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Polymers in Orthopedic Surgery and Tissue Engineering: From Engineering Materials to Smart Biofunctionalization of a Surface[1]

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Abstract—Polymeric materials are important for biomedical applications. Nevertheless, during the last few decades, the requirements on the used polymers have experienced significant changes. In the present review, the data illustrating the history of application of polymers in orthopedic surgery and tissue engineering are systemized and generalized. Special attention is given to the discussion of new problems which could be solved through creation of smart biologically functional polymeric structures able to control the behavior of cells.

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INTRODUCTION

Every year, tens of millions of people all over the world suffer from bone damage, most often, bone fractures, and one-fifth of the victims needs hospitalization [1, 2]. Many of people suffer from the pathology of bone destruction as a result of osteoporosis, and about 30 million people are in the high-risk group owing to loss of bone mass [3, 4]. More rare, but still sufficiently widespread, are the cases of genetically specified osteopsathyrosis and osteosarcoma [4]. Furthermore, the need to reduce or replace bones appears in the arthroplasty of joints, vertebral arthrodesis, and maxillofacial surgery [5, 6].

In all above-described cases, it is necessary to fill a defective portion of a bone in order to restore the structure and functions of the damaged tissue [7]. Although, since ancient times, the joining of bone fragments through application of splint bandages without operative intervention has been used in the case of bone breakage, this method ensures good results only in the cases of simple fractures. For heavy injuries and bone diseases, the progress in medical science made it possible to develop diverse treatment modes involving surgical interventions that provide the introduction (implantation) of different materials and articles into the place of fracture to joining or fill the cavities formed after the removal of bones or bone elements with such materials. *Autografts*, parts of bones taken from the body of a patient, have found limited applications [2, 5]. Among conventional nonbiological materials, metals and alloys, as well as ceramic and silicate materials, are widely used for bone endoprosthesis replacement. However, the mechanical properties of metals significantly differ from those of bone materials; under multiple cyclic stresses, this circumstance may lead to bone destructions [1, 8]. Ceramic articles demonstrate increased fragility and are applicable for prosthetics of predominantly small bone elements that moreover operate at low stresses [8, 14].

Although, at first glance, polymers seem to be incompatible with such a complex structure as bone tissue in terms of biochemical and biomechanical properties, already at the first stages of innovation in this field of medicine, they have received widespread attention from bioengineers, surgeons, chemists, and physicists engaged in the creation of new materials. Precisely polymers make it possible to change in a wide range the properties of materials through variation in their composition and structure. Initially, the use of polymers evoked interest associated with the possibility to replace heavy metal parts of endoprostheses with lightweight polymeric parts. However, during the last few decades, homo- and copolymers, as well as composite materials produced on their basis, have occupied an important place in the endoprosthetics of bone lesions [1, 8].

At the end of the last century, owing to the advancement of cytology (the development of methods for preparing stable cellular lines), genomics, and proteomics and the accumulation and extending knowledge on the processes of tissue healing, a new biotechnological scientific and practical branch, so-called *tissue engineering*, was born. This branch is best

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of all determined by its purpose: the design and creation of living functional components under laboratory conditions that may be used for the recovery of damaged or irregularly functioning tissues [2, 5, 8].

Investigations in this field predominantly deal with the development of materials for the regeneration of bone tissues. The design of three-dimensional porous frameworks, *scaffolds*, is one of the major problems in this direction. The scaffolds should satisfy biocompatibility requirements and should be able to support the growth of new tissue and provide the mechanical stability of this tissue until its growth is completed [2]. As evidenced by the current literature [2, 8, 9–11], polymers have taken leading positions in the design of these supporting media. Various research groups proposed many methods to form scaffolds on the basis of both purely polymeric structures and polymer–inorganic composites. However, the experience of clinical trials of proposed designs is still insufficient. Present-day investigations revealed that there is a need not only to create three-dimensional porous scaffolds for cell growth but also to develop methods for biological functionalization of synthetic materials that can cause their characteristics to approach the properties of native extracellular material (the *matrix*) [12]. It is not surprising that polymer science provides wide opportunities for the implementation of such ideas.

In this review, the data illustrating the history of application of polymers in orthopedic surgery and tissue engineering are systemized and new tasks are discussed. The solutions of these tasks may be achieved via creation of smart biologically functional polymers able to control the behavior of cells. Special attention is paid to the latter issue reflecting our own results and plans for the future.

POLYMERIC IMPLANTS FOR REPLACEMENT OF BONE TISSUE

The directions that are of main interest for possible applications of implants in bone systems are as follows: the endoprosthetics of removed bones, the use of fasteners and glues for joining of bone parts or for fastening in a bone–prosthesis system, and the packing of bone defects. Note that there are two directions in this area, namely, the use of polymers for permanent and temporary function in the body [1, 13].

Nondegradable Polymeric Implants

Endoprostheses of bones functioning in the body for life are fabricated from polymeric materials resistant to biodegradation. In this case, the main difficulties are associated with the permanent mechanical action of a device on surrounding tissues. Specifically, they are caused by its slip in a tissue medium that brings about hampered formation of a normal capsule, disturbances in metabolic processes, and necrotic changes. The improvement of engraftment of an implant resistant to biodegradation may be achieved via its nonspecific functionalization through endowment of porosity or via perforation of a material; this circumstance facilitates the intergrowth of new tissue into the implant mass and the formation of a normal tissue capsule [14, 15].

PMMA, polyolefins (PE and PP), polytetrafluoroethylene, PET, and PU belong to the group of widespread biologically inert medical polymers. The uses of such high-molecular-mass substances in orthopedic surgery were described in detail, for instance, in [1, 2, 8]. However, note that, at the first stages of development in this area, only commercially available polymers were applied. Engineering polymers became the first macromolecular substances that were employed as materials of spare parts for human. It is important that these materials should preserve their properties during the entire period of function because the physiological medium of the body may be considered sufficiently aggressive. This requirement is fulfilled owing to the stability of chemical bonds constituting backbones of the mentioned polymers, specifically, carbon–carbon or silicon–oxygen bonds, and owing to the hydrophobicity of macromolecules that prevents penetration of water into the material.

Biodegradable Polymeric Implants

In contrast to the above-mentioned polymers, macromolecules that are destroyed, for example, as a result of hydrolysis, exist. Until the 1960s, there was practically no demand for such polymers. In 1966, Kulkarni et al. [16] proposed to use poly(lactic acid) for fabrication of biodegradable surgical implants, because the hydrolysis of this polymer leads to the evolution of a natural metabolite of the Krebs cycle, lactic acid. A year later, Schmitt and Polistina [17] patented biodegradable suture threads based on poly(glycolic acid). Later on, these threads became known under the name Dexon®. Since that time, the rapid development of synthesis and application areas of biodegradable polymers has been observed. Implants based on these polymers temporarily function in the body and are replaced with the regenerated bone tissue during destruction. Such polymers are most frequently used to manufacture fastening elements of bone fractures and certain types of glue compositions and to replace bone elements able for rapid regeneration [18–22]. Polyesters of hydroxycarboxylic acids and polymers of cyanacrylates are the main types of synthetic biodegradable polymers suitable for the design of implants [1, 2, 23].

Thus, a number of polymeric materials have already found diverse applications in orthopedic surgery. It is important to note that polymers, whose properties may be altered in a wide range, depending on their chemical composition and structure, have made it possible to create implants and devices improving the quality of life of patients. However, it is evident that many problems exist, the main of which is

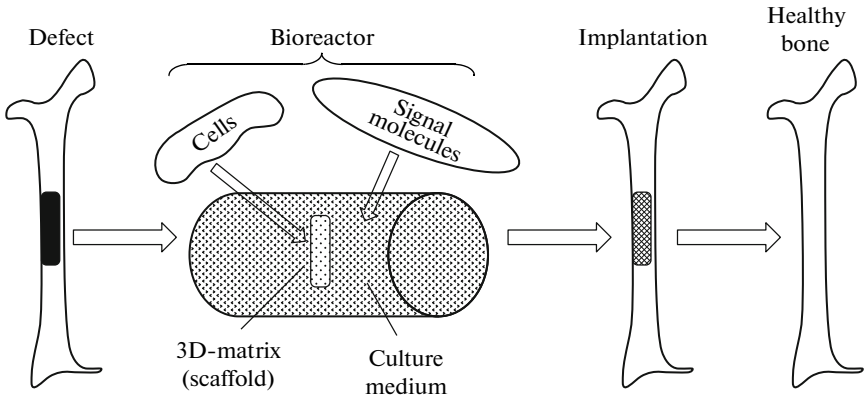
the reaction of the body to the introduction of alien objects [24]. Moreover, all modern orthopedic implants lack three of the most important characteristics of living tissues: namely, self-healing capability and the abilities to maintain the blood supply and to change the structure and properties in response to the environment [2, 8]. In addition, the growing demand for long-term orthopedic recovery suggests the displacement of emphasis from the replacement of tissues to their regeneration.

