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Perspective

Natural and Synthetic Polymers as Drug Carriers for Delivery of Therapeutic Proteins

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In order to cure and treat health-related disorders, therapeutic substance must reach its target site with a constant concentration over a long period of time. As oral administration is limited due to enzymatic degradation, most of the commercially available therapeutic proteins are usually being administered parenterally. However, because of their short biological half-life, daily multiple injections are required to maintain effective therapeutic levels of these drug candidates. To limit this drawback, a variety of polymers are being used to increase systemic bioavailability of therapeutic proteins and peptides. Development of protein-based therapeutic substances has tremendously increased the need for suitable polymeric-based carrier systems, guaranteeing safe and sustained delivery of therapeutic proteins to their target site. Here, we have briefly discussed two major types of polymers including natural and synthetic polymers that have been intensively studied for efficient delivery of various proteinous drugs. A wide variety of natural and/or synthetic polymers have been found to be useful and safe drug carrier systems for the delivery of therapeutic proteins which have been discussed over here in detail. To conclude, these polymers have been

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found to be compatible with most of the incorporated proteins and have shown to have minimal or no toxic profile.

Keywords Natural polymers, polysaccharide-based polymers, natural protein-based polymers, synthetic polymers, recombinant protein-based polymers, therapeutic proteins

1. Introduction

A decline has been occurring in the therapeutic use of many chemical therapeutic substances due to their potential hazardous effects; however, advancements in pharmaceutical biotechnology have synthesized various efficacious and disease-specific therapeutic proteins/peptides on a large scale.[1] These therapeutic proteins and peptides have gained considerable interest as they do not have serious side-effects; nevertheless, the major obstacle for the delivery of these therapeutic proteins and peptides is to reach the target site, as a majority of these agents are unstable in gastric environment and may undergo enzymatic degradation; however, many of them have short biological half-lives.[2,3] Hence the development of protein-based therapeutic substances critically requires an effective carrier system, guaranteeing the safe and sustained delivery of therapeutic proteins to the target site. Polymeric-based delivery of therapeutic proteins and peptides has been known to allow delivery of these therapeutic proteins at a controlled rate as per requirement of the treatment, depending upon the disease state.

The term polymer is derived from the two Greek words "poly" and "mer" which means many parts. Polymer can be defined as "a large molecule that is composed of repeated chemical units." The smallest repeating unit is called "mer" and the number of repeat units in a chain is called polymerization. Polymer chains can be chemically or physically connected to one another. These connections are known as cross-links and cause the connected chain to behave as a single unit. The polymer chains can also be chemically and/or physically connected to the desired therapeutic substance. After being connected, polymer is known to hold up therapeutic substance inside the polymer molecules. These polymers not only deliver the encapsulated therapeutic substance to its target site but also maintain its stability for a longer period of time.[4,5] Polymers are obtained from natural and/or synthetic resources. Polymers are recognized to be biocompatible and biodegradable having no known potential toxicity at optimal concentrations.[6] Recent advances and developments in the field of pharmaceutical biotechnology have enabled scientists to synthesize specific enzyme-sensitive polymers that possess the ability to release the incorporated therapeutic substance specifically at its targeted site.[7]

In this article, we have briefly discussed two major types of polymers including natural and synthetic polymers (Fig. 1) that have been extensively studied for the efficient delivery of proteinous drugs. Furthermore, we have subdivided natural polymers into polysaccharide-based and protein-based polymers, and synthetic polymers into polyesters, polyethers, ploxamers, and recombinant protein-based polymers. This article describes the use of different types of these polymers for delivery of therapeutic proteins and their possible limitations. In this article, we have discussed the polymers that are known to be biocompatible and inert with physiological fluids. Moreover, we have also discussed the clinical significance and toxicological evaluation of polymers, and stability of incorporated protein. We found that all the polymers discussed over here have shown to be compatible with incorporated proteins with non-toxic profile.

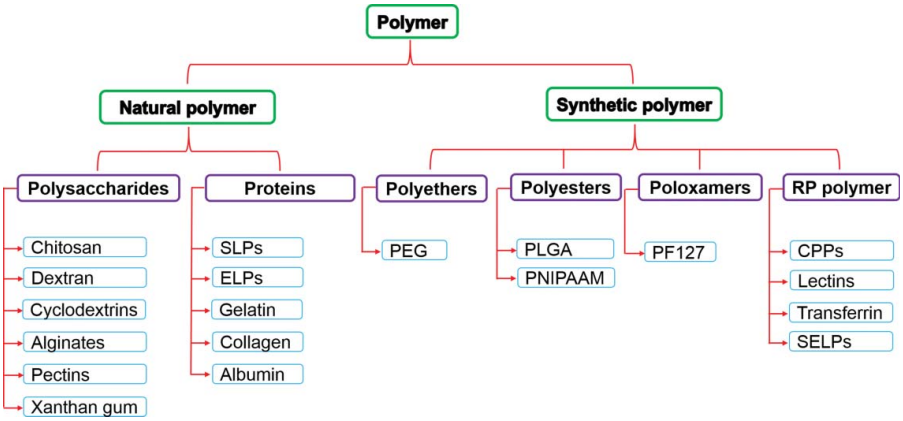

Figure 1. Classification of polymers. SLPs: Silk-like proteins, ELPs: Elastin-like proteins, PEG: Polyethylene glycol, PLGA: Poly (lactic-co-glycolic acid), PNIPAAM: Poly (N-isopropylacrylamide), PF127: Pluronic F127, CPPs: Cell penetrating peptides, SELPs: Silk-elastinlike protein.

2. Polymers as Carrier System for Delivery of Therapeutic Proteins

Therapeutic proteins have gained significant attention and found place in the pharmaceutical market due to diverse therapeutic characteristics, but a strong challenge in the development of these therapeutic proteins is their delivery to the target site. Another most challenging task during the development of therapeutic proteins is to handle the chemical and physical instabilities of proteins. Protein instability is one of the most important challenges due to which these proteins have been administered via the parenteral route as the oral route may cause enzymatic and/or proteolytic degradation of proteins in gastrointestinal tract (GIT), along with poor permeability across gastrointestinal mucosa and these proteins may also undergo first-pass hepatic metabolism.[8] Therapeutic proteins need to be protected from the gastric environment of GIT. Polymers as inert carrier system are known to be most suitable for protecting therapeutic proteins from such extreme conditions. A variety of natural and/or synthetic polymers have been intensively investigated for efficient delivery of different proteins and peptides.[6]

2.1. Natural Polymers

Natural polymers have distinct benefits to deliver therapeutic proteins to the target sites. These polymers serve as protein carriers and are known to play a significant role in the field of pharmaceutical drug development and technology. The significance of natural polymers in drug delivery systems is the presence of reactive sites that are amenable and help in cross-linking, ligand conjugation, and various other modifications that make these polymers ideal drug carriers for a wide range of therapeutic proteins.[9,10] Natural polymers have many advantages over synthetic polymers because of many reasons including their natural resources, being inexpensive, and having the capability of modifying chemically. Different types of therapeutic proteins and peptides have successfully incorporated in natural polymers.[6,9] In the following sub-sections, we have briefly discussed two main types of natural polymers (Fig. 1), namely polysaccharide- and protein-based polymers.

2.1.1. Polysaccharide-Based Polymers. Polysaccharide-based polymers due to their outstanding advantages have received considerable attention of pharmaceutical scientists

and researchers for incorporating and formulating different types of therapeutic proteins and peptides. They are obtained from algal, plant, microbial, and/or animal origin (Fig. 1). Because they have a wide range of molecular weight, large number of reactive groups, and varying chemical composition, polysaccharides exhibit diversity in their structures and properties. Due to the presence of derivable groups on the molecular structures, polysaccharides can easily be modified according to the demand and requirement.[11] Polysaccharides being natural biomaterials, are highly stable in the biological fluids, non-toxic, safe, and biodegradable.[12,13] Due to the presence of several derivable groups including hydroxyl, carboxyl, and amino groups on the molecular structure, polysaccharides are hydrophilic in nature and form non-covalent bonds with biological tissues (mainly epithelia and mucous membranes) by the phenomenon of mucoadhesion.[14] Due to mucoadhesive properties, polysaccharides are also called mucoadhesive polymers. Nanoparticles made up of mucoadhesive polysaccharides have shown to enhance the residence and absorbance time of incorporated therapeutic proteins.[15,16]

In the following sub-sections, we have briefly summarized the most important polymers belonging to the polysaccharides. These polymers have been intensively evaluated for the incorporation of different types of therapeutic proteins.

