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Review

Biomedical Applications of Polymeric Cryogels

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Featured Application: This study presents the comprehensive applications of polymeric cryogels in the field of biomedicine. To the best of our knowledge, this review is one of the most pioneering paper comparatively explains the biomedical applications of cryogels in detail.

Abstract: The application of interconnected supermacroporous cryogels as support matrices for the purification, separation and immobilization of whole cells and different biological macromolecules has been well reported in literature. Cryogels have advantages over traditional gel carriers in the field of biochromatography and related biomedical applications. These matrices nearly mimic the three-dimensional structure of native tissue extracellular matrix. In addition, mechanical, osmotic and chemical stability of cryogels make them attractive polymeric materials for the construction of scaffolds in tissue engineering applications and in vitro cell culture, separation materials for many different processes such as immobilization of biomolecules, capturing of target molecules, and controlled drug delivery. The low mass transfer resistance of cryogel matrices makes them useful in chromatographic applications with the immobilization of different affinity ligands to these materials. Cryogels have been introduced as gel matrices prepared using partially frozen monomer or polymer solutions at temperature below zero. These materials can be produced with different shapes and are of interest in the therapeutic area. This review highlights the recent advances in cryogelation technologies by emphasizing their biomedical applications to supply an overview of their rising stars day to day.

Keywords: supermacroporous cryogel; purification; separation; tissue engineering; bioreactor; cell separation; scaffold

1. Introduction

In recent years, there has been an increase in the number of studies using macroporous polymeric structures for the separation of biological molecules. High and rapid purification abilities of supermacroporous polymeric materials make them applicable in separation of different biological macromolecules. Cryogels have a wide range of applications in the biotechnology, bioseparation and biomedicine fields, taking part in many applications for the purification and immobilization of proteins, nucleic acids, and polysaccharides [1].

Cryogels are gel matrices formed by polymerization at temperatures below zero. While the polymerization process takes place in the unfrozen part, ice crystals formed act as pore forming agents. At the end of the polymerization process, after cryogels are thawed, supermacropores are formed within cryogel structure [2]. Since the interconnected macro pores provide a great advantage when working with viscous biological fluids, there has been a large increase in the number of studies using cryogels for the purification of natural source proteins over the last decade. Enzymes, nucleic acids

and carbohydrates can be separated from their source without back pressure problems and diffusion restriction due to the interconnected supermacropores. Large, interconnected supermacropores provide unique spongy structure to the cryogels. Elastic and spongy structure of cryogels makes them different from rigid monoliths [3].

Supermacroporous cryogels have a great deal of interest in biomedical, biotechnology and pharmacy. Supermacroporosity of cryogels allows them to be used without any diffusion problems when working with biological macromolecules. Cryogels can be used in the purification of macromolecules such as proteins and nucleic acids, as well as in the separation of cell organelles, viruses, plasmids, microorganisms and mammalian cells. In addition, since the porous structures are suitable for cell adhesion and mechanical stability of cryogels offers applicability in in vivo processes, cryogels are also used in tissue engineering studies [4]. Cryogels have a three-dimensional structure suitable for the proliferation of cells. In particular, the surface properties of the support materials used in tissue engineering greatly affect cell affinity. Hydrophilic and porous structure of cryogels make them suitable for cell affinity. This review describes the specific features of cryogels and refers to recent applications of cryogels in biomedicine and related area [5].

2. Preparation and Properties of Supermacroporous Cryogels

Monoliths, also called continuous stationary phases, are important tools for separating biological molecules. Although the studies on monoliths were started in the early 1970s, they gained importance as a big invention in the 1990s. Monolithic materials are characterized by low mass transfer resistance. In all studies involving biological macromolecules, monoliths perform much better than conventional chromatographic systems [6].

Monolithic materials have found wide use in chromatographic applications with the immobilization of different affinity ligands to monoliths [7]. The difference of monoliths from traditional filled columns is due to the one-piece porous structure. The interconnected pores in the structure of the monolith form a cross-channel network. Since the intra-channel mass transfer occurs with the flow, the transfer between the mobile phase and the stationary phase is very rapid. As a result, even very large molecules such as proteins can be separated from monolithic columns in a very short time. In addition, it is desirable to have low pressure during liquid flow in chromatographic systems. Since monoliths have smooth flow channels, high pressure does not occur, thus preventing any back pressure and/or clogging in the column [8].

Cryogel monoliths are gel matrices prepared using partially frozen monomer or polymer solutions. The most important features of the cryogels are their porous structure, non-specific interaction due to their hydrophilic structure, minimum mechanical properties and osmotic stability. The main disadvantage of cryogels is the low surface area. Cryogels characterized by their hydrophilic properties have supermacropores and are formed by the polymerization process performed at temperatures below zero. The formation of cryogels is given in Figure 1. During cryogel formation, the polymer precursors are first dissolved in a suitable solvent such as water. In the freezing phase, the solvent freezes and forms interconnected ice crystals. The polymer precursors present in the unfrozen portion polymerize to form a network around the ice crystals. After the polymerization is completed, the frozen mixture is brought to room temperature. Then, the ice crystals melt and the porous polymer network, called “cryogel”, is obtained. Solvent crystals act as pore forming agents. The melting of the ice crystals due to the surface tension of the solvent around the pore wall allows the pores in the cryogels to have a circular shape. The shape and size of the pores depend on many factors such as monomer concentration and temperature at which polymerization takes place are the most important factors. Micropore formations are also observed in the polymeric phase of the cryogels. Thus, in addition to supermacropores, there are also micropores in the cryogel structure [9]. The supermacropores in the structure of cryogels vary in size from 10 to 200 μm.

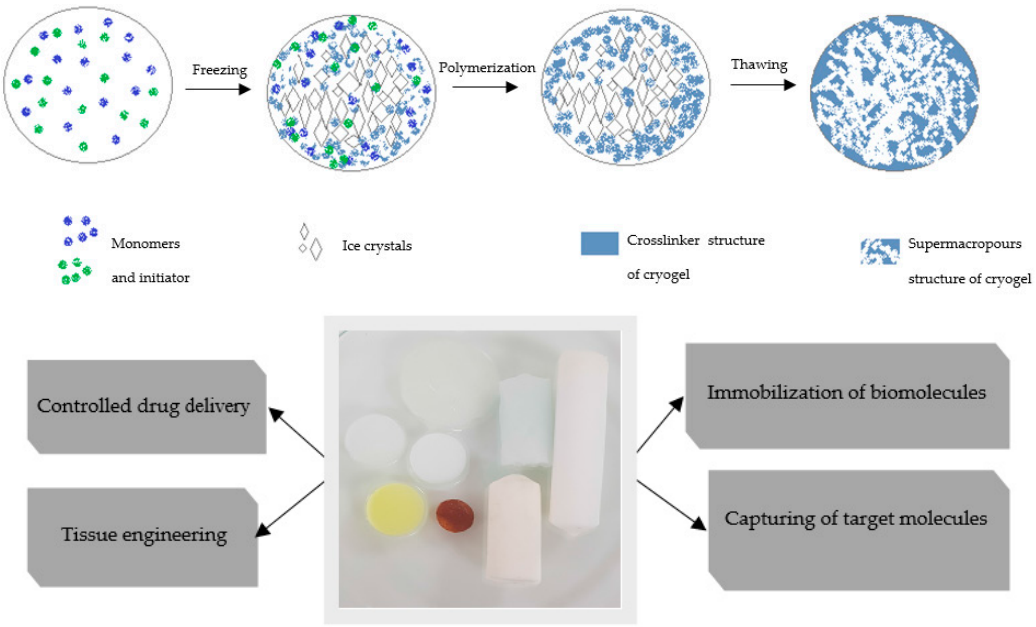

Figure 1. Schematic view of cryogel formation.

The pore size, the thickness and the density of the pore walls are the parameters that affect the performance of cryogels. The pore size affects the flow resistance. The thickness and density of the pore walls determine the macroscopic mechanical properties of the cryogel.

