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Biocompatible cryogels: preparation and application

Polymer cryogels are very promising for producing functional materials. Their porous structure makes them indispensable for some areas of medicine, catalysis, and biotechnology. In this review we focused on methods for producing cryogels based on biopolymers, interpolyelectrolyte complexes of biopolymers, and composite cryogels based on them. First, the properties of cryogels and brief theoretical information about the production of cryogels based on biopolymers were considered. The second section summarizes the latest advances in the production of cryogels based on complexes of biopolymers and composite cryogels. The features of the synthesis and the factors affecting the final properties of materials were considered. In the final part the fields of application of cryogels of the considered types in biotechnology, catalysis and medicine were studied in detail. In biotechnology cryogels are used to immobilize molecules and cells, as a basis for cell growth, and as chromatographic materials for cell separation. In catalysis cryogels are used as a matrix for the immobilization of metal nanoparticles, as well as for the immobilization of enzymes. Biocompatible cryogels and their composites are widely used in medicine for bone and cartilage tissue regeneration, drug delivery, providing a long-term profile of drug release in the body.

Keywords: cryogel, biocompatible, biopolymer, macroporosity, immobilization, biotechnology, catalysis, drug delivery, tissue engineering.

Introduction

Cryogels are porous polymer materials with the system of communicating pores. The term cryogel was first used by V.I. Lozinsky to refer to gels prepared in a frozen solvent medium [1]. Cryogels are synthesized by cryogelation (cryogenic gelation), based on the use of the effect of lowering the temperature below the freezing point of a pure solvent [2]. Visually, the mixture is a solid. The uncured zones of frozen multicomponent systems are called non-frozen liquid microphase (NFLMP). The polymer framework of the cryogel is formed in such unfrozen micro-regions [1]. When the frozen preparation is thawed, a macroporous cryogel is formed, the pore-forming agents are polycrystals of the frozen solvent.

The primary condition for the synthesis of cryogels is the content in the initial systems of structural elements that allow, as a result of forces of different nature (chemical bonds, Van der Waals forces, electrostatic interactions), to form three-dimensional agglomerates. The following groups of initial systems are distinguished [1, 3]: 1) colloidal sols; 2) solutions of monomers; 3) solutions of polymers with a crosslinking agent; 4) solutions of polymers capable of self-stacking; 5) solutions of polyelectrolytes, including low-molecular or polymer counterions.

The main difference between cryogels and other types of polymer materials is their morphology. The porous structure of the cryogel in combination with swelling, collapse, thermal and pH sensitivity opens up broad prospects for the use of these objects in various fields.

Porous materials are divided into 2 groups by origin [4]: addition systems (corpuscular) and subtraction systems (spongy). According to their structural and geometric characteristics, they are also divided into 2 types: regular porous structures with the same size of pores, channels, and walls, and stochastic bodies, in which the pore sizes, their location, wall thickness, and other parameters are random. According to this

classification, cryogels belong to stochastic subtraction systems, in which pores of random sizes are cavities, channels, or slits in a continuous matrix.

The review presents the data from the sources of recent 15 years on the preparation of cryogels based on biopolymers, mainly polysaccharides, and their application in different fields. During the making of this review article we focused on cryogels, which are prepared on the basis of complexes of polymers and biopolymers. We paid special attention to cryogels prepared on the basis of only natural polymers. Together, the review summarizes the methods for producing composite cryogels. In the subsequent sections of review the methods of applications of these cryogels are highlighted, especially in the field of biotechnology, catalysis and medicine.

Biopolymers based cryogels

Biopolymers usually contain a significant number of charged functional groups. This increases their bioavailability, biodegradability, and ensures their involvement in chemical processes occurring in a living organism. Thus, biopolymers are often polyelectrolytes. If a polyelectrolyte solution is used as the initial system, the formation of cryogels occurs as a result of the formation of sufficiently stable ion bridges between the polyelectrolyte units [1, 3].

An example of the formation of cross-linked systems by such a mechanism are gels based on gellan and guar gum [5]. However, the implementation of this mechanism for the production of cryogels is a difficult task. Since the rate of gelation is very high, when the critical concentration of gelation is reached, gelation in such systems usually occurs earlier than the freezing of initial monomer mixture. This leads to the fact that there is no cryoconcentration effect in the system and the resulting gels cannot be attributed to cryogels [1]. In [6] cryogels based on chitosan and calcium alginate were obtained by freezing the initial solution at –20 °C, followed by immersion of the frozen mixtures in alcohol solutions containing components that initiate the gelation process. In the case of chitosan it is NaOH, in the case of alginate it is Ca^{2+} ions. The researchers [7] obtained an alginate-based cryogel by sublimation of the initial mixture containing sodium alginate and gelatin, and then they kept the sublimate in a solution containing Ca^{2+} ions for 3 days. The obtained cryogels [6, 7] were used for cell growth.

