CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

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REVIEW ARTICLE

New-generation osteoplastic materials based on biological and synthetic matrices

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Abstract

Objectives. *The purpose of this analytical review is to evaluate the market for osteoplastic materials and surgical implants, as well as study the features of new-generation materials and the results of clinical applications.*

Methods. *This review summarizes the volumes of research articles presented in the electronic database PubMed and eLIBRARY. A total of 129 scientific articles related to biological systems, calcium phosphate, polymer, and biocomposite matrices as carriers of pharmaceutical substances, primary recombinant protein osteoinductors, antibiotics, and biologically active chemical reagents were analyzed and summarized. The search depth was 10 years.*

Results. *Demineralized bone matrix constitutes 26% of all types of osteoplastic matrices used globally in surgical osteology, which includes neurosurgery, traumatology and orthopedics, dentistry, and maxillofacial and pediatric surgery. Among the matrices, polymer and biocomposite matrices are outstanding. Special attention is paid to the possibility of immobilizing osteogenic factors and target pharmaceutical substances on the scaffold material to achieve controlled and prolonged release at the site of surgical implantation. Polymeric and biocomposite materials can retard the release of pharmaceutical substances at the implantation site, promoting a decrease in the toxicity and an improvement in the therapeutic effect. The use of composite scaffolds of different compositions in vivo results in high osteogenesis, promotes the initialization of biomineralization, and enables the tuning of the degradation rate of the material.*

Conclusions. *Osteoplastic materials of various compositions in combination with drugs showed accelerated regeneration and mineralization of bone tissue in vivo, excluding systemic side reactions. Furthermore, although some materials have already been registered as commercial drugs, a plethora of unresolved problems remain. Due to the limited clinical studies of materials for use on humans, there is still an insufficient understanding of the toxicity of materials, time of their resorption, speed of drug delivery, and the possible long-term adverse effects of using implants of different compositions.*

Keywords: *osteosynthesis, osteoplastic materials, regenerative medicine, tissue engineering, osteogenesis, chondrogenesis, recombinant osteoinducers*

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ОБЗОРНАЯ СТАТЬЯ

Остеопластические материалы нового поколения на основе биологических и синтетических матриксов

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Аннотация

Цели. *Цель литературного обзора – анализ остеопластических материалов и хирургических имплантатов нового поколения, изучение особенностей, характеристик и результатов их клинического применения.*
Методы. *Обзор суммирует объем научно-исследовательских материалов, представленных на порталах «PubMed» и «eLIBRARY». Проанализирован и обобщен материал 129 научных статей по следующим разделам: биологические, кальций-фосфатные, полимерные и биокомпозитные матриксы в качестве носителей целевых фармацевтических субстанций (рекомбинантных белковых остеоиндукторов, антибиотиков и биологически активных химических реагентов). Глубина поиска 10 лет.*
Результаты. *Среди всех видов остеопластических матриксов, применяемых в настоящее время в мировой хирургической остеологии, куда входит нейрохирургия, травматология и ортопедия, стоматология, челюстно-лицевая и детская хирургия, деминерализованный костный матрикс (ДКМ) занимает 26%. Полимерные и биокомпозитные матриксы сегодня представляются наиболее перспективными материалами в сравнении с ДКМ. Особое внимание в разработке новых видов матриксов уделяется возможности фиксации остеогенных факторов и целевых фармацевтических субстанций на материале-носителе с целью их контролируемого и пролонгированного выпуска на участке хирургической имплантации. Полимерные и биокомпозитные материалы способны замедлять время высвобождения фармсубстанций в месте имплантации, способствуя снижению токсичности и пролонгации терапевтического эффекта, являясь перспективной альтернативой аутогенной кости. Использование композитных носителей различного состава in vivo демонстрирует высокие показатели остеогенеза, способствует запуску биоминерализации и позволяет варьировать скорость деградации материала.*

Выводы. *Остеопластические материалы различного состава в сочетании с лекарственными средствами показали ускорение регенерации и минерализации костной ткани in vivo, исключая системные побочные реакции. И, хотя некоторые материалы уже зарегистрированы в качестве коммерческих препаратов, все еще сохраняется ряд нерешенных проблем. Из-за ограниченности клинических исследований материалов на людях остаются открытыми такие вопросы как недостаточное понимание токсичности материалов, времени их резорбции, скорости доставки лекарственного средства и его высвобождения, а также возможные неблагоприятные эффекты от использования имплантатов различного состава.*

Ключевые слова: *остеосинтез, остеопластические материалы, регенеративная медицина, тканевая инженерия, остеогенез, хондрогенез, рекомбинантные остеоиндукторы*

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Globally, ~2.2 million operations related to fractures and post-traumatic bone defects are performed annually, and this number is predicted to increase to 6 million by 2050 [1, 2]. In some cases, such as nonunion fractures of critical sizes or bone augmentation in dental implantology, the ability of the bone to self-regenerate is insufficient, and guided tissue regeneration is required, particularly when bone substitute materials are employed. The optimal osteoplastic material should have the following main biomedical characteristics:

– Biocompatibility: the material must interact with the cellular component of the bone without causing a toxic or immunological response.

– Osteoinduction: the ability of a material to induce the migration and differentiation of the recipient mesenchymal stem cells (MSCs) into osteoblasts and chondrocytes, which are the main cells of bone and cartilage tissue.

– Osteoconduction: the ability of the material to act as a supporting structure for the germination of blood vessels and structures of new tissue.

– Controlled resorption with the formation of nontoxic degradation products.

– Open bimodal porous structure (200–500 μm pores for germination into the material of the bone cells and vessels; micropores < 100 μm for interstitial fluids).

– The possibility of adhesion and chemical fixation of pharmaceutical substances on the structures of the carrier without reducing their activity.

– Preservation of biological characteristics during storage for extended periods.

– Manufacturability of the manufacturing process in commercial production [3–5].

In clinical regenerative medicine, the “gold standard” is the use of autografts. Autogenous bone grafts are osteoinductive, osteoconductive, and completely histocompatible materials [6]. However, autografts are limited to the amount of donor tissue available for transplant. The need for additional surgical intervention to harvest bone tissue, usually from the iliac crest, carries the risk of the patient developing long-term postoperative pain syndrome [4].