The pore volume of a chromatographic material is very important. An average of 3–4% of the total weight of a cryogel that is completely swollen by water is the dry polymer weight. 4–5% of the weight consists of polymer bound water. Thus, 90% of the remaining weight of the cryogel constitutes of water inside pores. Due to the elasticity of the cryogels, 70% of the water in the large pores can be removed by mechanical compression. This feature indicates that a large part of the cryogel volume consists of interconnected pores [10].

Many advantages of cryogels make them useful in the field of biochromatography. The large pores of the cryogels interconnected with each other offer advantages when working with viscose biological fluids such as blood, plasma, plant or animal tissue extracts. Thanks to the large pores, there is no diffusion limitation in cryogels, and the back pressure is low. Thus, cryogels are more advantageous than traditional polymeric spheres in the purification of biomacromolecules from natural sources. Denizli and his colleagues have provided purification and removal of many substances such as immunoglobulins, albumin, lysozyme, DNA, and cholesterol by immobilizing different ligands to cryogels. Lectins, protein A, tryptophan, triazin dyes and various metals have been used as ligands in these studies [11–17]. There are also some drawbacks of cryogels besides their desirable advantages. Cryogels have lower surface area than other polymeric structures such as microbeads and nanoparticles. By this way, cryogels have low ligand density which resulted in low adsorption capacity. Composite cryogels can be prepared to overcome this problem. Particle embedding and synthesis of cryogels using nanoparticles can be performed to increase the specific surface area of cryogels to be used in the capturing of target molecules [9].

Factors Affecting Properties of Cryogels

There are many factors affecting properties of cryogels. Temperature at which cryogelation occurs, polymerization time, type of crosslinking, contents of monomers used, and freezing rate are variables determine physical properties of cryogels. Lozinsky et al. have shown the effect of temperature on pore size. According to their study, lower cryogelation temperature results in smaller pore size. Thus, cryogels with different pore size distribution can be generated by changing cryogelation temperature. However, the temperature at which cryogels with maximum pore size are obtained, are preferred [18].

In a study, 2-hydroxyethyl methacrylate (HEMA)-based cryogel membranes were prepared at different polymerization temperature to observe the effect on the pore size of membranes. They reported smaller pore size formation at lower cryogelation temperature [19].

Monomer contents of cryogels and concentrations of reaction constituents significantly affect the cryogelation process. Monomers with lower molecular weights are expected to form larger pores when compared to monomers with larger molecular weights. In addition, increase in the amount of reaction constituents rises the rigidity of cryogels, thickness of cryogel walls and elasticity [20]. Also, rate of cryogelation increases and cryogelation occurs more efficiently with using of more concentrated polymer contents. Water soluble monomers are usually preferred for the preparation of cryogels. HEMA, *N*-isopropylacrylamide (NIPA), acrylic acid (AAc), *N*′,*N*′-dimethylacrylamide (DMA), *N*′,*N*′-diethylacrylamide (DEA) and acrylamide (AAm) are some monomer types used for cryogel preparation.

Freezing rate is also an important factor for optimal cryogel production process. To produce supermacroporous homogeneous cryogels, the crosslinking speed should be slower than the solvent freezing rate. If the crosslinking speed proceeds faster than the solvent crystallizes, no macro pores will be formed. Therefore, the typical supermacroporous cryogel structure will not occur [21].

Crosslinking degree affects physical properties of cryogels. Stiffness of the cryogel, elasticity and mechanical properties are the factors affected by crosslinking degree. Crosslinking degree also has an influence on swelling degree by affecting pore size with capability of keeping water [22]. *N*′,*N*′-methylene(bis) acrylamide (MBAA) and poly(ethylene glycol diacrylate) have been used as crosslinkers for preparation of different types of cryogels in the literature. Glutaraldehyde is also used for crosslinking of proteins via amine groups of peptide chains. In addition to proteins, glutaraldehyde can crosslink polysaccharides and synthetic polymers such as poly (vinyl alcohol) (PVA) and polyacrylamide in the preparation of cryogels [23].

Cryogels can be classified as supermacroporous hydrogels which are produced by cryogelation. There are some differences between hydrogels and cryogels. To obtain macroporous structure within hydrogels, different pore forming agents which are removed after gelation can be used. Removal of porogens and insufficient interconnectivity are main difficulties of conventional hydrogels in preparation process. Furthermore, chemicals that are used to remove porogens can stay in gel structure and affect binding of target macromolecules or cells. Porogen removal process can be time consuming and creates excessive waste. In addition, porogens which are not removed properly can cause unfavorable effects on target molecules in binding process. If porogens cannot be removed properly, they stay in gel structure and can prevent interconnectivity between macropores. Thus, non-toxic and easily removable porogens are preferred for producing interconnected macroporous gel structures. At this point, cryogels are attractive options as they are formed by using water as porogen-forming agent. Because water molecules can be easily removed in thawing process, cryogelation method is simple. Thus, cryogels have gained great interest in recent years and number of publications on cryogels have steadily increased [9,22].

3. Cryogels in Medical Applications

Cryogels are good alternatives to traditional protein-binding matrices with many different biomedical applications. The advantages of cryogels such as high blood-compatibility, high water content, resistance to degradation, non-toxicity, and pressure drop properties allow them to be used without any diffusion problems when working with biological macromolecules. The main applications of cryogel matrices are immobilization of biomolecules, capturing of target molecules, controlled drug delivery, cell separation, tissue engineering, bioreactor, cell separation, and scaffolds.

3.1. Immobilization of Biomolecules

In affinity-based separation and purification of biomacromolecules a specific ligand is immobilized onto cryogel matrices. The immobilization of ligand in cryogel matrices can be applied with two

approaches; (i) covalent coupling of the ligand to the prepared cryogel matrix and (ii) physical attachment of ligand in during the polymerization [24]. The low-pressure drop and lack of diffusion resistance are the main advantages of cryogels that causes to apply viscous samples.

Antibody immobilized cryogel matrices provide a selective removal of specific antigen from a complex mixture. In autoimmune diseases such as rheumatoid arthritis, plasma exchange treatment has been used to remove of autoantibodies produced during these diseases. The disadvantages of plasma exchange treatment can be altered by using affinity adsorption therapy for plasma exchange. For example, in a study, poly(2-hydroxyethyl methacrylate) (PHEMA) cryogel carrying protein A was used for the removal of immunoglobulin M (IgM) from human plasma [25]. The PHEMA cryogel was prepared by bulk polymerization, which proceeds in an aqueous solution of monomer frozen inside a plastic syringe. As seen in Figure 2a, the PHEMA cryogel has a continuous matrix with 10–200 μm pores. Pore volume in the PHEMA cryogel was 71.6%. Protein A was covalently immobilized onto the PHEMA cryogel via cyanogen bromide activation. The PHEMA cryogel was contacted with blood in vitro system for the determination of blood-compatibility (Figure 2b). IgM adsorption capacity decreased significantly with the increase of the plasma flow rate. An increase in the flow rate reduced the plasma volume treated efficiently until breakthrough point and therefore decreased the retention time of the PHEMA cryogel column. The maximum IgM adsorption amount was 42.7 mg/g. IgM could be repeatedly adsorbed and eluted without noticeable loss in the IgM adsorption amount. Separation of target molecule with high specificity and yield provide advantages to cryogels immobilized with monoclonal antibodies. Kumar et al. coupled anti-CD34 monoclonal antibodies to protein A-PVA cryogel beads for capturing human acute myeloid leukemia KG-1 cells expressing the CD34 surface antigen on the surface [26].