POLYMERS FOR BONE-TISSUE ENGINEERING

Method of Tissue Engineering

As regards bone tissue, the main task of tissue engineering is to combine principles of biology and engineering for creation of viable substitutes that recover and preserve functions of human bone. This kind of therapy differs from conventional methods based on the use of drugs and long-term implants by the fact that an implanted bone is integrated in the body of a patient and eventually leads to the complete elimination of defects [4, 5].

There are a variety of approaches to solving the above task [25–28]. Nevertheless, all these approaches involve the following key components: (i) borrowing of patient cells, (ii) recombinant signal biomolecules, and (iii) three-dimensional matrices. One of the most popular methods is based on the seeding of cells excorporated from a patient and the fixation of signal molecules (e.g., growth factors controlling the growth and changes in cell functions) on a biocompatible three-dimensional framework matrix (*scaffold*) having a shape precisely corresponding to that of the bone defect.



The purposes of the above-described procedure are to force the cells to attach to the framework surface, to accomplish three-dimensional growth, to undergo differentiation (that is, transformation from the nonspecific state into cells typical for the bone tissue), and to be organized into normal healthy bone tissue during biodegradation of the framework [23].

To date, the described approach represents one of the most promising and rapidly evolving fields of biotechnology. The application of this method in clinics will make it possible in future to eliminate the possibility of increased immune response of a recipient organism to introduction of the alien tissue [4, 5, 23].

There are many criteria whose fulfillment is necessary for creation of the so-called first-generation scaffolds [28]. To provide the possibility of tissue regeneration, the structure of scaffolds should ensure the growth of tissue in three dimensions and stimulate new growth in a form set by the framework. In order to ensure the three-dimensional growth of tissue, the structure of the matrix should possess a network of macro(supra)pores. The interconnected network of pores is needed to provide migration of cells inside and on the surface of scaffolds so as to facilitate the growth of tissue in the volume of the matrix. Moreover, during cultivation of cells in vitro, that is, outside a living body, as well as during tissue growth, the presence of this structure provides the unimpeded delivery of a nutrient medium to the cellular mass [29]. After implantation, the presence of a porous structure ensures the unimpeded access of blood to the cells. Finally, scaffolds should stimulate the growth of blood vessels (angiogenesis) inside the network of pores. In this case, it is necessary that the minimum diameter of pores should be greater than 10 μm [2, 28].

The material of the scaffold should not prevent the restoration of functions of tissues and their regeneration to the initial state. Hence, most polymers proposed for bone-tissue engineering are biodegradable [8, 28]. However, the problem is that the rate of scaffold destruction should be commensurable with the rate of tissue growth [30–32]. It is important to provide conformity between the mechanical properties of material and those of the bone tissue of the host organism [33]. Note that these properties are critical only for the final construction designed for implantation [8].

To optimize mechanical characteristics of the material, polymer–inorganic composites are developed [33–35].

Polymers for Fabrication of Scaffolds

Both synthetic and natural polymers are used for fabrication of scaffolds. Among the synthetic polymers that are in frequent use for creation of bone-tissue-engineering scaffolds are the above-mentioned (in the section devoted to biodegradable implants) poly(α-hydroxycarboxylic acids), specifically, poly(glycolic acid), poly(lactic acid), and their copolymers (poly(lactic-*co*-glycolic acid) (PMGAs)) [36–38], as well as polycaprolactone [39, 40], poly(3-hydroxybutyrate) [41, 42], polyorthoesters [43], and polyanhydrides [44]. All of them are plastics that can decompose at different rates via the chemical and enzymatic hydrolysis of chemical bonds of the backbone.

Among natural polymers, the extracellular-matrix proteins collagen [45] and fibrin [46], as well as polysaccharides, poly(hyaluronic acid) [47], chitosan [48], and alginate [49], are most often used to fabricate scaffolds. Such polymers are considered promising owing to their similarity with natural components of the cytoskeleton of the regenerated tissues. Nevertheless, their application is hindered by immunogenicity problems and by the inability to control their degradation.

In the up-to-date literature, much attention is given to the development of methods for preparing three-dimensional (3D) porous polymeric and composite structures of different architectures and morphologies. Among them are methods developed to prepare membranes (electrospinning [50, 51], thermally induced phase separation [52, 53], and washing-out of salts [54]) and methods that were specially elaborated for creating scaffolds (three-dimensional printing and solid free-form fabrication [28, 55]).

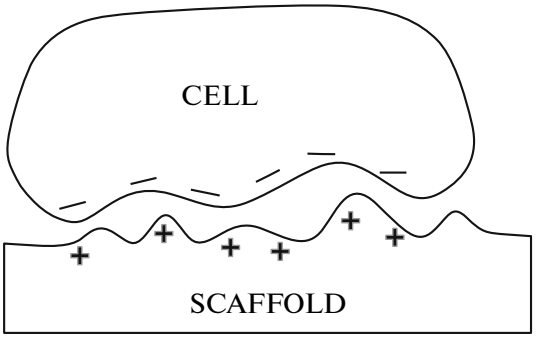
Thus, over the last two decades, the progress of polymer science has made it possible to create a significant number of polymers and methods for formation of porous biocompatible materials that possess the necessary physicochemical and mechanical properties and are suitable for application as media supporting the three-dimensional growth of a cellular mass. However, at present, it is clear that, for the growth of bone tissue under laboratory conditions, it is insufficient only to seed patient's cells on the biocompatible porous support. It is necessary to introduce not only stem cells or cells serving as precursors of osteoblasts but also special stimulating agents that control their adhesion [56], growth [57, 58], and differentiation [57, 59], that is, that are responsible for a gain of special functions by the cells. Thus, the above three-dimensional macroporous polymeric matrices cannot be unambiguously named scaffolds, because of their inability to independently support and regulate the said functions and, hence, to provide the formation of a new tissue. With the use of the English meaning of the term *scaffold*, i.e., building stages, it may figuratively be said that biologically inert three-dimensional macroporous matrices are *stages without builders*. Therefore, considerable attention in the up-to-date literature is paid to the biological functionalization of polymeric and polymer–inorganic frameworks.

Biofunctionalization as Nature Imitation

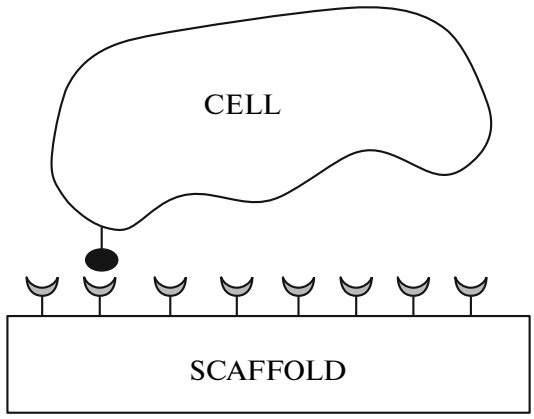
As was noted above, a great number of polymeric and composite materials that are nontoxic and may be used in biology and medicine have been created. Nevertheless, the character of interaction of such materials with cells and tissues is far from always being predictable; as a consequence, their clinical use is impossible. Therefore, invoking the known biological principles and relationships for controlling the properties of a material that favor the fulfillment of functions set by nature is the most important problem of modern science.

In the case of creation of bone-tissue-engineering scaffolds, it is necessary above all to control the most significant process, cell adhesion, that is, the attachment of cells to a material [2, 8, 56]. The effective interaction of cells with the material determines the feasibility of further growth of the cellular mass, the migration of cells, the gain of special functions, and the formation of a new tissue.

