SCAFFOLD- AND CELL SYSTEM-BASED BONE GRAFTS IN TISSUE ENGINEERING (REVIEW)

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The review considers the current trends in tissue engineering including maxillofacial surgery based on the use of scaffolds, autologous stem cells and bioactive substances. The authors have shown the advantages and disadvantages of basic materials used for scaffold synthesis — three-dimensional porous or fiber matrices serving as a mechanical frame for cells; among such materials there are natural polymers (collagen, cellulose, fibronectin, chitosan, alginate and agarose, fibroin), synthetic polymers (polylactide, polyglycolide, polycaprolactone, polyvinyl alcohol) and bioceramics (hydroxyapatite, tricalcium phosphate and bioactive glasses). There have been demonstrated the matrix techniques, special attention being paid to innovative technologies of rapid prototyping — the process of 3D-imaging according to a digital model. The most applicable of these techniques for biopolymers are laser stereolithography, selective laser sintering, fused deposition modeling, and 3D-printing. Great emphasis has been put on the use of bioactive substances in the process of obtaining scaffold-based bioengineered constructions — setting of stem cells on matrices before their transplantation to the defect area. Special attention has been given to a current trend of cellular biology — the application of multipotent mesenchymal stromal cells (most common marrow cells used in bone tissue regeneration), in particular, the available sources of their isolation and the variants of directed osteogenic differentiation have been presented. The review covers the characteristics and aims of bioactive substance inclusion in scaffold structure — not only to induce osteogenic differentiation, but also to attract new stem cells of a carrier, as well as promote angiogenesis.

Key words: tissue engineering; bone tissue defects; scaffold; multipotent mesenchymal stromal cells; cell therapy.

The treatment of bone tissue defects resulted from mechanical injuries, congenital abnormalities, or surgical interventions continue to be a relevant medical and social problem. Tissue engineering, which ranks among the key interdisciplinary spheres, concerns with injured bone tissue repair and replacement [1]. The first research studies on bone defect corrections can be referred to the 70s of XX c., when the attempts to use autologous bone were made. Currently, this technique is still widely used [2], however, its disadvantages are also well known: the limitation on graft volume, donor site damage, differences in the structure and biomechanics of different skeletal parts [3], infection and immunological graft rejection [4]. The use of synthetic bioinert materials (polymers, calcium phosphates, plastic masses, metals, etc.) of xenogenetic and alloplastic origin partially solve the problem of size and shape providing mechanical strength and other specific requirements for grafts, but their osteoinductive and osteoconductive properties are still concede to autologous or allogenic materials [2]. Today the problem is being dealt with the production of individual grafts with bioactive substances added. The bioactive substances are based on scaffolds from synthetic bioresorbable materials, which are inhabited by patient`s autologous stem cells, due to what they correspond to a damage to a high accuracy in structural and biomechanical characteristics, as well as have no immunologic rejection [5]. Thus, bone grafting combines complex technologies, scaffolds, with autologous stem cells and bioactive substances being their key elements [1].

Scaffolds in tissue engineering

Scaffolds are three-dimensional porous or fiber matrices, their primary function consisting in maintaining a mechanical frame for cells [6]. Ideally, scaffolds should exhibit a number of properties,

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which enable to form complete bone tissue. These characteristics are the following: the presence of adhesive surface promoting cell proliferation and differentiation; biocompatibility and no immunologic rejection; non-toxicity; biodegradation, its rate should correspond to self-tissue growth; optimal pore size for spatial cell distribution, vascularization, as well as the diffusion of nutrients and removal of wastes [7].

Basic materials for scaffold fabrication. Material selection is one of the most important stages of bone grafting. Relying on the fact that scaffolds perform the functions similar to those of extracellular matrix, the underlying factor in selecting the material is its capability to imitate partially extracellular matrix [1]. Generally, we can distinguish three major groups of materials used in scaffold fabrication: natural polymers, synthetic polymers, and ceramics [8, 9].

