

Recent Applications of Polyacrylamide as Biomaterials

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Abstract. The synthetic polymer, polyacrylamide derived from acrylamide monomer, was originally introduced for use as a support matrix for electrophoresis in 1959. Later, because of its applicability and economy, polyacrylamide has found widespread applications ranging from microanalysis to macro-fractionation for proteins, nucleic acid, and other biomolecules. On the other hand, recent years also witnessed increasing research interests in the potential of polyacrylamide acting as biomaterials. This review article will comprehensively present and discuss recent interesting patents classified according to the following highlights: (1) Enzyme immobilization within polyacrylamide gels; (2) Carriers for delivery of drugs and bioactive compounds; (3) Smart materials able to respond with stimulus; (4) Polyacrylamide-based matrices in extracorporeal toxin removal modalities; (5) Non-absorbable soft tissue fillers used for body contouring in reconstructive surgery or for cosmetic purposes. In summary, current researches and achievements of polyacrylamide have therefore well demonstrated its versatility and usefulness as biomaterials. Further developments of polyacrylamide-associated technologies will undoubtedly enhance the value and broaden the possibilities of applications of polyacrylamide in the field of biomaterials.

Keywords: Polyacrylamide, biomaterial, enzyme immobilization, drug delivery, extracorporeal toxin removal, smart materials, soft tissue fillers.

INTRODUCTION

Polyacrylamide, a synthetic polymer derived from acrylamide monomer, was originally introduced for use as a support matrix for electrophoresis in 1959 [1]. The polymerization reaction responsible for its synthesis can be rigorously controlled and standardized to produce uniform gels from highly purified reagents in a reproducible manner [2,3]. Generally, polyacrylamide gels result from polymerization of acrylamide with a suitable bifunctional cross-linking agent, most commonly, N,N'-methylenebisacrylamide (bisacrylamide) (Fig. 1). Gel polymerization is usually initiated with ammonium persulfate and the reaction rate is accelerated by the addition of a catalyst such as N,N,N',N'-tetramethylethylenediamine (TEMED). By adjusting the total acrylamide concentration (% T), polyacrylamide gels with a wide range of pore size can be readily made to suite size fractionation of a variety of proteins for practical purposes [4,5]. Because of its high resolving power, applicability to the entire molecular weight range, stability over wide pH intervals (pH 3-11), as well as simplicity and economy, polyacrylamide has found widespread applications ranging from microanalysis to macro-fractionation for proteins, nucleic acid, and other biomolecules, and become nowadays the medium of choice in all electrophoretic techniques [6-9].

In addition to electrophoresis using polyacrylamide gel, recent years also witnessed increasing research interests in the potential of polyacrylamide acting as biomaterials. This review will mainly cover the following highlights: (1) Enzyme immobilization within polyacrylamide gels; (2) Carriers for delivery of drugs and bioactive compounds; (3) Smart materials able to respond with stimulus; (4) Poly-

acrylamide-based matrices in extracorporeal toxin removal modalities; (5) Non-absorbable soft tissue fillers used for body contouring in reconstructive surgery or for cosmetic purposes.

ENZYME IMMOBILIZATION WITHIN POLY-ACRYLAMIDE GELS

Enzyme immobilization refers to the restriction of enzyme mobility within a fixed space [10] and has commanded increasing interests in a variety of applications because of its advantages over its counterpart, i.e., soluble enzymes, such as: (1) easy separation of enzyme from the product, which could minimize the effluent handling problems, (2) convenient enzyme preparation and reutilization, which would save the manufacturing cost, (3) establishment of a better microenvironment, which should improve the stability of enzyme activity as well as the quality of product [11-15]. Indeed, it is of particular interest to find that enzymes are normally tightly packed in cellular organelles for their action to take place as needed and some enzymes even only function when they are tightly bound to the membranes [16].

Initial attempts to immobilizing enzymes within polyacrylamide gels dates back to 1963. Berfeld and Wan prepared insoluble forms of enzymes by polymerizing acrylamide in the presence of antigens, enzymes, and other macromolecules materials to be embedded [17]. Since then, numerous efforts have been dedicated to the immobilization of enzymes using polyacrylamide as scaffold or matrix exploiting the above-mentioned advantages for attaining the desired purposes [18-25]. In this section, three current major methods for immobilizing enzymes, together with relevant patents regarding the use of polyacrylamide as matrix for enzyme immobilization are summarized and reviewed as follows.

(1) *Adsorption* is the attachment of enzymes on the surface of a support by weak physical forces, such as van der

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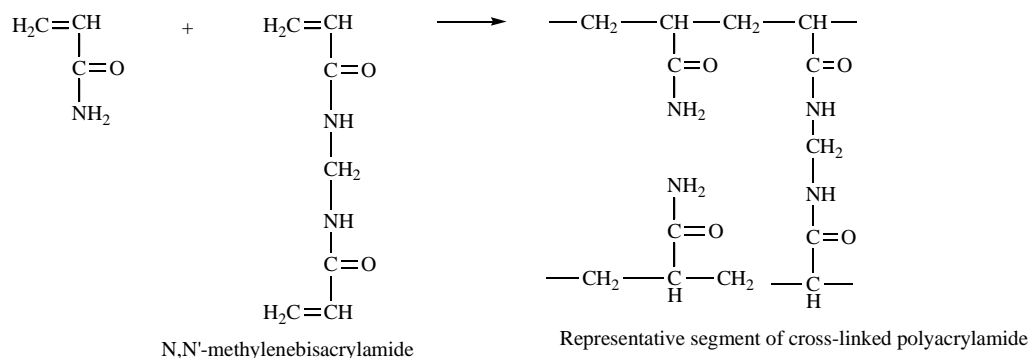


Fig. (1). The polymerization of acrylamide.

Waals force. The activity of adsorbed enzymes is almost not affected; however, desorption of enzyme during the course of reaction is inevitable, especially in the presence of strong hydrodynamic situations. To overcome this problem, adsorbed enzymes can be stabilized with glutaraldehyde treatment, albeit it could denature some enzymes [26-28].

Method for Immobilizing Dissolved Proteins

US4839419 describes methods for adsorbing an enzyme onto a small-particle support by washing said support with an aqueous solution of the protein and cross-linking said protein with a coupling component present in aqueous electrolyte-containing solution [29]. It has been found that the immobilization of dissolved proteins on a solid macroporous support which is water insoluble, or at most slightly swellable in water, can be carried out in a one step process if an aqueous protein solution containing an electrolyte and having an ionic strength of at least 0.15 mole/liter is allowed to wash over the support until the protein has been adsorbed thereon. If purely adsorptive immobilization is sufficient, the loaded support can simply be separated. As a rule, however, immobilization is stabilized by means of a cross-linking agent before, during, or after such adsorption.