Cryogels of cationic polyelectrolyte chitosan were prepared by crosslinking at subzero temperature. Glutaraldehyde (HA) [8], diglycidyl ethers of glycols [9] were used as crosslinking agents; the authors [10] used non-toxic biodegradable crosslinking agents-oxidized dextran and 1,1,3,3-tetramethoxypropane.

A new cryogel was prepared by cryopolymerization of salecan and acryloyloxyethyltrimethylammonium chloride using triallyl cyanurate (TAC) as a crosslinking agent [11]. The structure of cryogels was confirmed by IR spectroscopy and X-ray analysis. Adding more hydrophilic salecan inside of cryogels has significantly increased the water absorption. In vitro cytotoxicity analysis the non-cytotoxic nature of cryogels has been confirmed. They were biocompatible and maintained the adhesion, proliferation, and viability of L929 and 3 T3-L1 cells, as shown by cell proliferation and live/dead cell analysis. Overall, this work opens the door to the design and development of a mechanically robust salecan-based cryogel for cell adhesion and proliferation, as well as further applications in soft tissue engineering.

For the preparation of new biocompatible macroporous cryogels based on dextran and hyaluronan derivatives, the electron-beam reaction of free-radical crosslinking was used [12]. This approach ensures the production of high-purity materials with high porosity without the use of additional crosslinkers or initiators. It was found that the applied radiation dose and chemical composition strongly affect the properties of the resulting cryogel materials. Preliminary cytotoxicity tests illustrate the excellent in vitro cytocompatibility of the obtained cryogels, which makes them attractive as matrices for tissue regeneration procedures.

The use of non-toxic crosslinking agents in the production of biocompatible cryogels is also important. In [13] a single-stage method for producing chitosan or gelatin cryogels is proposed. For this purpose, nontoxic and biodegradable crosslinking agents such as oxidized dextran and 1,1,3,3-tetramethoxypropane are used. The chitosan cryogels prepared in this way had a degree of degradation ~2 times higher than the cryogels prepared by the two-stage method, i.e., reduced with borohydride. In addition, these cryogels showed significantly higher viability (~80 %) of fibroblast cells in vitro compared to cryogels crosslinked with glutaraldehyde (~40 %). Thus, cryogels prepared without the use of harmful crosslinking agents can be used as biocompatible and biodegradable scaffolds for cell culture and other biomedical applications.

A natural derivative of dialdehyde carboxymethylcellulose (DCMC) was used as a crosslinking agent for the production of spongy collagen cryogels by freezing-thawing [14]. Studies have shown that the crosslinking reaction and cryogenic treatment do not destroy the triple helix of collagen, but increase the thermal stability

of collagen; cryogels have a heterophase structure with interconnected macropores, and swell quickly. The swelling coefficient depends on the content of DCMC and on the medium pH. Tests for compatibility with blood in vitro showed that the introduction of DCMC does not cause a decrease in hemolysis and blood clotting compared to pure collagen. Thus, the resulting cryogels have great potential in tissue engineering and other biomedical applications.

Macroporous cryogels of hyaluronic acid (HA) with a tunable porous structure, viscoelasticity, and high mechanical strength were synthesized from methacrylated HA in aqueous solutions at a temperature of –18 °C by a free radical mechanism [15]. Poly(N,N-dimethylacrylamide) (PDMAA) was used as a filler. The porosity and average pore diameter decrease with increasing PDMAA content in cryogels due to a decrease in the amount of ice template during cryogeling. In addition, there is a reversible gel-sol transition due to the outflow and inflow of water through the pores. This flow-dependent viscoelasticity is of great interest, since it protects the cryogel network from damage during deformations, and therefore acts as a self-defense mechanism.

The review [16] considered the formation of various physically cross-linked cryogels from polysaccharides, such as hyaluronan, carboxymethylated cottage cheese, carboxymethylated cellulose, xanthan, β-glucan, locust bean gum, starch, maltodextrins, and agarose. Cryogels have tunable structural, mechanical, and biological properties, and therefore can have numerous applications.

Complex and composite cryogels of biopolymers

The synthesis of complex, composite, hybrid cryogels allows researchers to solve issues related to the improving the mechanical characteristics of materials, chemical properties of substances, as well as to give cryogels the ability to respond to changes in external conditions such as temperature, pH, and ionic strength. Therefore, cryogels based on pure polymers have a much smaller scope of application, and therefore are much less often used.