1. *Natural polymers.* As the term suggests, these materials are derived from natural sources. As a rule, they consist of polymer framework, which can contain up to 99% water. As consequence, these natural polymers are called hydrogels, their water regain capacity enables to simulate highly hydrated condition of living tissues [10, 11]. Another reason, wherefore natural polymers are of value in tissue engineering is their structure very similar to extracellular matrix structure that contributes to improving osteoinductive and osteoconductive properties of matrices [9]. A group of natural polymers includes polypeptides, polysaccharides, polyesters, and their combinations [12]. Let us consider the most common of them in detail.

Collagen is one of the most common natural materials, it serving the base for scaffold fabrication. It is fibrillar protein forming the base for connective tissue of the body and providing its strength and elasticity. Along with hydroxyapatite, collagen is one of two basic bone components [13]. Since collagen is "native" for mammalians it has become widely used both in biomedical and commercial technologies [14–20]. Such advantages as biocompatibility, adhesiveness, fibrillar structure, and high compatibility with other materials make it possible to use collagen for scaffold fabrication in tissue engineering [21–23]. The most frequently used is type I collagen [21, 24]. Both collagens can be applied as osteoplastic material: native collagen and also denaturated collagen, in the form of gelatin. Moreover, by processing native collagen, a variety of its forms can be derived for further scaffold fabrication: from porous sponges to fiber arrays [8, 21].

However, this material has essential drawbacks. Due to its protein nature, collagen is a biodegradable material, and under catabolic processes including the exposure to specific collagenases and phagocytosis it has very high rate of biodegradation. This disadvantage is avoided by including cross-links between polypeptide chains [25]. The second problem is that collagen triple helix has short non-helical end regions, telopeptides, which exhibit immunogenic properties [25–27]. Enzymatic protease extraction enables to get rid of unwanted domains leaving collagen triple helix unaffected. The third drawback includes poor mechanical characteristics, which are important to provide frame function of scaffolds. Now there are attempts to tackle the problem by chemical modification of the material [21, 25].

Cellulose is the most common natural polysaccharide, a basic component of cell walls of higher plants. It is a repetitive residue of β-glucose [28, 29]. Like collagen, cellulose, as a tissue engineering material, has shown high biocompatibility, cell adhesion capability, and high hydrophilic property [11, 29, 30]. In addition, this material has high tensile strength threshold, and easily mechanically processed [31].

The chief fault of cellulose is its poor biodegradation due to the lack of specific hydrolytic enzymes in human for cellulose cleavage and high density of nanofibrils that restricts occupation of scaffold by requisite cells [11, 30, 32]. To eliminate these drawbacks cellulose derivatives are used as engineering material, they are carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose and acetyl cellulose [33, 34].

Fibronectin is one of key proteins of intercellular matrix, structural glycoprotein synthesized and extracted in intercellular space by many types of cells. It consists of two identical polypeptide chains connected by disulfide bridges at C-termini. Fibronectin is capable to bind collagen, proteoglycans, hyaluronic acid, carbohydrates of plasma membranes, heparin, transglutaminase, therefore, it can perform an integrating function when organizing intercellular substance, as well as enhance cell adhesion due to its ability to bind to transmembrane integrines [35, 36]. Controlled biodegradation rate, non-toxicity and inflammatory reactions permit the use of fibronectin in tissue engineering [12].

Chitosan is a natural derivative of linear polysaccharide, its macromolecules consisting of randomly bind β-(1-4) D-glucosamine links and N-acetyl-D-glucosamine. This material is usually extracted from chitin occurring as part of crustacean shell, insect cuticle and fungus cell wall [8, 37].

Due to many advantages chitosan is widely used in tissue engineering. In contrast to synthetic materials, this natural polymer is dissolved at pH<5.5 and, therefore, does not require particular processing conditions [8, 11]. The presence of side cationic groups to be attached to other molecules enables to combine chitosan with various bioactive substances. In addition, this polymer shows high biocompatibility, the lack of immunologic rejection; in addition, antimicrobial properties are of great importance in relation to some bacteria and fungi [38–40]. The mechanism determining this ability is not fully understood. There are hypotheses that cationic groups of chitosan can bind with anionic groups of a bacterial cell wall causing substance transport failure

and suppressing biosynthesis resulting in bacteria death [41].

The main disadvantage of this natural polymer is low mechanical strength, though it can be dealt with by combining chitosan with other materials [8].