What is particularly surprising is that the method of the invention makes it possible readily to obtain high absolute activities and outstanding activity yield even on widely differing nonspecific supports, which absolute activities and yields otherwise could be obtained only after a complicated activation of the support. Once cross-linked, the enzyme remains bound to the support even at low ionic strength and can be reused many times without appreciable losses of activity. One of examples illustrating the embodiment of this invention is binding of penicillinamidase to Oxirane-polyacrylamide resin with 85% activity yield and 172 U/g moist weight.

(2) **Covalent bonding** is the retention of enzymes on support surfaces by covalent bond formation. This method results in a stable combination of enzymes and supports; however, it suffers a disadvantage of possible enzyme deactivation because of direct chemical modification [30-32].

Method for Immobilizing Enzyme

US4073689 relates to a method for immobilizing an enzyme utilizing the specific chemical reactivity of N-

halogenoamide group [33]. The N-halo-genoamide compound is formed as an intermediate compound in the Hofmann's decomposition of acid amide. That is, when an amide compound, e.g., homopolymers and graft polymers of acrylamide or methacrylamide, is reacted with a hypohalogenoxyacid ions (OX⁻) under strong alkalinity, an amine is formed through an N-halogenoamide compound. In an illustrative embodiment, α -amylase was immobilized in the gel of polyacrylamide. The enzyme is immobilized to the polyacrylamide chains not only by physical but also by chemical bond and therefore, will not flow out even by repeated washings.

Immobilized Aminoacylase Enzyme

US4608340 describes methods for immobilizing aminoacylase by covalent bonding of amino-acylase to a partially hydrolyzed Akrix P type acrylamide-N,N'-methylene-bis (acrylamide) copolymer [34]. Covalent bonding is carried out by partially hydrolyzing the copolymer with a base or an acid to form carboxy groups, activating the carboxy groups with a carbodiimide and coupling aminoacylase to the activated carboxy groups. An immobilized aminoacylase enzyme product with binding capacity of 6.14 milliequivalents/g was prepared by the claimed process.

Crosslinked Polyacrylamide-Sulfhydryl Polymer for Immobilization of Biologically Active Substances

US4898824 relates to the preparation of a water-insoluble, cross-linked polyacrylamide copolymer having recurring monomeric residues of acrylamide, bisacrylamide or analogs thereof, and N,N'-bisacrylyl-cystamine or analogs thereof [35]. The cross-linked copolymer can be formed into discrete particles having cross-linking disulfide groups on their external surface which can be reduced to provide activated particles having exposed chemically active sulfhydryl groups. The activated poly-acrylamide particles are free of residual amino and carboxylic acid groups and can be coupled with suitable biologically active substances such as haptens, antigens, antibodies, lectins, enzymes, receptor proteins. An immobilized reagent comprising a glycopeptide covalently bound to the external sulfhydryl functional groups of the crosslinked polyacrylamide-sulfhydryl gel particles was prepared.

(3) **Entrapment** is the physical enclosure of enzymes in suitable matrices. When immobilizing in a polymer matrix,

enzyme solution is mixed with polymer solution before polymerization. Subsequently, polymerized gel-containing enzyme is either extruded or a template is used to shape the particles from a liquid polymer-enzyme mixture. With optimal pore size and proper configuration, the support matrices can keep firm hold of intact enzymes, while permit selective passage of small substrate molecules. Enzyme activity and stability are retained to a satisfactory extent under favorable microenvironmental conditions [36,37].

Enzyme Immobilization by Entrapment in a Polymer Gel Matrix

US4978619 provides a method which comprises dispersing a fine powder containing an enzyme in a solution having a polymerizable monomer or a prepolymer dissolved in an organic solvent, then polymerizing the monomer or prepolymer thereby giving rise to a gel, subsequently displacing the aforementioned organic solvent with an aqueous solvent, and enabling the dispersed and immobilized enzyme to be entrapped within the reticulated gel [38]. Consequently, the enzyme in an activated form is entrapped in the gaps formed in the gel. By optimizing the size of the meshes of the gel destined to form the aforementioned gaps, the ratio of immobilization of the enzyme is heightened, the ratio of leakage of the enzyme is lowered, the immobilization of a plurality of enzymes of different types is attained with ease, and the enzyme is allowed to come into ample contact and react efficiently with a substrate under treatment. One of examples illustrating the embodiment of this invention is entrapment of a freeze-dried formic acid dehydrogenase (FDH) within polyacrylamide. Initially, freeze-dried FDH, 2.5 units, was comminuted and dispersed in 2 ml of dimethyl sulfoxide containing 360 mg of acrylamide and N,N'-methylenebisacrylamide. After polymerization, the gel consequently formed was chopped into cubes of about 0.2 mm with a cutter and washed by stirring overnight in 1 liter of a 0.1M tris-hydrochloride buffer (pH 7.5) to effect displacement of the solvent in the gel. The washed gel cubes were suction filtered for removal of the washings. Consequently, an immobilized enzyme (3.3 g) was obtained. The activity tests for the the washings of gel and immobilized FDH indicated that immobilized enzyme consequently obtained suffered substantially no leakage of enzyme and the activity of the immobilized FDH was substantially the same as that acquired at the time of preparation. Thus, the immobilized enzyme produced herein possessed high stability.

CARRIERS FOR DELIVERY OF DRUGS AND BIOACTIVE COMPOUNDS

To overcome the inherent limitations and problems associated with the conventional drug administration, the development of drug delivery system (DDS) is gaining increasing interest for the past decades. The drug delivery systems are comprised of a therapeutic agent incorporated in a carrier, which maintains desired plasma level, i.e., without reaching a toxic level or dropping below the minimum effective level, over the required duration. Compared to the conventional single dose administration, DDS typically require smaller and less frequent dosages and minimize concomitant side effects. Ideally, reproducible and predictable kinetics of releasing drug has been obtainable with the

development of DDS products already available on the market [39-43].