In [17] the features of the formation of cryogels of interpolyelectrolyte complexes (IPEC) based on chitosan and sodium alginate were studied. Complexation occurs by the mechanism of electrostatic interaction between oppositely charged carboxyl groups of pyranose cycles of L-guluronic acid of neighboring alginate polymer chains and chitosan amino groups, as well as due to numerous hydrogen bonds. It is shown that the conformational state of the lyophilizing component, which is in excess in the system, has a decisive influence on the mechanism of IPEC formation. It was found that changes in the degree of binding of chitosan and alginate significantly affect the formation of the inner surface of cryogels based on them. It is shown that the most developed mesoporous structure is obtained when a denser gel is formed in the system.

Cryogels based on pectin and chitosan were prepared by cryotropic gelation. A 1 % solution of pectin was layered on a frozen solution of chitosan and $CaCl_2$, the mass ratio of pectin and chitosan was 3:1. The cryogel was formed for 4-6 hours at a temperature of 15-22 °C with slow thawing of the chitosan and $CaCl_2$ solution. According to SEM data, cryogels have a macroporous leaf-like structure [18]. It was found that cryogels based on *Heracleum* pectin are more resistant to degradation *in vitro* compared to cryogels from apple pectin. The inclusion of chitosans with a high degree of deacetylation in the composition of cryogels increases the time of their degradation [19].

pH-Sensitive cryogels based on two biodegradable polyelectrolytes (chitosan and 2-hydroxyethylcellulose (HEC)) were prepared by cryogenic treatment of semi-diluted aqueous solutions and UV-induced cross-linking in the frozen state. H_2O_2 and N,N'-methylene bisacrylamide, were used as the photoinitiator and cross-linking agent respectively. The resulting cryogels were opalescent spongy materials that rapidly release/absorb water due to their open porous structure [20].

New porous films based on xanthan and polyvinyl alcohol (PVA) were obtained by a universal and non-destructive freezing/thawing method. The stability of the films depends on the crystal zones created by the PVA during the freeze/thaw treatment. Cryogels with increased mechanical strength were synthesized by increasing the number of freeze/thaw cycles from three to seven, and pore stability was improved by applying grape pomace. The resulting film showed excellent antioxidant and antimicrobial activity, which indicates the possibility of using these systems in food packaging [21].

A new cryogels consisting of various compositions of chitosan and hyaluronic acid (0, 10, 20, 30 and 50 wt. % hyaluronic acid) were prepared. Morphological studies have shown that the porosity of cryogels is 90–95 %. It is noted that the mechanical properties of the cryogels are better than those of pure chitosan cryogels. The new cryogels do not have a significant cytotoxic effect and can be used in tissue engineering [22].

Applications of biocompatible cryogels

The physical and chemical properties of cryogels, such as macroporosity, elasticity, water permeability, and ease of chemical modification, are of great practical interest in various fields, such as biotechnology, catalysis, regenerative medicine, bioremediation, and water purification.

Application in biotechnology

The use of cryogels in biotechnology as chromatographic materials, templates for the immobilization of molecules and cells and the basis for cell growth is associated with high biocompatibility, non-toxicity, and excellent mechanical characteristics [23].

The separation of protein mixtures on cryogels was carried out in [24–26]. It should be noted that cryogels have a relatively low sorption capacity relative to proteins (less than 100 mg/g), which limits their wide use in protein separation processes compared to classical chromatographic methods [26].

Cryogels are used for the production of chromatographic columns, for this purpose, the starting materials must have the following properties [27]:

– high porosity;

– high capacity for the retained substance;

– low cost of manufacture and ease of filling the column.

Cell separation on chromatographic columns is a common application of cryogels [28]. When cells come into contact with the column material, multiple interactions of different nature occur, as a result of which the cells can become so firmly fixed in the column volume that their removal is impossible [23]. The use of macroporous cryogels as the column material reduces the multiplicity of bonds formed between the material and the cell. This is achieved by selecting a cryogel material that has a small supply of functional groups that bind cells. Reducing the activity of cell binding by functional groups of cryogel can be achieved by changing the external parameters or by preliminary functionalization of the cell surface [26]. The advantage of cryogel chromatographic columns in comparison with classical ones is their elasticity. This property allows the removal of bound cells by mechanical action on the column (Fig. 1) [29, 30].