Alginate and agarose are linear polysaccharides extracted from red and brown algae. Generally, these polysaccharides are used together to improve mechanical and adhesive properties [8, 42]. Alginate is the combination of such monomers as β-D-mannuronic acid and α-L-guluronic acid, and agarose contains β-D-galactopyranose and 3,6-ahydride-α-1-galactopyranose. In the presence of bivalent cations such as Mg^{2+}, Ca^{2+}, Ba^{2+} and Sr^{2+} these polysaccharides are exposed to ionotropic gel formation [43]. Upon the whole, the material exhibits high biocompatibility, pore volume optimal for cell migration and nutrient delivery, as well as chondrogenic differentiation maintenance [8, 42, 44]. However, biodegradation of alginate and agarose is a rather slow and low-controlled process [8].

Fibroin is a fibrillar protein forming the base for threads of cobweb and insect cocoons, in particular, mulberry silk. This protein exhibits some properties, which make it attractive for tissue engineering to regenerate bone tissue [45]. The characteristics include high biocompatibility, high rigidity and strength, biodegradation. It is universal when processed for biomedical application [46–48]. The size of pores and mechanical properties of fibroin scaffolds can be controlled by changing fibroin concentration and the size of porogene particles. Moreover, this material requires no additional stabilization by chemical cross-links. The disadvantage of fibroin matrices is low biodegradation rate, which is not always adequate to replace a defect by newly formed bone tissue, but the problem is solved through chemical modification [46, 48].

2. *Synthetic polymers.* The analysis of natural polymers shows that the disadvantage common for them is low mechanical strength. Considering the fact that a framework function is the primary function of scaffolds, this drawback presents the chief problem in bone tissue regeneration [6]. A synthetic polymer is the alternative to natural materials. The necessary form, as well as a wide range of physicochemical properties of matrices can be easily obtained due to a great number of synthesis and processing techniques for materials [49].

Synthetic polymers are generally divided into two groups: biodegradable and nonbiodegradable. Biodegradable materials include polylactide, polyglycolide, their copolymers: polylactoglycolide, polycaprolactone, polycyanoacrylate, etc. Nonbiodegradable polymers are the following: polyvinyl alcohol, polyhydroxyethylmethacrylate, poly-N-isopropylacrylamide, etc. [49].

Polylactide is a biodegradable, thermoplastic, aliphatic polyester, which can be synthesized by polycondensation of lactic acid and lactide — lactic acid dimer. A combination of these methods is usually used in production [50]. A lactide has optical activity and can exist in the form of stereoisomers of L-lactide, D-lactide and racemic LD-lactide forms. Therefore, polylactide characteristics depend on the forms it consists of. L- and D-lactides have high crystallinity and similar physicochemical properties, while LD-lactide is a non-crystalline material [51]. L-lactide-based polymer has very low biodegradation rate (complete degradation can take several years). LD-lactide-based polylactide — an amorphous material — by contrast, has very high biodegradation rate, though its mechanical properties are rather low. L-lactide and LD-lactide copolymers provide polylactide with mechanical strength and reasonable degradation rate [52]. One more advantage of polylactide is its high biocompatibility [49].

The disadvantage of the polymer consists in low wetting ability and therefore, irregular cell distribution. Its degradation products are CO_2 and water, it resulting in local acidification. If polylactide enters the body, inflammatory reactions may occur. Moreover, this material shows inadequate compressive strength [49, 53].

Polyglycolide is the simplest linear aliphatic polyester, glycolic acid polymer [54]. In contrast to polylactide, polyglycolide has high crystallinity due to the lack of side methyl groups and low degradation rate. For bone tissue regeneration polylactoglycolide — a copolymer of polylactide and polyglycolide — is generally used. This copolymer is a rather attractive material for tissue engineering due to its high biocompatibility, and the possibility to modulate biodegradation rate. In addition, under the influence of the cells added, polylactoglycolide biodegradates into monomers — natural metabolites, such as lactic and glycolic acids; though it can cause adverse effects due to unwanted acidification [45].

Generally, the use of different combinations of polylactide stereoisomers, copolymerization of polylactide and polyglycolide aim at the regulation of scaffold biodegradation rate [45].