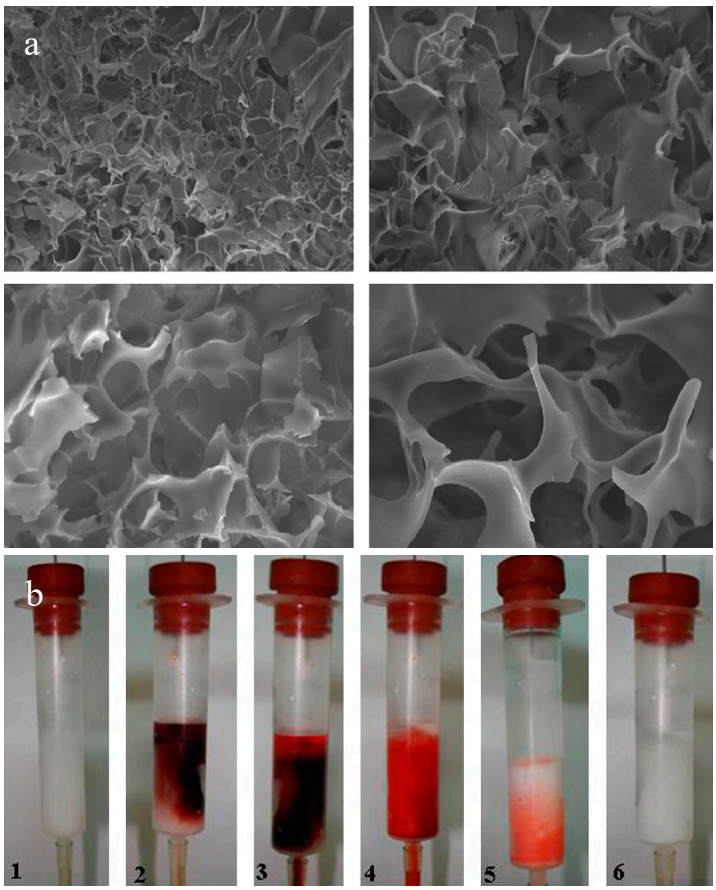

Figure 2. Scanning electron microscopy (SEM) images of the PHEMA cryogel (**a**), Flow behavior of whole blood through a PHEMA cryogel column (**b**) [25].

In another study, apoproteinB100, a ligand against the main protein component of low-density lipoprotein (LDL) was used for the selective removal of harmful LDL without decreasing useful high-density lipoprotein (HDL) in hypercholesterolemia. Bereli et al. prepared protein A immobilized cryogels carrying Anti-LDL antibody. They successfully removed approximately 59% of the initial LDL from the hypercholesterolemic plasma [16].

3.2. Capturing of Target Molecules

Capturing of a target molecule from a complex medium is an important factor in modern biotechnology. The effective separation of the target can be achieved by using supermacroporous materials and longtime physically and chemically stable sorbents. Lower mass transfer resistance and pressure drop properties of cryogels make them useful as sorbents for the separation of target molecules from a complex medium [10]. Traditional gels are delicate and fragile. Whereas, cryogels are elastic, soft, sponge-like materials without deformations. Therefore, these materials can be used in the field of biochromatography. Cryogel matrices are widely used in bioseparation technology such as separation and removal of proteins, peptides, nucleotides and even toxic metals. The removal of dominating proteins such as human serum albumin (HSA) and immunoglobulins from the biological sample is crucial to allow the analysis of less components. The promising cryogels can be used as matrices to make these minor components more accessible. Different technologies such as immunoaffinity, dye affinity, and molecular imprinting can be applied with cryogel-based matrices for the effective separation and removal of target molecules.

Avcıbaşı and co-workers used HEMA and *N,N*-bis (2,6-diisopropylphenyl) perylene-3,4,9, 10-tetracarboxylic diimide (DIPPER) monomers for preparation of P(HEMA-DIPPER) monolithic cryogels and used these cryogels for capturing of albumin [27]. The maximum adsorption for albumin was reported 40.9 mg/g. Monolithic cryogels are even more attractive with shorter processing times, and they have better ease use and higher overall ease of capacity. The dominant interaction between matrices and target molecules could be attributed to the hydrophobic interactions due to the hydrophobic character of DIPPER.

Bereli and co-workers synthesized supermacroporous PHEMA-based cryogel with *N*-methacryloyl-(L)-histidine methyl ester (MAH) as the specific ligand for purification of immunoglobulin G (IgG) from human plasma. Supermacroporous cryogels were produced by free-radical polymerization initiated by tetrametiletilendiamin and ammonium persulfate pair in an ice bath. Supermacroporous cryogels with 38.6 m^2/g surface area and containing 113.7 μmol MAH/g were used in the purification of IgG from human plasma. They reported 24.7 mg/g maximum adsorption capacity of IgG. The adsorption capacity of supermacroporous cryogels was significantly improved due to the MAH incorporation into the cryogel. They obtained 97.3 mg/g IgG adsorption capacity from human plasma [28]. Also, 94.6% purity of IgG by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) has been indicated in this study.

In another study, Bakhshpour et al. used immobilized metal affinity-based cryogels for the separation of IgG. They used poly(ethylene imine) (PEI) as the chelating ligand covalently coupled with PHEMA cryogels after activation and then Cu(II) ions were chelated to the PEI bound PHEMA cryogels. The separation was selectively achieved up to 72.28 mg/g cryogel. As a conclusion, they have reported that IgG separation was performed by immobilized metal affinity-based cryogel column successfully and selectively (Figure 3) [29]. In another study, they prepared and characterized two different combinations for the purification of IgG. They synthesized thiophilic PHEMA-based and thiophilic composite cryogel membranes via attachment of 2-mercaptoethanol as an affinity ligand. They reported 27.5 mg/g and 68.7 mg/g maximum adsorption capacities for thiophilic PHEMA and thiophilic composite cryogel membranes, respectively [30].

Besides immobilization-based affinity chromatography, it is also possible to separate biomolecules with molecularly imprinted cryogels and then to use them for the separation of molecules. Molecular imprinting-based cryogels (MIC) are prepared with high affinity and specificity binding sites to

the target molecules. In a typical molecular imprinting process, the functional monomers and the template molecules are interacted to form complexes and then, the complexes are polymerized with appropriate crosslinkers. After the removal of template molecules selective cavities are obtained which are complementary to template molecules in dimension, shape and functional groups.

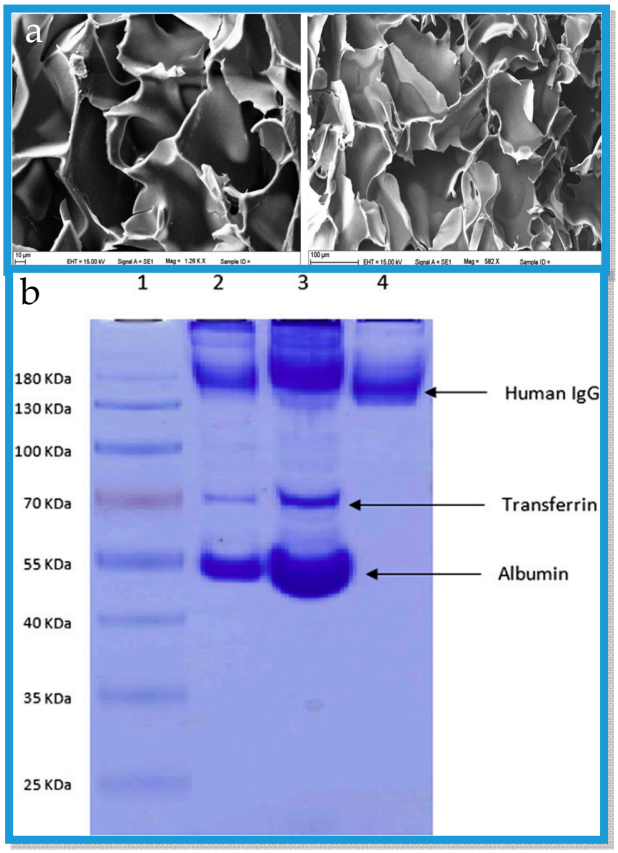

Figure 3. SEM images of PHEMA cryogels (**a**), and SDS-PAGE of IgG separation on the cryogel (Lane 1: Molecular mass markers, Lane 2: After adsorption of human plasma diluted (1/10), Lane 3: Before adsorption human plasma, and Lane 4: Bound eluted fractions (**b**) [29].