Since the first demonstration that polyacrylamide could be used as an implantable carrier for sustained delivery of insulin to significantly lengthen the life of diabetic rats [44], various drug delivery systems based on polyacrylamide have been developed [45,46]. Furthermore, polyacrylamide also finds useful applications as carrier for other bioactive macromolecules and cells to produce the desired effects [47-50].

Typically, the mechanism of drug release from carrier can be diffusion-limited, chemically controlled erosion of carrier and others. These mechanisms can be illustrated by the following patents related to the application of polyacrylamide to drug delivery systems.

Polymeric Microspheres

US6720007 features core-shell microsphere compositions, hollow polymeric microspheres, and methods for making the microspheres [51]. The micro-spheres are characterized as having a polymeric shell with consistent shell thickness. Uniform hollow polymeric microspheres were made by using surface confined living radical polymerization. Using the silica microsphere as a sacrificial core, hollow microspheres were produced following core dissolution. First, a controlled/living polymerization was conducted using an initiator attached to the surface of silica microparticles to initiate atom transfer radical polymerization. This procedure yielded core-shell microparticles with a silica core and an outer layer of covalently attached, well-defined, uniform thickness polymers. The silica cores were subsequently dissolved by chemical etching, resulting in hollow polymeric microspheres. Shell thickness was controlled by varying the polymerization time. This method is applicable for preparing a variety of hollow polymer microspheres. This approach may allow for the fabrication of different shapes of hollow polymeric materials produced from a variety of templates.

Such an approach can be applied to the fields of materials encapsulation and drug delivery. Drugs such as tranilast or ibuprofen are encapsulated in polymeric microspheres [45]. The spheres are used to slowly release drug over time in the digestive tract. Biocompatible hydrogels such as polyacrylamide-chitosan are useful for sustained antibiotic release [52]. For example, microspheres with a core size of approximately 3 micrometers are used for drug delivery. Microspheres produced by living polymerization are more advantageous for drug delivery applications because of the consistency in shell thickness and porosity. In contrast, the shell thickness of the microspheres produced by existing technology cannot be controlled during the polymerization. Being able to control the shell thickness and therefore the rate of drug release from the microspheres is a significant advantage of the microspheres of the invention. Microspheres produced for delivery of therapeutic products are washed with water or a physiologically-compatible buffer (e.g., phosphate-buffered saline) following the etching procedure to remove the silica template and residual etching agent. The microspheres are then contacted with a thera-

peutic agent in solution phase. The microspheres are loaded with the agent by diffusion.

Hydrogels and Water Soluble Polymeric Carriers for Drug Delivery

US7186413 includes carriers for drug delivery, methods of making such carriers and for associating them to drugs, the resulting carrier and drug combination and methods for drug delivery, particularly controlled or sustained release delivery, using such carrier and drug combinations [53]. A part of this invention involves modifying polymers, e.g., polyacrylamides, so that they can reversibly bind multiple molecules of drug per molecule of polymer. Any polymer which is biocompatible, water-soluble, preferably of less than 80,000 dalton molecular weight and can be bonded by a degradable covalent bond to a drug, can be used. The conjugate of drug and polymer can be injected as a prodrug which will give a sustained release of active drug over time, i.e., as the degradable bond hydrolyzes. This method can be used as a means to decrease the toxicity of the free drug, economize on the amount of drug given by increasing circulation time, and help to solubilize hydrophobic drugs. For example, because the active form of the drugs are released over time, the concentration of the active form of the drug at any given time can be minimized to levels where it is not substantially detrimental to certain organs. Further, conjugation with the polymer can be used to prevent a large portion of the drug from being eliminated through the kidneys before it has been able to act on the desired area. The linking of the polymer to the drug can be carried out through any of a number of chemistries which will provide a degradable covalent bond between the polymer and the drug or a prodrug which is degradable covalently bonded to release the active drug. For example, for the water-soluble polymeric drug carriers aspect of the invention, economical poly-hydrazides can be synthesized from low molecular weight polyacrylamides (Fig. 2). Hydrazinolysis of aqueous 50% w/w polyacrylamide in water (molecular weight average=10,000, 50 ml) by refluxing for three hours with aqueous hydrazine (35% w/w, 100 ml) yielded the poly(acrylic hydrazide-co-acrylamide) in quantitative yield. The product was precipitated by the addition of an equal volume of ethanol. The product was dried under reduced pressure. The solid was re-dissolved in water and dialyzed extensively until the dialysate gave a negative TNBS test. The solution was frozen and lyophilized to yield a white powder. TNBS analysis of the product indicated that 52% of the amide groups of polyacrylamide were transformed to hydrazide groups. The degree of substitution of amide groups can be controlled by varying the temperature, time, and equivalents of hydrazide used.

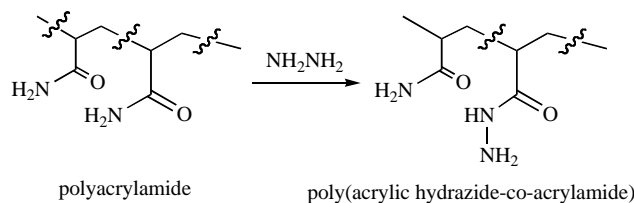


Fig. (2). Synthesis of poly-hydrazides from low molecular weight polyacrylamides.

Enhanced Skin Penetration System for Improved Topical Delivery of Drugs

US5874095 involves pharmaceutical compositions comprising certain specific non-ionic polymers which may be applied topically to the skin and which result in improved transdermal penetration of the drugs through the skin [54]. These compositions also have a high solvent tolerance, i.e., high level of solvents such as alcohol and other water-soluble components which may be necessary to solubilize the active can be included in the compositions. The non-ionic polymers useful in the present invention are polyacrylamides and substituted polyacrylamides, branched or unbranched. These polymers are non-ionic water-dispersible polymers which can be formed from a variety of monomers including acrylamide and methacrylamide which are unsubstituted or substituted with one or two alkyl groups (preferably C₁-C₅). These non-ionic polyacrylamides having a molecular weight of from about 1,000 kDa to about 30,000 kDa are present at a level from about 0.05% to about 20%. Compositions for anti-acne and/or analgesic, dermatological disorders, sunless tanning, are made utilizing conventional mixing techniques and display skin penetration of the actives as well as improved skin feel and residue characteristics together with excellent moisturizing, emolliency, rub-in and absorption characteristics.