2.1.1.1. Chitosans. Chitosans are cationic polysaccharides which are derived from naturally occurring polysaccharide, chitin and most interested mucoadhesive polymers. Chitosans are the second most abundant polymer in nature after cellulose.[8] Chitosans are composed of D-glucosamine and -acetyl D-glucosamine that contain abundant amino and hydroxyl groups (Fig. 2). They are known to promote the absorption of large molecular weight therapeutic proteins through intestinal epithelial mucosa. These are non-toxic, biocompatible, and FDA approved polymers that can enhance the intestinal absorption of large molecular weight therapeutic proteins by increasing paracellular permeability.[17,18] Due to their high molecular weight, chitosans are not absorbed from the gut that limits the possibility of chitosans-related side effects.[19] Moreover, chitosans are known to be safe at their effective concentrations.[20] Chitosans have been used to enhance the absorption of insulin.[19] Their mucoadhesive property is dependent on the electrostatic interaction between their negatively charged amino groups and positively charged sialic acid groups of mucin glycoproteins.[18,21,22] But chitosan-based carrier systems are highly labile in gastric environment, as their amino groups are easily protonated in very low pH values.[8] Various approaches have been utilized to improve the stability of chitosan-based particles in gastric environment. Lin et al. have made an attempt to improve the stability of chitosan/poly-γ-glutamic acid (γPGA) in a broader pH range by imparting tripolyphosphate (TPP) and magnesium sulfate to γPGA nanoparticles.[23] Another attempt has also been made by encapsulating freeze-dried insulin-loaded chitosan/γPGA nanoparticles. Orally administered insulin-loaded chitosan/γPGA nanoparticles increased the relative bioavailability up to 20.1% in comparison with subcutaneous administration of the free form of insulin.[24] Stability of chitosan-based carrier systems has also been increased by

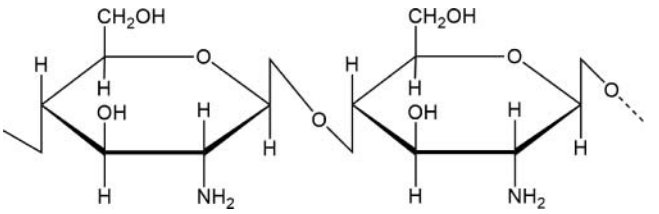

Figure 2. Structure of chitosan.

associating pH-sensitive polymers including alginate[25] and/or hydroxypropyl methylcellulose phthalate with chitosans.[26]

Despite gastric instability, chitosan-based carrier systems are unable to show their mucoadhesive properties at higher pH values in the intestinal region, one of the major absorption sites.[27,28] This limitation of chitosan-based carrier systems has been overcome by developing various types of derivatives of chitosans. Among different derivative of chitosans, quaternized derivatives have gained significant interest as these derivatives are known to enhance the intestinal absorption of therapeutic proteins in a wide range of pH values.[27] Many research groups have evaluated the mucoadhesive properties of N-trimethyl chitosans (TMC), which is a partial methyl-quaternized derivative of chitosan.[22,29–31] The mucoadhesive properties of TMC depend upon the degree of quaternization.[28,32] Yin et al.[30] reported that TMCs may increase the *in vitro* transportation of insulin by increasing the degree of quaternization. However, beyond the optimal degree of quaternization, TMCs may cause toxicity instead of increasing the absorption of therapeutic proteins.[32,33]

Mucoadhesive property of chitosans can also be increased by conjugating the chitosans with desired therapeutic proteins. Lee et al.[34] conjugated low-molecular-weight chitosans (LMWCs) with insulin which showed an increase of oral bioavailability of insulin as compared to native chitosan-based oral delivery of insulin.[35] Lee et al.[36] enhanced this technique to improve the solubility and stability in gastric environment which increased the oral bioavailability of taxane.

Thiolation of chitosan and/or its derivatives, i.e., TMCs is known to increase their mucoadhesion property via covalent disulfide bonding with mucin glycoproteins resulting in increased retention time of therapeutic substance at the site of absorption.[37] Yin et al.[30] and later, Dunnhaupt et al.[38] also reported that thiolation of TMC and chitosan or PAA significantly improved mucoadhesion and *ex vivo* permeation of insulin. Furthermore, when insulin encapsulated with thiolated chitosans was administered either orally or directly to the ileum, it produced distinct hypoglycemic effects as compared to the non-thiolated form of corresponding carriers.[30,39] Despite enhancing the mucoadhesion property of chitosans, thiomers also triggered the permeation of therapeutic proteins via inhibition of protein tyrosine phosphatase and intestinal P-glycoprotein.[40,41]

Despite having mucoadhesive properties, chitosans have also been used as thermosensitive gels for sustained delivery of therapeutic proteins and peptides. Bhattarai et al.[42] were able to form a thermoreversible gel by incorporating PEG into chitosan with no additional crosslinking agents. PEG grafting into chitosan improved the solubility of chitosan in water and gelation at physiological pH values. Moreover, they also investigated the controlled release of albumin from PEG-grafted chitosan. The initial burst release followed by steady-state release of albumin for about 3 days was observed.[43]

Similarly, another attempt has also been made by Yoo et al.[44] to prepare photo-cross-linked PF127/Chitosan based thermosensitive gel. Gelation temperature of PF127/Chitosan-based thermosensitive gel was dependent on chitosan concentration. PF127/Chitosan-based thermosensitive gels were photo-cross-linked by UV irradiation above their GTs. PF127/Chitosan-based thermosensitive gels with long photo-cross-linking time exhibited low degradation rate. Thereafter, they mixed rhGH with the mixtures of PF127/chitosan and subjected to photo-cross-link via UV irradiation to prepare rhGH loaded PF127/Chitosan-based thermosensitive gel. Thermosensitive gels of rhGH with long photo-cross-linking time and high chitosan content prevented the initial burst release and resulted in sustained release of rhGH in a diffusion-controlled mechanism. Chitosan possesses multiple sites for acrylation while PF127 acrylated possessed those sites only at either ends of each molecule. Acrylated chitosan significantly increased the formation of

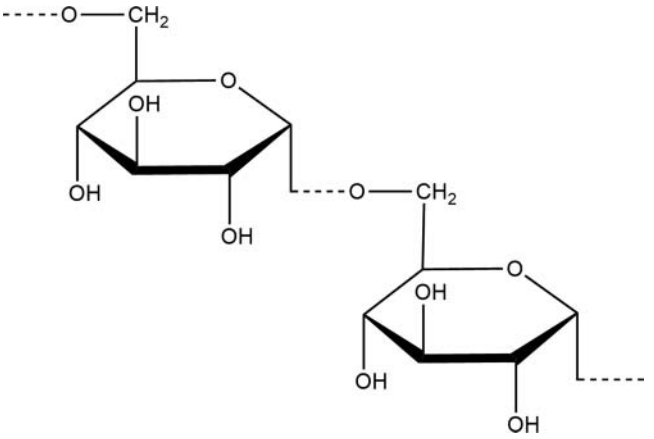

Figure 3. Dextran contain a linear backbone of $\alpha(1\rightarrow6)$-linked d-glucopyranosyl repeating modified with small side chains of d-glucose branches linked to the backbone with $\alpha(1\rightarrow2)$, $\alpha(1\rightarrow3)$, and $\alpha(1\rightarrow4)$-linkage.

interconnected networks as compared to di-acrylated PF127 in rhGH thermosensitive gel of PF127/Chitosan. Multiple-acrylated chitosan also increased the interconnectivity of PF127/chitosan-based thermosensitive gels and resulted in the high content of chitosan that decreased the degradation of encapsulated rhGH compared to low chitosan contents thermosensitive gels of rhGH.