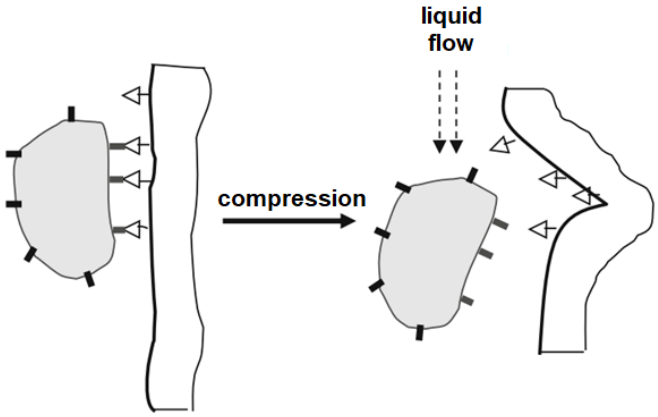


Figure 1. Mechanism of removal of bound cells from the cryogel column under mechanical action [30]

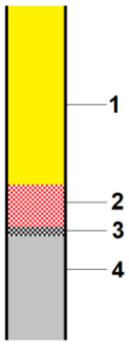
Mechanical actions break the bonds between the cryogel and the substrate and allow removing most of the bound particles.

Cell immobilization in PVA-based cryogels is widely used for cleaning environmental objects from pollutants in analytical practice [26]. When immobilizing cells in cryogels, they do not use direct cell culture on the cryogel, but add spores of microorganisms to the initial mixture. After immobilization of the spores in the resulting cryogel, cell growth is initiated [31].

The authors [32] showed that immobilized cells don't lose the ability to secrete various hydrolytic enzymes — amylases, proteases, and lipases. Cryogel-immobilized cells are used in the waste water treatment processes of the food industry. Fats and oils inhibit the metabolism of the active biomass used to treat such wastewater by forming a hydrophobic film on its surface. Preliminary treatment of wastewater using the proposed biocatalysts reduces the level of oxygen consumption required for oxidation by 2.7–3 times and increases the efficiency of wastewater treatment.

Application in catalysis

Catalysis is one of the most promising applications of cryogels. This fact is due to the large surface area per unit volume of the cryogel material. The elasticity, the possibility of varying the pore size, the ease of functionalization-all this opens up wide prospects for the use of cryogel materials in catalysis. One of the most promising developments related to the use of cryogels in catalysis is the so-called flow-through catalytic reactor (Fig. 2) [33, 34]. A special feature of this development is that the reaction mixture is pumped through the volume of the cryogel, while the reaction occurs on the surface of the cryogel pores, which are saturated with catalytically active groups (nanoparticles, enzyme molecules, etc.). This approach allows to get the finished product in one stage without further cleaning it from the catalyst particles.



1 — initial mixture; *2* — cryogel-catalyst; *3* — Schott filter; *4* — mixture of reaction products

Figure 2. Schematic structure of a flow-through catalytic reactor

In [34, 35] the results of the use of a macroporous amphoteric cryogel based on methacrylic acid (MAA) and dimethylaminoethyl methacrylate (DMAEM) crosslinked with methylene bisacrylamide (MBAA) for the immobilization of gold nanoparticles (GNP) are presented. The resulting DMAEM-MAA/GNP composite was used as a flow-type catalytic reactor for the reduction of 4-nitrophenol. The high stability of the prepared catalysts, which withstood at least 100 catalytic cycles, is shown.

Cryogels based on poly-1-vinylimidazole (p-VI) were synthesized by cryopolymerization [36]. After modification with dihaloidalkyl, the synthesized cryogels were used as templates for in situ production of cobalt and nickel metal nanoparticles (Fig. 3A). Poly(1-vinyl imidazole) (p-VI)/metal composites are also used as a catalyst for the hydrolysis of $NaBH_4$ to produce hydrogen. The cryogel matrix based on p-VI showed good operational properties even after 5 catalytic cycles, and the catalyst based on the p-VI/Co composite provided 100 % substrate conversion with a slight loss of catalytic activity. In addition, the proportion of nanoparticles in the cryogel matrix compared to hydrogel and microgel matrices was significantly higher (Fig. 3B).