The limitations associated with obtaining autogenous grafts can be overcome with allografts obtained from other donors. Today, allografts constitute 25% of the osteoplastic matrices used in surgical osteology [6]. In the United States alone, ~1 million allogeneic matrices are implanted annually [7]. Their main advantages over autogenous implants are the unlimited donor material and the ability to receive grafts of various shapes and sizes [6]. However, the risk of transmission of bacterial and viral infections is the main drawback of this material [8]. Additionally, the limited osteoinductive capacity of allografts is the main cause of recurrence or nonunion of bone tissue, which occurs in 15–20% of cases [6]. Osteoinduction activation of allogeneic bone matrices can be achieved by adding recombinant osteoinductive proteins [9]. However, the fixing of recombinant bone morphogenetic proteins (rhBMPs) on an allogeneic matrix results in uncontrolled excessive bone formation that goes beyond the field of corrected pathology, which is attributed to their uncontrolled release from the matrix framework [10].

Modern technological solutions involve the use of natural and synthetic polymers and calcium phosphates and their derivatives, including in combination with osteoinductive growth factors (Fig. 1). These materials are considered the most promising for use in osteoplasty, since they allow the setting of the required characteristics at the stage of producing the implant [3].

Even though the demand for plastic materials and surgical implants is expected to increase annually, the development of a universal osteoplastic material that could meet all the above requirements remains a major challenge.

In this review, we consider the characteristics of osteoplastic matrices that show potential in surgical osteology use and their clinical use cases.

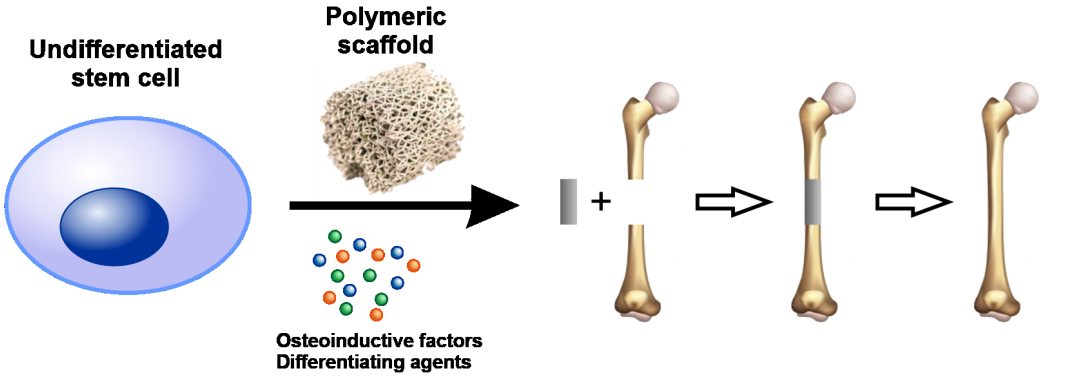

Fig. 1. Tissue engineering approach to bone treatment: undifferentiated stem cells are seeded on a polymer scaffold together with differentiating agents and growth factors, followed by implanting *in vivo*.

OSTEOPLASTIC MATRICES BASED ON BIOCERAMICS

Ceramic materials based on calcium phosphates have pronounced osteoconductive characteristics, which result in increased local interaction with the recipient's bone in corrected pathology; additionally, they are manufactured in block, granule, pasty, and injectable forms [11]. Synthetic calcium phosphates in a biological system, due to the metabolism of body cells, break down into calcium and phosphorus ions, which are further included in the structure of the regenerated bone tissue [12].

Hydroxyapatite

The most well-known calcium phosphate material is hydroxyapatite (HAP). It is the main inorganic component of bone tissue and tooth enamel, well absorbed by the human body, and widely used in orthopedics, traumatology, and dentistry to correct bone tissue defects [11].

The chemical formula of HAP is $Ca_{10}(PO_4)_6(OH)_2$. In the crystal lattice, HAP molecules are distinguished by two structural frameworks. The first, the "apatite channel," is formed by OH^- groups located inside the lattice, which is bound by columns of Ca^{2+} and PO_4^{3-} ions, while the "backbone," which can accommodate F^-, Cl^-, OH^- and CO_3^{2-} ions, can isomorphically substitute PO_4^{3-} groups [12, 13].

The HAP is electrically neutral; it has a stable ionic lattice and is a stable compound. However, depending on the amount of calcium ions in the HAP structure, it can carry both positive and negative charges [13]. Further, chemical instability is a major disadvantage associated with using HAP in osteoplasty. The slow and incomplete resorption of synthetic HAP limit the formation of new bone tissue [14]. The resorption of calcium phosphate materials depends on the Ca/P molar ratio in their composition. The lower the Ca/P ratio, the higher the rate of material resorption [15].

Due to the nonstoichiometric composition of HAP and the possibility of performing anionic or cationic substitutions in the crystal lattice, the value of the Ca/P ratio in the HAP composition can vary from 1.5 to 1.67 [12, 15]. The introduction of substituent ions into the HAP structure induces the distortion and deformation of the crystal lattice, which subsequently leads to an increase in the solubility and bioresorbability of the substituted HAP in comparison with pure HAP [14].

HAP-based materials can be modified by a covalent attachment of collagen to transfer and deliver various therapeutic agents (antibiotics, growth factors), enabling their prolonged release at the injury site [16]. The use of recombinant growth factors of bone tissue, such as bone morphogenetic proteins (BMP) immobilized on osteoplastic carriers, allows for the highly efficient and rapid correction of complex congenital and acquired pathologies of the human musculoskeletal system [10].

Covalent crosslinking using (*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide) (EDC) hydrochloride and *N*-hydroxysuccinimide (NHS) hydrochloride is widely employed to obtain composite materials with increased biocompatibility, a high potential for cell differentiation [17], and increased resistance to enzymatic degradation [18]. This method allows one to obtain "zero-length" amide crosslinks between carboxylic acid groups and amino groups [19].

To modify the surface of HAP with collagen and immobilize the recombinant growth factors on it, the HAP is incubated in a solution of bovine serum albumin (BSA) and collagen, in the presence of a mixture of EDC/NHS reagents. Thereafter, the HAP–collagen

composite material is incubated in a solution with recombinant bone morphogenetic protein 2 (rhBMP-2) [20]. The protein is adsorbed on the surface of the carrier through non-covalent interactions [11, 20]. The reaction scheme for the modification of the HAP surface and the immobilization of rhBMP-2 on it is shown in Fig. 2.

Tricalcium phosphate

Another class of orthophosphate materials that have found use in osteoplasty is tricalcium phosphates. Materials based on tricalcium phosphate are characterized by a higher rate of resorption compared to the materials based on HAP [21]. They can also be used as components of composite materials together with HAP, which enables the control of the material resorption rate [22].