Alcohol-Free Transdermal Insulin Composition and Processes for Manufacture and Use Thereof

US7033998 discloses a dermal delivery system composition comprising an aqueous base vehicle including American Emu oil, Isopropyl Palmitate (PROTACHEM IPP), PEG-8 (a polyethylene glycol available under the trade name PROTACHEM 400), methylsulfonylmethane (MSM) and SEPIGEL 305 (a combination including polyacrylamide/C₁₃-C₁₄ Iso-paraffin and Laureth-7) in combination with a therapeutically affective amount of at least one species of insulin, and to processes for the manufacture and use thereof [55]. Application of a transdermal insulin delivery system/composition in accordance with this invention demonstrated that the insulin transdermally entered the circulatory system in therapeutically effective quantities, thereby affecting a change in sugar levels in the body.

Cross-Linked Polymeric Nanoparticles and Metal Nanoparticles Derived Therefrom

US7189279 provides a process for preparing internally cross-linked, stable polymeric materials, in the form of substantially spherical particles, each particle consisting essentially of a single macromolecule [56]. They can be prepared by dissolving polymeric material in a solvent system to form a solution of the polymeric material at a concentration therein of less than the critical concentration for the polymer, causing the polymeric material to contract into an approximately spheroidal conformation in solution, cross-linking the polymeric material in solution in said spheroidal conformation so assumed, and recovering stable, cross-linked approximately spheroidal polymeric particles from the solution. Polymer particles of very small size, average diameter in the range 0.1-10 nanometers (nanoparticles), can be made in this way. Examples of useful polymers in the present invention include polymers and

copolymers derived from such monomers as styrene, vinyl naphthalene, styrene sulphonate, vinyl naphthalene sulphonate, acrylic acid, methacrylic acid, methylacrylate, acrylamide, methacrylamide, acrylates, methacrylates, acrylonitrile, N-lower alkyl acrylamides and the like. One preferred embodiment of the invention involves the use of polymers having a critical solution temperature, i.e. a temperature below which they are soluble in water, and above which they are insoluble in water. Using the process of the present invention, such polymers can be dissolved in water, caused to assume a condensed, spheroidal conformation and internally cross-linked as described. They can then be used for delivery and controlled release of other organic compounds such as drugs. The drug can be dispersed in a suspension of the cross-linked polymer at a temperature above the critical solution temperature, at which the drug will be absorbed by the polymer in its collapsed-particulate form. When the temperature is reduced below the critical solution temperature, the polymer particle swells and slowly releases the drug. Polymers having critical solution temperatures include polymers of N-isopropylacrylamide (NIPAM), which has a lower critical solution temperature (LCST) of 31°C (Fig. 3). In a specific example, 100 mg of polyNIPAM with a molecular weight of 200,000 g/mole was dissolved in 100 ml water at 20°C. and was cross-linked with 10 megarads of γ radiation. After isolation and purification, the internally cross-linked 5-10 nm nanoparticles can be used for the controlled delivery of other organic compounds. For example the drug can be absorbed by the collapsed particle in a water dispersion above LCST. After removal of the unabsorbed drug, the dispersion will remain stable until the temperature of the water is reduced below LCST, at which point the particle swells and slowly releases the drug. Since the size of the internally cross-linked nanoparticle is extremely small (~10 nm) it can access almost any part of the human body including the smallest blood capillaries which makes it of interest in a variety of medical therapies.

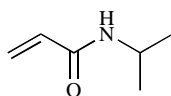


Fig. (3). Structure formula of N-isopropylacrylamide.

SMART MATERIALS ABLE TO RESPOND WITH STIMULUS

As the reasons mentioned in the previous section, controlled drug delivery systems have been used to overcome the shortcomings of conventional dosage forms. Nevertheless, it could be more beneficial if the amount of drugs or active agents released can be affected according to physiological needs. These include the delivery of insulin for patients with diabetes mellitus, antiarrhythmics for patients with heart rhythm disorders, gastric acid inhibitors for ulcer control, nitrates for patients with angina pectoris, etc. [57] Recently, increasing research attention has been given to the development of synthetic polymers that can respond with dramatic property changes to small changes in their environment. They are known as “stimuli-responsive” or “smart” polymers [58-62]. Smart polymers can be classified according to the stimuli they respond to as: temperature-,

pH-, ionic strength-, light-, electric- and magnetic field-sensitive. Some polymers respond to a combination of two or more stimuli. The response mechanism is primarily based on the chemical structure of the polymer network, e.g., the functionality of chain side groups, branches, and crosslinks. For example, polyacrylamide microgels derivatized by saponification of the -CONH₂ group to the -COOH group are responsive to pH and ionic strength of the external medium [63]. Polyacrylamide that contains rationally designed single-strand DNA (ssDNA) as the crosslinker can shrink and swell in response to ssDNA samples and recognize a single base difference in the sample [64].

Among these polymers that can respond to external stimuli, poly-N-isopropylacrylamide (PNIPAA) has been widely examined as a smart drug delivery material because of its unique phase separation behavior upon external temperature change. The following patents are related to the application of polymers of acrylamides or its derivatives as smart materials.

Gel Pad Arrays and Methods and Systems for Making Them

US6682893 features a method of providing a gel having a substance disposed within the gel [65]. The method includes:

- (1) providing a substrate on which is disposed a gel, e.g., a gel pad or an array of gel pads, and wherein said gel is an intelligent gel, capable of existing in an expanded and a contracted state;
- (2) contacting the intelligent gel, while in the expanded state, with the substance, e.g., a solute in a solution, and allowing the substance to enter the gel;
- (3) causing the expanded intelligent gel to contract, wherein upon contraction molecules of the substance remain in the gel, thereby forming a gel having a substance disposed, e.g., concentrated or captured, within the gel.

In a preferred embodiment the intelligent gel includes, an enzyme, e.g., glucose oxidase, and the reaction of the enzyme with its substrate, e.g., glucose oxidase with glucose, changes the pH of the gel. Thus, in the presence of the analyte, e.g., glucose, in a sample solution which is brought into contact with the gel pad, the gel pad will shrink. A gel pad can be provided adjacent to a piezocrystal, such that changes in gel pad swelling produce a piezoelectric signal, which can be detected and correlated with the glucose concentration. In a preferred embodiment the gel is chosen from the group of N-alkylacrylamides polymers, e.g., N-isopropylacrylamide (NIPA) and N,N-diethylacrylamide (DEAAm). The gel pad arrays can be used for sequencing by hybridization (e.g., where the pads include nucleic acid strands immobilized within the gel matrix), for cell based assays (e.g., where the pads include, or are adjacent to and contacting, living cells), and for other uses which will be apparent to one of ordinary skill in the art.