2.1.1.2. Dextran. Dextran is a non-toxic and highly water-soluble polysaccharide. It predominantly contains linear α-1,6-linked glucopyranose units with some degree of 1,3-branching (Fig. 3). The main source of its production is the sucrose-rich environment of *Lactobacillus, Leuconostoc*, and *Streptococus*. Commercially, it is available with different molecular weights. The degree of branching and molecular weight are known to affect the physicochemical properties of dextran.[45] Dextran is known to have a wide range of therapeutic applications.[46] Clinically it has been used in plasma volume expansion, peripheral blood flow enhancement, thrombosis prophylaxis, and as artificial tears. Low molecular dextrans have short biological half-life (8 h) and are secreted from the kidneys, whereas high molecular weight dextrans exhibit longer half-lives and are subsequently degraded by reticuloendothelial system.[45] Moreover, dextrans are also metabolized by enzymes (α-1-glucosidases) in various parts of the body.[46]

Dextran-based carrier system has gained significant interest over the recent decades in which therapeutic proteins can be incorporated in a variety of ways. Dextran-based carrier systems can be obtained either by chemical and/or chemical cross-linking.[45] Till now, a large number of therapeutic proteins have been successfully incorporated in dextran-based carrier systems and significant therapeutic outcomes have been obtained either from *in vitro* or *in vivo* experimental studies.[47–51] Most of the studies conducted on dextran-based carrier systems for delivery of therapeutic proteins have shown that dextran is biocompatible with incorporated proteins.[52–54]

2.1.1.3. Cyclodextrins. Cyclodextrins (CDs) are cyclic oligosaccharides that contain 6-D-(+) glucopyranose unit linked with α-(1,4) glucosidic bonds.[55,56] The outer part of CDs is hydrophilic in nature whereas, the inner part is hydrophobic. There are several types of CDs but the most commonly used CDs in pharmaceutical biotechnology are α-CD, β-CD, and γ-CD in which they contain six (α), seven (β), and eight (γ) glucose in their main structure respectively (Fig. 4). These CDs have been known to have the ability

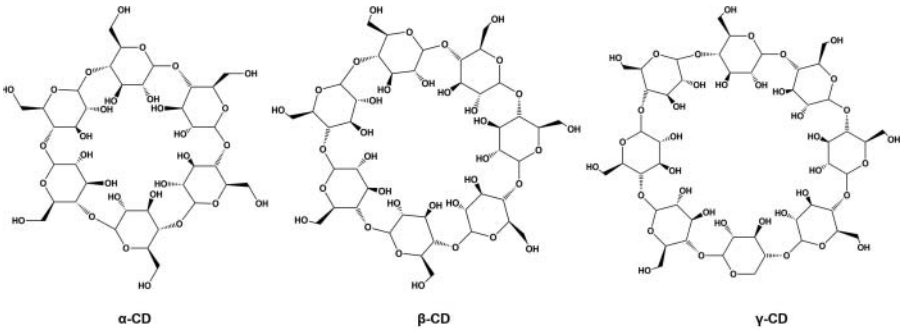

Figure 4. Cyclodextrins composed of α-1,4-linked glucopyranose (glucose units) arranged in ring-form. The cyclodextrins family is made up of three cyclodextrins: α-, β-, and γ- cyclodextrins, containing six, seven and eight glucose subunits, respectively.

to form inclusion complexes by interacting with guest molecules.[57,58] CDs act as potential carriers by interacting with biological membranes for large molecular weight therapeutic proteins.[59] β-CD has been used in the formulation of alginate microspheres of insulin.[60] β-CD significantly increased the uptake of insulin from microspheres in GIT compared to the controlled alginate microspheres of insulin. CDs have been extensively evaluated for efficient delivery of different types of therapeutic proteins, peptides, and genes.[61-63]

2.1.1.4. Alginates. Alginate is also known as align and/or alginic acid and is anionic polysaccharide that is widely distributed in the cell walls of brown algae. It is mainly extracted from three different species of brown algae (*Laminaria hyperborean, Ascophyllum nodosum*, and *Macrocystis pyrifera*) and is composed of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues (Fig. 5). Alginate has attained considerable attention due to its excellent mucoadhesive property, biocompatibility, and biodegradability.[64] Recently it has been used as a component of a carrier system for efficient delivery of therapeutic proteins and peptides.[25,65] Alginate requires only a mild condition for fabrication in aqueous solutions, which is favorable for heat-sensitive therapeutic proteins. Moreover, alginate have shown to protect the labile proteins and peptides from gastric environment and delivers them safely to the intestine.[16] Despite its wide range of pH-sensitivity for labile proteins, alginate has some limitations as a protein carrier system including drug loss during preparation of beads and/or leaching of drug through the pores in beads. To cope with this problem, many modifications have been made in the structure of alginate.[8]

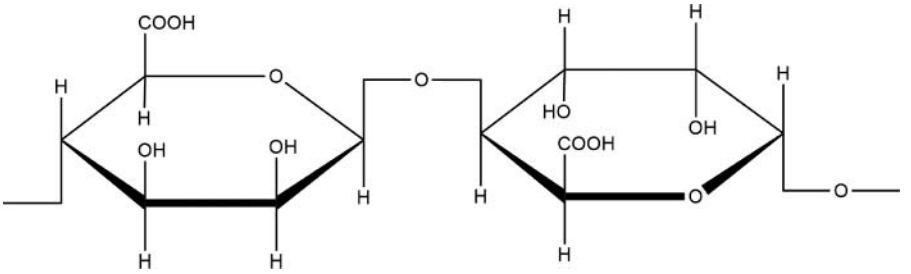

Figure 5. The chemical structure of alginate constitute of random sequences of chains of β-D-mannuronic and α-L-guluronic acids.

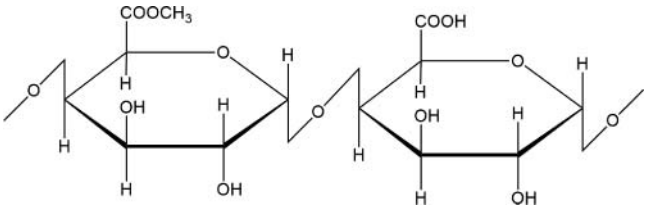

Figure 6. Chains of 300 to 1000 galacturonic acid units are joined with a variable number of methyl ester groups forming the chemical structure of Pectin.

Alginate has been extensively studied for efficient delivery of therapeutic proteins and peptides.[66–70] Gombotz and Wee have also reviewed the encapsulation of therapeutic proteins and peptides using alginates alone and/or with other copolymers.[64] This high degree of flexibility of alginate help deliver the therapeutic proteins and peptides over a time period ranging from minutes to months.