Polycaprolactone is biodegradable, semi-crystalline, aliphatic polyester, and caprolactone serves as its monomer [55]. This material exhibits good mechanical properties, biocompatibility, as well as it is easy to process [56]. However, due to its internal hydrophobic structure and the lack of bioactive functional groups, polycaprolactone is not very favorable medium for cell growth. It limits the application of the polymer in tissue engineering [55, 57]. Currently, many techniques are aimed at modification of polycaprolactone surface by adhesive materials [55, 56].

Polyvinyl alcohol is the material belonging to a group of nonbiodegradable synthetic polymers. It is a thermoplastic polymer obtained by hydrolysis of polyvinyl ester [49]. Polyvinyl alcohol is mechanically stable and flexible material dissolving in water at sufficiently high

temperatures — about 70°C [49, 58]. Its advantages also include high hydrophilic property and semi-permeability for oxygen and nutrients [59].

Despite excellent mechanical characteristics, the lack of biodegradation common to all materials of this group is significant limitation for application [49].

3. *Bioceramics.* They include a group of inert and semi-inert materials of ceramic nature. Such ceramic materials as hydroxyapatite, tricalcium phosphate, bioactive glasses are the materials most frequently used in tissue engineering [49]. All materials of this group exhibit high biocompatibility due to their presence in the mineral phase of bone tissue [60].

Hydroxyapatite is the basic mineral constituent of bone tissue [61]. As the material for bone regeneration, this mineral shows high biocompatibility, as well as osteoconductive and osteoinductive properties [49, 62, 63]. Pure hydroxyapatite is not used for scaffold fabrication due to poor mechanical characteristics, lack of porous structure, low biodegradation rate and brittleness [49, 63]. Currently, hydroxyapatite is widely used in tissue engineering as an accessory material to improve osteoconductive and osteoinductive properties of scaffolds.

Tricalcium phosphate is tertiary calcium phosphate, also known as bone ash. This phosphate is a rich source of calcium and phosphorus, which are in the form available for cells [64]. In this regard, in contrast to hydroxyapatite, tricalcium phosphate is a well-biodegradable material [65]. In other respects, the material has the properties similar to hydroxyapatite [49].

Bioactive glass is a group of surface-active glass-ceramic biomaterials, SiO_2, Na_2O, CaO and P_2O_5 being their basic components [66]. After grafting, a number of specific reaction have been shown to occur on the surface of bioactive glasses resulting in the formation of amorphous calcium phosphate or crystalline hydroxyapatite that is favorable for osteogenesis [67]. In addition, this material is capable of releasing critical concentrations of Si, Ca, P and Na ions, which induce osteogenesis; and by changing the concentration of different components one can adapt scaffold degradation rate [67, 68]. Their low strength and brittleness is a limiting factor of using bioactive glasses. The problem is now solved by optimization of the composition, processing and sintering conditions [67].

4. Combination of materials. It should be noted that one component is rarely used to synthesize scaffolds. Most often, a combination of several materials is used to optimize the parameters and obtain the necessary characteristics; these materials exhibiting different properties [69]. For example, stability of polymers with insufficient mechanical strength can be enhanced by adding rigid polymers. Similarly, more bioactive polymers can be added to the materials with poor osteoconductive and osteoinductive properties [11].

Scaffold techniques. The second important step after selecting the material is to make a decision on using a certain technique to obtain scaffolds with given properties. Now there is a vast number of techniques enabling to obtain matrices of the right shape, size and composition.

1. *Techniques based on the use of solvents and high temperatures.* To produce fiber and porous structures from biodegradable natural and synthetic polymers the following methods are used: electrospinning, phase separation, lyophilization, gas-foaming, leaching [70].

Electrospinning enables to spin nanofibers of the necessary diameter from fluid or hot melt and form scaffolds under electrostatic forces [71]. The mechanical strength degree of such fiber matrices is not high, and the size of pores is limited by fiber diameter [72].

If create certain conditions, a *phase separation* method can help obtaining two fractions: polymer-lean and polymer-rich, which are distributed diffusely relative to each other. After solvent release, and consequently, polymer-lean fraction, the remaining polymer-rich fraction hardens forming fiber and porous structures [73]. The control over internal morphology of scaffolds obtained by this technique is difficult to maintain [74].