Temperature-Responsive Polymer/Polymer Complex

US6863437 provides a polymer mixture which forms an inter-polymer complex, by being responsive to temperature even under neutral to alkaline conditions [66]. The polymer

mixture contains a poly-N-acetylacrylamide or polyvinyl alcohol and polyethylene glycol, polyacrylamide or polymethacrylamide. According to the invention, by fixing a ligand having the ability to recognize molecules, the aforementioned inter-polymer complex can be broadly applied to separating agents, assay reagents, immobilized enzymes, denatured protein modifiers, separation method or concentration of microorganisms, purification or concentration of nucleic acids, drug-releasing microcapsules and the like. One of examples illustrating the embodiment of this invention is the preparation of an inter-polymer complex of poly-N-acetylacrylamide and polyacrylamide. When 1.3 g of poly-N-acetylacrylamide having a molecular weight of about 13,000 was mixed with 1.4 g of polyacrylamide having a molecular weight of about 14,000 in 10 g of purified water, an inter-polymer complex was formed. The thus obtained inter-polymer complex showed lower critical solution temperature (LCST) in the aqueous solution, and the temperature was 23°C both at the time of temperature up and temperature down. In this connection, measurement of LCST was carried out after adjusting pH of the aqueous solution to 7.4.

Gels with a Shape Memory

US6538089 relates to a physically cross-linked copolymer comprising hydrophobic monomers, hydrogen bonding monomers, and thermosensitive monomers, said thermosensitive monomers having a distinct phase change at its lower critical solution temperature (LCST) when existing as a homopolymer [67]. This completely novel, non-chemically cross-linked, thermo-sensitive polymer can separate from the solution on increasing temperature at or over its lower critical solution temperature (LCST) to form a physically cross-linked temporary gel, to yield the shape of the holding vessel. This gel can shrink substantially but still maintain the shape of the vessel. The shrunken polymer gel can be reshaped in another form, simply by dissolution on cooling below its lower critical solution temperature (LCST) and repeating the casting process using a different shaped vessel. Preferably, the thermosensitive physically cross-linked gel is formed only at a narrowly defined combination of three monomers, 1) N-isopropyl acrylamide (48-58%); 2) N-acryloyl hydroxy-succinimide (23-33%) and 3) styrene (19-23%). The physically cross-linked copolymer is used for drug delivery system or for enzyme delivery system. The physically cross-linked copolymer is also used for casting shapes of cavities, for production of miniaturized, detailed micro-parts or micro-machine parts, or for production of thermal switches.

Interactive Molecular Conjugates

US5998588 relates to the combination of the capabilities of stimuli-responsive components such as polymers and interactive molecules to form site-specific conjugates which are useful in a variety of assays, separations, processing, and other uses is disclosed [68]. The polymer chain conformation and volume can be manipulated through alteration in pH, temperature, light, or other stimuli. The interactive molecules can be biomolecules like proteins or peptides, such as antibodies, receptors, or enzymes, polysaccharides or glycoproteins which specifically bind to ligands, or nucleic acids such as antisense, ribozymes, and aptamers, or ligands

for organic or inorganic molecules in the environment or manufacturing processes. The stimuli-responsive polymers are coupled to the recognition biomolecules at a specific site so that the polymer can be manipulated by stimulation to alter ligand-biomolecule binding at an adjacent binding site, for example, the biotin binding site of streptavidin, the antigen-binding site of an antibody or the active, substrate-binding site of an enzyme. Binding may be completely blocked (i.e., the conjugate acts as an on-off switch) or partially blocked (i.e., the conjugate acts as a rheostat to partially block binding or to block binding only of larger ligands). Once a ligand is bound, it may also be ejected from the binding site by stimulating one (or more) conjugated polymers to cause ejection of the ligand and whatever is attached to it. Alternatively, selective partitioning, phase separation or precipitation of the polymer-conjugated biomolecule can be achieved through exposure of the stimulus-responsive component to an appropriate environmental stimulus. Examples demonstrate formation of a site-specific conjugate by genetically engineering a protein, streptavidin, to insert a coupling site, then coupling a temperature-responsive polymers, polyNIPAAm, to the coupling site. The physical relationship of the polymer to the biotin binding site of the streptavidin is controlled by altering the temperature of the reaction; i.e., at low temperatures, 100% of the biotin is bound, at higher temperatures, 37°C, significantly less biotin is bound.

POLYACRYLAMIDE-BASED MATRICES IN EXTRACORPOREAL TOXIN REMOVAL MODALITIES

An extracorporeal toxin removal modality refers to the device placed outside the body where blood totally or partially diverted from the heart or arterial system is subjected to processing to remove unwanted toxic substances, and subsequently returned to the circulation. The extracorporeal devices have been conceived to be simple, efficient and economical to the patients; their easy accessibility, lower chances of infection and immune rejection, as well as the avoidance of a major traumatic surgical procedure have made these devices popular [69-72].

The role that polyacrylamide plays in an extracorporeal toxin removal modality is similar to that it play in enzyme immobilization. That is, to provide a support matrix for immobilization of the functional parts or ligands. Because of its chemical inertness as well as the stability over various conditions, polyacrylamide has been employed, whether clinically or under development, to serve as a useful matrix for several types of extracorporeal toxin removal devices, as can be exemplified by the following patents.

Method for Eliminating Potentially Toxic and/or Harmful Substances

WO02081006 describes a method for eliminating potentially toxic and/or harmful substances, wherein particles which are capable of binding, taking up and/or carrying the toxic and/or harmful substances, are removed from a body fluid in an extracorporeal step or in an extrinsic or exogeneous device [73]. A particular advantage of the method according to the present invention is that toxic therapeutic agents can be removed after their peak effect by means of the particulate carrier from a suitable body fluid, in