Chun and co-workers added human serum into the monomer solutions to synthesize molecularly imprinted polymers; thus, some abundant proteins were imprinted in the polyacrylamide cryogels. Albumin, serotransferrin, and most globulins were depleted with the columns packed with the molecularly imprinted polymers. Meanwhile, enrichment of less abundant proteins was carried out. After depletion, less abundant proteins were revealed by SDS-PAGE, peptide fingerprint analysis, and identified by Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS). This is an example that a "per se imprint" protocol enables to gradually dimidiate proteomes, simplify sample complexities, and facilitate further proteome profiling or biomarker discovery [30]. In Table 1 some different technologies for the separation of proteins published were listed.

Table 1. Some examples of cryogel applications for the separation of proteins.

Target	Matrix	*Functional Monomer/Ligand*	Method	Adsorption Capacity	Reference
Human serum albumin	Poly(acrylamide-allyl glycidyl ether) [poly(AAm-AGE)] cryogel	*Dye ligand Cibacron Blue*	Dye-affinity- based	74.2 mg/g	[31]
Human serum albumin	PHEMA cryogel	*Dye ligand Cibacron Blue*	Dye-affinity- based	950 mg/g	[12]
Human serum albumin	Poly(glycidyl methacrylate) beads embedded PHEMA composite cryogels	*Dye ligand Cibacron Blue*	Dye-affinity- based	342 mg/g	[15]
Human serum albumin	PHEMA-based cryogel	*N-Methacryloyl-Lphenylalanine (MAPA)*	Molecular imprinting	390.2 mg/g	[32]
Hemoglobulin	PHEMA cryogel	*N-methacryloyl-L-histidine methyl ester*	Molecular imprinting	11.4 mg/g	[33]
Ovalbumin	N-[3-(2-aminoethylamino) propyl] trimethoxysilane	*N-isopropylacrylamide and p-vinylphenylboronic acid*	Molecular imprinting	21 mg/g	[34]
Lysozyme	Polyacrylamide cryogels	*N,N-Methylene bisacrylamide*	Molecular imprinting	36.3 mg/g	[35]
Heparin	HEMA-based monolithic cryogel	*L-lysine*	Affinity-based	40,500 IU/g	[36]
Immunoglobin G	Poly(GMA) beads embedded PHEMA composite cryogels	*Iminodiacetic acid (IDA)-Cu^{2+}*	Immobilized metal affinity chromatography based	257 mg/g	[29]
Immunoglobin G	Fc fragment-imprinted PHEMA cryogel	*N-Methacryloyl-(l)-cysteine methyl ester (MAC)/ Immobilization of anti-hIgG*	Molecular imprinting	86.9 mg/g	[37]
Immunoglobin G	PHEMA composite cryogel disks	*Thiophilic ligand*	Thiophilic affinity-based	68.7 mg/g	[29]
Immunoglobin G	PHEMA cryogel	*Poly(ethylene imine) (PEI) -Cu^{2+}*	Immobilized metal affinity chromatography based	72.28 mg/g	[30]
Bilirubin	PHEMA composite cryogel	*N-methacryloyl-(L)-tyrosinemethylester*	Molecular imprinting	10.3 mg/g	[38]
Bilirubin	PHEMA cryogel	*N-methacryloyl-(L)-tyrosinemethylester*	Molecular imprinting	3.6 mg/g	[39]
Heparin	PHEMA cryogel	*N-[(3-dimethylamino)-propyl]methacrylamide)*	Molecular imprinting	9.36 mg/g	[40]
Cholesterol	PHEMA cryogel	*Anti-human β-lipoprotein antibody*	Affinity-based	129 mg/g	[16]
Anti-dsDNA antibodies	PHEMA cryogel	*Herring testes DNA*	Affinity-based	70,000 IU/g	[41]
IgM-antibody	PHEMA cryogel	*Protein A*	Affinity-based	42.7 mg/g	[25]

Besides the separation and removal of proteins, removal of metal ions plays important role in the treatment of diseases. The toxic effects of many heavy metals are hazardous to human health. Aluminum is a known neurotoxicant which can contribute to Alzheimer's disease [42]. Aluminum enters the brain through the blood-brain barrier, contributing to Alzheimer's disease and other neurodegenerative diseases. Demirçelik et al. prepared Al(III)-imprinted poly(hydroxyethyl methacrylate-*N*-methacryloyl-Lglutamic acid) (PHEMAGA–Al(III)) supermacroporous beads by a suspension polymerization technique for removal of Al(III). They reported maximum Al(III) adsorption capacity as 0.76 mg/g. They also reported relative selectivity coefficients of the PHEMAGA–Al(III) supermacroporous beads for Al(III)/Fe(III), Al(III)/Cu(II) and Al(III)/Zn(II) as 4.49, 8.95 and 32.44 times greater than those of the non-imprinted PHEMAGA beads, respectively.

Aslıyüce et al. [43] prepared iron imprinted PHEMA cryogel to remove excess Fe(III) ions from beta-thalassemia patient plasma. β-thalassemia is an autosomal recessive disorder characterized by a deficiency in the synthesis of β-chains. Therefore, impaired production of β-chain and unbalanced synthesis of α and β-chains leads to the destruction of red blood cells (RBCs). Chronic iron overload may occur in thalassemia. Iron intoxication is a life-threating form of poisoning. They complexed Fe(III) ions with *N*-methacryloyl-L-cysteine (MAC) monomer and the resulting MAC-Fe(III) complex were cryopolymerized in the presence of HEMA monomer. They reported maximum Fe(III) adsorption capacity onto cryogel as 75 μg/g. The Fe(III) imprinted poly(hydroxyethyl

methacrylate-*N*-methacryloyl-L-cysteine (PHEMA-MAC) cryogel was shown to be selective for the Fe(III) ions. They also reported relative selectivity as 10.5 and 2.3 for Fe(III)/Cd(II) and Fe(III)/Ni(II), respectively. The cryogels offer great alternatives via advantages of large pores, short diffusion path, low-pressure drop, and very short residence times for both adsorption and elution. Some different technologies for the removal of metal ions are listed in Table 2. It should be noted that there are many different approaches such as specific interactions, group-specific affinity, and molecular imprinting technology applied to remove metal ions.

Table 2. Some examples of cryogel applications for the removal of metal ions.

Metal Ion	*Functional Monomer/Ligand*	Method	Adsorption Capacity	Reference
Iron(III)	*N-methacryloyl-(l)-cysteine methyl ester*	Ion-imprinted cryogel	75 μg/g	[43]
Iron(III)	*N-methacryloyl-(L)-histidine methyl ester*	Affinity-based cryogel	1.79 mg/g	[44]
Aluminum(III)	*N-methacryloyl-L-glutamic acid*	Ion-imprinted polymer	0.76 mg/g	[42]
Iron(III)	*N-Methacryloly-(l)-glutamic acid*	Ion-imprinted polymer	92.6 μmol/g	[45]

Derazshamshir and co-workers synthesized novel cryogels for the depletion of hemoglobin from human blood. They synthesized MAH as a functional monomer for complexing with hemoglobin. The hemoglobin imprinted cryogel was prepared by free-radical polymerization below 0 °C. Figure 4 shows the morphology and porosity of hemoglobin imprinted supermacroporous cryogels.