2.1.1.5. Pectins. Pectin is another important polymer having distinct mucoadhesive property on the intestinal epithelium.[71] These are linear polysaccharides that are extracted from the plant cell walls. These are mainly composed of α-(1-4)- linked D-galacturonic acid residues interrupted by 1,2- linked L-rhamnose residues (Fig. 6). The carboxylic groups present in pectin are responsible for showing a mucoadhesive property by interacting with functional groups present on the mucus layer and remain intact in the physiological environment of GIT.[72] The mucoadhesive strength of pectin depends upon its molecular weight and degree of esterification.[71] Despite the molecular weight and degree of esterification of pectin, the mucoadhesive property of pectin also depends on the site of intestine.[62] These are non-toxic and generally considered inert for physiological fluids.[73,74] Pectin prevents the enzymatic proteolysis of incorporated proteins and significantly increases the intestinal absorption of several therapeutic proteins and peptides.[75–77] It has also been found that pectin delivers a variety of therpaeutic proteins to the target sites via different routes.[78]

2.1.1.6. Xanthan Gum. Xanthan gum (XG) is high molecular weight anionic extracellular polysaccharide (Fig. 7) and is produced from *Xanthomonas campestris*. It has a wide range of therapeutic applications including food, cosmetics, and pharmaceuticals.[79] Despite being used for the delivery of non-proteinous drugs,[80–83] XG has also been evaluated for the delivery of therapeutic proteins and peptides.[79,84,85] Sodium carboxymethyl xanthan gum, a derivative of XG has also shown to prolong the sustained release of incorporated therapeutic protein as compared to XG and maintained the integrity of therapeutic protein.[84]

2.1.2. Protein-Based Polymers. Among natural polymers, protein-based polymers (Fig. 1) have also gained incredible consideration mainly owing to their characteristics including abundance, ease of availability, low toxicity, ease of modification due to their complex heterogeneity, and versatile routes of administration.[86] The most commonly used proteins as drug carriers for delivery of therapeutic proteins are silk, elastin, collagen, gelatin, and albumin. Though stability of protein-based polymers is to be a great challenge for the use of these polymers as ideal therapeutic polymers, however, several techniques have been proposed to prevent the degradation of protein-based polymers.[87–89]

2.1.1.2 Silk-Like Proteins. Silk-like proteins (SLPs) are naturally occurring proteins. They contain various hydrophilic and hydrophobic blocks within their structure due to which SLPs act as block copolymers.[90] Hydrophobic blocks of SLPs are composed of

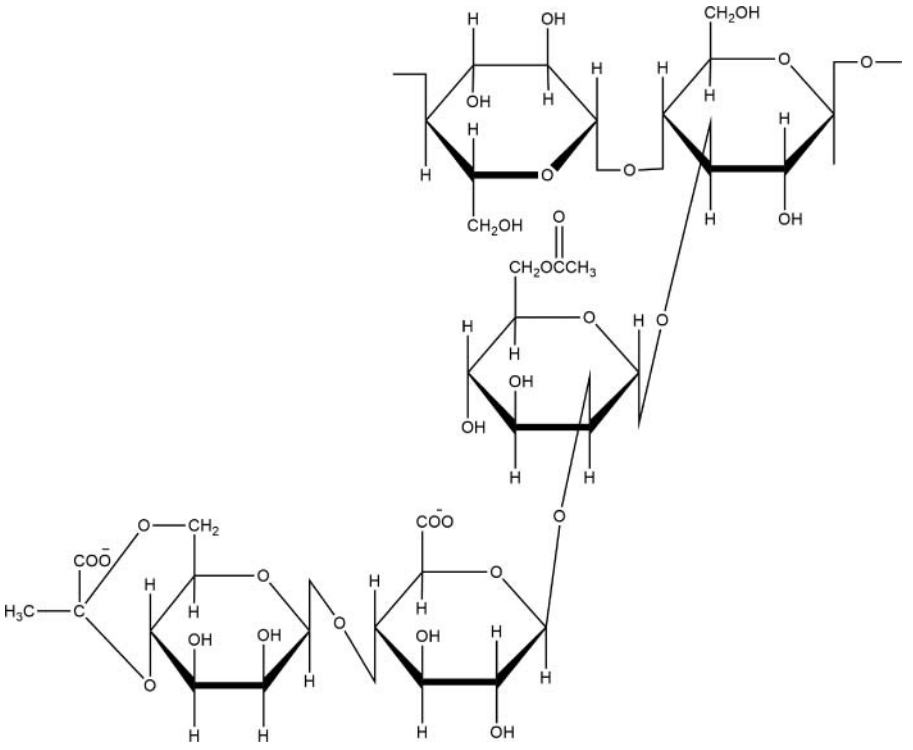

Figure 7. Xanthan has a similar backbone as cellulose containing β-(1-4)-D-glucose. Every alternate glucose consists 3 sugar side chain containing 2 mannose residues and 1 glucuronic acid residue.

conserved repeating sequences of short-chain amino acids such as glycine and alanine, whereas the hydrophilic blocks are composed of shorter domains with non-repetitive sequences and charged or bulkier side chains amino acid residues. The most studied recombinant sources of SLPs come from natural silk fibroin domains of the cocoons of silkworm *Bombyx mori or* from the dragline of the spider *Nephila clavipes*.[91,92] Self-assembly of SLPs has the ability to form many structures that facilitates the delivery of specific therapeutic substance. These SLPs are known to have excellent biocompatibility and biodegradable characteristics. Various delivery strategies have been investigated utilizing SLPs as block copolymers for efficient delivery of therapeutic substances.[93,94] Moreover, for efficient delivery of genes to the target site, spider silk-based SLPs have been produced on the length of repeating proteins.[93,95,96]

Cell penetrating peptides (CPPs) are being utilized to enhance the delivery of therapeutic proteins through cellular membrane penetration. CPPs have been genetically engineered with SpI-based SLPs to produce a delivery vehicle that is up to forty-five times more efficient in transfection than poly (ethyleneimine) at low pDNA concentrations.[96] A combination of CPPs with SLPs utilizing genetic engineering tools for efficient delivery of non-viral gene vectors to cells in a safe and efficient manner is known to be highly biocompatible and might be utilized for efficient delivery of other therapeutic peptides.

2.1.2.2. Elastin-Like Proteins. Elastin-like proteins (ELPs) are extracellular matrix proteins which have distinctive mechanical property that allow repetitive extensibility followed by elastic recoil. ELPs are replicative polypeptides that are resultant of amino acids sequences found in the hydrophobic domain of tropoelastin. The most frequently

used motif for ELPs consist of replicates of the sequence (VPGXG)n where "X" can be the guest residue having any amino acid other than proline and "n" represents the number of pentapeptide replicates in the ELPs.[97] There are some other variants of ELPs that are composed of pentapeptide replicate sequences KGGVG or LGGVG to heptapeptides with the sequence LGAGGAG and nonapeptides with the sequence LGAGGAGVL.[98–100] Although all of these ELPs exhibit elastin-like properties but among all of them, the most important and most commonly used ELPs is (VPGXG)n. ELPs that are composed of VPGXG pentapeptide repeats demonstrate to be thermally responsive polypeptides that exhibit a reversible inverse temperature phase transition.[101] ELPs reveal up to 25-fold increase in its intra-articular biological half-life when compared to that of soluble non-transitioning ELPs. Moreover, systemic exposure of ELPs are also known to decrease due to its phase transitioning property.[102] The phase transitioning property of ELPs conjugated to drug makes it suitable for generating potentially valuable intra-articular drug depot at the site of injection. The formation of drug depot allows slow resolubilization of drug at the site of administration that would ultimately extend the drug longevity at targeted site. The biological role of ELPs has made it a versatile protein for therapeutic delivery of various proteinous drugs and tissue engineering.[103–105]

2.1.2.3. Gelatin. Gelatin is a protein-natured biopolymer that has thermoreversible properties.[106] Being protein in nature, gelatin allows easy modification on the amino acid level. It is also biocompatible and biodegradable.[107] Below room temperature aqueous solution of gelatin becomes semisolid rigid mass forming triple helices and a rigid mass three-dimensional network. Above 30°C, conformational changes in gelatin convert helix form of gelatin to more flexible coiled-formed gelatin, rendering the gel to be liquid again.[108] Researchers have improved the thermogelation behavior of gelatin close to body temperature by combining gelatin with NiPAAM, silk fibroin, and monomethoxy poly(ethylene glycol)–poly(D,L-lactide) (mPEG–DLLA) block copolymers in an effort to produce a thermosensitive gel.[107,109,110] Due to its versatile characteristics, it is widely used for delivery of therapeutic molecules via targeted drug delivery systems.[111–113]

2.1.2.4. Collagen. Collagen is the most abundant protein in the living orginism. Collagen has a wide-range of applications in different fields and has also been intensively investigated for the delivery of therapeutic proteins and to prolong the sustained release of incorporated proteins.[114,115] In one study conducted on db/db mice as an experimental animal model, it has been found that collagen can effectively result in sustained release of human growth hormone (hGH) resulting in better wound healing with a single administration of hGH.[115]