Lyophilization is based on sublimation. Polymer solution is exposed to low temperatures followed by solvent removal in a vacuum chamber by subliming or sublimation [75]. Pore size can be regulated by changing freezing rate of polymer solution and pH, but the main advantage of the technique lies in the fact that it does not require high temperatures and a separate leaching stage. Long processing time and relatively small size of pores restricts lyophilization application [75, 76].

By *gas-foaming* porous scaffolds are fabricated without using organic solvents and high temperatures. The method consists in the following: a polymer is aerated in a high-pressure chamber followed by foaming under decreased pressure. In this manner the structure is formed, its porosity being controlled by pressure release rate and the amount of dissolved gas [77]. The advantages of the technique are poor mechanical properties of scaffolds and insufficient interconnectivity of pores [78].

By means of *leaching* polymer solution is obtained, which is mixed with water-soluble salts (sodium chloride, sodium citrate, etc.). Then the mixture is poured into a container of proper shape, the solvent is removed by evaporation or lyophilization. Salt particles are washed off forming porous structure. The technique is easy-to-use, and in addition, the pore size of scaffolds is easy-to-control. However, the pore shape is limited by cubic shape of salt crystals, and the difficulty of solvent removal from scaffold internal pores limits its thickness [79, 80].

Generally, all techniques based on the use of solvents and high temperatures are characterized by several common disadvantages. Firstly, the formation of scaffold inner structure is not a well-controlled process, and it is impossible to obtain matrices of exactly specified

morphology. Secondly, many technologies require organic solvents, such as chlorophorm or methylene chloride [81]. The removal of these substances and their traces from polymer structures requires costly and not always effective procedures, which, furthermore, are conducted at high temperatures resulting in the loss of physicochemical and mechanical properties of matrices. Moreover, the use of high temperatures limits the addition of bioactive substances.

Currently, there is a group of highly relevant techniques aimed at the solution of the problem — rapid prototyping [82].

2. Rapid prototyping. The technique is the process of three-dimensional object formation of almost any form according to a digital model. Rapid prototyping can be performed in a variety of ways using various materials, but any of the techniques is based on layer-by-layer solid object synthesis [82]. Currently, there is a great deal of prototyping technologies, laser stereolithography, selective laser sintering, fused deposition modeling and 3D-printing [83–85].

Laser stereolithography is based on photopolimerization. This technique uses liquid photopolymer to produce matrices. Liquid photopolymer is able to harden by laser radiation. Scaffolds undergo layer-by-layer formation, the first layer of the radiation-exposed photopolymer is attached to a moving platform, which after each polymerization cycle moves one-layer up for further processing [85, 86]. The biopolymer suitable for laser stereolithography can be both modified synthetic polymers (polycaprolactone, polylactide, polypropylene fumarate), and also some natural polymers (alginate, chitosan, hyaluronic acid, fibrin), which, however, require accessory agents added for intermolecular cross-links [86–88].

There is an improved laser stereolithographic technique — two-photon photopolimerization. In this case material polymerization is resulted from nearly simultaneous absorption of two photons when exposed to femtosecond laser radiation [86, 89, 90]. Two-photon absorption is realized in very low 3D-volume around a focus making it possible to achieve high space resolution of an object formed [91].

Selective laser sintering like the previous method enables to form 3D-objects with precisely specified structure according to a digital model. For scaffolds fabrication this technique requires a powdery thermoplastic material fusible under infrared laser radiation. The material is placed on a platform, and a laser beam forms a lower layer of the specified object. Subsequently, the platform is moved one-layer down, and the cycle is repeated [92, 93]. Spatial resolution of an object depends on laser beam diameter. Polyamide, polystyrene and polypropylene are the basic thermoplastic materials for selective laser sintering. Due to a small number of appropriate polymers to fabricate scaffolds, the modifications of the technique are being developing [92].

Surface selective laser sintering is a new approach to form objects, the method lacking temperature effect directly to a polymer that enables to use a great number of materials including bioactive additives [94]. The critical distinction of this approach is in the fact that small amount of a sensitizing substance (for example, carbon) is applied on the surface of polymer particles. The sensitizer is capable of intensive absorption of laser radiation of the near infrared region. Laser has its direct effect only on sensitizer particles, while polymer and included bioactive substances remain unchanged [94, 95].