The osteoplastic matrix based on β-tricalcium phosphate (β-TCP) has received considerable attention in scientific clinical studies. β-TCP, with the chemical composition of $Ca_3(PO_4)_2$, unlike other polymorphic modifications of tricalcium phosphates, is stable at temperatures below 1100°C, and it has a lower Ca/P ratio than that of HAP; consequently, it exhibits increased biodegradability and biocompatibility [23].

To obtain an osteoplastic material based on β-TCP, a suspension of crystalline hydrate ($CaHPO_4 \cdot H_2O$) and calcium carbonate ($CaCO_3$) is mixed in the presence of zirconium dioxide (ZrO_2), dried, and subsequently calcined at 750–900°C, at which point HAP is converted into β-TCP. After sintering the preformed β-TCP at 1050°C for 1 h, a β-TCP block with a porosity of 75% is formed [24, 25]. The chemical reactions are described by Eqs. 1 and 2.

$$4CaCO_3 + 6CaHPO_4 \times H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 8H_2O + 4CO_2\uparrow \quad (t = 750–900°C) \quad (1)$$

$$Ca_{10}(PO_4)_6(OH)_2 \rightarrow 2Ca_3(PO_4)_2 + Ca_4P_2O_9 + H_2O \; (t < 1050°C) \quad (2)$$

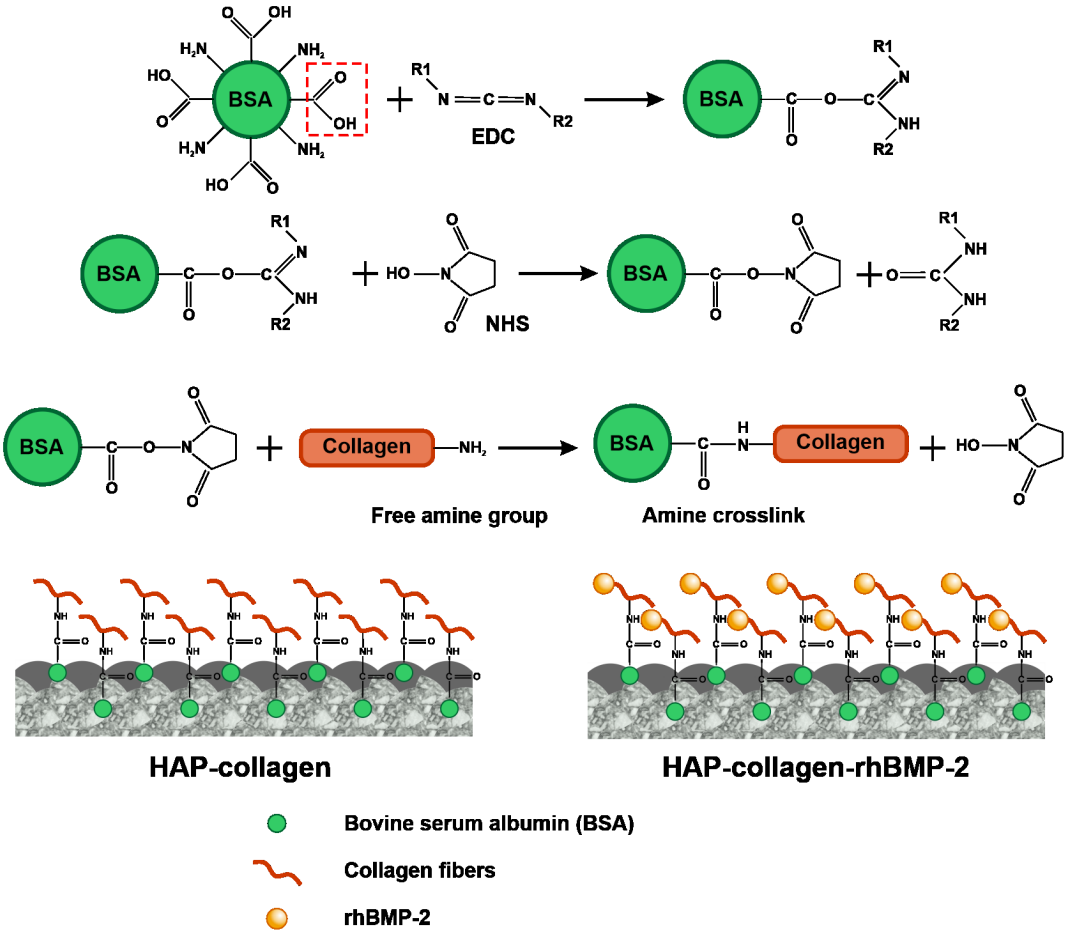

Fig. 2. Illustration of the reaction mechanism of BSA and collagen chemical crosslinking for the subsequent immobilization of the rhBMP-2 osteoinducer on a hydroxyapatite matrix;
EDC: 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride, NHS: *N*-hydroxysuccinimide.

Another common approach to obtain matrices from β-TCP involves calcining chemically synthesized calcium-deficient HAP. At temperatures of 700–800°C, it loses water and transforms into the low-temperature polymorph, β-TCP, used in osteoplasty (Eq. 3). Further heating to a temperature of ~1150°C leads to the transformation of β-TCP to a high-temperature polymorphic α-$Ca_3(PO_4)_2$ material, which is highly soluble in water [26].

$$Ca_9(HPO_4)(PO_4)_5OH \rightarrow 3Ca_3(PO_4)_2 + H_2O\uparrow \ (t = 700–800°C) \quad (3)$$

The β-TCP structure allows one to perform isomorphic substitutions of calcium ions for ions of monovalent and divalent metals or silicate ions. Silicate ions in the β-TCP structure accelerate the differentiation of MSCs on the matrix at the implantation site [27]. Zn, Cu, and Ag metals impart antibacterial properties on the β-TCP based material. Additionally, the β-TCP matrix substituted with Zn ions retards the formation of osteoclasts (cells that destroy bone tissue) on its surface and accelerates the work of osteoblasts, contributing to the formation of the bone matrix [28].

In clinical usage, β-TCP has already demonstrated complete regeneration of bone defects over several years and replacement of the osteoplastic matrix with newly formed tissue. The partial resorption of the β-TCP implant in a clinical setting is observed 2–3 weeks after surgery, and complete degradation occurs from 1.5 to 5 years, depending on the patient's age. It was noted that in cancellous bone defects, β-TCP resorption and bone formation occurs faster than the in the case of cortical bone defects [24].