2.1.2.5. Albumin. Albumin is a biocompatible and non-toxic metabolizable protein. Commercially, albumin is available as ovalbumin, bovine serum albumin, and human serum albumin. Owing to its abundant availability and high affinity, it has been widely investigated as a drug carrier system for delivery of therapeutic substances.[116,117] Albumin contains a large number of binding sites and charged amino acids that make it ideal for the binding of both negatively and positively charged molecules.[118] Although, a majority of protein-based polymers are unstable, but albumin has the advantage over the other protein-based polymers, as albumin-based carriers are known to be naturally stable and have the ability to deliver therapeutic proteins across the nuclear membrane as well as blood-brain barrier and do not show any change in the particle size when stored either in aqueous solution and/or as lyophilized powder.[118–121]

2.1.2.6. Lectins. Lectins are sugar-binding proteins having the ability to recognize and bind to specific glycoproteins on the mucosal membranes.[122] They can also enhance the active transportation of large molecular weight therapeutic proteins from intestinal

epithelium.[123] The most important derivative of lectin is WGA, which is isolated from *Triticum vulgaris*. WGA specifically bind to *N*-acetyl-D-glucosamine and sialic acid residues present on the intestinal mucus and can also be taken up by enterocytes.[124] WGA bound to carbapol has been investigated for efficient delivery of therapeutic protein via oral router. WGA have significantly shown improved adhesion on Caco-2 cell monolayer *in vitro* and membrane permeation *in vivo*. WGA has also shown to increase the blood concentration of calcium threefold greater than the carbopol liposome without WGA modification.[26]

2.2. Synthetic Polymers

Synthetic polymers have attained significant consideration from the formulators for delivery of therapeutic proteins and peptides. These polymers have shown to increase the pharmacokinetics and circulation times of incorporated therapeutic substances. Synthetic polymers often provide a passive function as drug carriers. In synthetic polymers, we have discussed the polyethers, polyesters, poloxamers, and recombinant protein-based polymers (Fig. 1).

2.2.1. Polyethers. Polyethers are synthetic compounds with more than one ether group in their molecular structure. These are generally known as synthetic polymers because of having ether group as a functional group in their main chain. The most commonly known polymer belonging to this class is polyethylene glycol (PEG) that has attained incredible consideration of pharmaceutical researchers due to its outstanding therapeutic applications.

2.2.1.1. Polyethylene Glycol. PEG is the most extensively studied polymer for efficient delivery of therapeutic proteins and peptides via invasive and non-invasive routes. This polymer (Fig. 8), known to be non-immunogenic, nontoxic, non-antigenic, and highly soluble in water, is approved by FDA. Most importantly, a conjugation phenomenon is involved to conjugate therapeutic proteins with PEG. This phenomenon is known as PEGylation. PEGylation of therapeutic proteins prevents enzymatic degradation and protein immunogenicity, prolonging the residence time in body and improving the stability, pharmacokinetics, and therapeutic activity of PEGylated proteins by altering various physicochemical properties.[125–129] Till now, several therapeutic proteins and peptides have been PEGylated. A majority of these PEGylated therapeutic proteins and peptides have been represented in Table 1.

PEG/PLA-based copolymers have diverse thermoreverable characteristics. Various derivatives of these block copolymers have also been synthesized to obtain the desired thermogelation behavior.[145] PEG/PLA block copolymers have been intensively evaluated for the delivery of therapeutic proteins and peptides using different delivery systems and routes.[146–149]

PCL is hydrophobic in nature with controlled biodegradability and biocompatibility. It has gained significant interest for delivery of therapeutic substances.[150,151] Owing to

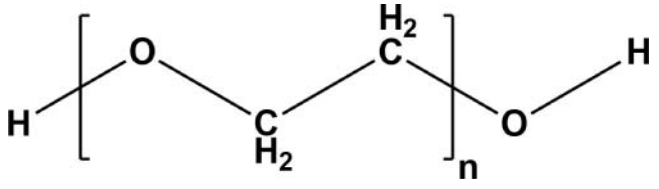

Figure 8. Structure of PEG. Here n represents repeated element in parentheses.

Table 1
Overview of available PEGylated therapeutic proteins and peptides

Protein	Animal model	Route	Therapeutic outcomes	Ref.
Insulin	Balb/c mice	Oral	PEGylated insulin maintained the biological activity, decreased blood glucose levels	125
Insulin	Diabetic rats	SC	PEGylated insulin PLGA microparticles lowered blood glucose levels and maintained for 9 days	126
Insulin	Diabetic rats	SC	Bioactivity of mono-PEGylated insulin was mostly preserved and increased of its resistance to proteolysis	127
IGF-I	C57BL/10ScSn mice	SC	PEGylated IGF-I ameliorated the pathophysiology in models of severe muscular dystrophies	128
UC-r	Rabbit and Balb/c mice	SC	PEGylated UC-r was stable at 70 °C, preserved the native-like secondary structures of UC-r, sufficient reduction of the immunogenicity	129
TIMPs	Mice	IP	Complete inhibitory activity toward the MMP-3 catalytic domain and partial inhibitory activity toward full length MMP-9, PEGylation extended the plasma half-life of rhTIMP-1	130
rhGH	SD-rats	Tail vein	PEGylation preserved the native-like secondary structures of hGH, increased half-life 4.5-fold with respect to native hGH	131
rhTSH	SD-rats	SC	showed a prolonged duration of action compared to rhTSH	132
SAK	Balb/c mice	SC	PEGylated SAK maintained the bioactivity, increased the plasma half-life, decrease the proteolytic sensitivity and immunogenicity	133
GLP-1	*db/db* mice	IP	PEGylated GLP-1 analogues were resistant to degradation and enhanced biological potencies of these analogues to lower blood glucose	134
GLP-1	SD-rats	IV, SC	PEGylated GLP-1 improved the half-life and improved the mean plasma residence time	135
GLP-1	db/db mice	IP		136

			PEGylated GLP-1 preserved insulinotropic activity, increased proteolytic stability and glucose-stabilizing capability	
GLP-1	db/db mice	IN	PEGylated GLP-1 had longer half-life than native GLP-1 and maintained hypoglycemic activity than GLP-1	137
GLP-1	db/db mice	IP	PEGylated GLP-1 preserved antidiabetic activity	138
Exenatide	Swiss Albino mice, SD-rats	IP	PEGylated exenatide improved *in vivo* glucoregulatory activity compared with exenatide	139
pGLP-2	BALB/c mice	IP	PEGylated pGLP-2 was resistant to degradation and reduced the severity of colonic injury	140
Exendin-4	Wistar rats	IP	PEGylated exendin-4 displayed glucose-lowering and insulin-stimulating action	141
Exendin-4	SD-rats	-	PEGylated exendin-4 prolonged the half-life of exendin-4 and improved anti-diabetic activity	142
CCK	Swiss Albino mice	IP	PEGylated CCK enhanced the therapeutic potential of CCK	143
Interferon α-2a	Athymic nude mice	SC	PEGylated interferon α-2a enhanced anti-tumor activity, showed no immunogenicity, increased serum half-life and mean plasma residence time	144

Abbreviations: **TIMPs**: Tissue inhibitors of metalloproteinases, **IP**: Intraperitoneal, **hGH**: Recombinant human growth hormone, **SD**: Sprague-Dawley, **rhTSH**: Recombinant human thyroid stimulating hormone, **SC**: Subcutaneous, **UC-r**: recombinant uricase, **SAK**: staphylokinase, **GLP-1**: glucagon-like peptide-1, **IV**: Intravenous, **IN**: Intranasal, **pGLP-2**: porcine glucagon-like peptide-2, **CCK**: Cholecystokinin.