particular from blood, after they were applied for therapeutic purposes in the form of suitable and, in general, known particulate carrier systems. The toxicity of the agent is reduced as a by-effect, whereby the improved tolerability and reduced toxicity of conventional, site-specific and/or target-directed drug delivery systems based on an agent/carrier unit act in combination with the very efficient option of eliminating these macroscopic agent carriers. Moreover, the toxic substances are removed from the natural clearance cycle and/or physiological metabolism which spares the organs specialized on natural detoxification such as the liver, bile, kidneys, etc. Depending on the size and type of the particles to be eliminated from a body fluid, processes based on the precipitation, filtration, chromatography and/or adsorption of the particles are particularly suited for eliminating said particles. According to the invention, it has been found that for instance conventional blood apheresis procedures can be used for this purpose. Apheresis procedures of this type are known for instance for the selective reduction of the low-density lipoprotein (LDL) content of blood in the treatment of hypercholesterolemia. One option based on the adsorption technique involves that the adsorption is mediated by electrostatic interactions between the particles containing charged groups or being ionizable, and the adsorbent material carrying the corresponding opposite charge or being oppositely ionizable. Suitable materials for this adsorption principle include polycationic or polyanionic adsorbent materials for eliminating the particles carrying oppositely (negative or positive) charged or ionizable groups. The polycations or polyanions may be ligands that are covalently bound to the corresponding adsorption carrier materials, such as polyacrylamide. In this context, reference can be made to the DALI, apheresis procedure i.e., direct adsorption of lipoprotein, because it is particularly well-suited and efficient [74-78]. Although hitherto known only for treatment of hypercholesterolemia, this procedure can also be applied for eliminating carrier particles, as those specified by the present invention, for toxic substances or agents other than LDL particles, e.g. liposomes. The main advantage of the procedure is that the purification step can be performed on whole blood. The approach of the DALI technique is based on a combination of adsorption and size exclusion chromatography (gel chromatography). For this purpose, the small adsorber beads are provided to be porous and have a mean diameter of 150-200 μm with a coating of polyanions, in particular polyacrylate, adhering to the inner and outer surfaces (of the pores) as adsorbent materials.

Method for Formation of a Stationary Phase in an Immunoabsorption Wall

TPI248827 provides a method for formation of a stationary phase in an immunoabsorption wall based on polyacrylamide which is aimed for removal of certain toxins from blood [79] (Fig. 4). This method, i.e., a partially incomplete two-stage polymerization method [80-83], comprises of steps of (1) forming a supporting gel layer, (2) loading a stacking solution with immunoabsorbents onto top of the supporting gel layer, (3) completing a sedimentation process

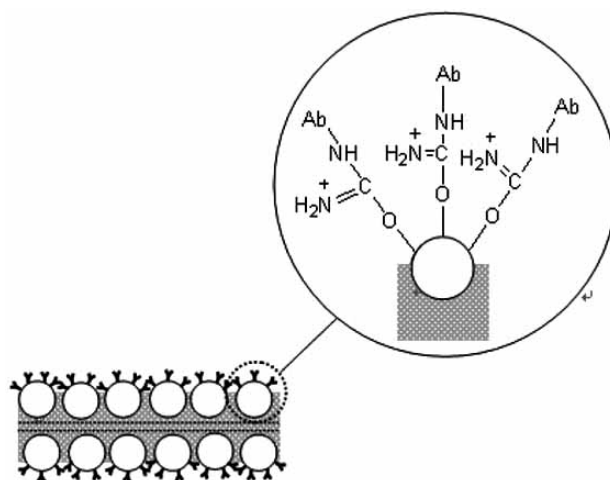


Fig. (4). Schematic representation of an immunoabsorption wall.

of the immunoabsorbents while the stacking solution remains in an unpolymerization state, (4) creating a non-uniform concentration of monomer within the settled immunoabsorbents, (5) converting the remaining stacking solution to a polymerization state, and flushing away the unpolymerized portion to form a stacking gel layer with exposed immunoabsorbents. Furthermore, the step of creating a non-uniform concentration of monomer within the settled immunoabsorbents further includes a step of removing part of the supernatant and rinsing the surface of the remaining stacking solution with a buffer for dilution. In accordance with the concept of the partially incomplete two-stage polymerization method, three approaches to the embodiments of this patent are provided, including (1) antibody-coupling procedure performed prior to the formation of an immunoabsorption wall (post-coupling), (2) antibody-coupling procedure performed post the formation of an immunoabsorption wall (pre-coupling), and (3) formation of multiple immunoabsorption walls with minimal thickness of stacking gel layer in series. Effective removal of selected middle molecular toxin, β -2-microglobulin, in neutral phosphate buffer can be experimentally attainable with these three embodiments.

Extracorporeal Reactors Containing Immobilized Species

US4863611 relates to a reactor containing immobilized species on a substrate having a very high surface area which is capable of treating biological solutions, especially blood, at high flow rates without damaging the biological materials [84]. The reactor can contain immobilized species such as enzymes, antibodies, receptors, drug binding molecules or cofactors and thus can be made highly specific for the compound of interest. Alternatively, or in addition, non-specific solid phase adsorbents can be used to remove chemical species. The reactor consists of a chamber with an inlet and an outlet which is fitted with a mesh at the outlet of the device for restraining porous particular supports within the chamber. These provide a high internal surface area, up to several orders of magnitude higher than the equivalent volume of hollow fibers or planar sheets, for the binding of

large quantities of protein with the potential for high capacity removal.

Up until the present invention, the use of particular particles for extracorporeal reactors has been limited by the packing of the solid phase in the device. In order to use an extracorporeal reactor with particles at clinically useful blood flow rates, ranging from 100-1000 ml/min, a method of maintaining the beads in a fluidized state is required. In the present invention, the particular supports are maintained in suspension in the reactor by a combination of high speed recirculation and multi-directional agitation away from the direction of recirculation. The particles are formed of a biocompatible material which is selected for stability both to the biological solution and to the agitation. The particles are limited to a size range between that which can be freely recirculated and agitated by the reactor and that which can be restrained within the reactor. The maximum flow rate is limited by the stability of the particles. Suggested particle materials include agarose, cross linked dextran, polyhydroxyl ethyl methacrylate, polyacrylamide, cellulose, and derivatives or combinations thereof, preferably in the form of porous spheres. In the primary application of a device constructed according to this method, heparin is removed from blood in series with an extracorporeal device such as a dialysis unit or a blood oxygenator.