Notably, materials based on calcium phosphates have low tensile strength, and their Young's modulus is, on average, 10 times higher than that of bone tissue [3]. However, the mechanical characteristics of calcium phosphate materials can be varied during the manufacturing step. As the porosity of the material decreases, the compressive strength increases; thus, β-TCP with 60% porosity has a compressive strength of 22 MPa, which is almost seven times higher than that for β-TCP with 75% porosity. However, the resorption rate for the β-TCP with 60% porosity is lower than that for the β-TCP with 75% porosity [29].

Bioactive glass

Biologically active glasses (BGs) have gained significant interest in the fields of hard- and soft-tissue engineering. This is due to their ability to induce the expression of genes that regulate the processes of osteo- and angiogenesis, thereby enhancing the production of the corresponding growth factors [30].

The first type of these biologically active inorganic materials, known as Bioglass-1 45S5 (BG-1), was discovered by Larry Hench in the late 1960s at the University of Florida. BG-1, with the composition of $45SiO_2$–$24.5CaO$–$24.5Na_2O$–$6P_2O_5$ (wt %), binds to living tissues, forming a stable and densely structured surface; thus, it is effectively used as a filler in bone fractures [31].

The term, "biological activity," in the context of these special glasses indicates the ability of the bioglass surface to direct the crystallization of calcium phosphate salts toward the formation of HAP, thereby facilitating the connection between the artificial material and body tissues [32]. The biosilicate mineralization process occurs in several stages and is shown in Fig. 3. First, the surface of the bioglass turns into a silica gel with an open structure, which exchanges ions with biological body fluids (Stages 1–3, Fig. 3). Subsequently, the calcium and phosphate ions form an amorphous calcium phosphate layer (Stage 4, Fig. 3). Afterward, the Ca–P layer adds hydroxyl and carbonate ions, which facilitate the crystallization of hydroxycarbonate apatite (Stage 5, Fig. 3) [33].

Bioglass is categorized based on three different types of inorganic oxides, including structure-forming (SiO_2, B_2O_3, and P_2O_5), modifying (Na_2O, CaO, MgO, K_2O), and intermediate compounds (Al_2O_3, ZnO, ZrO_2, and TiO_2) [34]. According to the principle of the main structure-forming oxide, bioglasses are divided into glass families based on silicates, borosilicates, borates, and phosphates [35]. Additionally, BGs doped with a small amount of biologically active metal ions have been developed, and they exhibit various therapeutic effects (stimulating osteo- and angiogenesis, anti-inflammatory, and antiseptic) (Table 1) [36]. Mesoporous BGs obtained by sol–gel processes have the porosity (2–50 nm) suitable for the immobilization of various therapeutic agents in nanopores with their subsequent local release in a controlled manner [37]. Alloyed and mesoporous BGs are considered as separate classes of the bioglass family.

In vitro and *in viv*o studies have shown that such therapeutic functions of BGs, including improving the cell growth and proliferation, biomineralization, stimulation of angiogenesis, anti-inflammatory and antibacterial activity, are associated with the release of metal ions and growth factors from the glass structure, after which the bioglass itself undergoes resorption [36].

The use of biocomposite osteoplastic scaffolds based on a BG and a polymer matrix provides additional advantages, such as the launch of biomineralization, which contributes to the formation of a bond between the newly formed tissue and the material; improvement of the initial mechanical properties of the polymer phase; and the ability to fine tune the rate of material resorption [30].

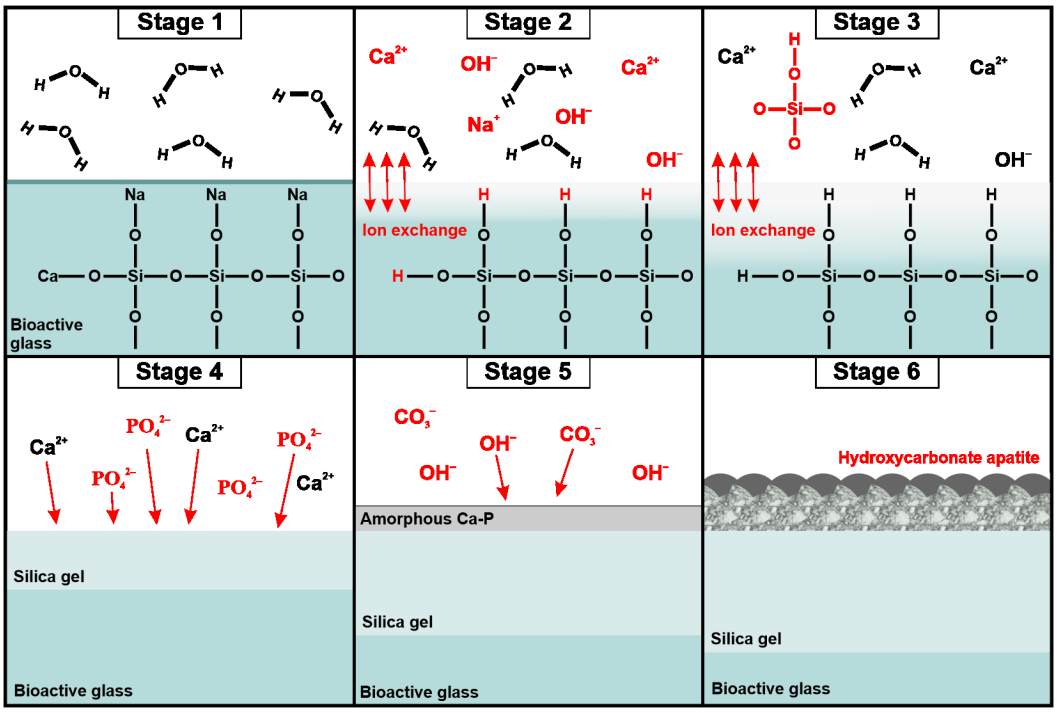

Fig. 3. Formation mechanism of hydroxycarbonate apatite on the surface of bioactive glass.

Table 1. Therapeutic effects of doped bioglass based on various biologically active ions

Therapeutic effect	Metal ions
Angiogenesis	Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, B^{3+}, Si^{4+}, P^{5+}
Antibacterial	Ag^{+}, Cu^{2+}, Zn^{2+}, Ga^{2+}, Mn^{2+}, Fe^{3+}, Ce^{3+}
Osteogenesis	F^{-}, Li^{+}, Sr^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, Ga^{2+}, Si^{4+}, Nb^{5+}
Anti-inflammatory	Li^{+}, Mn^{2+}, Zn^{2+}, B^{3+}

To date, several studies have been published on the use of BG frameworks [38] and composite carriers of the polymer/BG composition [39, 40] in the field of bone tissue engineering. Results of these studies indicate that PLA/BG scaffolds are suitable candidates for achieving optimal bonding between material and tissues, the latter being both soft and hard [41]. Therefore, several studies are actively underway that suggest the use of these systems in areas where the device must simultaneously connect to both soft and hard tissues (for example, middle ear implants or joint implants) [36].