Fused deposition modeling consists in object formation by layer-by-layer arrangement of a fused thread from the material used. For this purpose a polymer is put in a discharge head and heated up to necessary condition. Then the discharge head to high precision places the material on a platform, which afterwards moves one-layer down. A fused thread application process is repeated [96, 97]. Practically, any thermoplastic polymers are appropriate for this technique. Some studies demonstrate the opportunity to obtain the samples from such materials as polycaprolactone, polypropylene, and their combination with hydroxyapatite and tricalcium phosphate [98–100]. However, fused deposition modeling still faces an open problem of a destructive effect of high temperatures on bioactive substances.

3D-printing. Now the terms 3D-printing and rapid prototyping are interchangeable, though initially 3D-printing meant one specific technology to obtain three-dimensional objects. The technique consists in elective application of binding liquid by a printing head on a powder layer of a proper material. Further, a new layer is applied on the bonded area of a 3D-object, and the process is repeated [82, 97]. In general, by means of 3D-printing a great number of various materials can be processed (synthetic polymers, ceramics, composites, etc.). The key point of this approach is the selection of an adhesive agent. Both organic (chloroform), and nonorganic (aluminium nitrate, silver) substances can serve as an adhesive agent [82, 101]. Objects fabricated by 3D-printing generally have significant porosity, and require post-processing that can be a drawback in some cases [101].

Cell systems and bioactive substances in tissue engineering of bone grafts

Cell approach in tissue engineering consists in preliminary stem cell put on scaffolds before matrices are transplanted in defect area. Such bone grafts have been shown to have better integration with host tissues, and there is no immune response due to the fact that cells are autologous [1, 3].

Available sources of adult stem cells. Multipotent mesenchymal stromal cells (MMSC) of bone marrow are the most common cells used in bone tissue

regeneration [3, 102]. As the term suggests, the source of this type of cells is bone marrow. One of basic functions of mesenchymal cells of bone marrow is the formation of stromal and hematopoiesis-induced microenvironment. General characteristic of marrow MMSC is fibroblast-like morphology, high proliferative capacity, adhesive capacity, easy-to-induce differentiation in osteogenic, chondrogenic and adipogenic directions [102]. There have also been shown the opportunity to obtain cardiomyocyte- and neuron-like cells [103, 104]. One more essential characteristics of marrow MMSC is their capability to secrete a wide range of bioactive substances enhancing tissue regeneration [102].

Adipose multipotent mesenchymal stromal cells can be an alternative to marrow MMSC. Adipose tissue is composed of adipocytes and a heterogeneous cell population — stromal vascular fraction, which surrounds and supports adipocytes. This fraction, in its turn, contains pre-adipocytes, endothelial cells of vessels and their progenitors, T- and B-lymphocytes, mast cells, macrophages, and the main thing — mesenchymal stromal cells [105]. Adipose and marrow MMSC have similar immunophenotype, morphology, as well as target differentiation capability [3].

There is one more MMSC source, which is less studied — tooth pulp [3, 106]. These cells are shown to be capable of osteogenic differentiation and bone tissue regeneration, and their proliferative activity is as good as that of marrow MMSC [106]. The material to extract such cells is easily drawn during dental surgeries [3].

Currently, the most frequently used MMSC source is bone marrow. However, the procedure of cell extraction is an invasive and painful procedure leading to a new defect formed in a donor site. By contrast, adipose tissue can be obtained less invasively — by lipectomy or liposuction.

Both cell types have been found to express classical MMSC markers (CD73, CD90, CD105, CD166), as well as angiogenic cytokines [107–109]. Moreover, a number of studies suggest that adipose MMSC have higher proliferative capacity and lower ageing coefficient than marrow MMSC, as well as genetic and morphological stability in a long-term culture [105, 110].

However, some studies comparing osteogenetic potential of stromal cells from different sources report controversial results [105, 111–117]. Some researches show marrow MMSC to have higher osteogenic potential than adipose MMSC [105, 113–115]. On the other hand, some authors state that capability to osteogenic differentiation of marrow stromal cells is not significantly higher, and probably, even lower than stromal cells of adipose tissue [105, 111, 112, 116, 117].