Removal of Metabolic Components from Blood

US7066900 relates to various methods and apparatus for removing unwanted components from blood or plasma using membrane-based electrophoresis [85]. In one aspect, a system for removing or reducing concentration of a metabolic component from blood or plasma includes a first and second ion-permeable barrier, each having a defined pore size, which is disposed in an electric field area. A treatment chamber is formed between the first and second ion-permeable barrier. A structure cools the blood or plasma. Another structure provides dialysate to a cathode zone and an anode zone. A transporting device provides blood or plasma to the treatment chamber. Upon application of the electric potential, a metabolic component from the blood or plasma moves through at least one barrier into one of the cathode or anode zones. Preferably, the barriers are hydrogel membranes formed from polyacrylamide or any other suitable polymer. In another aspect, a method for removing or reducing concentration of a metabolic component in blood or plasma includes placing blood or plasma from the subject in a treatment chamber of an electrophoresis system. Applying an electric potential between the cathode and anode causes movement of a metabolic component from the blood or plasma through a barrier into at least one of the electrode zones. This application step is maintained until the desired amount of removal of the metabolic component from the blood or plasma is achieved. The blood or plasma in the treatment chamber is returned to the subject. This method does not result in heating the blood or plasma above physiological temperature.

In current renal dialysis therapies, phosphate and problematic middle molecules are not adequately removed. To identify that the *in vitro* process based on the electrophoresis system technology with polyacrylamide membranes was capable of removing uremic wastes and that the measured

biocompatibility markers demonstrated no significant adverse effect, an animal study was conducted. The results of uremic toxins (urea, uric acid, creatinine, phosphate and β -2-microglobulin) removal experiments from the phase 1 animal study have identified that the electrophoresis system was capable of being used as a medical device. The animal study established the *in vivo* biocompatibility of the claimed electrophoresis system in an ovine model.

NON-ABSORBABLE SOFT TISSUE FILLERS IN RECONSTRUCTIVE SURGERY OR FOR COSMETIC PURPOSES

The use of mini-invasive techniques is well-known in modern reconstructive plastic and cosmetic surgery. Many of these techniques involve the use of fillers. An ideal filler should be safe and effective; it should be biocompatible, nonimmunogenic, easily obtainable, nonreabsorbable, low in cost, and easily stored. It should also be easy to remove if necessary. Biocompatibility is considered to be an indispensable condition for dermal fillers, and it is basically determined by the host tissue response to implants [86-89].

Polyacrylamide gels have been widely used in Europe and China for more than 10 years as implants for reconstructive surgery and soft tissue augmentation [90-92]. Approximately 30,000 patients underwent injection of polyacrylamide hydrogel for soft tissue augmentation during this period. Retrospective analyses were conducted indicating that polyacrylamide hydrogel is well tolerated and does not cause evident soft-tissue reactions. The biocompatibility and toxicity of the polyacrylamide component have been well characterized. For example, according to Kebuladze *et al.*'s reports [93], polyacrylamide hydrogel has been well tolerated in the subcutaneous compartment and in glandular breast tissue, and unlike other products used for augmentation, such as silicone and collagen gel, polyacrylamide hydrogel stays in place at the injection site without being degraded or displaced. Furthermore, capsule shrinkage caused by the development of a thick, firm, disfiguring connective tissue capsule, which may be found around silicone prostheses, has never been reported or observed. The amount of injected polyacrylamide hydrogel varies from 80 to 360 ml/breast, and biopsy specimens from the breast tissue surrounding the gel have given evidence of very modest tissue reactions, even up to 10 years after the injection.

The following recent patents give us more examples regarding applications of polyacrylamide gels as soft tissue fillers.

Polyacrylamide Hydrogel and its Use as an Endoprosthesis

European Patent EP1418188 relates to the biostable hydrogel obtainable by (1) combining acrylamide and methylene bis-acrylamide in amounts so as to give about 0.5 to 25% by weight polyacrylamide, based on the total weight of the hydrogel; (2) radical initiation; and (3) washing with pyrogen-free water or saline solution. The bio-stable hydrogel typically has a molecular weight between 0.01×10^6 and 20×10^6 [94]. The polymer is resistant to biological degradation and is not permeable through biological membranes. The polyacrylamide hydrogel of the invention is

fully biocompatible (according to ISO standard test ISO-10993). The polyacrylamide hydrogel does not have cytotoxic effect on human fibroblasts, is non-toxic, non-carcinogenic, non-allergenic, non-mutagenic, and resistant to enzymatic and microbiological degradation. Furthermore, the polymer is not water-soluble. These hydrogels are useful as injectable or implantable endoprosthetic devices for use in mammoplasty, soft tissue filling, penile augmentation, facial correction, lip augmentation, body contouring, arthritis, incontinence, reflux oesophagitis, and vesicoureteral reflux.

Filling Material for Soft Tissue Implant Prostheses and Implants made Therewith

US5941909 discloses a new soft tissue implant filling material, such as breast and testicular implants and tissue expanders, and more specifically, is directed to a filling material for such implants [95]. The surgically implantable soft tissue implant filling material has good aesthetic properties, as measured by viscosity and elasticity, and good elimination properties. One embodiment of such a new filling material is a gel which comprises three components. The first component is a water soluble polymer or hydrogel that has a high molecular weight in the range of 200,000 to 1.5 million. Alternatively, the first component is both crosslinked and has a high molecular weight. This component of the filling material is the minor polymeric component. However, it provides the majority of the physical properties of the filling material. The second component of the filling material is a water-soluble polymer that has a molecular weight and a molecular weight distribution that is essentially under the renal threshold molecular weight of the polymer and is eliminated rapidly through the kidneys upon subcutaneous release. This component of the filling material is the major polymeric component. It provides enhanced radiolucency to the filler material and provides a synergistic viscosity effect with the first component. The third component of this filling material is saline. Saline is used to dissolve the polymer and to adjust the osmolality of the polymer. In addition, a buffer may be added to the filling material to adjust the pH of the gel. One gel formulation of the present invention, in its preferred embodiment, consists of polyacrylamide and derivatives thereof.

The utilization of such a composite gel as a filling material has the following advantages. The filling material of the present invention has a viscosity and an elasticity similar to that of a silicone gel. In addition, it has elimination properties that are similar to saline. In essence, the composite gel will, upon implantation, provide good aesthetic qualities while providing the assurance of safety after implantation because it can be quickly eliminated from the body upon the rupture of the shell. The composite gel further provides increased radioalucency. The polymers used to make the composite gel can be any water soluble polymer or hydrogel. In a preferred embodiment, the first component is a polyacrylamide and the second component is a linear polyacrylamide. The first component of the gel may be a linear or a crosslinked polymer, and can range from approximately 1% to 9% of the total weight of the gel. The second component of the gel can range from approximately 4% to 36% of the total weight of the gel.