There are two main types of material–cell interactions: specific



and nonspecific



Usually, it is difficult to control nonspecific interactions because they are based on properties common for many types of cells. Such properties include the partial negative charge of the surface of a cellular membrane [60] and the lipophilic character of extracellular-matrix proteins that is responsible for the nonspecific interaction of cells with the surface of a

hydrophobic polymer. In contrast, the specific interactions are considerably more controllable because they are related to the interaction of objects possessing definite chemical structures [61]. Usually ligand–cellular-receptor interactions denoted as a key for a lock are regarded as specific or complementary interactions. Materials on whose surfaces biological molecules capable of similar specific interactions with cells are localized may be referred to as bioactive or biomimetic [62]. In other words, biomimetic materials are materials that imitate the natural biological environment and receive the necessary cellular response facilitating the execution of experimental tasks [63, 64]. It is significant that complementary interactions of the ligand localized on the framework surface with the corresponding receptor of the cellular membrane are not features of biomimetic materials. An important point is that the result of such interactions should be accomplishment of a specific function, such as attachment to cells of a definite type or creation of a specific scaffold structure directed toward formation of a certain tissue. These tasks may be solved with the use of both specific and nonspecific factors.

Current experiments show that, for the successful in vitro growth of complex tissues, the targeted functionalization of the scaffold surface should be performed; that is, biomolecules able to control biomaterial–cell interactions should be incorporated [65]. Under the influence of these ideas, the biocompatibility concept experienced significant changes. For example, in a recently published review [9], Molly Shoichet, a living classic of biomaterials science, adduced the citation of Williams related to the biocompatibility mechanism: *The biocompatibility of a scaffold or matrix for a tissue engineering product refers to the ability to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signaling systems, in order to optimise tissue regeneration, without eliciting any undesirable local or systemic responses in the eventual host.* This definition indicates that, in the creation of modern frameworks, emphasis should be placed on support and regulation of cell functions with the use of stimulating factors. Such an approach should eventually lead to the successful preparation of the living tissue.

With consideration for the fact that bone tissue is a structure with an extremely complex organization, the strategy of scaffold biofunctionalization for its reproduction via the bioengineering method must be based exactly on the approach for creation of biomimetic materials. In this case, attention should be concentrated on two main types of specific factors:

(i) factors of cell adhesion—extracellular matrix proteins (collagen [66], fibronectin [67], vitronectin [68]) and peptides (the most widespread are the so-called RGD peptides [69–71] containing the arginine–glycine–aspartic acid sequence that is the active center of interaction with integrin cellular receptors in the above-mentioned proteins);

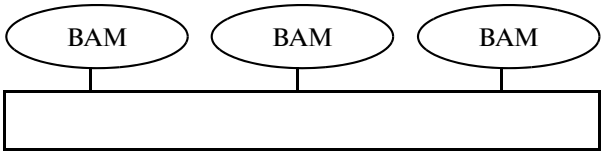
(ii) factors of growth and differentiation of cells—relatively low-molecular-mass proteins responsible for the regulation of proliferation, mobility, and differentiation of cells [72–74].

These factors are introduced with the use of polymers executing the function of delivery of a biologically active signal molecule to cellular receptors. Hence, the development of this direction may be assigned to the transition from the use of polymers in tissue engineering as engineering materials to their application as functional carries of biological substances.

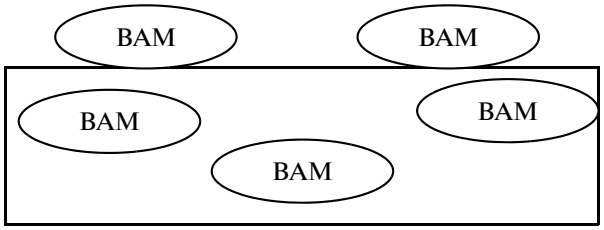
POLYMERIC STRATEGIES FOR DESIGNING BIOFUNCTIONAL SCAFFOLDS FOR BONE-TISSUE ENGINEERING

At present, a considerable body of papers devoted to the biofunctionalization of scaffolds for tissue engineering, specifically, for bone-tissue engineering, have been published. The methods and approaches proposed in those papers differ significantly and can hardly obey common classification. Summation of the data of investigations related only to synthetic polymers and to discussion of methods for introduction of biologically active molecules makes it possible to distinguish the following strategies for creation of biofunctional scaffolds for bone-tissue engineering:

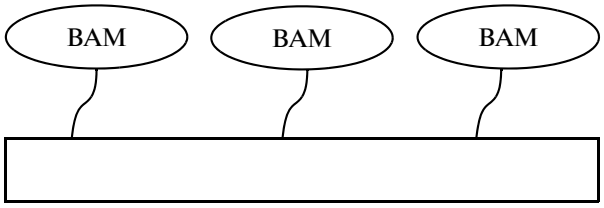
(i) immobilization (adsorption or covalent binding) of biologically active molecules (BAMs) on the surface of a biodegradable polymeric material;



(ii) creation of biodegradable polymers containing grafted RGD peptides and preparation on the related materials capable of specific cell adhesion;

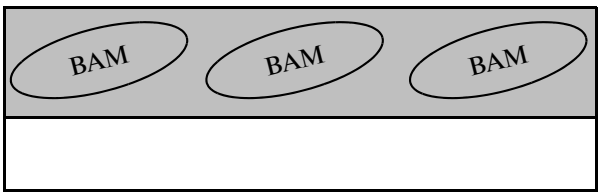


(iii) use of macromolecular compounds primarily based on PEG for introduction of RGD peptides or risk factors into the structure of the scaffold surface;



and

(iv) creation of special layers on the surfaces of scaffold materials for the targeted delivery of biologically active molecules.



Immobilization of RGD Peptides on the Surface of a Biodegradable Polymeric Material

In the tissues of the living body, cells are immersed into the extracellular matrix, which is a coacervate of glycosaminoglycans and proteins with different mechanical and signal functions. The specific adhesion of cells on the surfaces of structural components of the extracellular matrix proceeds via interactions between transmembrane cellular receptors (integrins) and special proteins, such as collagen, fibronectin, laminin, and vitronectin [75]. A considerable body of studies [76, 77] deal with the biofunctionalization of scaffolds performed through adsorption of the indicated proteins on the surface with the aim to improve cellular adhesion. Nevertheless, owing to the restricted possibility to control the adsorption of proteins and the low stability of adsorbed layers, the covalent binding of ligands responsible for this process is of interest to researchers.

At present, several short peptide sequences have been separated from adhesion extracellular-matrix proteins. These sequences can bind integrins and do not rank below corresponding proteins in specificity [69, 70]. In the case of covalent modification of biomaterials, short peptides have advantages over proteins owing to the absence of denaturation and immunogenicity as well as lower susceptibility to proteolysis [56, 78]. As was noted above, the peptides containing RGD (arginine–glycine–aspartic acid) sequences are of prime interest. The modification of biomaterials with RGD peptides brings about an increase in the adhesion of cells and activates the cascade of biochemical reactions related to binding with integrins. This discovery led to a significant growth of a number of studies devoted to the immobilization of such peptides on the surfaces of different materials performed with the aim to stimulate the specific attachment of cells and thereby to impart biomimetic properties to them.

Marletta et al. [79] showed that the adsorption of RGD peptides on the surface of polycaprolactone has the minimum effect on the adhesion of cells. However, irradiation of the surface of polyester with He^+ ions followed by the adsorption of RGD peptides led to a change in the expression of integrins involved in the growth and function of human osteoblasts [80]. The majority of these methods suffer form significant drawbacks, specifically, a small quantity of peptide molecules accessible for interaction, insufficient control of the arrangement of peptide molecules on the surface, and the possibility of appearance of unknown degradation products. Moreover, in the discussed method, biologically active molecules are bound with the surface only via adsorption interactions. This implies that they may be desorbed, the cell culture may change, or RGD peptides may appear in the body after implantation in vivo. Such a development of the situation may lead to complications associated with the marked toxicity of RGD peptides [69, 70].