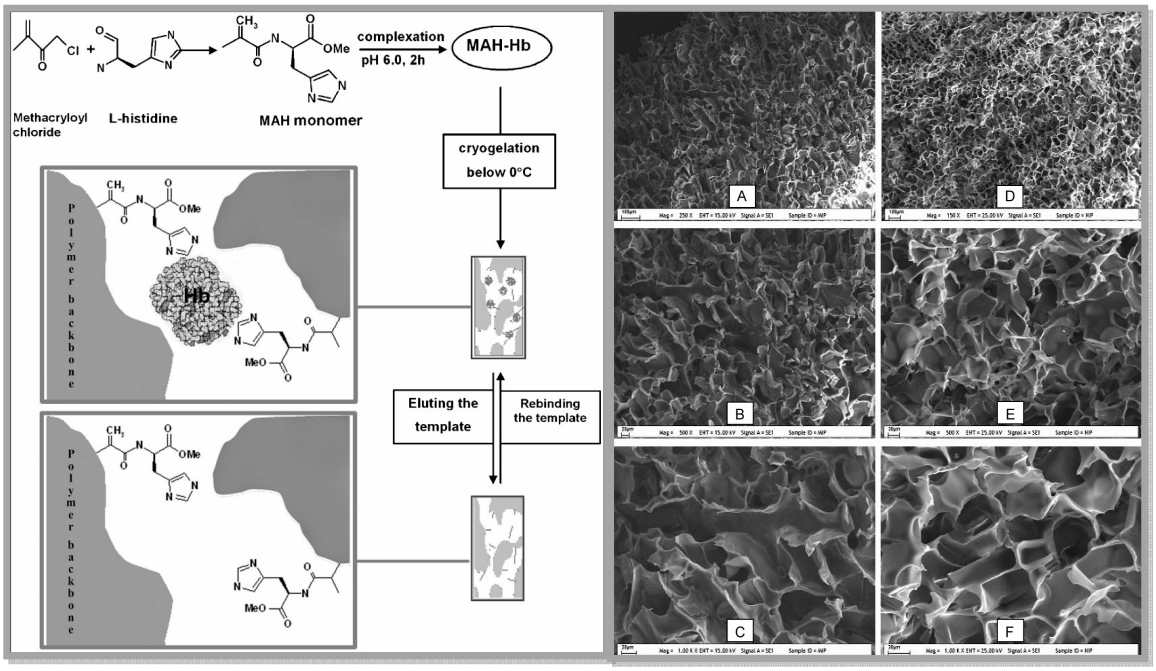

Figure 4. Schematic illustration of preparation of hemoglobin imprinted supermacroporous cryogels and SEM photographs of hemoglobin imprinted supermacroporous cryogels [33].

3.3. Controlled Drug Delivery

Drug delivery systems (DDs) are designed to deliver various drugs by using polymeric carriers at a certain time and specific sites. DDs are methods for applying a pharmaceutical compound to achieve a therapeutic effect. DDs improve therapeutic effects and reduce the toxicity of drugs. DDs have recently gained great attention of scientists in the fields of biomedicine and pharmacy. Four types of DDs can be reported such as diffusion-controlled, swelling-controlled, erosion-controlled and

stimulus-controlled. Various polymeric biomaterials have been developed to deliver drugs to human body [46]. DDs are classified as polymeric capsules, micelles, liposomes and gels according to dosage formulation of drugs. A core containing therapeutic drug and a polymer membrane outside the core are defined for a polymeric capsule. Thus, drug can be released by diffusion from the core through the membrane [47]. Supermacroporous cryogels are developed to deliver drugs to human body. The biocompatibility and controllable permeability of cryogels have caused extensively using in DDs for bioactive compounds in the last few decades. Stimuli-responsive cryogels have attracted a great deal of attention of researchers because of their sensitive response to a variety of physical and chemical changes. The structure of cryogels has been defined as smart DDs to fulfill the desired drug release profiles [19].

Supermacroporous polymeric structures have decent advantages such as high water content, non-toxicity, user-friendly, mild and efficient nature. Their reusability and easy storage offer added advantages. Therefore, these materials can be used in drug delivery due to their mechanical and chemical robustness, easy production, mechanical strength, and low cost. Cryogels offer distinctive three-dimensional structure materials for local delivery of therapeutic drugs. High blood-compatibility and resistance to degradation are great properties of these materials. These materials have good alternatives for implantable systems to deliver a large dose of drugs close to the tumor for sustained drug delivery. These materials also greatly decrease the toxic effects of the drug, and increase the therapeutic effect of chemotherapy [48].

Presently, researchers are working on the design of effective and low-cost gel-based drug delivery systems. Dinu et al. prepared three-dimensional biocomposites based on chitosan and clinoptilolite by cryogelation and investigated their potential application as drug carriers for diclofenac sodium and indomethacin. They reported the cumulative release of diclofenac sodium from the monoliths lower than 5% at pH 1.2 and higher than 70% at pH 7.4. In addition, they reported cumulative release 6% of indomethacin within the first hour in phosphate buffered saline (PBS) from composite cryogels [48]. The group of Denizli [19,49] prepared mitomycin C (MMC) imprinted cryogels for the delivery of MMC. They synthesized MMC imprinted poly(hydroxyethyl methacrylate-*N*-methacryloyl-(L)-histidine methyl ester)–Cu(II) [PHEMAH–Cu(II)] and MMC imprinted PHEMAGA cryogel membranes by free-radical bulk polymerization under partially frozen conditions. They synthesized these materials in different molar ratios of HEMA and MBAA at fixed amount of the template molecule for showing the effect of cross-linker in drug release rate. The increase of cross-linker ratio in the cryogel network resulted in the construction of more rigid and crosslinked polymers. They studied the release rate of MMC with different amounts of template and showed an increasing cumulative release of MMC with the increasing amount of MMC.

Caka et al. synthesized poly(hydroxyethyl methacrylate-*N*-methacryloyl-L-phenylalanine poly(HEMA-MAPA) membranes used for the controlled release of curcumin via UV-polymerization technique. Curcumin is used as a folk remedy and a drug for some certain diseases and cancers. They reported 7.4 optimal pH and 37 °C temperature for controlled release of curcumin. They obtained optimum values which were very compatible with in-body usage without any extreme release factors [50]. Çetin and Denizli have prepared 5-fluorouracil (5-FU) imprinted cryogel discs using a coordination complex between metal-chelate monomer MAH and 5-FU with the assistance of Cu(II) ion [51]. The complex was copolymerized with HEMA to produce PHEMAH cryogel discs. It was observed that the cumulative release of 5-FU decreased by the increasing amount of cross-linker, and 5-FU transport mechanism was found to be non-Fickian. In another study, Çetin et al. prepared an implantable and degradable molecularly imprinted cryogel for pH-responsive delivery of doxorubicin (DOX) [36]. The release rate of DOX from cryogel discs increased in more acidic conditions. Kinetic studies have shown that the general release behavior of molecularly imprinted cryogel discs was a combination of diffusion and erosion control. In another study, Kostova et al. prepared temperature-sensitive poly(ethoxytriethyleneglycol acrylate) cryogels for controlled releasing

of the hydrophilic drug verapamil hydrochloride. The controlled releasing of verapamil hydrochloride reported over a period of more than 8 h [52].

3.4. Cryogels for Tissue Engineering

The extracellular matrix (ECM) is a significant part of the cells and it has a great importance in maintaining support, arranging cell-cell communication, by the way, regulating cell growth, differentiation and survival [53]. ECM mainly contains of nm-sized fibers [54] and some macromolecules such as collagen, fibronectin, laminin and elastin [55]. These fibrous proteins play an essential role in cell coordination by enabling signal delivery, matrix reconstruction, etc. Cell-ECM interactions rely on signal transmission occurs in the cell membrane, by the way, organization between cytoskeleton and related molecules. When two-dimensional (2D) and three-dimensional (3D) structures of the ECM are compared, it has been proven that there is a dramatic well-defined difference in cell behavior in the form of 3D. Therefore, designated matrices should mimic the 3D structure of native tissue ECM. These matrices are conducted to support cell-ECM communication mentioned above [53].