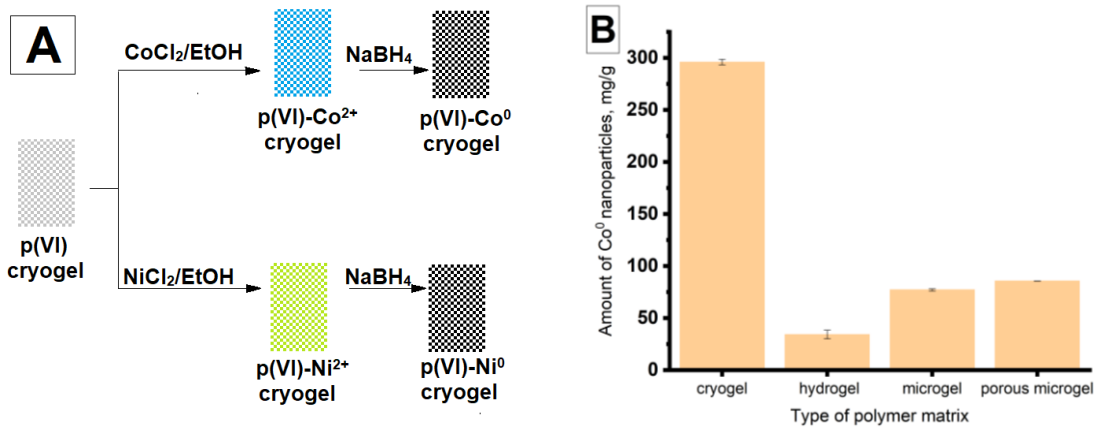


Figure 3. (A) Scheme for the production of cobalt and nickel nanoparticles in a matrix of cryogels based on poly-1-vinylimidazole. (B) The dependence of amount of Co^0 nanoparticles on the type of polymer matrix [36]

A cationic cryogel based on poly(3-acrylamidoproply)trimethylammonium chloride (p-APTMACl) was used to stabilize Co and Ni nanoparticles [37].

A series of papers are devoted to the immobilization of enzymes in the cryogel matrix [35, 38–40]. Enzyme immobilization is a promising method for a variety of applications, including biotechnology, medicine, biochemistry, and environmental protection. Enzymes have a very high sensitivity to external conditions and are quickly deactivated when optimal conditions are violated, which, in turn, leads to the impossibility of their repeated usage [38, 41]. However, the immobilization of enzymes in cryogel matrix significantly expands the possibilities of their application.

The resulting composite poly(methyl methacrylate-glycidyl methacrylate (p-MMA-GMA)/amylase is used for the catalytic hydrolysis of starch to produce glucose. It was found that the rate of starch hydrolysis by amylase immobilized in the cryogel matrix is 4 times less than in the case of free amylase, but the stability of the catalyst exceeds the stability of free amylase.

The authors [38, 41] note the prospects of the developed catalysts in comparison with the available analogues, since cryogel-immobilized enzymes allow to prepare the finished product, avoiding the stage of purification and separation of the substrate and the enzyme (Figure 4).

An interesting method of amylase immobilization in a PVA-based cryogel is described in [42]. An aqueous solution of PVA and amylase was frozen and then lyophilized. It is known [1] that cryogenic treatment of aqueous PVA solutions leads to the formation of PVA cryogels. It was found [42] that such treatment of PVA-amylase solutions also leads to the formation of cryogels, and the amylase is automatically integrated into the PVA cryogel matrix. The resulting cryogels were tested in the starch hydrolysis reaction. The substrate conversion rate averaged 70-90 %. Based on the obtained PVA-amylase cryogels, microreactors were constructed by freezing the initial mixture in the capillary. It is shown that the conversion of the substrate in the case of using a microreactor, with rare exceptions, did not exceed 30 %.

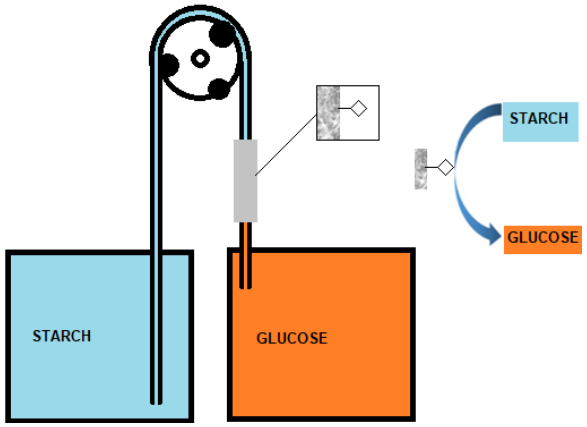


Figure 4. Scheme for the production of glucose from starch on the p-MMA-GMA/amylase catalyst [41]

The laccase enzyme (broad-spectrum oxidase) was immobilized in a cryogel based on polyethylene glycol methacrylate (PEGMA) and tetraethylene glycol diacrylate (TEGDA) [43]. Redox mediators were introduced into the initial mixture: lilac aldehyde or 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid. The polymerization reaction was initiated by electron beam irradiation. The resulting cryogels were used as a bioreactor in the oxidation reaction of bisphenol A, as a model wastewater pollutant. It was found that the cryogel-laccase biocatalytic reactor is effective in disinfection of wastewater and completely decomposes the pollutant bisphenol A in the model wastewater within 24 hours.

Cryogels based on functionalized polyacrylamides and alginate were also used to immobilize laccase [44]. It has been shown that the immobilized laccase enzyme successfully removes 70 % of phenolic compounds, more than 55 % of dyes from wastewater and provides 93-99 % of the discoloration of some dyes in solution.