In contrast, the chemical methods directed toward the covalent binding of RGD peptides with the surface of biomaterials demonstrate a sizeable advantageous effect of bioactivation of the polymer surface. The reactive groups are introduced into the surface structure of biodegradable polyesters through such reactions as hydrolysis [81, 82] and aminolysis [81] alongside with plasma treatment [83–85]. These processes are based on the partial destruction of the surface layer of biodegradable polymer to yield carboxylic, amino, or hydroxyl groups. Moreover, as a result of this treatment, the hydrophilization of the polymer surface occurs.

Thus, via treatment of the surface of biodegradable polyester with diamine, amino groups that were further used for the covalent binding of RGD peptides with the use of carbodiimide, glutaric aldehyde, and diethylene glycol diglycidyl ether [86] were grafted. The authors of [86] convincingly demonstrated an increase in the cell adhesion both on the aminated polyester and on the surface of peptide-modified polymer. The former effect is explained by an increase in the hydrophilicity of the surface and the simultaneous appearance of positive charge that attracts partially negatively charged cell membranes. Moreover, it was shown that there is sufficiently strong adhesion and that the spreading of cells on the surface modified with RGD peptides occurs.

In addition, reactive groups are formed on the surface of different biomaterials with the use of polymer coatings that are produced via the plasma-deposition method. This approach was employed in [87] for deposition of a polyacrylic acid film on the surfaces of glass rods. As a result, surfaces saturated with carboxylic groups were obtained. These groups may be used for further modification with specific biomolecules.

Despite positive effects, all results presented in a number of publications as methods for biofunctionalization of bone-tissue-engineering scaffolds were developed for two-dimensional structures, thin films, and plates. However, as was described above, three-dimensional, that is, pore-penetrated, matrices should be used for the in vitro growth of bone tissue. As a consequence, problems arise about how to extend the developed methods to macroporous materials. Moreover, the steric accessibility of adhesion peptides for interaction with active centers of integrin cellular receptors is an important issue. In addition, the authors of [88] mentioned that peptides immobilized

on the surface of biodegradable polymeric matrix may be detached during degradation of the material both in vivo and in vitro; as a consequence, the adhesion of cells should decrease owing to the blockage of cell receptors responsible for adhesion of free peptides.

Immobilization of Growth Factors on the Surface of a Biodegradable Polymeric Material

Of interest are studies devoted to the immobilization of growth factors on the surfaces of polymeric scaffolds for bone-tissue engineering. The majority of these molecules that are either small proteins or polypeptides were discovered in experiments on their effect on cell growth; however, the biological function of these biocompounds is much more complex [72, 73, 75]. It was found that the growth factors also regulate the migration, differentiation, and apoptosis of cells. Therefore, they might be called factors of development regulation; however, the initially adopted term is usually used. Growth factors are secreted by cells of a tissue. They diffuse for short distances and affect the function of neighboring cells. Being water-soluble proteins, growth factors cannot penetrate through the lipid layer of the cytoplasmic membrane; hence, their action is based on the transmembrane transfer of a signal [75].

In the studies on the regeneration of bone tissue, most often *bone morphogenetic proteins* (BMPs) are used. They are a group of proteins belonging to the superfamily of *transforming growth factors*-β. As was shown in experiments on animals, BMPs exhibit the strongest selective effect on the regeneration of bone defects relative to those of other agents of this superfamily. This effect is especially pronounced in the case of the BMP-2 factor, which is in most frequent use now [59, 73].

The simplest method for delivery of growth factors includes the introduction of their solutions directly into the place where a tissue defect is located (e.g., a bone fracture). However, the recovery of tissue in this specific case often does not proceed, owing to the rapid metabolism of protein in the body [74]. Of special current interest is the strategy based on the strong immobilization of growth factors on a scaffold surface.

Thus, BMP-2 was immobilized on the porous surface of a scaffold based on the copolymer of lactic and glycolic acids treated with oxygen, carbon dioxide, and ammonia plasma [89]. It was found that the treatment with oxygen plasma provides the maximum saturation of the surface with BMP-2 molecules. In accordance with [89], this effect may be explained by hydrophilization of the surface as well as by the formation of surface carboxyl and carbonyl groups that attract positively charged protein groups. The data obtained in experiments with the cells showed that the positive influence of BMP-2 on the proliferation of cells is noticeable only at the initial period and that this influence disappears during the experiment. Thus, the desorption of protein molecules immobilized via the above-described method from the support surface cannot be excluded.

In later investigations, the covalent immobilization of BMP-2 molecules was performed on matrices based on polycaprolactone [90] and chitosan [91]. The authors of these studies mentioned a significant growth in the capacity of the support with respect to those of protein molecules as well as an increase in the stability of protein relative to its stability during physical adsorption. Good results on the proliferation of cells and the expression of alkaline phosphatase were demonstrated also. Among the disadvantages of this approach, a partial loss in the activity of protein associated with its covalent attachment to the surface should be mentioned. This effect in turn makes the suggested method extremely expensive with consideration for the high cost of the recombinantly produced BMP-2.

The Use of Biodegradable Polymers Containing Grafted RGD Peptides

As opposed to the previous variant of peptide grafting onto the surfaces of polymeric materials, this method involves the creation of reactive sites and performance of the reaction of polymer biofunctionalization prior to formation of a material.

Thus, the triblock copolymer poly(ethylene glycol–*block*-lactic acid–*block*-glutamine acid) was synthesized [92, 93]. The terminal carboxyl group of the glutamic acid block was modified with RGD peptide. The resulting copolymer was introduced into a composite porous matrix consisting of the poly(lactic-*co*-glycolic acid) copolymer (PLGA) and nanoparticles of hydroxyapatite. The authors of [92, 93] justified the use of this modified copolymer not only by the need to introduce a factor of nonspecific cell adhesion but also by an increase in the compatibility of the matrix with hydroxyapatite nanoparticles due to their interaction with glutamic acid blocks. Moreover, those authors were sure that the mentioned polymer makes it possible to ensure steady-state adhesion of cells even during gradual destruction of the material. The above results show that the developed materials actually favor the adhesion of cells. However, for the growth of bone tissue to be successful, molecules of the factor of growth and differentiation of cells (BMP-2) should be periodically introduced also. At present, many studies are devoted to the synthesis of similar copolymers suitable for creation of biofunctional polymeric scaffolds for bone-tissue engineering [94–96].

The Use of Conjugates of Hydrophilic or Amphiphilic Macromolecules with Biologically Active Molecules

As was noted in a great number of recent investigations, one of the significant problems of tissue engineering is related to nonspecific interactions of proteins and cells [9, 97]. To provide the physiological integration into the surrounding tissues and to create

functioning tissues, it is necessary to perform the surface modification that prevents the nonspecific adsorption of proteins and undesirable adhesion of cells but makes it possible to immobilize specific signal molecules. A similar strategy allows switching off of undesirable cell–biomaterial interactions and switching on of only interactions that are necessary for the effective regeneration of new tissue. PEG is usually used as a polymer that prevents the undesirable nonspecific adhesion of proteins and masks the surface of the material from the immune system. It is assumed that macromolecules of this polymer structure the water around their chains; as a result, a hydrate shell preventing the adsorption of proteins is formed [98]. Alongside PEG, certain polysaccharides, for example, hyaluronan [99, 100], possess similar properties. Nevertheless, PEG is used in the most studies in which biological properties are imparted to the surface of a scaffold material.

For example, the grafting of PEG molecules on the surface of polyester containing carbonyl reactive groups was performed in [101]. PEG macromolecules containing aminooxy groups were used for grafting. This circumstance made it possible to perform reactions under mild conditions with a high yield. The authors of [101] showed that the reaction of keto groups of the polymer surface with aminooxy-PEG is chemoselective. Similar reactions gave rise to the *polymer brush* of PEG molecules covering the surface of biodegradable polyester. In this case, the thickness of the grafted layer is determined by the molecular mass of PEG molecules. Subsequently [95], the authors showed that the external terminal groups of PEG molecules may be modified with the RGD peptide. Moreover, it was noted that the immobilization of growth factors offers promise. Thus, the authors of the cited studies demonstrated the success of a strategy directed at prevention of the nonspecific adsorption of proteins and at an increase in the specific adhesion of cells.