In this regard, a question which MMSC type is more appropriate for tissue engineering remains open.

Targeted osteogenic differentiation of MMSC. *In vitro* MMSC differentiation largely depends on their culture conditions. One can achieve the maintenance of targeted osteogenic differentiation by adding different osteogenesis-inducing substances in culture medium. These substances are dexamethasone, ascorbic acid, organic phosphates, in particular: β-glycerophosphate, dihydrohyvitamin D_3, and some proteins belonging to a family of bone morphogenetic proteins (BMP) [118].

Dexamethasone refers to glucocorticosteroid hormones. This hormone is considered to induce osteogenesis due to gene expression stimulation of Osterix (OSX) protein, zinc-containing transcription factor, the lack of which results in sudden retardation or termination of bone matrix formation [119, 120]. Organic phosphates support osteogenesis playing a certain role in mineralization, as well as regulation of osteoblasts due to the induced gene expression of some osteogenic markers, such as osteopontin. Ascorbic acid and dihydroxyvitamin D_3 are likely to have an effect on osteogenesis through alkaline phosphatase activity increase and enhanced osteocalcin synthesis [118, 121]. Another group of substances for targeted osteogenic differentiation includes some proteins of BMP family. In general, BMP are multifunctional cytokines belonging to a superfamily of transforming growth factor (TGF-β) and being of great importance in the regulation of proliferation, differentiation and apoptosis of various cell types. BMP2 and BMP7 are regarded to be of key importance in osteogenesis induction [122].

Inclusion of bioactive substances in scaffold structure. One more object of tissue engineering is the inclusion of bioactive substances in scaffold structure for their sustained release in the process of material bioresorption.

Ideally, bioactive substances should not only induce osteogenic differentiation, but also attract new stem cells of a carrier, and promote angiogenesis. These substances mainly include different growth factors (TGF-β, including BMP, IGF, FGF, PDGF, VEGF, etc.) [123–125]. Generally, systemic injection of growth factors is inefficient, and sometimes even dangerous due to their short life-time (especially, in physiological media), nonselective biodistribution, potential toxicity and carcinogenic activity risk [125]. Thus, the inclusion of bioactive substances in scaffold completes several key tasks: localizied delivery of growth factors with optimal concentration inside a graft, maintenance of biological activity of molecules, controlled release of substances within the necessary period of time [123].

The first variant of growth factor delivery includes their direct incorporation in scaffold structure [123]. For this purpose proteins are usually immobilized through covalent or non-covalent binding with matrix polymer. Non-covalent binding includes physical entrapment, absorption or ion complex formation. However, the use of such systems is limited due to the complicated control over the release of large protein molecules [125].

The second variant of inclusion of bioactive substances in scaffold structure consists in encapsulation of

growth factors in drug delivery systems (microspheres, liposomes, hydrogels, etc.) [125, 126]. As well as scaffolds themselves, delivery systems should have controlled biodegradation rate, nontoxicity, and no effect on matrix structure [126]. In general, the same biodegradable polymers used in matrix synthesis can serve as the materials for microspheres (polylactide, polyglycolide, polyethylene glycol, gelatin, cellulose, etc.) [127–129].

It should be noted that the inclusion of bioactive substances in scaffold material significantly limits the range of polymer processing techniques due to the fact that solvents and high temperatures have a damaging effect on biomolecules.

The combination of cell approach with inclusion of bioactive substances — growth factors suggests that, upon the whole, these techniques aim at populating scaffolds with osteogenically pre-differentiated MMSC, attraction of autologous stem cells, osteogenesis and vascularization of a graft.

Conclusion. Repair of normal bone tissue architecture and functions in case of damage is a challenge in tissue engineering. The success of the operation for defect correction depends on a great number of factors. Experimental studies, as well as profound knowledge of biochemical body reactions play a critical role. Moreover, the findings of complex application of different techniques of scaffold fabrication, the works on cell systems and osteogenesis promotion provide strong evidence that the integration of a large number of interdisciplinary disciplines are relevant for successful completion of these tremendous tasks.

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