MATRICES BASED ON SYNTHETIC POLYMERS

Synthetic biodegradable polymers appear to be promising materials for use in various tissue-engineered structures, mainly of composite composition [42].

The most used resorbable synthetic polymers for the manufacture of osteoplastic matrices are saturated poly (α-hydroxyesters), including polylactic acid (PLA) and polyglycolic acid (PGA), as well as polylactic acid glycolide copolymer (PLGA) [43].

The chemical composition of these polymers allows for hydrolytic degradation by deesterification. After resorption, the monomeric components of each polymer are excreted from the recipient's body naturally. PGA is converted to metabolites or removed via other mechanisms, and PLA can be purified through the tricarboxylic acid cycle [44].

PGA is a hydrophilic and highly crystalline polymer with a relatively high degradation rate. Although PLA is structurally very similar to PGA, it exhibits different chemical, physical, and mechanical properties due to the presence of a pendant methyl group on the α carbon (Fig. 4) [45].

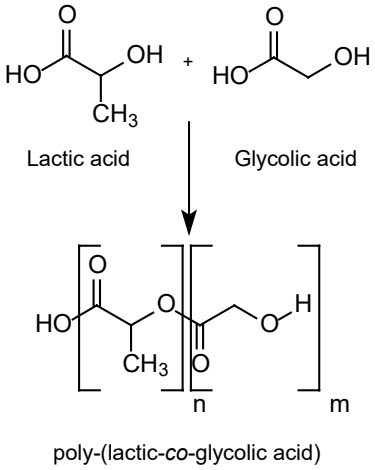

Fig. 4. Chemical structure of PLGA and monomers PLA and PGA.

The PLGA copolymer is preferred over its constituent homopolymers for the manufacture of bone implants, since the physicochemical properties of PLGA allow one to control the rate of decomposition of the material, and PLGA can be obtained in block, fiber, hydrogel, and nanoparticle forms [44].

The rate of resorption of synthetic polymer matrices is influenced by the following factors:

1) The molecular weight of the polymer: degradation rates vary from several weeks to several months.

2) The LA/GA ratio: PLGA copolymers with a high LA content are less hydrophilic; consequently, they absorb a low amount of water and degrade slowly.

3) Stereochemistry: mixtures of D- and L-lactic acid monomers are often used for the preparation of PLGA, since the rate of penetration of water molecules in the D- and L-regions is high, which leads to accelerated degradation.

4) The structure of end groups: polymers with ester residues at the ends have longer half-lives than those with free carboxylic acid [46, 47].

Furthermore, polyethylene glycol (PEG) [48, 49], polyanhydrides [50], poly-ε-caprolactone (PCL) [49, 51], polypropylene fumarate (PPF) [51], and poloxamers [52] are considered synthetic polymer carriers. The advantages of these resorbable polymer carriers are hydrolytic and enzymatic resorption, zero risk of bacterial and viral contamination, and the ability to regulate the mechanical strength by manipulating the polymer structure [53].

Due to their flexible design and controlled degradation rate, biodegradable synthetic polymers in the form of nanoparticles are considered as carriers for the delivery of recombinant protein osteoinducers and pharmaceutical substances. A system for delivery of the growth factor, rhBMP-2, was demonstrated based on the PLA–PEG copolymer; a carrier in the form of a viscous liquid or polymer granules was implanted at the site of surgical correction of bone pathology [54]. According to the results of the study, the PLA–PEG complex was recognized as an effective transport matrix for the prolonged release of the recombinant osteoinducer, rhBMP-2. The efficacy of rhBMP-2 in various animal models was shown when it was immobilized on the matrices of PLA [55], PGA [56], and their copolymer, PLGA [57].

Even though the low pH of the medium created by the products of acid cleavage accelerates the degradation of PLGA due to autocatalysis, this factor is simultaneously a disadvantage of synthetic polymers [58]. This acidification of the medium and the hydrophobic nature of the polymers have a negative effect on the stability of the protein immobilized on the surface of the carrier [59] and increase the risk of inflammatory reactions and delayed clearance [60].

In bone tissue engineering, a combined approach is used, which consists of the synthesis of block copolymers to manipulate the characteristics of the polymer delivery system, e.g., the kinetics of the release of pharmaceutical compounds immobilized on an osteoplastic polymer carrier [61, 62].

Synthetic polymer matrices based on PLA and PGA can be combined in various ratios with calcium phosphate materials (CaPs) to create composite materials with or without chemical modifications of the surface [63]. When CaPs are combined with polymers to form a composite framework, the rate of their resorption is reduced in comparison with that of the pure polymer [64].

Park *et al.* demonstrated the effectiveness of using PCL composites with the addition of β-TCP under mechanical loading conditions, comparable to the modulus of compression of the human trabecular bone. The earliest differentiation of MSCs and high expression of osteogenic markers were noted in PCL/β-TCP composites with a content of 30% β-TCP [65].

Additionally, a high level of osseointegration was demonstrated by the PLA composite containing tricalcium phosphate microspheres with a size of 60–140 μm (PLA/β-TCP). Due to the formation of an ordered porous structure of the composite material, PLA/β-TCP, 16 weeks after implantation into the femur of rabbits, the vascularization of the implant and growth of newly formed tissue into its pores were observed [66].

BIOCOMPOSITE FRAMEWORKS

Composite frameworks with mesoporous silicon

From the viewpoint of clinical efficacy, biocomposite carriers of various pharmaceutical substances created based on nanotechnologies are the most promising materials for tissue engineering [67].

Mesoporous silicon nanoparticles (MSNs) accelerate bone formation by increasing the osteoblast activity and decreasing the bone resorption due to a decrease in the osteoclast activity [68]. MSN-based materials can deliver pharmaceutical molecules of various structures and masses to the injury site due to their pore size and morphology, as well as the possibility of modifying the MSN surface [67]. The variability and flexibility in the design of silicon nanoparticles allow one to choose the dosage of a pharmaceutical substance and control the kinetics of its release in accordance with the functional groups of the molecule that will be adsorbed on the MSN surface [69, 70].

Take the delivery of ibuprofen, which has a –COOH group in its composition, as an example. There is an increase in the adsorption of ibuprofen on the surface of MSN modified with polar molecules as compared to silicon nanoparticles with nonpolar modifications [70]. Consequently, prolonged release of the pharmaceutical substance and a lasting therapeutic effect are observed [70].