It is necessary to mention the approach to the biofunctionalization of inorganic materials that is based on the use of star-shaped PEG containing six arms that have one terminal isocyanate group each [97]. The backbones of such polymers consist of ethylene glycol and propylene glycol taken at a ratio of 4 : 1. For the covalent immobilization of star-shaped molecules of PEG–NCO, the amination of the surface of an inorganic substrate with *N*-[3-(methoxysilyl)propyl]ethylenediamine was performed. Then, the method of thin-film deposition with the use of centrifugal force (*spin coating*) was employed to apply the star-shaped PEG–NCO on the surface from its solution in a THF–water mixture. The presence of the aqueous medium favored the hydrolysis of a part of the isocyanate groups into primary amino groups. Thus, during film formation, not only the attachment of the star-shaped PEG–NCO to the surface but also the crosslinking of its molecules with star-shaped PEG–NH_2 molecules should occur. In this case, a part of the isocyanate groups directed toward the environment remain intact in the above processes. The authors of [97] implemented the covalent modification of these groups with RGD-peptide molecules and studied the adhesion of human fibroblasts. With the use of optical and fluorescent microscopy, it was shown that adhesion on the films modified with RGD peptides proceeded to a noticeable degree, while in the case of the films of the nonmodified star-shaped PEG, adhesion was practically absent. Similar data were reported in [102, 103].

Creation of Layers for the Delivery of Growth Factors on the Surface of the Scaffold Material

As was mentioned above, for the successful growth of bone tissue, it is necessary to use not only peptides responsible for the adhesion of cells but also special proteins referred to as growth factors. The advantageous and disadvantageous aspects of the immobilization of growth factors directly on the polymeric scaffold were discussed as well. In recent investigations, successful attempts were made to create hydrophilic polymer layers of the hydrogel type with the encapsulated BMP-2 growth factor.

In [104], rapid layered prototyping (solid free-form fabrication technique) was used to fabricate a porous matrix on the basis on the PLGA copolymer. The conjugate of hyaluronic acid (HA) and PLGA (PLGA–HA) required for encapsulation of the BMP-2–PEG complex was deposited on the surface of this matrix. The conjugate was synthesized in DMSO via the use of PLGA activated with *N*,*N*-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide and GA modified with adipic acid dihydrazide. Then, with the use of the solid free-form fabrication technique, the BMP-2–PEG complex was prepared and then encapsulated into PLGA–HA. As was shown by in vitro experiments, the excretion of the BMP-2 growth factor into the cellular mass was observed for a month. The experiments were performed also in a culture of osteoblast precursors and in vivo in rats. It was shown that the prepared scaffold can induce the differentiation of osteoblast precursors to osteoblasts and can facilitate tissue regeneration in vivo. Nevertheless, the authors mentioned the preservation of the immunogenicity of BMP-2 in vivo, which may be associated with its excretion outside the regenerated tissue.

In [105], the triblock copolymer PLGA–*block*-PEG–*block*-aspartic acid was prepared and used for the physical or covalent binding of specially synthesized peptide sequence P24, similar in composition to the active center of BMP-2. The authors of [105] reported their successful experiments in a cell culture and in vivo.

Similar approaches for the delivery of growth factors were reported in [106, 107].

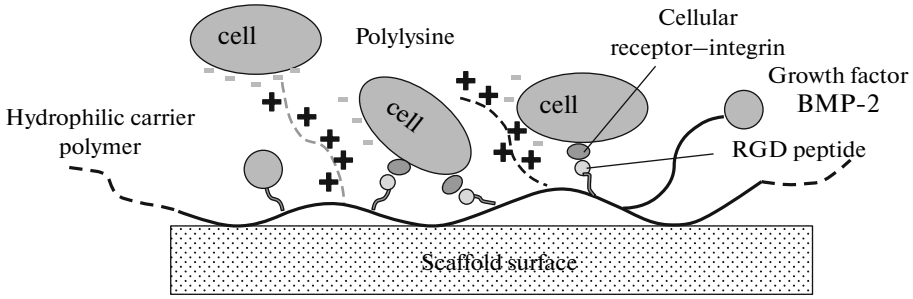


Fig. 1. Strategy of smart biofunctionalization of the scaffold surface.

STRATEGY OF SMART BIOFUNCTIONALIZATION OF THE SURFACE OF HYBRID SCAFFOLDS WITH THE USE OF A HYDROPHILIC POLYMERS AS CARRIERS

Consideration of the above polymeric strategies of biological functionalization of bone-tissue-engineering scaffolds makes it possible to draw several conclusions. First, in all cases except those which are based on the conjugation of peptides with PEG, a loss in the biological activity of the bound biomolecules is possible. During their direct immobilization on the matrix surface, such a loss should occur owing to an increase in steric hindrances to the contact of biologically active molecules with their biological partner located in the cell membrane. In the case of physical binding or encapsulation, a biologically active substance (BAS) will inevitably excrete in the environment and, hence, may be absorbed by the cells via endocytosis. Second, most strategies, including those based on the use of PEG, are directed at introduction of BAS only with a single function: either RGD peptides for adhesion or BMP-2 for growth and differentiation. When PEG is used, the quantity of bound BAS is limited because PEG macromolecules contain only terminal reactive groups. Third, the primary aim of researchers is to minimize nonspecific interactions, although these interactions, together with the specific signals, may lead to a synergetic effect [108].

In order to perform the smart biofunctionalization of the scaffold surface, biologically active substances should be introduced with a minimal loss in activity and the combination of BASes of various functionalities should be provided. In this case, the term *smart* relates above all to the possibility to control the behavior of cells through control of the nature, set, and concentration of different biomolecules, phenomena that should finally lead to formation of a new tissue.

The authors of the present review proposed to draw attention to a great body of experimental and theoretical data on the synthesis and application of hydrophilic polymers as carriers of different biological ligands [109–113].

It is known [114–120] that the interaction of biomaterials with the physiological medium of the living body may be changed with the aid of hydrophilic polymers. For example, the adsorption of PVP on poly(ester sulfone) membranes [119] or silica particles [120] may be used for restriction of protein adsorption and an increase in hemocompatibility. The developed carrier polymers are as a rule nontoxic and, if their molecular masses do not exceed 30000, may be easily removed from the body. Most hydrophilic carrier polymers are obtained via the method of free-radical polymerization. Thus, monomers that form the basis of a hydrophilic polymer may be involved in copolymerization with monomers carrying reactive groups. Hence, with specially prepared copolymers with a controllable number of reactive sites, it is possible to control the quantity of attached biomolecules. Moreover, in some, the controlled quantity of reactive groups may be introduced through polymer-analogous transformations.

Thus, it was proposed to introduce BASes into the scaffold with the use of hydrophilic (co)polymers containing a certain quantity of reactive groups capable of binding signal bioligands [121]. Similar polymers modified with bioreactive molecules may play the role of biofunctional intermediates (*biofunctional vector*) between the scaffold material and cells (Fig. 1).

Synthesis of a Hydrophilic Reactive Polymer

In our first studies [121], the well known copolymer of *N*-vinylpyrrolidone (VP) and acrolein diethyl acetal was selected as a carrier polymer. This copolymer was previously used to design prolonged-action drugs [122]. Moreover, new polymers under the common name poly(vinyl saccharides), which have been intensely studied during the last two to three decades [123–126], have become the focus of our attention. This type of hydrophilic polymer has the carbochain nature but, at the same time, contains saccharide side

fragments. The synthesis of vinyl saccharides was described in detail in [127].