PAA-based cryogel was used to produce a series of modified cryogels that exhibit the properties of cationic (allylamine), anionic (acrylic acid), and amphoteric (allylamine-acrylic acid) cryogels for use as catalysts in the production of biofuels. For this purpose, cryogels of various compositions were mixed with a mixture of methanol and oleate. It is shown that the activity of the cationic catalyst is significantly higher than that of the anionic and amphoteric ones. The relatively high stability of the catalyst over 5 cycles was established [44].

Thus, catalysts based on cryogels and nanoparticles, enzymes, and microorganisms immobilized in their matrix can be successfully used as wide-spectrum catalytic systems.

Application in medicine

Biocompatible cryogels and their composites are used in medicine for drug delivery, wound healing, and as materials for bone and cartilage tissue regeneration [45, 46]. The frames made by cryogelization are spongy, highly porous, mechanically stable, elastic, and can be easily cut into any desired shape. Therefore, cryogel materials are of great interest in tissue engineering.

Restoration of bone and cartilage tissue

Bone and cartilage are relatively rigid structures compared to other types of tissue. Therefore, materials for the restoration of such tissues must have appropriate mechanical characteristics, as well as be suitable for the germination of osteocytes (bone tissue cells) and chondrocytes (cartilage tissue cells). Cryogels are very promising materials for use in cartilage and bone engineering due to their porosity and functionality. The necessary mechanical strength is achieved by introducing inorganic fillers. For example, in [47], the authors used a polyelectrolyte chitosan/chondroitin sulfate complex modified with nanobio-glass based on silicon, calcium, and phosphorus oxides. An increase in the mass content of nano bio-glass in the composite leads to an increase in the mechanical strength of the composite and a decrease in the pore size. *In vivo* studies have shown excellent bioactivity: increased bioapatite formation, suitable pore size, porosity, and suitable mechanical strength in biological conditions.

Also cryogel chitosan/gelatin crosslinked with glutaraldehyde or genipine was used for bone tissue regeneration by the authors of [48]. For the synthesis the cryogels used covalent crosslinking of macromolecules of chitosan with glutaraldehyde and genipin. The formation of a porous structure is provided by the method of lyophilic drying. The use of genipin provides high biocompatibility of cryogels, however, when studying cell infiltration, it was found that cryogels crosslinked with genipin do not reach the desired level of infiltration and do not provide conditions for cell proliferation.

A composite cryogel based on the chitosan/gelatin-hydroxylapatite system crosslinked with glutaraldehyde was also prepared by freezing and thawing [49]. The cryogel was saturated with hydroxyapatite at a temperature of 37 °C, pH 7.4 in a synthetic body fluid medium (Figure 5). With an increase in the gelatin content in the cryogel, it leads to an increase in the content of hydroxyapatite. The cryogels do not have cytotoxicity against fibroblasts, which was proved in the experiment on rats.

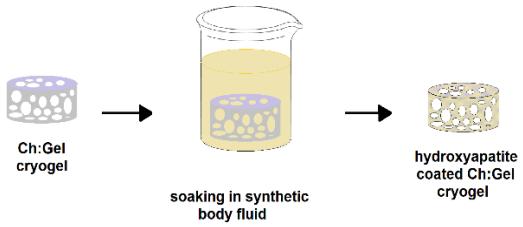


Figure 5. Scheme of preparation a composite cryogel chitosan/gelatin-hydroxylapatite [50]

Modification of hydroxylapatite with cerium and zinc was carried out in [50]. Modified cryogel chitosan/gelatin-Ce-Zn-hydroxylapatite has higher protein adsorption rates, lower biodegradation and cell germination compared to cryogel chitosan/gelatin-hydroxylapatite.

Restoration of cartilage tissue

Physical cryogels have an advantage over synthetic ones in the processes of cartilage tissue repair, since they do not require the use of toxic crosslinking agents and do not harm cells [51]. In addition, the lack of blood vessels in the cartilage tissue is a serious limitation of the creation of cartilage substitutes [46]. Elastic macroporous cryogel framework gelatin/chondroitin-6-sulfate/hyaluronan (GCH) is used for the restoration of cartilage tissue.

By replacing 20 % of gelatin by chitosan a new GCH-chitosan cryogel has been synthesized with larger pores, higher ultimate strain (stress) and elastic modulus, and a lower stress relaxation percentage comparing to GCH cryogel. Chondrocytes proliferate and differentiate in cryogels. Implantation of a cryogenic chondrocyte/GCH-chitosan structure into a full-thickness articular cartilage defect regenerates cartilage with a modulus of elasticity similar to native cartilage [52].