The efficacy of doxorubicin delivery using MSNs surface-modified with PEG has been demonstrated in a mouse malignant tumor model [71]. On the 12th day, the animals were withdrawn from the experiment, and the comparable growth rates of tumor volumes were evaluated. The effect of doxorubicin, expressed as the degree of inhibition of the tumor growth rate, was 68.7% for the MSN–PEG loaded particles, compared to 42.5% for pure silicon nanoparticles [71]. This result is due to the improved stability of the doxorubicin molecule on the MSN–PEG surface and the prolonged circulation of the nanoparticles with the pharmaceutical substance in the blood.

In recent studies, significant attention has been paid to composite frameworks based on MSN nanoparticles crosslinked with methacrylate gelatin as part of hydrogel membranes [72]. A recombinant osteoinducer, rhBMP-2, is immobilized on the surface of the mesoporous bioglass through an amide bond. It was shown *in vitro* that the release of rhBMP-2 from the matrix during the first 4 weeks of the experiment significantly stimulated the osteogenic differentiation of cells, and the resorption of the composite carrier to calcium and silicon ions promoted cell adhesion and osteogenic differentiation over a long period [73]. *In vivo* hydrogel membranes based on mesoporous bioglass crosslinked with gelatin demonstrated high rates of bone tissue osteogenesis in a defect in a rat's skull of critical size [72, 73].

Composite frameworks with carbon nanotubes

Biodegradable composite scaffolds based on PLA and PGA polymers in combination with carbon nanotubes (CNTs) are a promising development for a wide range of applications in bone tissue engineering, particularly in cases where the implanted material mainly handles high loads [74]. This combination of composites is particularly effective, since it allows one to achieve self-assembly of CNT fibers and create a network structure in the polymer matrix, and it improves the mechanical strength, thermal stability, and electrical conductivity of the material at low CNT concentrations [75].

Mikael *et al.* presented an efficient method for the preparation of composite frameworks from PLGA microspheres and multi-walled carbon nanotubes (MWCNTs) with various surface modifications [76]. Such scaffolds showed high *in vitro* cell adhesion, cell proliferation, and mineralization, as well as signs of a connection with soft tissues.

A similar approach was tested on composite frameworks with single-walled carbon nanotubes (SWCNTs). It was shown that the PLGA/SWCNT combination led to an even higher gene expression and cell proliferation for the formation of new muscle tissue, compared with that for the composite carrier of PLGA and MWCNTs [77]. It is assumed that such a cellular activity is a consequence of the increased expression of transmembrane cellular receptors, integrins, which may be caused by the topographic features of SWCNTs. This activity is essential for achieving enhanced interaction of the polymer framework with biological components [77].

Another quality of CNTs in composite materials is their ability to change the thermal and electrical properties of PLA [76, 78]. This approach can be used to increase the reactivity of stem cells seeded on the polymer through electrical stimulation, thereby improving tissue regeneration in the long term [79].

A composite material based on a CNT/sodium hyaluronate complex demonstrated a high potential for the restoration of bone tissue defects in rats [80, 81]. This composite induces the expression of genes involved in bone tissue regeneration, such as osteocalcin and BMP-2 [80]. An increase in the expression of type I collagen, as well as the vascular endothelial growth factor, was also observed. When using the CNT–sodium hyaluronate composite in tibial defects, histo-morphometric analysis showed an increase in the number and organization of bone trabeculae, in comparison with the case in the control group [81].

However, carbon nanostructures raise serious concerns when used as components of biomedical devices due to the lack of data on their carcinogenicity and the accumulation of decay products in the human body [78].

Composite frameworks with metal oxides

Composite systems of PLA/metal oxide composition, including zinc oxide (ZnO), magnesium

oxide (MgO), and iron oxides (Fe_2O_3 and Fe_3O_4), have interesting and promising characteristics suitable for application in surgical osteology [82, 83]. Each of these metals has properties suitable for a variety of tissue engineering applications. Compared to clinically used PLGA materials, metal oxide composite structures can reduce inflammation and simultaneously stimulate osteogenesis and osseointegration [84].

The ZnO in the osteoplastic matrix inhibits bacterial attachment and stimulates cell differentiation in the direction of the myocyte phenotype [85]. When the oxide is integrated into the PLLA/ZnO composite system (ZnO in the form of ~40 nm nanorods), the composite slowly releases zinc ions into the environment [86]. Nanorods act as catalytic nuclei, slightly accelerating the polymer degradation. This observation is of key importance as it improves the connection between differentiated myocytes and the implant [85].

MgO is used in composite materials as an alternative to BGs to improve biomineralization and retard PLA degradation [87]. MgO particles incorporated into the polymer matrix buffer the ambient pH, thereby reducing the rate of PLA hydrolysis and weakening the autocatalytic effect of the polymer. The characteristics of the porous PLA/MgO composite framework have been studied in the field of dental bone grafting [88]. The authors reported high compressive and tensile strength, prolonged material resorption time, proliferation of bone marrow MSCs *in vitro*, and bone tissue regeneration *in vivo* in a dog model [89].

Fe_2O_3 and Fe_3O_4 have a unique property that can be used to improve the bond between tissue and biomaterial—supermagnetism [90]. The use of supermagnetic iron oxide particles, particularly in the treatment of cancer and many other drug delivery systems, is a new trend in the field of regenerative medicine [91, 92].

Studies have investigated the incorporation of superparamagnetic iron oxide nanoparticles (γ-Fe_2O_3 and $FeO{\cdot}Fe_2O_3$) into a PLGA matrix, followed by the application of a static magnetic field to the composite structure during cell culture. Magnetic stimulation, similar to nanoparticles obtained separately, promoted the differentiation of osteoblasts [93].

The explanation of this phenomenon consists of two aspects: first, the stimulation by the application of a static magnetic field due to the diamagnetic properties of the cell membrane changes the flow of ions through the membrane; second, iron oxide nanoparticles reduce the intracellular production of H_2O_2, thereby accelerating the progression of the cell cycle. These two stimuli act synergistically, which leads to a significant increase in the proliferation, differentiation, and secretion of MSCs, promoting the formation of a bond between tissue and material [90, 91, 93].

COMPOSITE MATRICES FROM NATURAL POLYMERS

Since the implant used in bone tissue engineering must, to a certain extent, mimic the characteristics of cartilage and bone tissue, natural polymers appear to be an intuitive choice for the initial matrix [94]. Natural polymers can be classified according to their origin (animal, plant, or microbiological) and chemical structure (proteins, polysaccharides, polynucleotides) (Fig. 5) [95].