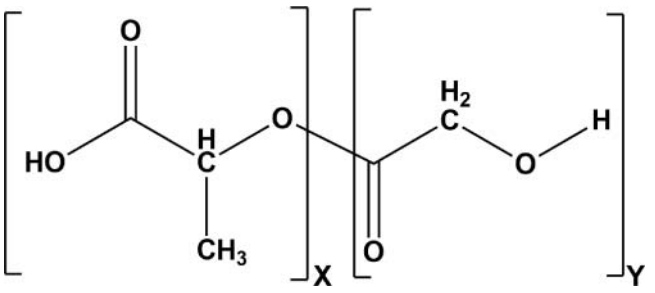

Figure 9. Two different monomers; glycolic acid and lactic acid constitute the structure of PLGA. Here the x represents the number of lactic acid units and y represents the number of glycolic acid units.

the high degree of hydrophobicity PCL degrades slowly and is less biocompatible with soft tissue, which restricts its clinical application. Therefore, the modification of PCL has been proposed. PEG for being non-toxic, hydrophilic, and lacking immunogenicity and antigenicity, is usually attached to PCL, forming PEG-PCL copolymer. Thus, their hydrophilicity, biodegradability, and mechanical properties can be improved.[152-154] PEG-PCL-PEG shows its sol-gel-sol transition behavior with the increase in the temperature.[155-158] Thermoreversible behavior of PEG-PCL-PEG depends upon the PEG/PCL ratio, block length of PEG, and PCL.[159] PEG-PCL-PEG block copolymer has been extensively evaluated for the delivery of a variety of therapeutic proteins, peptides, genes, and vaccines.[160–163]

2.2.2. Polyesters. Polyesters belong to the category of synthetic polymers. These polymers contain ester group in their main chain. There are many polyesters but the most important polymers being intensively investigated as drug carriers for delivery of therapeutic proteins and peptides are mentioned in the following sub-section.

2.2.2.1. Poly(lactic-co-glycolic acid). Poly (lactic-co-glycolic acid) (PLGA) (Fig. 9) is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). PLGA is an acronym for poly D,L-lactic-coglycolic acid in which D- and L-lactic acid forms an equal ratio. PLGA is biodegraded by hydrolysis of the ester linkages. The biodistribution of PLGA follows a non-linear and dose-dependent profile.[164] It belongs to the family of FDA-approved biodegradable polymers that has excellent biocompatibility and changeable biodegradability. A considerable amount of research has been conducted on PLGA for the delivery of therapeutic proteins and peptides since PLGA has exhibited immense potential as therapeutic substance carrier.[165] PLGA has been extensively studied for delivery of many therapeutic proteins, peptides, and other macromolecules such as DNA and RNA via different routes.[166–168]

There are certain limitations including low entrapment efficiency, initial burst release, instability of entrapped protein, and incomplete release profile that limit PLGA to be an ideal polymer.[166,169] These shortcomings can be overcome by copolymerizing other polymers with PLGA.[170] Various types of block copolymers of PLGA with PEG have been developed including PLGA-PEG, PLGA-PEG-PLGA, PEG-PLGA-PEG.[171–174] These block copolymers of PLGA behave as thermoreversible block copolymers. They have flowing properties at or below room temperature and rapidly convert into the gel at body temperature. These block copolymers undergo gel-sol transition in water; therefore, triblock of PEG-PLGA-PEG are used for the encapsulation of therapeutic substance.[175] PEG-PLGA-PEG triblock copolymer forms gel as the temperature increases and works as sustained drug delivery depot *in vivo*.[176] The critical gelation

concentration is controlled by changing the composition of PEG-PLGA-PEG copolymers. It has been found that *in situ* gel formed after subcutaneous administration of PEG-PLGA-PEG into the rats can persist more than one month.[174] These block copolymers are biodegradable in nature and their biodegradability has been well documented.[177,178] They are biodegraded into non-toxic small molecule monomers. These block copolymers have been extensively studied for efficient delivery of therapeutic proteins and peptides.[179–182]

2.2.3. Poly (N-isopropylacrylamide-co-propylacrylic acid) copolymers. Poly (*N*-isopropylacrylamide-co-propylacrylic acid) copolymers (PNIPAAM) and its derivatives are the most invasively investigated polymers for delivery of therapeutic substances.[183–186] Though, PNIPAAM has a great potential for delivery of therapeutic proteins and peptides,[187] but the clinical use of PNIPAAM and its derivatives is limited as these are non-biodegradable and upon contact with blood, they activate the platelets.[188,189]

2.2.4. Poloxamers. Poloxamers are non-ionic triblock co-polymers. The central part of the poloxamer is hydrophobic in nature that is made up of polypropylene oxide (PPO), whereas the central part is surrounded by the polyethylene oxide (PEO) which is hydrophilic in nature. Poloxamers are also known by their trade name, pluronics.[190,191] These polymers are thermosensitive in nature and have been intensively studied for sustained delivery of therapeutic proteins. Due to their temperature-responsive behavior, these are also known as thermosensitive polymers.[175,192–195] These polymers are inert in nature and are known to maintain the stability of incorporated therapeutic proteins and peptides with an increase in their survival period as compared to other sustained release drug delivery systems.[145,196,197] Other than having the efficient property to deliver therapeutic substances to the targets, these polymers have a diverse range of applications. In the following subsections, we have described the most commonly used polymer, i.e., pluronic F127 (PF127) for efficient delivery of therapeutic proteins and peptides.

2.2.4.1. Pluronic F127. PF127 is made up of repeating units of PEO and PPO having PPO as a central region. PEO is hydrophilic in nature which surrounds the hydrophobic part; PPO of PF127 (Fig. 10). PF127 has shown to be non-irritant and cytocompatibile with various cell types, and has been approved by FDA as a biomaterial for delivery of therapeutic proteins and peptides.[198,199] PF127 maintains the thermostability of incorporated proteins. Incorporated therapeutic proteins are known to be completely recovered from PF127-based thermosensitive gel after the dissolution of gel in excess buffer at body temperature and/or biological fluid.[200] Because of its thermo-reversible characteristics, PF127 remains in liquid state at room temperature and rapidly converts into a semi-solid, rigid gel at body temperature depending on the concentration used.[201] Due to its sol-gel transition characteristic, PF127 is easily administered into the body via an invasive route. PF127-based thermosensitive gel possesses excellent biocompatibility with biological fluids. Beside its suitability to be easily injected into the body, its method of drug formulation is

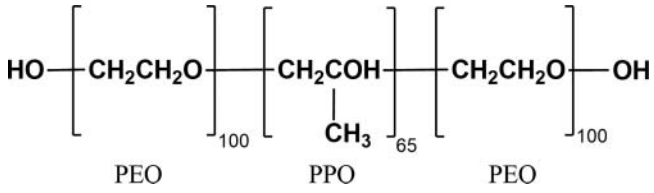

Figure 10. Structural formula of PF127 block copolymer. PEO: Polyethylene oxide, PPO: Polypropylene oxide.

also very easy, as a simple mixing process is required below room temperature without the involvement of overheating which may cause the denaturation of therapeutic proteins. This simple method of preparation has the advantage over other polymer-based techniques which include complicated and multiple steps in their preparation method.

After being administered into the body, PF127 immediately converts into a semi-solid rigid gel. Once the gel is formed inside the biological fluid, it maintains the integrity of incorporated protein for a desired period of time depending upon the concentration of PF127 used.[177] PF127 does not alter the physiological functions of the body and is easily cleared out via the renal route in the form of unimers.[202] Type 2 diabetes mellitus (T2DM) is an auto-inflammatory syndrome and conventional therapeutic agents may have certain limitations.[203,204] Various dietary- and herbal-based treatment strategies are being used to cure T2DM.[205,206] Interleukin-1 receptor antagonist (IL-1Ra) has shown its anti-diabetic effects in GK-rats.[207,208] Various delivery strategies have been investigated to prolog the half-life and sustained release of IL-1Ra using polymers.[97] Recently, IL-1Ra has been incorporated in PF127-based thermosensitive gel and investigated *in vitro* and *in vivo* effects. PF127 significantly prolonged the sustained release of IL-1Ra in a dose dependent manner.[201] Moreover, PF127 also maintained the stability of IL-1Ra and kept the conformational integrity of IL-1Ra throughout the stability study period.[200] Thereafter, a one month study has also been conducted on diabetic GK-rats to investigate the anti-diabetic effects of IL-1Ra loaded in PF127.[209] At the end of the treatment period, no alteration in the normal physiological functions of the kidneys of GK-rats treated with PF127 was observed.

