hydroxybutyrate is more suitable for the cultivation of olfactory ensheathing (Schultze) cells whereas poly-3-hydroxybutyrate-Co-3-hydroxyvalerate – for MSC [22].

The interrelationship of PHA and the macroorganism *in vivo*. The acute and long-term experiments on laboratory animals demonstrated that the PHA degradation depends on the chemical structure of the polymer, on the place of implantation and the form of the product; it occurs slowly via humoral and cellular pathways, mainly, from the surface of the product without the formation of local defects and sharp decline in solidity. The PHA degradation involves the macrophages and gigantic cells of foreign bodies with the high activity of acid phosphatase, correlating with the activity of the enzyme in the blood serum of animals. The main targets of polymer particles are the tissues of liver, kidneys and spleen. The most active destruction of microparticles of the polymer matrix occurs in spleen and liver. PHA may be used for the period of several months up to a year; they neither cause inflammatory, necrotic, sclerotic and any other negative reactions in the surrounding tissues nor prevent the reparation *in vivo*, which is especially valuable for surgical sutures, endoprostheses and osteoimplants. The degradation of the polymer structure becomes evident if the experiment lasts for 12 and more weeks [29, 30]. The experiments on the reparative osteogenesis demonstrated that the implants made of PHB have pronounced directional osteoplastic properties [21].

The works of Fedorov *et al*. [31] dedicated to the investigation and production of the PHB-based fibrous and film materials proved the reasonability of the use of this polymer to apply the sheath on surgical sutures. The obtained surgical material meets all the requirements of the modern surgery. The mentioned requirements are quite numerous, namely torpidity, mechanical performance, atraumatic nature, *i. e*. the suture should not interrupt the blood supply and cause the development of necrosis, inflammation in the sutured tissues (the latter is achieved via sterilization, a lower capillarity and prolonged bactericide properties). The suture material should be nonhygroscopic, its biodegradation should not occur prior to the terms, specified by the process of wound healing.

It should be noted that in the literature along with the results, testifying to high efficiency of the PHA application for biomedical purposes, there are opposite data, indicating poor biodegradation or even the complete absence of lysis of this class of polymers in the living organism [32–34].

The morphological and radiovisiographic methods were used to study the regeneration of the damaged part of the lower jaw bone of rats after the PHA application (copolymer of 85 % of PHB and 15 % of PHV). The opening in the bone, where PHA was applied, did not change for five weeks of observation. No signs of the PHA consolidation with the edge of the bone defect were found. The polymer was surrounded by the fibrous tissue with numerous cellular elements. There were no signs of degradation of the artificial material at any moment during the experiment [32].

The controversial data were obtained while studying the processes of regeneration of the damaged knee joint cartilage of rats after the PHB/PHV implantation. After the PHA application the destructive changes in the damaged joints were much more pronounced than in case of natural healing. No PHA were found between the joint surfaces during the observation. However, sometimes PHA were revealed in lateral folds of the joint capsule. More often small PHA fragments were located in the soft tissues around the joint; they were encapsulated by the actively proliferating fibrous tissue and deformed. There were neither macrophage and leukocyte reactions to the foreign body nor the signs of developing granulomatous inflammatory process. At the same time, there was no evidence of the PHA degradation [34].

The morphological methods were used to study the reaction of the organism of rats after the implantation of materials made of PHB/PHV. It was revealed that the polymer implantation into the abdominal cavity is followed by the active adhesive process, which results in the formation of fibrotic folds between PHA and the intestinal loops. The implanted PHA films under the skin and in the muscle tissue are encapsulated by the thick fibrous capsule. At the implantation of PHA superfine fibers, the granulomas of the foreign body with perifocal inflammation and sclerosis of the adjacent tissues

are formed in all the tissues. In these granulomas the fragmentation of the polymer and phagocytosis by macrophages occur with the formation of gigantic cells of foreign bodies. The conclusion was made that after the implantation into the organism, the PHA materials cause active and pronounced chronic granulomatous inflammatory reaction and are destroyed very slowly by macrophages [33].