Scaffolds can be identified as 3D biomaterials fabricated to carry out many functions. Desirable scaffolds acting as an artificial ECM should have some unique properties. They must be biocompatible by allowing cell adherence and movement towards onto the surface with stimulating remissible immune response. They also should be biodegradable for maintaining the substitution of own tissue of the cells. In addition, they must possess robust mechanical feature for efficient surgical processes and their structure should be coherent with the region into which it is planned to be implanted [56]. On the other hand, their interconnected pores provide the transportation of the nutrients within the cycle of cell-ECM-scaffold and enable the removal of wastes from the scaffolds [53,56,57].

Different methodologies are assembled to construct functional scaffolds serving as a cell supportive in tissue engineering. Therefore, scaffolds have potential use in the replacement/regeneration of tissues/organs and in the meantime regenerative medicine has gained great attention providing novel treatment methods for various steps of several diseases. Recently, several attempts were made to establish in vitro fundamentally target part of the body [56].

Scaffolds could be prepared in various structures such as microbeads, cryogels, hydrogels, ceramics and nanofibers. Many of them have been explored for a wide variety of applications including bladder, airway, skin, bone, ligament, tendon, liver, kidney, pancreas, intestine, esophagus, nerve, heart muscle and valve, cartilage regeneration [53]. It is noteworthy to highlight that the chosen scaffold construction method has a crucial role in the production of effective and accomplished materials, since scaffolds have similarity to their intended tissue. In recent years, cryogelation has become a rather novel approach which aims to improve tissue engineering. Among many scaffold structures, cryogels are considered as good templates and have high potential for the regeneration of indicated tissue parts. They are interconnected supermacroporous polymeric materials with 3D and hydrophilic property. Therefore, they can absorb high volume of tissue fluids and so that they got the edge over other groups of synthetic polymer networks with the advantage of swelling character and soft biocompatibility. It should be also noted that their gel formed structure with high porosity facilitates cell penetration, contact and migration preserving the chemical and mechanical stability. In addition, the preparation step of cryogels is very simple and cost-effective [53]. Considering all these features, it is not surprising that these interesting and individual polymeric materials has come up in tissue engineering in recent decades.

Natural and/or synthetic polymers, widely used for scaffold production, have been promising tools in biomedical applications. Natural polymers such as agarose, alginate, hyaluronic acid, chitosan, gelatin, collagen and silk show biocompatibility with eliciting minimal immune response. They also stimulate cell-cell interaction and have low toxicity. On the other hand, synthetic polymers such as poly-glycolic acid, PVA, poly-l-lactic acid, poly-caprolactone, polyorthoester have intelligently tailored unique features and manageable characteristic behavior. However, their usage can be problematic in terms of injection and leading infection; besides that, harmful byproducts may occur in course of

degradation. Apart from this polymeric material prepared via combination of natural and synthetic polymers, polymeric scaffolds and composites could be applied for tissue engineering applications. In this section, recent advances and current approaches in the usage of cryogels are briefly summarized by emphasizing the tissue engineering fields in which they situated: bioreactors, cell separation and artificial ECMs [53].

3.4.1. Bioreactors

Bioreactors are defined as devices which support biological reactions. They have a great importance in the cell cultivation providing control and monitoring of pH, temperature, oxygen and nutrient dissociation in microbiology. When tissue engineering was taken into consideration, bioreactors can be applied to develop new functional tissue in vitro with the production of regulatory signals in an appropriate physical environment. By this way, they stimulate cell proliferation, modification with the advantage of uniform cell distribution on 3D matrices and ECM generation before implantation step. Scaffolds are particularly well-designed candidates to store and deliver of the cells. Cryogels have an important role in the development of bioreactor matrix enabling high amount of mass transfer and have been attractive alternatives as bioreactors.

There have been several research publications highlighting the vital roles of cryogels in bioreactor platforms. A previous study regarding with the storage and transportation ability of the cells reported the application of both HEMA-agarose (HA) and gelatin cryogels for this purpose. Firstly, HA cryogel matrices seeded with C_2C_{12} cells, after five days, it was indicated that C_2C_{12} cells were successfully protected with high viability. In terms of gelatin cryogels, it was shown that C_2C_{12} cells, human hepatocellular liver carcinoma cell line (HepG2), human umbilical vein endothelial cells (HUVECs) were sustained by cryopreservation [58]. Efremenko, E.N., et al. were examined the capability of PVA cryogels in the storage of both gram-positive and gram-negative bacteria, yeasts, and filamentous fungi. This study revealed that the viability and effective reproductive potential of the microbial cells were kept at -18 °C for two years [59].

Polymeric cryogel matrices have been prominent tools for the cultivation of a large number of cells as well. In a previous study, copolymer of acrylonitrile and *N*-vinyl-2-pyrrolidone and interpenetrated polymer networks of chitosan and NIPA were prepared as cryogel matrices. These two types of cryogels with supermacropores the size of 20–100 μm provided operative transportation; besides that, they were triggered in vitro cultivation of fibroblasts and HepG2. On the other hand, both hemocompatibility and biocompatibility of these cryogels were proved. Consequently, cryogels as efficient bioreactor matrices enable hepatocyte immobilization and their ability for detoxification of plasma was clinically demonstrated in acute on chronic liver failure [60]. In another study, it was aimed to establish cryogels and use them as bioreactor systems to hydrolyze starch. For this purpose, the immobilization of alpha-amylase from *Aspergillus oryzae* was performed by cryopolymerization of AAm monomers. The effect of different experimental conditions (pH, temperature, concentration of starch solution) on starch hydrolysis were investigated. The results obtained in this study supports that the alpha-amylase immobilized cryogels are applicable for industrial processes [61]. In a study carried out by Jain, E. et al., different cryogels consist of polyacrylamide-chitosan (PAAC), poly(NIPA)-chitosan, polyacrylonitrile, and poly(NIPA) were investigated for hybridoma cell (6A4D7, B7B10, and H9E10) growth and monoclonal antibody (mAb) production. PAAC was demonstrated better activity on mAb production and cell growth. PAAC cryogel was also prepared as monolith, disks, beads and was applied for continuous packed-bed bioreactor. The monolith one has a good performance on antibody production with markedly high concentration. The data reported by this study suggests that the productivity of these systems may be broadened by different polymeric blends [62].

Cryogels have been represented to withstand cell behavior modification and stimulation of molecule generation. In a study, incorporation of polyallylamine plasma on the polyvinyl alcohol-tetraethylorthosilicate-alginate-calcium oxide (PTAC) biocomposite cryogel surface was

examined. Plasma polymerization was applied for obtaining different physical and chemical surface functionality to make changes on bone regeneration potential of human osteoblast cells [63].

3.4.2. Cell Separation

Cell separation was one of the preliminary applications of supermacroporous monolithic columns including cryogels. The accepted strategy known as fluorescence-activated cell sorting (FACS) was used for investigating cell populations. This method relies on fluorescent labeling, by this way, the cells could be divided into sub-populations. Another alternative approach for cell sorting identified as magnetic-activated cell sorting (MACS) allows separation and capturing of targeted cells from complex media. Apart from these two cell sorting standards, affinity-based cryogels have superior efficiencies with their more portable and cost-effective properties. In addition, cryogel adsorbents provide high surface area for the cell attachment and high liquid flow with low flow resistance [53].

In a study, microbial cell (histidine tagged *Escherichia coli*) separation have been performed using Cu^{2+}-IDA supermacroporous monolithic columns with continuously preserving cell viability [64]. Another example from literature consists of the ligand immobilization to the support matrices offers effective cell separation. 2-(Dimethylamino)ethyl methacrylate (DMAEMA), MBAA and AAm was preferred to synthesize of cryogel matrix using ion-exchange ligands. Amino groups of the ligands show affinity against *E. coli* cells with a 70–80% recovery ratio after desorption step. SEM photographs of matrices with bound *E. coli* cells were given in Figure 5 [65]. To give an example for virus capturing, streptavidin immobilized monolith was prepared to allow single-step capturing of biotinylated Moloney Murine Leukemia Virus (MoMuLV) from cell cultures. These matrices were synthesized using acrylamide, MBAA, and glycidyl methacrylate via cryopolymerization [66].