Cryogels of the composition gelatin/hyaluronic acid, gelatin/chondroitin sulfate modified with methacrylate were prepared in [53]. It was found that cryogel gelatin/chondroitin sulfate *in vitro* showed significant stimulation of cartilage tissue. In addition, when placed in the subcutaneous tissue of the mouse for 6 weeks, the cryogels showed a uniform distribution of cells with the preservation of the normal phenotype. And when implanted in the osteochondral defect of the New Zealand white rabbit, complete integration with the host tissue and cell germination were observed.

Cryogel based on chitosan and gluconic acid was synthesized by freezing-thawing without the use of crosslinking agents [54]. To prepare materials of cartilage tissue substitutes an average pore diameter of 100 to 300 microns is a prerequisite. The authors established the optimal synthesis temperature and the initial ratio of the components to achieve the desired pore diameter. In experiments on the stability of cryogels in the cellular environment and the proliferation of DNA and glucosaminoglycans, the superiority of a cryogel without a crosslinking agent over a chemically crosslinked cryogel of a similar structure was shown.

The authors of [55] used platelet lysate and oxidized dextran to produce cryogel as a material for cartilage tissue. The complete biodegradability of the synthesized materials under *in vivo* conditions was demonstrated in experiments with rats.

Cryogels in drug delivery systems

Cryogel-based drug and biomolecule delivery systems are the subject of intensive research. Biopolymers are widely used for the construction of such materials by combining them with synthetic polymers [56, 57], forming polymer complexes [58] and individually [59]. It is important to note that for drug delivery systems, an important condition is not only and not so much the porosity of the gel material, but the binding of the drug with the polymer matrix as well. Often, hydrolytically cleavable bonds such as simple and ester bonds are used to conjugate the drug with the matrix. These types of bonds provide a long-term drug release profile and increase the half-life of drugs in the body [60].

Three-dimensional (3D) biocomposites based on chitosan and clinoptilolite were obtained by cryogelation. Biocomposites were studied as carriers of the medicinal substances sodium diclofenac and indomethacin. It has been shown that drug delivery preferably occurs at pH 7.4 (intestinal environment), and at pH 1.2 (stomach environment) there is a decrease in drug release [59].

Chitosan cryogel scaffolds including Hypericum perforatum (HP) vegetable oil have been developed, which exhibit unique antimicrobial and antioxidant properties [61]. The composition showed the greatest antimicrobial activity against *E. coli* and *L. pneumophila*. The resulting cryogel scaffolds are promising materials as wound dressings for exudative and long-term healing wounds.

Collagen cryogels with polysaccharide functional components (dextran and carboxymethylcellulose) showed good bio- and hemocompatibility. These cryogels can be used as potential scaffolds for use in tissue engineering and regenerative medicine [62].

Cryogels based on apple pectin and chitosan are used as anti-adhesive barrier materials [63]. The anti-adhesive effect is provided due to the short time of biodegradation of cryogels based on apple pectin, non-degradable cryogels based on hogweed pectin do not have an anti-adhesive effect.

A series of cryogels based on glycol chitosan and ε-polylysine significantly reduce bleeding. The inclusion of ε-polylysine significantly increases the ability to kill a multidrug-resistant bacterial infection (MDR). The effectiveness of wound healing, treated with cryogel, was significantly higher compared to the control. Polysaccharide-peptide cryogels can become competitive multifunctional wound dressings for the control of bleeding and healing of MDR-infected wounds [64].

Examples of medical application and initial polymers for the synthesis of cryogels are shown in Table.

T a b l e

Composition of cryogels and areas of their application for medical purposes

Chemical composition of cryogel	Application area	Reference
1	2	3
Chitosan/Chondroitin sulfate	Prosthetics and bone regeneration	46
Chitosan/Gelatin crosslinked with Glutaraldehyde or Genipine		47
Chitosan/Gelatin-Hydroxylapatite crosslinked with Glutaraldehyde		48
Chitosan/Gelatin-Ce-Zn-Hydroxylapatite		49
Collagen/Hydroxylapatite		65, 66
Gelatin/Hydroxylapatite		67

Continuation of Table

1	2	3
Gelatin/Nanohydroxylapatite	Prosthetics and bone regeneration	68
Hyaluronic acid/Gelatin		69
Alginate		70, 71
Chitosan-Agarose-Gelatin	Restoration of cartilage tissue	72
PVA/Chitosan		73
Polyhydroxyethylmethacrylate-Gelatin		74
Hyaluronic acid/Polyethylenimine		75
Hyaluronic acid		76
Gelatin /Hyaluronic acid		77
Carrageenan/Alginate		78
Sodium Alginate/ acetylated Dextran	Drug delivery	79
Nanocellulose/Gelatin		80

Conclusions

Thus, based on the literature data, it can be concluded that cryogels based on biopolymers, especially polysaccharides, due to their unique properties, namely, macroporosity, elasticity, biodegradability, biocompatibility, and biological activity are promising materials for application in biotechnology, catalysis, and medicine.