This contradiction is likely to occur because of different interpretation of the data obtained. For instance, Shishatskaya *et al*. [29, 30], based on the active macrophage reaction in the place of PHA implantation, made a conclusion about the PHA degradation. They stated that it is rather a long-term process, the start of which may be revealed only after 12 weeks.

Maiborodin *et al*. [33, 34], based on the same long-term infiltration of peri-implant tissues by macrophages, stated about the absence of the degradation of these polymers. Due to the fact that these authors found the PHA fragments in the tissues of rats one year after the implantation, their conclusions seem more grounded.

The perspective of further studies on PHA. Recently special attention is paid to the elaboration of new ways of production and modification of PHA, aimed at the improvement of their properties [6, 7, 9, 23, 35–38]. In particular, Ruth *et al*. [36] and Mauclairea *et al*. [8] studied new types of PHA, poly-3-hydroxyoctanoate (PHO) and poly-3-hydroxyundecanoate (PHUA), with antibacterial properties.

There are some reports about the possibility of creating biodegradable carriers for MSC made not just of PHB and PHV [21–24], but also of the composites of these polymers with calcium phosphate [39, 40]; on the basis of polyhydroxybutyrate-Co-hydroxyvalerate (PHBV) with wollastonite (W). It was revealed that the introduction of wollastonite into the construct improves the adhesion, the proliferation of the progenitor cells and their differentiation into osteoblasts even in the non-osteogenetic medium [41].

There are the works, confirming the best suitability of 3D constructs of the mixture of 3-hydroxybutyrate, 3-hydroxyvalerate and 3-hydroxyhexanoate (terpolyester) for the cultivation of the nervous cells, if compared to the copolymer (3-hydroxybutyrate/3-hydroxyhexanoate) and to the polymer of the other class – polylactic acid (PLA) [27, 42].

Conclusions. At the modern stage of the development of regenerative medicine there is the urgent necessity of implementation of new biocompatible functional materials, which allow construction of the systems, capable of reproducing biological functions of the living organism. It could be, in turn, a prerequisite of creating bioartificial organs and tissues.

The literature contains numerous data on the reasonability of using PHA in the reconstructive medicine, cell and tissue engineering. However, noteworthy is the deficiency of information on the contradictions and complications, whereas only based on the thorough analysis of complete scope of available knowledge, the reliable conclusion can be made about the spheres of PHA application. Additionally, there are still unsolved issues on the kinetics and controllability of the PHA biodegradation, the mechanisms of interaction of PHA products with different cells and tissues *in vivo*.

Взаємодія полімерів групи полігідроксиалканоатів з клітинами і тканинами

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Резюме

У літературі останніх років, присвяченій результатам вивчення, отримання і застосування полігідроксиалканоатів (ПГА), міститься безліч експериментальних і клінічних даних щодо ефективності і безпеки використання цих полімерів для медико-біологічних потреб. Проте застосування ПГА як самостійно, так і в комбінації з іншими препаратами і речовинами, поза сумнівом, повинно мати свої переваги і свої недоліки. Варто відмітити повну відсутність у науковій літературі відомостей про протипоказання і ускладнення за використання ПГА, а також недостатню кількість даних стосовно закономірностей деградації ПГА у живому організмі і морфологічних процесах, з цим пов'язаних.

Ключові слова: полігідроксиалканоати, реконструктивна медицина, біодеградація, клітинні технології.

Взаимодействие полимеров группы полигидроксиалканоатов с клетками и тканями

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Резюме

В литературе последних лет, посвященной результатам изучения, получения и применения полигидроксиалканоатов (ПГА), содержится множество экспериментальных и клинических данных об эффективности и безопасности использования этих полимеров в медико-биологических целях. Однако применение ПГА как самостоятельно, так и в комбинации с другими препаратами и

веществами, несомненно, должно иметь свои преимущества и свои недостатки. Следует отметить полное отсутствие в научной литературе сведений о противопоказаниях и осложнениях при использовании ПГА, а также недостаточное количество данных о закономерностях деградации ПГА в живом организме и морфологических процессах, с этим связанных.

Ключевые слова: полигидроксиалканоаты, реконструктивная медицина, биодеградация, клеточные технологии.

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