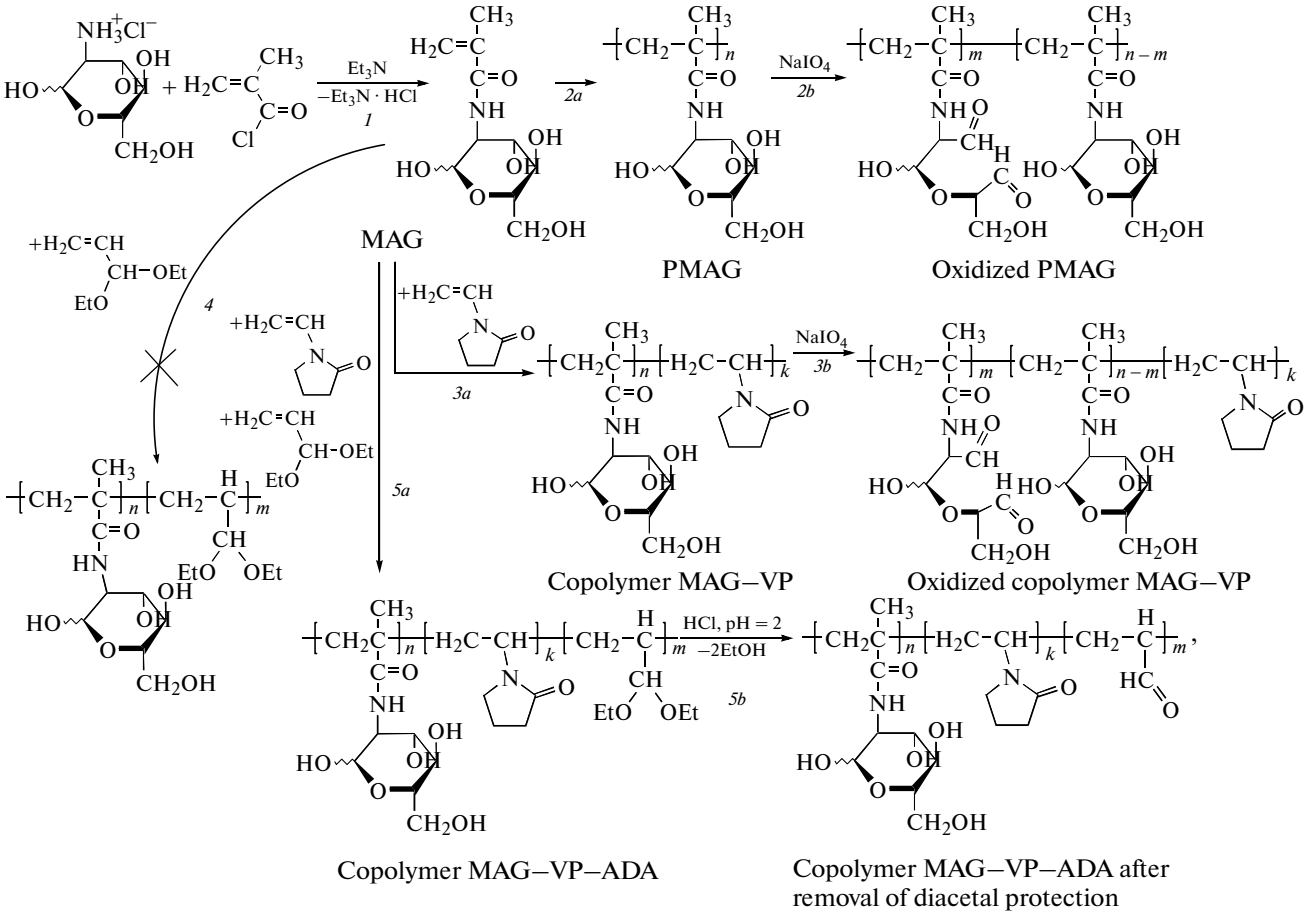
The use of poly(vinyl saccharides) as a basis for the creation of biofunctional supporting media for bone-tissue growth seems extremely promising because the presence of sugar fragments in the structure of these polymers makes it possible to expect that these media will possess an affinity for cell membranes. Moreover, it is possible to use vinyl derivatives of sugars featuring different biological activities, for example, revealing nonspecific or specific affinity for cells of a certain type.

In [128, 129], 2-deoxy-*N*-methacryloylamido-*D*-glucose (MAG) was employed as a basis for the synthesis of a carrier polymer. The selection of this vinyl saccharide was due to sufficient experience in the field of synthesis of its (co)polymers [128–131] and by the fact that the polymeric products are nontoxic [132]. Moreover, it is important that the accessible and inexpensive starting material, glucosamine derived from chitin, is used for the synthesis of monomer MAG. The synthesis of MAG was performed as described in [130], namely, via the reaction of glucosamine with methacryloyl chloride.

Along with the main monomer for creation of a hydrophilic carrier polymer, it is necessary to select reactive groups that are the most useful for the covalent binding of BASes. Among a wide set of chemical groups and methods suitable for providing conjugation, particular attention has been given to the reductive-amination reactions proceeding between aldehyde groups of the carrier polymer and amino groups of BASes [122, 133]. These reactions may be conducted rapidly (for 1–2 h) under mild conditions, namely, at room temperature in aqueous buffer solutions [121].

The synthesis of aldehyde-containing carrier polymers based on MAG was described in [134–136]. The main parameters controlled at the stage of polymer synthesis are the quantity of introduced aldehyde groups and molecular mass, which should not exceed values (usually within (10–30) × 10^3) during the unhampered removal of the polymer from the body. In the case, when the polymer enters the blood flow, it is removed via filtration through the kidneys.

Aldehyde-containing MAG-based polymers are prepared trough two techniques: polymer-analogous transformations in homopolymer (PMAG) chains and the copolymerization of MAG with an aldehyde-containing comonomer, for example, acrolein diethyl acetal (ADA).



Here, *1* is the preparation of MAG, *2a* is the homopolymerization of MAG, *2b* is the periodate oxidation of PMAG, *3a* is the copolymerization of MAG with VP, *3b* is the periodate oxidation of the copolymer of MAG with VP, *4* is the copolymerization of MAG with ADA, *5a* is the copolymerization of MAG with VP and ADA, and *5b* is the removal of diacetal protection.

With consideration for the fact that the hydrocarbon fragment of the MAG unit contains an α-diol group, the specific oxidation of *vic*-glycols with sodium metaperiodate is of interest as the first way of preparing the discussed polymers. The use of a relatively low monomer concentration and a sufficiently high initiator concentration makes it possible to synthesize PMAG with a molecular mass of 21 000, which is necessary for further experiments. As the $NaIO_4$-to-MAG molar ratio was varied from 0.3 to 2, poly(vinyl saccharides) containing from 10 to 55 mol % aldehyde groups were synthesized [135].

In order to implement the second way of preparing the reactive carrier polymer based on MAG, the copolymerization of this monomer with ADA was performed [134, 135]. It is noteworthy that ADA is of interest for the preparation of aldehyde-containing carrier polymers because the aldehyde group of this monomer is in the form of acetal. Such a structure ensured the protection of aldehyde groups in copolymerization and excluded the feasibility of polymerization through C=O bonds as well as their protection from oxidation by air oxygen during polymer storage. The copolymers containing ADA may be readily activated, that is, converted into aldehyde-containing derivatives through treatment with a hydrochloric acid solution (pH 2) immediately before the procedure of bioligand binding. However, attempts to prepare such copolymers via the direct copolymerization of MAG with ADA turned out to be unsuccessful. As far as MAG is a methacrylamide-type monomer and is very active in free-radical polymerization, whereas ADA features low activity in these reactions and is capable of degradative chain transfer, it is reasonable to suggest that the copolymerization of MAG with ADA should lead only to the homopolymer of MAG containing possibly only trace quantities of ADA units. At the same time, it is well known that VP readily enters into the copolymerization with ADA [122]. In addition, MAG–VP copolymers of various compositions are described in [124]. Thus, the ternary copolymerization of MAG with VP and ADA is of interest here. The experimental details of this process are reported in [135]. It turned out that variation in the quantity of VP introduced into the reaction makes it possible to vary the quantity of ADA involved in copolymerization and thus to control the quantity of introduced reactive aldehyde groups. The resulting polymers had molecular masses not above 2×10^4, owing to the inhibitory effect of the ADA monomer.

Construction of a Polybiofunctional Polymer Vector

The strategy of smart biofunctionalization of scaffolds for bone-tissue growth (Fig. 1) involves the immobilization of a hydrophilic polymer on their surfaces that are modified with biomolecules of different natures and functions. Such a macromolecular conjugate was referred to as a *(multi)biofunctional nondegradable polymer vector*. The construction of this vector consists in the covalent modification of the above-described aldehyde-containing MAG-based carrier polymers with special ligands. These ligands are signal biomolecules that are capable of undergoing specific interactions with components of cell membranes and affecting the processes of adhesion, growth, and differentiation of cells.