PF127 is among the most widely studied polymers for efficient delivery of therapeutic proteins and peptides. Despite its outstanding multiple biomedical applications reviewed by Akash et al.[210] for efficient delivery of therapeutic proteins and peptides (Table 2), there are some limitations such as short residence time due to its week mechanical properties. This might be due to rapid degradation in physiological fluids.[196,217,236] Pharmaceutical scientists have tried to evade this limitation of PF127 by making few physical and chemical modifications using other biodegradable polymers which may increase the residence time and mechanical properties that ultimately help prolong the sustained release of incorporated therapeutic proteins for a longer period of time compared to PF127 alone.[195,229,237–239] Mechanical strength and the bioadhesive properties of PF127 have been also increased by conjugating PF127 with other mucoadhesive polymers.[44,226,240,241]

2.2.5. Recombinant Protein-Based Polymers. Specific receptors such as enterocytes or M cells are present on intestinal epithelium that decreases the absorption of therapeutic proteins and peptides. Advances in genetic engineering have made it possible to develop genetically engineered polymers with desired monomer sequence and polymer length that may help in modifying these receptors and/or transporters on intestinal epithelium to increase the intestinal absorption of therapeutic proteins and peptides.[122,242,243] The most commonly used protein-based polymers are CPPs, lectins, and transferrin, and silk-elastinlike protein (SELP) polymers.[242,244] Recombinant protein-based polymers consist of repeating units of natural and/or engineered amino acid sequences that are produced by biological synthesis and genetic engineering.[245,246] These protein-based polymers have been extensively evaluated for the successful delivery of therapeutic proteins and peptides.[244] Protein-based polymers have many distinct properties as compared to other polymers as they are composed of amino acids and are known to biodegrade through natural degradation pathways. Their biodegradation products are

Table 2
List of PF127/copolymer-based therapeutic proteins and peptides

Protein	PF127/copolymer	Animal model	Route	Therapeutic outcomes	Ref.
	PF127/-	—	—	Maintained total protein content and stability	200
	PF127/-	Wistar rats	SC	Increased sustained release of and decreased peak plasma concentration of IL-1Ra. Maintained *in vitro* protein stability and *in vivo* bioactivity.	201
	PF127/-	GK-rats	SC	Produced hypoglycemic effects and did not influence the normal physiological functions of the kidneys and skin.	209
IL-2	PF127/-	SD-rats	IM	Increased sustained release of and decreased peak plasma concentration of IL-2	211
rHV2	PF127/-	SD-rats	SC	Maintained *in vitro* stability and *in vivo* bioactivity of rHV2 also prolonged the therapeutic effects of rHV2.	212
Insulin	PF127/PLGA	Wistar rats	SC	Exhibited prolonged hypoglycemic effects.	213
	PF127/CaA	SD-rats	SC	Prevented initial burst release and maintained *in vivo* release over 48 h.	214
	PF127/MC	Diabetic rabbits	SC	Exhibited goo *in vitro* release characteristics and maintained basal plasma level of insulin over 10 days	215
	PF127/UFA	Wistar rats	Rectal	Increased rectal absorption of insulin	216
	PF127/UFA	Wistar rats	Buccal	Profound and remarkable hypoglycemic effects	217
	PF127/MC/HPMC	—	—	Prolonged *in vitro* release and permeation of insulin	218
	PF127/HPMC	SD-rats	Buccal	Maintained hypoglycemia for 8 h	219
	PF127/CE	SD-rats	TD	Maintained long-term stability of insulin. Improved permeation of insulin and decreased plasma glucose concentration	220
Deslorelin/GnRH	PF127/-	Cows	—		221

(*Continued on next page*)

Table 2

List of PF127/copolymer-based therapeutic proteins and peptides *(Continued)*

Protein	PF127/copolymer	Animal model	Route	Therapeutic outcomes	Ref.
				Prolonged the sustained release of two hormones. Decreased the plasma levels of luteinizing hormone	
rhEGF	PF127/HP-β-CD	Rabbits	Eyes	Maintained *in vitro* stability and prolonged *in vitro* release of rhEGF. Increased residence time in pre-corneal area.	222
	PF127/COS	C57BL/6 mice	Wound site	Maintained *in vitro* biological activity and significantly enhanced the epidermal differentiation at wound healing site	223
rhGH	PF127/-	SD-rats	SC/IM	Prolonged *in vitro* and *in vivo* release	224
	PF127/-	Mixed breed dogs	SC	Maintained plasma concentration of hormone till 132 h	225
	PF127/ST-n	—	—	Prolonged the sustained release of hormone over 13 days	226
	PF127/HA			Showed sustained release pattern of hormone	227
	PF127/Chitosan	—	—	Prevented initial burst release and prolonged *in vitro* sustained release of hormone	44
Lentiviral vector	PF127/-	SD-rats	Brain	Delivered lentiviral vector to its target site without causing any injury	228
Plasmid DNAs	PF127/PL61	C57Bl/6, Balb/C mice, SD-rats	IM	Increased the expression of gene levels without causing any known toxicity	229
pCMV-Luc	PF127/HA	—	—	Retarded the initial burst release and prolonged the sustained release of pCMV-Luc over 10 days	230
IgG	PF127/-	—	—	Prevented the adsorption of IgG on solid surfaces	231
Ag85A	PF127/CpG	Balb/C mice	PR	Increased the residence time of Ag85A in lungs and increased the immune response	232
TT	PF127/Chitosan	Balb/c mice	INR	Significantly increased the immune response	233

BBD	PF127/CMs	ICR-mice	INR	Exhibited highest levels of BBD-specific antibody	234
TT, DT, rPA	PF127/Chitosan	Balb/C mice	IP	Significantly increased the immune response of these three antigens	235
Vit. B12	PF127/PEG	—	—	No polymer-drug interaction was found.	236

Abbreviations: **IL-2**: Inter leukin-2, **IL-1Ra**: Interleukin-1 receptor antagonist, **rHV2**: Recombinant hirudin variant 2, **CaA**: Calcium alginate, **PF127**: Pluronic F-127, **SD**: Sprague Dawley, **GK**: Goto kakizaki, **IM**: Intramuscular: **SC**: Subcutaneous, **MC**: Methylcellulose, **UFA**: Unsaturated fatty acids, **HPMC**: Hydroxypropylmethyl cellulose, TD: Transdermal delivery, CE: Chemical enhancer, **GnRH**: Gonadotropin-releasing hormone, **rhEGF**: Recombinant human epithelial growth factor, **HP-β-CD**: Hydroxypropyl- β-cyclodextrin, **COS**: Chitooligosaccharide, **rhGH**: Recombinant human growth hormone, **ST-n**: Stereocomplexed multi-blocks copolymers of poly(L-lactic acid) and poly(D-lactic acid), **HA**: Hyaluronic acid, **PL61**: Pluronic L61, **pCMV-Luc**: Plasmid DNA coding for luciferase, **IgG**: Immunoglobulin G, **Ag85A**: *Mycobacterium tuberculosis* antigen A85, **PR**: Pulmonary route, **TT**: Tetanus toxoid, **INR**: Intranasal route, **BBD**: Bordetella bronchiseptica antigens containing dermonecrotoxin, **CMs**: Chitosan microparticles, **DT**: Diphtheria toxoid, **rPA**: Anthrax recombinant protective antigen, **PEG**: Polyethylene glycol.

small peptides that can easily be eliminated from the body without causing any inherent toxicity.[247] The desired functional features in protein-based polymers such as hydrophobicity, secondary structures, and biorecognizable motifs can be easily produced by utilizing genetic engineering tools.[248] In the following sub-sections, we have briefly discussed the role of these protein-based polymers for efficient delivery of therapeutic proteins and peptides.