Porous scaffolds composed of natural polymers stimulate the osteogenic differentiation of MSCs [94]. However, the strength characteristics and resorbability of these matrices under the conditions of the recipient's organism are insufficient, and these matrices are inferior to synthetic resorbable polymer matrices [96].

Chitosan-based matrices

Chitosan is a biodegradable natural polymer obtained by the deacetylation of the natural polymer of chitin [97]. Chitosan has pronounced bactericidal properties, and due to its ability to enhance the absorption of hydrophobic macromolecules, it is used as a carrier to achieve prolonged local release of pharmaceutical substances [98].

Composite systems of the chitosan/PGA, chitosan/HAP, and chitosan/gelatin compositions can serve as effective osteoplastic carriers [99, 100]. In *in vitro* experiments, biological membranes based on chitosan nanofibrils with the addition of rhBMP-2 demonstrated a high biological activity expressed in the osteogenic differentiation of MSCs, high alkaline phosphatase activity, and calcification for 4 weeks with 50% preservation of the immobilized rhBMP-2 on the membrane [101].

Due to their mucoadhesive cationic nature, chitosan nanoparticles (NPCS) are used to reduce the toxic effect and increase the activity of drugs, since they allow the therapeutic agent to be delivered to the immediate vicinity of the injury site [102]. NPCS are usually modified to increase their effectiveness. For example, 2*N*-,6*O*-sulfated chitosan (2,6SCS) forms a polysaccharide similar in structure to heparin, which can successfully bind to the rhBMP-2 domain region (Fig. 6A). Modified NPCS retard the release of the growth factor and increase its biological activity [103, 104].

Gelatin-based matrices

Gelatin is a hydrolyzed form of collagen obtained by heat treatment. The use of gelatin as the only material in the composition of a carrier for pharmaceutical substances is complicated because it tends to undergo rapid biodegradation in the recipient's body [105]. The prolongation of the biodegradation time is achieved by chemical

"crosslinking" of collagen fibers with glutaraldehyde; however, a cytotoxic effect is noted, indicated by the retardation of the osteogenic differentiation of MSCs in *in vitro* studies [106]. A decrease in toxicity can be achieved after 4 days of washing the crosslinked matrix from glutaraldehyde [107].

A biocomposite material based on gelatin and β-TCP demonstrated improved biodegradability under the influence of collagenase with a large amount of gelatin and high osteoinduction, expressed as an increase in the level of alkaline phosphatase activity *in vitro* [108].

The photochemical process involving tris-(2,2'-bipyridine) chloride of ruthenium(II) $[Ru(bpy)_3]Cl_2$ and persulfate ion allows the covalent crosslinking of tyrosine-rich proteins (rubber, gelatin, and fibrinogen) because of the formation of dityrosine bonds and to obtain biopolymer materials with variable biomechanical and tissue-adhesive properties pre-set at the stage of material creation [109, 110]. The tendency of tyrosine-rich proteins to self-organize polymer fibers and interact with extracellular matrix proteins enables the application of the biopolymers crosslinked via this route as surgical sealants or drug delivery systems [111, 112].

The thus obtained photopolymerizable gelatin hydrogel (PH) possesses the porosity required to load it with modified NPCS [103, 113]. The direct introduction of growth factors into the PH does not have a significant effect, since the hydrogel swells and decomposes rapidly, and the complete release of rhBMP-2 is observed after 7 days (Fig. 6B) [103]. However, the composite PH system including 2,6SCS nanoparticles (PH/rhBMP-2/NPs) shows the best results for the stepwise release of therapeutic agents. The first intense rhBMP-2 release is recorded within the first 2 weeks after implantation, and it is associated with the swelling of the hydrogel. Thereafter, there is a gradual release over 42 days, due to the slow degradation of the PH (Fig. 6C) [103].

Collagen osteoplastic matrices

Collagen is the most abundant protein in the human body and a non-mineral biological component of the skeleton. It can be easily isolated and enzymatically purified from various types of xenogeneic matrices for use as a supporting scaffold for cell proliferation in bone tissue engineering [114, 115].

Collagen osteoplastic scaffolds are manufactured in the form of powder, membrane films, aqueous forms, gels, nanofibers, and absorbent sponges [116].

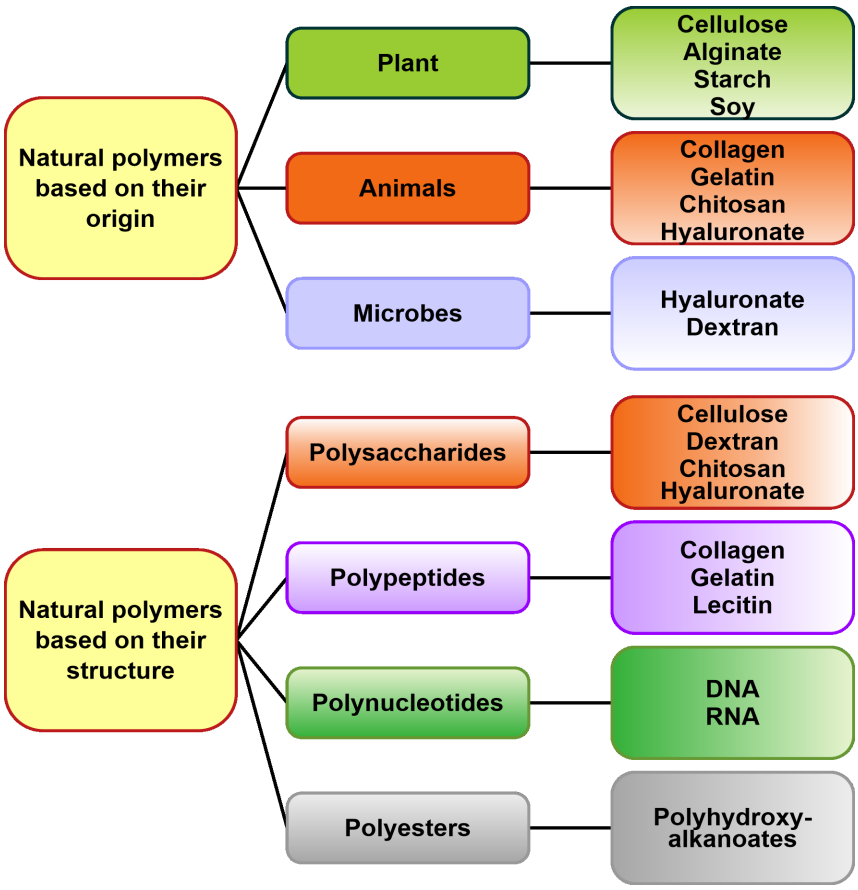

Fig. 5. Classification of natural polymers based on their origin and chemical structure.