The controllable modification of a surface with the above-mentioned biomolecules (RGD peptide, the BMP-2 growth factor) is a significant problem of smart biofunctionalization of scaffolds. In [137], oligo- or poly(*L*-lysines) were used to increase the electrostatic adhesion of cells.

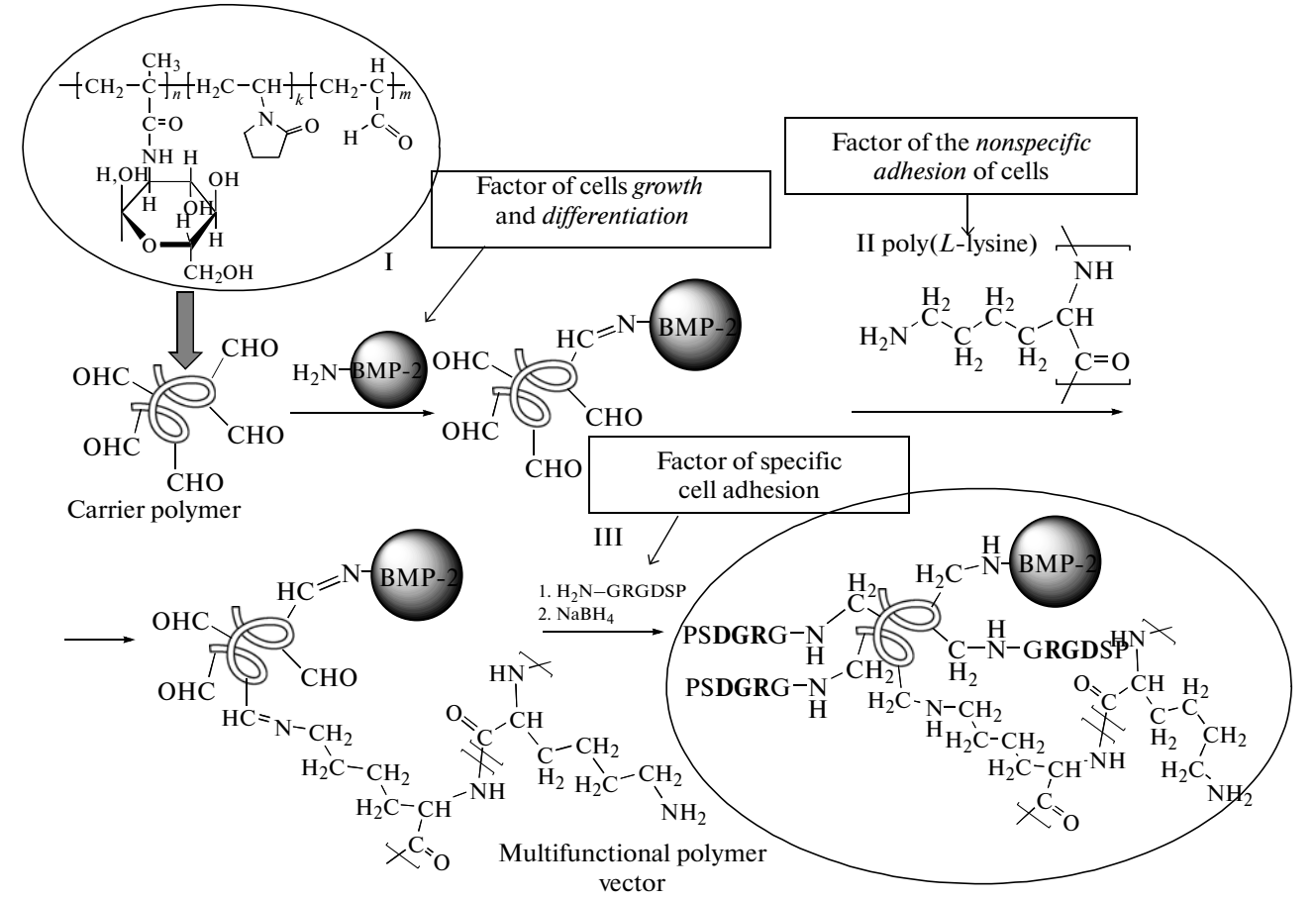
The surface was modified through the method of quantitatively controlled binding of ligands of different natures with the carrier polymer. Biofunctionalization of the hydrophilic polymer was conducted in solution, that is, before its immobilization on the ceramic support. In this case, creation of a multifunctional polymer vector that contained all three types of the indicated molecules and thus could affect the cells in a complex manner was the key purpose of research into modification processes of the synthesized polymers [134, 137].

To study the possibility of controllable introduction of ligands of different natures into the structure of a carrier polymer, the formation of single conjugates by model substances was studied. For example, instead of the extremely expensive BMP-2 growth factor, ribonuclease A, which possesses close physicochemical properties (the molecular mass and size of a molecule and the isoelectric point), was taken. The quantity of bound molecules was determined through a very sensitive fluorescent-labeling method. In this case, it was important to select the tactics of further synthesis of the multifunctional polymer vector, namely, simultaneous or stepwise addition of the mixture of ligands and their covalent attachment to the polymer chain.

The precise regulation of the conjugation process for both high-molecular-mass (ribonuclease and poly(*L*-lysine) and low-molecular-mass (GRGDSP peptide) ligands with the synthesized aldehyde-containing poly(vinyl saccharides) was performed in [137]. It is important that the nature of the molecule attached to the polymer chain had the decisive effect on the final result. The reaction of the polymer with protein is limited by steric factors associated with the macromolecular structure of reacting substances. This circumstance causes the need to use a significant excess of aldehyde groups of the polymer in order to

attain a more complete binding of protein. In the case of the reaction of the polymer with poly(*L*-lysine), this effect is not so pronounced, because of the presence of a large quantity of accessible amino groups in polycation molecules. The reaction of the polymer with low-molecular-mass RGD peptide is limited to a larger extent by the diffusion of peptide molecules through a macromolecular coil toward aldehyde groups. Therefore, the excess of peptide must be taken to increase conversion in this particular case.

On the basis of the above results, a step-by-step strategy was proposed for the creation of a polybiofunctional polymeric vector.



At the first stage, when aldehyde groups are in excess, the binding of protein occurs. Then, after purification of the reaction mixture through dialysis, another macromolecular ligand, polylysine, is involved in the conjugation reaction. At the final stage, the residual aldehyde groups of the polymer are modified with an excess of the RGD peptide.

It was shown that the strict quantitative variation in the polymer-to-bioligand ratio makes it possible to vary the quantity of ligands introduced into the hydrophilic polymer. The unreacted aldehyde groups may be deactivated via treatment with sodium borohydride. In this case, the labile double bonds of the Schiff base formed as a result of interaction of amino groups with aldehyde become stronger owing to transformation into CH–NH bonds. Thus, the concentration of bound biomolecules and the possibility of varying a set of conjugated bioligands are determined by the presence of reactive groups in the polymer, that is, are preset at the stage of carrier-polymer synthesis [137].

Immobilization of a Multifunctional Polymer Vector on the Surface of a Macroporous Scaffold

The technique of immobilization of the obtained macromolecular construction on the surface depends on the type of material. Macroporous matrices for bone-tissue engineering may be organic (polymeric) or inorganic (bioceramic). In the first case, the surface of the material is as a rule sufficiently hydrophobic; therefore, the covalent immobilization is preferable. For macroporous bioceramic structures, which possess hydrophilic surfaces and a surface charge, it is reasonable to use adsorption interaction with the surface.