2.2.5.1. Cell Penetrating Peptides. CPPs have attained considerable interest for efficient delivery of therapeutic proteins and peptides.[249–251] CPPs enhances the low permeability of large molecular weight therapeutic proteins by increasing the absorption of therapeutic proteins through intestinal epithelium. Several studies have demonstrated that CPPs have potential to increase the permeability of poorly permeable therapeutic proteins.[252–255] CPPs use lipid bilayer of the cell membrane or the process of endocytosis to transport therapeutic proteins and peptides into the cytoplasm.[256,257] HIV-1 Tat, penetratin, and oligoarginine are important CCPs that have been extensively studied for intracellular delivery of therapeutic proteins.[250] CPP linked to insulin have demonstrated to increase the intestinal absorption of insulin as compared to normal insulin across Caco-2 cell monolayers.[258] CPPs have also been evaluated for the successful delivery of various macromolecules inside cells.[259,260] The length of oligoarginine as CPPs imparts its significant effect on the intestinal absorption of therapeutic proteins. One study has been conducted in which oligoarginine composed of six, eight, and ten arginine residues were coadministered with insulin.[261] D-form oligoarginine, comprising of eight arginine residues, showed the most potent absorption enhancing effect. This exhibited more than threefold increase of relative bioavailability as compared with other two forms of oligoarginine. Similarly, penetratin has also been shown to be a potent intestinal absorption enhancer for therapeutic proteins and peptides.[253,255]

2.2.5.2. Transferrin. Transferrin is an endogenous iron-binding glycoprotein. The main role of transferrin is to transport iron by transferrin receptor-mediated endocytosis into cells. Transferrin receptors are highly expressed on the intestinal epithelial cells. These receptors provide transferrin a way to facilitate the absorption of drug through intestinal epithelium. Transferrin have shown to increase the intestinal absorption of granulocyte colony stimulating factor, insulin, and human growth hormone.[262–264]

2.2.5.3. Silk–Elastinlike Protein. SELPs are composed of recombinant SLPs and ELPs. SELPs have specific physicochemical properties of SLPs and ELPs. The silk sequences of SELPs are obtained from *Bombyx mori* silkworm silk, GAGAGS, and the elastin repeating sequences of SELPs in each monomer are obtained from mammalian tropoelastin, GVGVP.[244] Physicochemical properties of SELPs depend upon the number and sequence of these repeats in each monomer. SELPs are soluble proteins in nature and undergo an irreversible phase transition to form densely cross-linked hydrogels. The silk part of SELPs imparts the mechanical stability of protein network whereas; the elastin part exhibits viscoelastic properties of the gel. Different delivery strategies have been investigated using these SELPs for efficient delivery of therapeutic proteins and peptides.[265–270] The hydrogel network formed by SELP controls the release of incorporated therapeutic proteins from hours to days depending upon the concentration of SELP, charge on the released agents and ionic strength of the release media.[268,271,272]

3. Clinical Significance of Polymers

All the polymers enlisted in this article have been approved from FDA for their use as a biomaterial for delivery of therapeutic proeins in human beings reflecting there clinical

significance.[6,273–275] Many therapeutic proteins incorporated in polymers have also been approved from FDA for the treatment of different diseases and syndromes. The most important therapeutic proteins approved from FDA include recombinant human growth hormone (Nutropin Depot), insulin (insulin lispro), pegademase bovine (Adagen®), L-asparaginase (Oncaspar®), interferon α-2b (Pegintron®), and interferon α-2a (Pegasys®).

4. Toxicological Evaluation of Polymers

Generally speaking, the possible toxicity concern regarding the use of polymers is one of the major challenges during the use of polymeric-based medicines for human beings. The possible toxicological events could involve physiological, physicochemical, and molecular considerations.[276–278] Physico-chemical characteristics of the polymers might influence the interactions that occur at the interface between polymers and biological systems. Moreover, the biodistribution of polymer-based therapeutic substance to a specific target site is mostly influenced by the physicochemical characteristics of the polymer along with biocompatibility and biodegradability.[279] There are three main factors including biodistribution, phagocytosis, opsonization, and endocytosis that may cause the potential toxicity of the polymer.[280] The particles taken up dendritic cells after opsonization activate NALP3 inflammasome that potentiate both innate and antigen specific cellular immunity. Along with the activation of inflammasome, interaction with the vascular components of the blood may also potentiate local inflammation. Moreover, interaction of drug carriers with mitochondria may also induce the production of reactive oxygen species that can ultimately lead to a wide range of toxic cellular events including apoptosis, inflammation, and induction of signaling pathways.[281] Despite the activation of various immune systems, protein-based polymers may also cause the downregulation of the immune system.[282]

Although the potential toxicity associated with the tissue accumulation and metabolism of biodegradable polymers is considerably less, but upon the interaction with blood constituents, these polymers may induce minor level of cytotoxicity, hemotoxicity, inflammation, and oxidative stress. Similarly, anionic polymers have been reported to be biocompatible[283] whereas, cationic polymers may cause cytotoxicity by inducing apoptosis and, when in direct contact with blood, can activate the blood coagulation pathway.[284,285] To reduce toxicity and improve hemocompatibility, cationic polymers require surface modification.[286–288]

5. Stability Issues for Therapeutic Proteins Incorporated in Polymers

Stability of therapeutic proteins incorporated in polymers is a big challenge for the pharmaceutical scientists for the commercialization of these polymeric-based protein therapeutics. Generally, there are three main critical factors that may affect the stability of therapeutic proteins incorporated in polymers including (i) chemical, (ii) physical stability of the incorporated protein and polymer, and (iii) drug release characteristics. Among these critical factors, chemical stability is a major concern that needs considerable attention from the formulators and researchers as it involves the drug-polymer interactions that may cause the degradation of incorporated protein and results in production of untoward effects. Similarly, physical instability is also a second big hurdle for the success of therapeutic proteins. Storage conditions may also potentiate the certain factors that may result in physical instability. Moreover, physicochemical properties may also influence

the chemical and physical stability of incorporated proteins. These factors are very critical and should also be considered during the development of therapeutic proteins.

6. Future Perspectives

Despite considerable research and development in recent years, the question of availability of an ideal polymer for the delivery of therapeutic proteins still needs to be addressed. Presently, a small number of polymers, either biodegradable or non-biodegradable, have been successfully evaluated for the delivery of therapeutic proteins using either an invasive or a non-invasive route; however, there is a need to develop advanced methodologies for the characterization of therapeutic proteins incorporated in polymers. Protein stability and compatibility is another concern for the researchers and therefore, development of *in vitro-in vivo* correlation is mandatory for better evaluation of compatibility of therapeutic proteins with polymers. Another critical concern that requires attention to be paid upon is the immunological reactions of the therapeutic proteins incorporated in polymers. This requires a better understanding of the pattern of protein release from the polymer and its presence in the immune system. Overall, the successful future of polymers as ideal drug carrier systems for therapeutic proteins depends on the pharmaceutical researchers and formulators to develop effective polymeric-based particles of therapeutic proteins. Although, significant advancements have been made till now, still there is a need for more investigations in order to make therapeutic proteins commercially available in the market at reasonable and affordable prices for the common people.

7. Conclusions

Therapeutic efficacy of proteins and peptides also depend upon the suitability of polymers used for the delivery of these therapeutic proteins and peptides. Enzymatic degradation, poor absorption, stability, short biological half-life, and rapid elimination of therapeutic proteins and peptides are the major obstacles that limit the use of therapeutic proteins and peptides for the treatment of life-threatening diseases. Nowadays, pharmaceutical scientists are focusing on polymer-based therapeutics using natural and/or synthetic polymers as an ideal drug carrier to achieve desired therapeutic effects. These polymers are more or less commonly inert in nature, biocompatible with biological fluids, biodegradable, and eliminate from the body as inert biodegradable products. Advancements in genetic engineering and pharmaceutical biotechnology has made it possible to synthesize recombinant protein-based and enzyme-specific polymers that may help in the release of therapeutic proteins to the targeted diseased cells and/or tissues.

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