Process for Cross-Linking an Acrylic Polymer or Copolymer or an Acrylic Derivatives of a Copolymer and the Cross-Linked Polymer Thereby Obtained

US6770711 describes a process for preparing a cross-linked acrylic polymer from water-soluble acrylamide monomers and catalysts whose cross-linking is subject to reaction during and after the polymerization stage, comprising (1) preparing an aqueous polymerization solution comprising an acrylamide monomer and a catalyzing agent; (2) polymerizing the monomer present in the polymerization solution by agitating and heating the polymerization solution to obtain a cross-linked acrylic polymer [96]. The polymerization is conducted in the presence of gaseous oxygen. Preferably the aqueous polymerizing solution comprises the acrylamide monomer, a derivative of it such as methylol-acrylamide, one or more monomers chosen from N,N'-methylene-bis-acrylamide and N,N'-ethylene-bis-acrylamide, catalyzing agents and, possibly, a chelating agent such as ethylene-bis(oxyethylene nitrilo)-tetracetic acid. The cross-linked polymer prepared according to this patent has been tested at several research centres. The tests have shown not only the high degree of biocompatibility of the material but also its total incapacity to stimulate cell growth. The chemical and physical properties of the cross-linked polymer are such that it can maintain its structural stability even in time. Once implanted, a permanent capsule is formed which isolates the compound from the surrounding tissue. Thanks to these properties, the cross-linked polymer undoubtedly constitutes a novelty. It can be injected into a patient's body (to form a prosthesis endogenously) in much larger quantities than the average quantities normally used up to now. As much as several hundred grams of it can be implanted without risk to the patient's health.

Mammary Prosthesis Made of Polyacrylamide Hydrogel

US6955690 relates to a mammary prosthesis made of polyacrylamide hydrogel [97]. Said prosthesis include a shell which is made of medical high polymer elastic material, such as silicone, and said shell have a round curved surface. The shell is filled with polyacrylamide hydrogel, or with hydrogel powder, and the weight of the filled powder is matched with the volume of the circular shell, that is to say, each 100 ml volumes of the shell could be filled with about 2.5-5 g hydrogel powder. The mammary prosthesis feels good and guarantees safety, whether it is ruptured or not. United States Patent 5632774 relates to improved surgically implantable mammary tissue expanding prosthesis comprising an elastomeric shell that serves as a hollow soft container for discrete biocompatible hydrophilic cross-linked polymeric bodies or units, such as poly-acrylamide, as a new filler material. In more detail, the inventive new filler material is made from polymeric hydrogel macro unit or units at their smaller dehydrated state which are placed into the open shell to occupy a very small fraction of the total shell volume before sealing the implant. Later on, as it is required, water will swell hydrogel into the shape of the full sized shell as the hydrogel is hydrated by injecting sterile water into deflated shell either at manufacturing site or in operating room after breast implant is inserted in the patient. These hydrated hydrogel units are non-bleeding while conforming to the shape of the shell by assuming different

Table 1. Relative Advantages of Polyacrylamide Compared with Other Biomaterials

Applications	Relative Advantages	Compared with :
Enzyme immobilization	<ul style="list-style-type: none"> enhanced stabilities for operation or against heat treatment, changes in pH, and urea denaturation better mechanical stability, preservation of enzymatic activity, longer half-life time better operational stability 	<ul style="list-style-type: none"> none (soluble enzyme)[18-25] calcium alginate[103] chitosan [104]
Carriers for delivery of drugs and bioactive compounds	<ul style="list-style-type: none"> enhanced drug potency with a sustained action more mechanically stable, greater drug loading capacity, better biocompatibility More suitable for replacement and immunoisolation 	<ul style="list-style-type: none"> conventional dosage forms[52] liposomes [48] agarose and alginate [105]
Smart materials	<ul style="list-style-type: none"> temperature-induced transition over broad and useful compositional and pH range 	<ul style="list-style-type: none"> copolymers of polyethylene glycol and poly(methacrylic acid) [106]
Extracorporeal toxin removal modalities	<ul style="list-style-type: none"> No plasma separation (applicability to whole blood) Good biocompatibility Time - efficient Minimal possible risk of infection 	<ul style="list-style-type: none"> dextran sulfate, agarose [75-77]
Soft tissue fillers	<ul style="list-style-type: none"> good tolerability little lymphocyte infiltration, no serious complications nonabsorbable longer lasting effect low incidence of granuloma formation reduced antigenic properties easy-to-use and effective 	<ul style="list-style-type: none"> silicones [107,108] gelatin, collagen, autologous fat, acrylic gels [91]

viscoelastic packing arrangements as shell deforms in different positions when breast implant receiver moves in different direction. Accordingly, it would be a significant contribution to the medical device art to eliminate the major problem of bleeding or leakage and transudation from mammary prosthetic surgical implant by providing a non-bleeding harmless filler material capable of being used with existing mammary device manufacturing and breast surgical procedures practiced in the U.S. or elsewhere.

CURRENT & FUTURE DEVELOPMENTS

Nowadays, polyacrylamide not only remains the most popular medium for use in all electrophoretic methods, but also has demonstrated relative merits compared with other biomaterials in various applications, as shown in Table 1. Indeed, it is easily perceived that there is still enormous space and opportunity for research and development of polyacrylamide-related technology in the field of biomaterials. The interests of immobilizing enzymes to/within polyacrylamide matrix for processing of products could be further directed to biomedical aspects such as in biosensor or diagnostic kits using the unique advantageous properties of immobilized enzymes [98]. Also, the combination of stimulative materials based on polyacrylamide or its derivatives with nanotechnology would improve the efficacy of conventional drug delivery system [47,99,100]. Moreover, with the advent of new concept and creativity, it is of

particular promise that polyacrylamide might help resolve some critical issues long existing in conventional extracorporeal toxin removal therapies [82,101]. Lastly, the search for an ideal filler material for use in reconstructive and cosmetic surgery has been an ongoing effort. Although polyacrylamide has been used as a soft tissue filler for decades in some countries, its long term efficacy and safety remain controversial to date [102]. Therefore, improvement of its biocompatibility as well as careful evaluation of follow-ups with larger sample size would be necessary before polyacrylamide can be more acceptable and find approval in more countries [103-108]. In summary, current researches and achievements of polyacrylamide have therefore well demonstrated its versatility and usefulness as biomaterials. Further developments of polyacrylamide-associated technologies will undoubtedly enhance the value and broaden the possibilities of applications of polyacrylamide in the field of biomaterials.

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