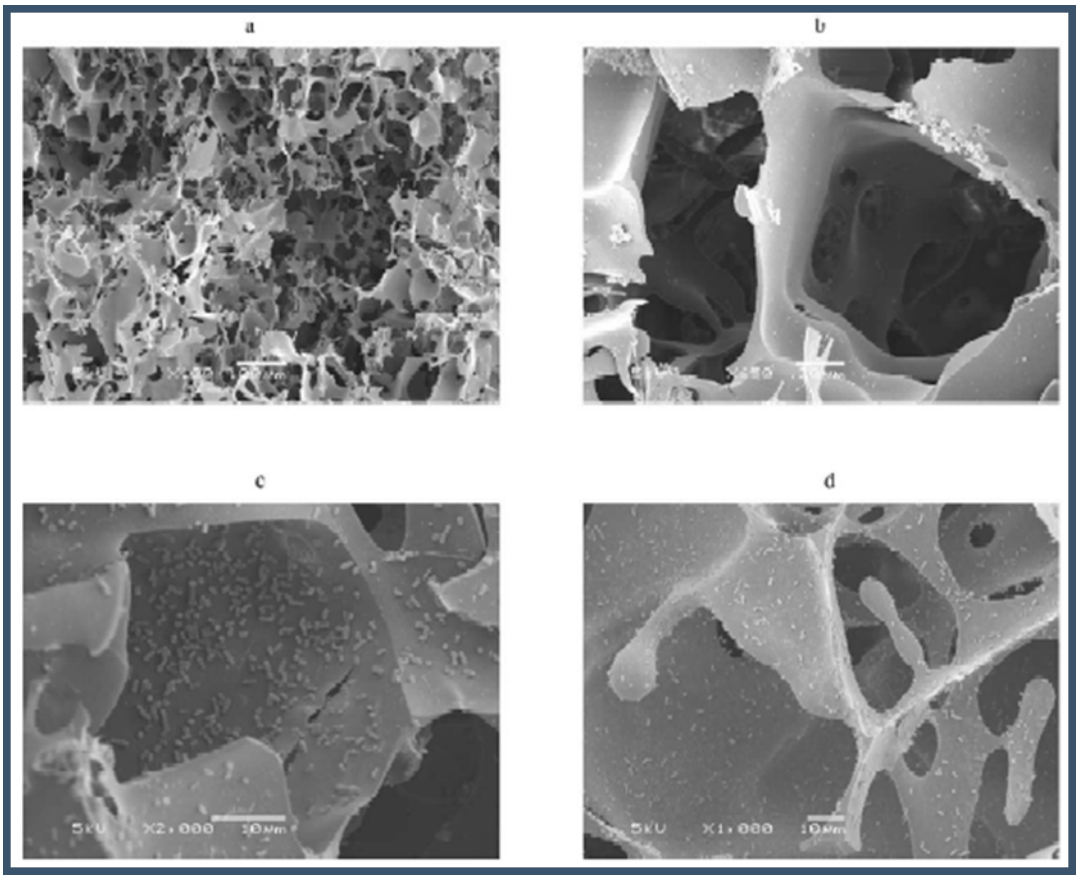

Figure 5. SEM photographs of matrices with bound *E. coli* cells [65].

Supermacroporous cryogels make it easy to process human body fluids including blood. These monoliths offer a high flow rate, enabling cells to pass through the porous matrix. In a previous study, bacteriophage immobilized poly(dimethylacrylamide) monoliths were applied for von Willebrand factor (vWF) capturing from blood samples [67].

Immunoglobulins playing crucial roles in immune system functioning are attractive molecules for the development of affinity-based cryogels. Antibody-affinity-based cryogels have been known as effective matrices for cell separation due to their specificity and variety. The interactions between immunoglobulins and antigens present on the cell surface enable specific cell separation such as T- and B-lymphocytes separation. In a study conducted in our research group, poly(hydroxyethyl methacrylate)/poly(ethylene imine)-Cu(II) (PHEMA/PEI-Cu(II)) cryogels were prepared for IgG purification from human plasma with immobilized metal affinity chromatography [30].

Separation of mammalian cells considers the availability of appropriate matrices necessary. Cryogels can well serve as adsorbents for the separation of mammalian cells. In a previous study, Kumar et al. (2004) prepared PVA cryogel beads and protein A immobilized dimethyl acrylamide (DMAAm) monolithic cryogel to specifically capture human acute myeloid leukemia KG-1 cells and fractionate human blood lymphocytes. The cell capturing capacity of protein A immobilized dimethyl acrylamide (DMAAm) monolithic cryogel was found to be higher (90–95%) than that of cryogel beads (76%). On the other hand, the separation of T- and B-lymphocytes was successfully performed with high yields.

3.4.3. Scaffolds

Tissue engineering have emerged for the repair, replacement and regeneration of defected bone, cartilage and skin tissues. Scaffolds have gained popularity as promising materials for these purposes. Critical-sized bone damages have been become an important problem. Their treatment using bone grafts bring along with some challenges such as high cost, integration and rejection problems, infection risk and morbidity. Cryogels have been introduced as a good choice to bone grafts [53].

A study reported that (PTAC) biocomposite cryogels have been explored as healing tools for critical-sized cranial bone defects in Wistar rats for 4 weeks. The result of this study emphasizes the significant role of cryogels in bone regeneration and even osteoblastic differentiation [68]. In a previous study, HEMA–lactate–dextran (HEMA-LLA-D)-based biodegradable cryogel scaffolds were applied in bioreactors for the 3D ingrowth of bone cells. Osteoblast-like cell line (MG63) was used to examine the effect on cell adherence and ingrowth [69].

Bone structure mainly consists of collagen and hydroxyapatite (HA) (osteoconductive component of bone. Therefore, HA-based cryogels were used for the reproduction of bone matrix. In particular, these cryogels incorporated with collagen were constructed with the advantages of human osteoblast-like cell proliferation, human bone marrow stromal cell differentiation. In another study, HA-gelatin-based cryogels were produced and physical features were examined indicating the applicability of these materials in bone regeneration [53].

Cryogels have also been introduced as treatment agents for several bone diseases with the usage of additives. In addition, fibrous protein has been inquired into cryogelation for bone healing and they provide increasing porosity and formation of elastic scaffold structure with remaining mechanical strength [53].

Injectable cryogels have recently gained great attention due to offering appropriate matrix for the attachment and growth of the cells with the combination of physicochemical characteristics of HA and adhesion features of gelatin. To give an example research, 3T3 cell adhesion could be improved by cultivation in hyaluronic acid-co-gelatin cryogels. In addition, these matrices possess a negligible influence on bone marrow dendritic cell activation, in other words, cytocompatibility was confirmed [70].

Cartilage consists of chondrocytes, progenitor cells, and ECM, and it is essential for minimizing surface friction on the site of bone. Cartilage damages could be treated by autologous chondrocyte

implantation (ACI) in which two operations even one is open are necessary with additional disadvantages of high cost, morbidity, iatrogenic pneumothorax atelectasis. Therefore, it is a requirement to use scaffolds including cryogels as a better alternative for the treatment [71]. In this field, cryogels could be modified by additives to enhance their applicability and they have also been implanted to observe their ability in treatment of cartilage damages.

Skin wound repair has become a critical challenge since burns, chronic wounds and accidents lead to substantial skin loss. Cryogels as tissue engineered scaffolds provide cell migration and serve as an attractive alternative to skin grafts. Furthermore, their spongy structure eliminates fluid gathering in wound. Especially chitosan and gelatin-based cryogels were mainly preferred for the treatment of skin wound. Cryogel scaffolds used in tissue engineering for bone, cartilage and skin tissues have been demonstrated in Table 3.