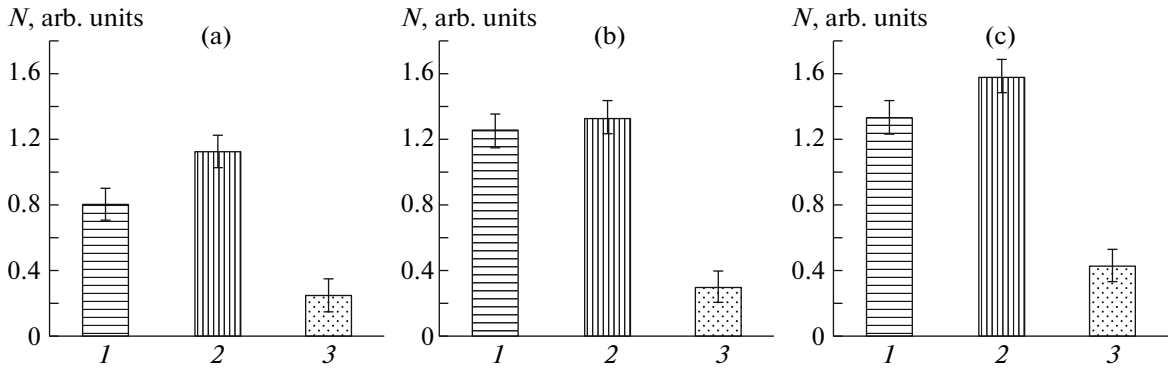


Fig. 2. Fluorescent-analysis data on the adhesion of dyed DAPI cells of the MT3C3-E1 line on the surface of the Sponceram substrate: (*1*) substrate covered by the PMAG conjugate with the GRGDSP peptide; (*2*) substrate covered by the PMAG conjugate with the GRGDSP peptide and polylysine; and (*3*) substrate without covering. Cultivation times are (a) 1, (b) 2, and (c) 24 h; *N* is proportional to the quantity of dyed cells.

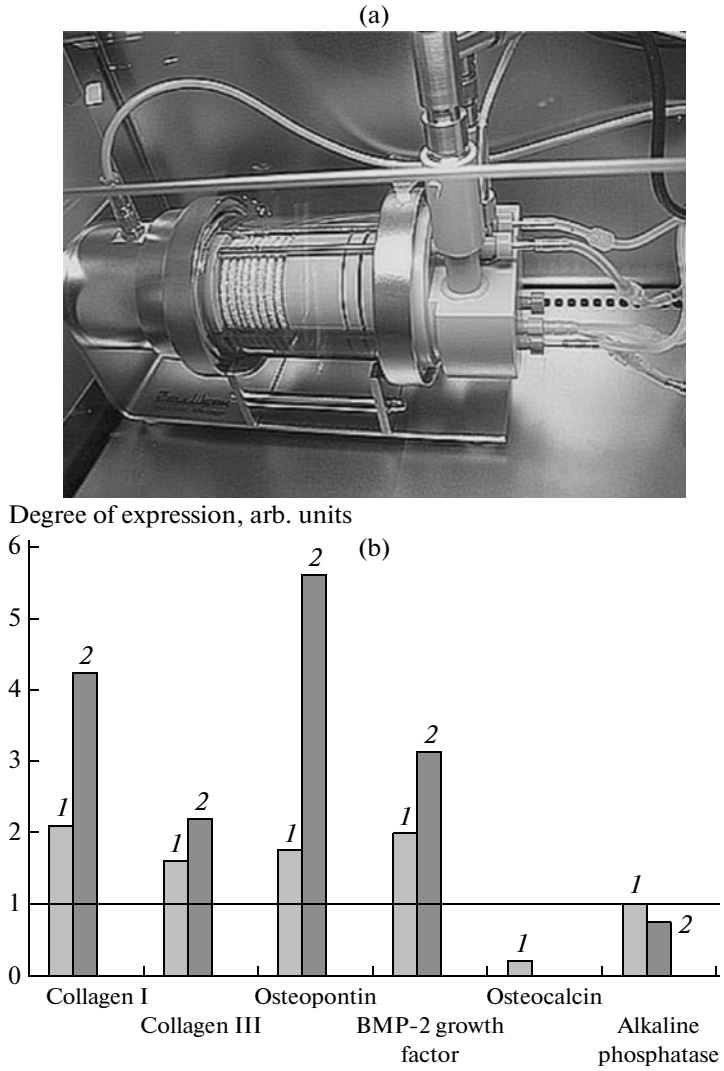


Fig. 3. (a) Flow bioreactor (Zellwerck GmbH) and (b) the data of experiments performed there on the polymerase-chain-reaction analysis of the degree of protein expression indicating the formation of bone tissue: (*1*) Sponceram/BMP-2 and (*2*) Sponceram/polymer BMP-2; the bold line parallel to the abscissa axis relates to the control experiment.

In our studies [134–138], a Sponceram® (Zellwerck GmbH, Germany) commercial bioceramic supraporous material was used as a supporting substrate; in accordance with the proposed strategy, the adsorption layer of the polymer vector was deposited on this substrate. This material was zirconium dioxide doped with calcium hydroxyapatite. The study of adsorption of the initial polymers [135, 136] and the obtained conjugates [137] showed that the process proceeds rapidly and the interaction of the polymer with the ceramic surface is strong. The observed dependences are evidently associated with a strong affinity of polymer chains for the surface. Nevertheless, the introduction of polylysine into the chemical structure of the polymer led to an increase in the saturation of matrix surface with the polymer; this effect may be explained by its partially negative charge.

Studies of the reverse process, specifically, the desorption of adsorbed conjugates in a phosphate salt buffer and in a cell cultivation medium revealed the absence of any noticeable quantities of both initial polymers and their biofunctionalized derivatives in solution after scaffold incubation under physiological conditions for four weeks. The presence of adsorbed layers of polymers and conjugates was confirmed by the method of X-ray photoelectron spectroscopy.

The experiments of the osteoblast precursors in the cell culture [137–139] demonstrated that the obtained hybrid polymer–inorganic scaffolds can intensify the adhesion of cells (Fig. 2). The use of the PMAG conjugate with the GRGDSP peptide and polylysine led to a more pronounced adhesion than that in the case of conjugates with a single ligand. Thus, one may speak about the manifestation of a synergetic effect of the introduced sites of interaction with the cell. Moreover, it was demonstrated that the proliferation of cells on all hybrid matrices proceeds more intensely than that on the initial Sponceram ceramic carrier.

The experiments performed in a laboratory dynamic bioreactor (Fig. 3) showed that, in the case of BMP-2 conjugated with the polymer and adsorbed on Sponceram, a more pronounced differentiation of stem cells into the cells of bone tissue is observed. This effect was registered from a greater level of excretion of special marker proteins that indicate the formation of bone tissue, namely, collagen I and osteopontin, and BMP-2 growth factor. Thus, the results of biological experiments verify the operability of the strategy proposed in our studies.

At present, this line of research is being developed with the use of biodegradable polymers as porous carriers. In this case, the creation of a biodegradable supraporous monolithic matrix designed according to the *molecular lego* principle and containing dispersed micro- or nanoparticles of hydroxyapatite is promising. On the basis of reasoning from the necessity of intermolecular crosslinking of macromolecules for creation of such a framework as well as covering it with a covalently attached multibiofunctional polymer vector, the creation of active sites in the structure of the biodegradable matrix is required. The unsaturated bonds of different natures may serve as such sites.

CONCLUSIONS

The possibilities and specific features of the application of polymers in orthopedic surgery and bone-tissue engineering that have been considered in this review make it possible to state that there has been considerable evolution of this scientific and practical direction that in many respects coincides with the development of polymer science. In the epoch of polyolefins and nondegradable polymers produced through polycondensation, high-molecular-mass compounds were primarily used as engineering materials, namely, as implants that can replace lost bone tissue. After the appearance of tissue engineering, polymers have found wide applications in the design of three-dimensional macroporous biodegradable structures. Later on, it became obvious that the in vitro growth of bone tissue with a complex organization needs fine tuning of polymeric or polymer-inorganic frameworks through the targeted biofunctionalization of their surface. This observation led to the application of polymers as carriers of signal molecules facilitating the adhesion, growth, and differentiation of the seeded cells and their transformation into the corresponding tissue.

At present, the development of the chemistry and physics of macromolecules in this field is directed toward creation of approaches to the smart biological biofunctionalization of the surfaces of scaffolds. Of particular interest is a strategy that allows the controllable introduction of biological molecules of different natures and functionalities into the structure of the surface of the designed supporting medium. In this case, the behavior of cells at the stage of scaffold creation may be controlled through combination of different types of ligands and variation in their quantities. The works of the authors collected in this review demonstrate such a possibility.

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