In the field of cryogel synthesis, it is necessary to develop technologies that make it possible to prepare a biocompatible material without the use of harmful and toxic substances. In this regard, cryogels based on interpolyelectrolyte complexes of natural polymers seem promising.

According to the authors, the most promising areas of development of cryogel technologies are the development of biocatalytic systems and tissue engineering. The most important task of researchers for a breakthrough in these areas in the near future will be to develop a method for producing biocompatible cryogels of the desired strength. This approach will make it possible to obtain cryogels that are comparable in mechanical strength to bone and cartilage tissues. Despite the complexity of the task, the authors believe that in the foreseeable future the technology of 3D-printing cryogels will be developed in order to produce catalysts, as well as joint prostheses based on biocompatible durable cryogels.

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Биоүйлесімді криогельдер: алынуы және қолданылуы

Полимерлі криогельдер пайдалы функционалды материалдарды алу үшін өте перспективалы заттар болып табылады. Криогельдердің кеуекті құрылымы оларды медицинаның, катализдің және биотехнологияның кейбір салаларында баға жетпес материал етеді. Осы шолуда биополимерлерден және де биополимерлердің интерполиэлектролитті комплекстерінен алынатын криогельдерге және оларға негізделген композиттік криогельдер алу әдістеріне ерекше назар аударылған. Алдымен, криогельдердің ерекше қасиеттері туралы және биополимерлер негізінде криогельді материалдарды өндіру туралы қысқаша теориялық мәліметтер берілген. Шолудың екінші бөлімінде биополимерлер мен олардың комплекстеріне негізделген композиттік криогельдер өндірудегі әлемдегі соңғы жетістіктер жинақталған. Криогельдерді синтездеу ерекшеліктері және де синтезделетін криогельді материалдардың қажетті болатын қасиеттеріне әсер ететін факторлар қарастырылды. Шолудың қорытынды бөлімінде қарастырылатын типтегі полимерлі криогельдерді биотехнологияда, катализде және медицинада қолдану салалары егжей-тегжейлі зерттелген. Биотехнология саласында криогельді материалдар молекулаларды және де биологиялық жасушаларды иммобилизациялау үшін, жасуша өсуінің негізі ретінде, сонымен қоса жасушаларды өзара бөлу үшін хроматографиялық материалдар ретінде пайдаланылады. Катализ саласында полимерлі криогельдер металл нанобөлшектерін иммобилизациялау үшін, сондай-ақ ферменттерді иммобилизациялау үшін матрица ретінде қолданылады. Биологиялық үйлесімді криогельдер мен олардың композиттері медицина саласында сүйек және шеміршек ұлпаларын қалпына келтіру үшін, сондай-ақ дәрі-дәрмектерді адрестік жеткізу үшін кеңінен қолданылады, бұл организмде дәрі-дәрмектерді бөліп шығарудың ұзақ мерзімді профилін қамтамасыз етеді.

Кілт сөздер: криогель, биоүйлесімді, биополимер, макрокеуектілік, иммобильдеу, биотехнология, катализ, дәрі-дәрмекті жеткізу, ұлпалық инженерия.

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Биосовместимые криогели: получение и применение

Полимерные криогели являются весьма перспективными веществами для получения функциональных материалов. Пористая структура делает криогели незаменимыми материалами в некоторых областях медицины, катализа и биотехнологии. В данном обзоре авторы сосредоточились на методах получения криогелей на основе биополимеров, интерполиэлектролитных комплексов биополимеров и композитных криогелей на их основе. Сначала рассмотрены свойства криогелей и краткие теоретические сведения о способах получения криогелей на основе биополимеров. Во втором разделе обзора обобщены последние достижения в производстве криогелей на основе комплексов биополимеров и композитных криогелей. Рассмотрены особенности синтеза криогелей и факторы, влияющие на требуемые конечные свойства криогелевых материалов. В заключительной части обзора подробно изучены области применения криогелей рассматриваемых типов в биотехнологии, катализе и медицине. В биотехнологии криогелевые материалы используются для иммобилизации молекул и биологических клеток, в качестве основы для роста клеток, а также хроматографических материалов для разделения клеток. В катализе криогелевые материалы применяются как матрицы для иммобилизации наночастиц металлов и ферментов. Биосовместимые криогели и композиты на их основе находят широкое применение в медицине для регенерации костной и хрящевой ткани, а также для адресной доставки лекарственных средств, обеспечивая долгосрочный профиль высвобождения лекарственных средств в организме.

Ключевые слова: криогель, биосовместимый, биополимер, макропористость, иммобилизация, биотехнология, катализ, доставка лекарств, тканевая инженерия.

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