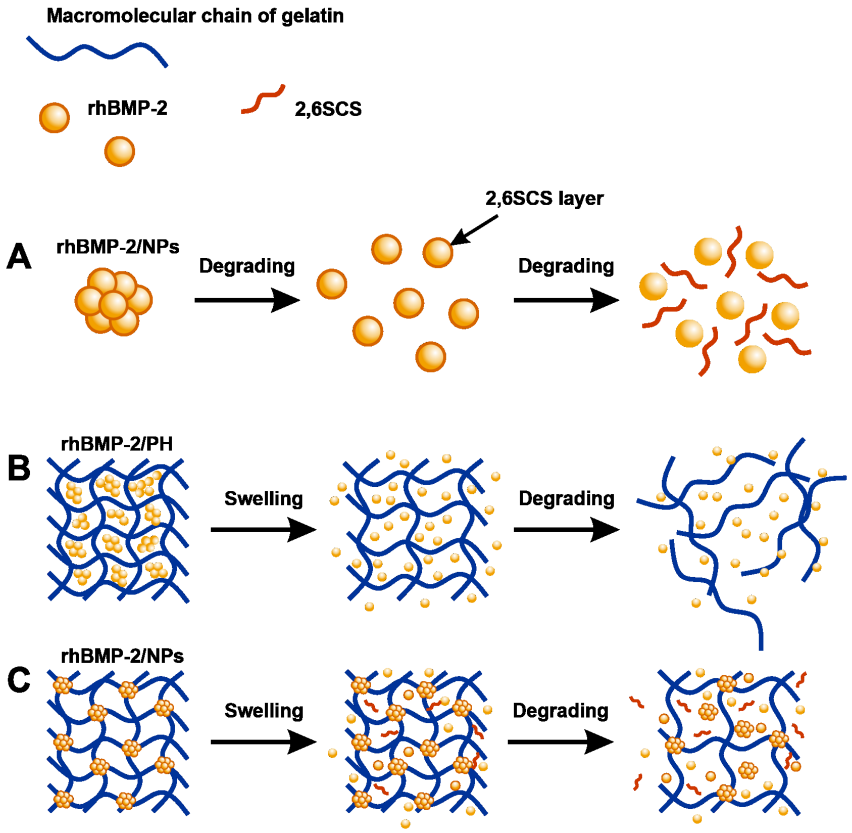

Fig. 6. Illustration of the mechanisms of rhBMP-2 release from (A) sulfated chitosan nanoparticles (NPCS), (B) photopolymerizable gelatin hydrogel, and (C) a complex of a hydrogel with NPCS.

The versatility, hygroscopicity, and ease of use of collagen sponges have led to their widespread clinical use for the localization and delivery of targeted pharmaceutical substances [117, 118]. Since 2002, the United States Food and Drug Administration has approved the commercial preparation of INFUSE with recombinant rhBMP-2 on an ACS collagen plate at a concentration of 1.5 mg/mL [119].

In surgical osteology, INFUSE is used as an alternative to the autologous iliac crest for the single-level fusion of the vertebral bodies in the lumbar spine and to accelerate the fusion of open tibial fractures with intramedullary fixation [119]. Additionally, INFUSE is widely used as an alternative to autologous bone implants for the limited enlargement of the alveolar sinus and treatment of defects associated with bone loss in dentistry [120, 121].

Despite its high biocompatibility, collagen has several disadvantages. It is mechanically unstable, and therefore, upon implantation into an environment, where the sponge is compressed by the surrounding muscles and tissues, there is a local excess release of osteoinductive proteins immobilized on the carrier [114]. Collagen resorption is unpredictable and difficult to control, which also leads to undefined kinetics of recombinant growth factor release. *In vivo*, it was shown that after 2 weeks, only 5% of rhBMP-2 remains in the collagen sponge [122].

An increase in the collagen resorption duration can be achieved by crosslinking collagen molecular chains with chemical agents, such as glutaraldehyde, carbodiimide, and genipin, or by physical exposure, such as UV radiation or dehydrothermal treatment. However, due to cytotoxicity, chemical crosslinking agents adversely affect the biocompatibility and regenerative potential of the material [116, 123].

Additionally, collagen extracted from the xenogeneic matrix with insufficient and ineffective chemical cleaning demonstrates pronounced immunogenicity; in 20% of patients who received an implant from a collagen sponge, antibodies to type I collagen were found [114, 124].

Another disadvantage of using collagen scaffolds is the difficulty of sterilizing them, since heat sterilization causes the partial or complete, irreversible denaturation of collagen fibers [125, 126]. Thus, gas sterilization with ethylene oxide is used to sterilize collagen sponges [127]. However, with this method of sterilizing a collagen sponge with rhBMP-2 immobilized on it, an unpredictable change in the kinetics of the growth factor release and a decrease in its biological activity were noted [128, 129].

CONCLUSIONS

Despite all the advantages of an autologous bone, in the presence of cellular elements of the bone marrow, presence of growth factors, and local blood supply, synthetic and biocomposite osteoplastic matrices can be a real alternative to an autologous bone graft, particularly in the variants of transport systems for the prolonged local release of target pharmaceutical substances.

Although positive scientific and practical results have been achieved in the study of new-generation osteoplastic matrices, many unresolved issues remain, and the main ones are as follows:

– Optimization of the resorption time of the osteoplastic matrix.

– Selection of an effective technology to facilitate the resorption of the osteoplastic matrix, synchronized in time with the process of bone regeneration.

– Stabilization of the matrix to exclude a pronounced macrophage reaction of the recipient's body.

– Solving issues related to the certification and registration of new options for osteoplastic surgical implants in supervisory medical organizations.

Experimental and clinical studies on osteoplastic matrices are underway in most countries. The participation of many leading research centers, as well as the connection of significant material and financial resources, increases the possibility of achieving significant research and production success in this field of regenerative medicine.

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Authors' contribution

D.D. Lykoshin – work idea, selection of publications, analysis and description of search results, preparation, design of the article, and writing the text of the article;

V.V. Zaitsev – selection of publications and analysis and description of search results;

M.A. Kostromina – preparation and design of the article and work with graphic materials;

R.S. Esipov – preparation and design of the article and writing the text of the article.

The authors declare no conflicts of interest.

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