Table 3. Cryogels as scaffolds used in tissue engineering for bone, cartilage and skin tissues.

Scaffolds	Outcome	Reference
	Bone	
Collagen-nanoHA biocomposite cryogels	In vitro: Differentiation of human bone marrow stromal cells into osteoblastic phenotype. In vivo: Reduced inflammatory response by new tissue production	[72]
Collagen-nanoHA biocomposite cryogels	In vitro: Adherence and spreading of osteoblast cells along with high cell reproduction	[73]
Gelatin-HA cryogels	In vitro: Cell viability In vivo: Biocompatibility at the end of implantation within bone	[74]
Chitosan-gelatin composite cryogels incorporated with HA	Excellent cell compatibility and high mineralization with the usage of bone char cryogels	[75]
Silk fibroin, chitosan, agarose and HA with/without bioactive glass biocomposite cryogels	In vitro: No advantage of bioactive glass Reproduction of C_2C_{12} myoblasts and mesenchymal stem cells. Potential carriers for recombinant human bone morphogenicprotein-2 (rhBMP-2) and zoledronic acid (ZA)	[76]
Gelatin-HA cryogels with/without local vascular endothelial growth factor (VEGF)	Successful grafting of critical-sized bone defects in white rabbits. Favorable effect in early fracture healing	[77]
Gelatin and silk fibroin cryogels incorporated with Manuka honey (MH)	Long-term inhibition of bacterial growth with 5% MH-silk fibroin cryogel. Promising alternatives for chronic bone infections	[78]
Silk fibroin cryogels	Showing good performance on compressive modulus (50 MPa) and alternatives for bone scaffolds	[79]
Electrically stimulated PTAC biocomposite cryogels	Supporting the movement of C_2C_{12} myoblasts towards osteogenic lineage. In vivo and in vitro verification of osteoinductive and osteoconductive characteristics	[80]
3D Hyaluronic acid-based cryogels	Recommendable for bone tissue repair confirmed with optimization experiments	[81]
Poly(ethylene glycol) diacrylate-co-*N*-acryloyl 6-aminocaproic acid cryogels	Osteogenic differentiation of human mesenchymal stem cells (hMSCs) In vitro: Cell viability and proliferation of hMSCs In vivo: Promoted ectopic bone formation	[82]
Gelatin cryogel discs	Confirmation of electromagnetic stimuli effect on mesenchymal stromal cells isolated from the bone marrow High cell reproduction and osteogenic differentiation Increased production of bone matrix components	[83]

Table 3. *Cont.*

Scaffolds	Outcome	Reference
	Cartilage	
Collagenase incorporated cryogel	Isolation of chondrocytes and triggered proliferation in a sheep model	[71]
2-hydroxyethyl methacrylate–lactate–dextran (HEMA-LLA-D)-based cryogels	Synthesis of type II collagen Verification of biocompatibility with cartilage cells Chondrocyte attachment and infiltration 73.7 ± 3.3% cell viability after 15 days	[84]
Chitosan-gelatin cryogels incorporated with chondroitin sulfate loaded microparticles	Encouraged chondrocyte reproduction and differentiation Elevated matrix formation	[85]
Gelatin-oxidized dextran cryogels with bone morphogenetic protein-7 (BMP-7)	Improved matrix synthesis	[86]
Chitosan-agarose-gelatin cryogels	In vivo: Confirmed biocompatibility and production of hyaline cartilage in rabbits after implantation	[87]
Biomimetic gelatin/chondroitin-6-sulfate/ hyaluronan cryogel incorporated with chitosan	Up-regulation of glycosaminoglycans (GAGs) and type II collagen (COL II) secretion	[88]
	Skin	
Fibrinogen and gelatin containing cryogels	High migration of human dermal fibroblast by elevated fibrinogen concentration	[89]
Gelatin-fibrinogen cryogel	Reproduction of fibroblasts and migration of them within the scaffolds	[90]
Chitosan-gluconic acid conjugate/poly(vinyl alcohol) cryogels	High infiltration of inflammatory cells into wound In vivo: Healing of partial-thickness wounds with incorporation of basic fibroblast growth factor	[91]
Keratinase and enrofloxacin loaded pectin PVA cryogel	Controllable release of antibiotics	[92]
Bilayer cryogels, Top layer: antiseptic layer Polyvinylpyrrolidone-iodine (PVP-I) Bottom layer: regenerative layer Gelatin cryogel	Cell infiltration and attachment Proliferation of fibroblasts and keratinocytes In vivo: Regeneration ability was proven in implanted rabbits Resembling to commercial skin regeneration product Neuskin-F	[93]

3.4.4. Other Tissue Engineering Applications

Cardiovascular grafts usage has not been appropriate because of donor availability. If there is a treatment process, patients can be faced with such risk factors including hyperplasia, calcification and inflammation. In addition, using heart valves brings along with some challenges in terms of degeneration in the time period and calcification. Therefore, cryogels have been received attention for these applications. In a previous study, (PVA)-gelatin cryogels were preferred as an alternative to arterial grafts due to their mechanical usefulness. It was indicated that (PVA)-gelatin cryogels were applicable for the arterial endothelialization under shear stress leading high nitric oxide (NO) levels [94]. A different study reported alginate-based cryogels seeded with rat embryo cells have promoted angiogenesis and ECM formation when they were implanted into rats. Furthermore, these findings were supported in pigs [95].

Intervertebral disc problems could be occurred when the discs were damaged resulting in prolonged pressure, lowered vascularization, degenerative diseases. Cryogel applications have become an alternative to spinal surgery. In a study, PVA cryogels were applied by their ability to mimic artificial lumbar intervertebral disc with the advantage of their mechanical property. Optimization experiments were performed, and it was shown that compressive stress relaxation and creep levels decreased in high levels of PVA [96]. In another studies, silk fibroin incorporated PVA cryogels were constructed to enhance biocompatibility and gelatin-poloxamer 407 biocomposite cryogel were produced to extend water retention [97].

Another field of cryogel usage is neural cell adhesion. In a study, incorporated laminin gelatin/dextran cryogels were examined and it was reported that laminin supported cell attachment

and movement of neurons. In addition, there were no immune response during their implantation into brains of rat [98].

Cryogels have been also alternative biomaterials for muscle regeneration and functionalization. In a previous study, myoskeletal cell lines were cultured on PHEMA-gelatin cryogels and their potential on biocompatibility and cell reproduction were confirmed. Their highly porous structure and mechanical stability make them applicable in both skeletal and cardiac tissue engineering [99].

4. Conclusions

Cryogels are the polymeric materials synthesized by cryotropic gelation with the advantages of easy and cost-effective preparation. Physical and chemical parameters have significant effect on the functionalized cryogel formation. Cryogels have unique features such as interconnected and supermacroporous structure, and both mechanical and chemical stability. These desirable properties make them attractive materials for isolation, immobilization and purification of biomolecules, separation of whole cells. In addition, they have potential to be used in the field of environmental separations and even for heavy metal removal. On the other hand, there have been many well-documented studies performed regarding the construction of marketed cryogels for tissue engineering. Obtained results from those studies indicated that cryogels have been promising tools and applicable as scaffolds in a variety of tissue types. These materials also can be used in cell cultures and they are of great interest for both in vitro and in vivo applications. In such cases, they directly affect the improvement of cell migration, proliferation and differentiation with the advantage of enhanced mechanical stability. Evidently, cryogels with shape memories produced by molecular imprinting undoubtedly provide advantages for a wide range of biomedical applications.

Future trend research recently emphasized that it is possible to improve the potential of cryogels' usability and applicability by some modifications. When viewed from this side, incorporation of some additives to gain novel cryogel structures and functionalized matrix surfaces for several purposes and applications are described above.

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