Amandeep Singh*, Kamlesh Kumari and Patit Paban Kundu

Synthesis of biopolymer-polypeptide conjugates and their potential therapeutic interests

Abstract: Biopolymer-based conjugates are widely used for numbers of biomedical applications. Materials scientists have become progressively interested in compounding biological-relevant entities with biopolymers into polymeric biohybrid framework. Biopolymer are conjugated with various fragments such as enzymes, proteins, nucleic acids as well as their analogues, peptidomimetics, peptides, fluorescent composites, avidin or streptavidin, biotin, polyethylene glycol, and various other bioactive compounds in order to serve a particular functionality in biomedical applications. In current chapter, a summary of various methods to synthesize biopolymer-peptide biohybrid conjugates and their prospective applications in biomedical field is presented.

Keywords: bioactive hybrids; peptide-containing biopolymers; protein mimetics

1 Introduction

Conceptually, the biopolymer-polypeptide conjugates (BPCs) are bio-based or bio-derived macromolecular building blocks. The self-assemblies of BPCs into diversified supramolecular architectures, i.e., chains of polymers, anisotropic one-dimensional array, two dimensional arrays, and more complicated three-dimensional cross networks are well known in linear, coiled-coil, and cyclic form. The building blocks based on the peptides are predominantly interesting for formulating the completely mimics because of the synthetic convenience as well as their compatibility with aqueous media and assembly proprieties.

Peptides as well as proteins are structurally linear polymers comprised of α-amino acids monomers. The proteins are comparatively longer polymer as compared to the peptides. The protein designates biological macromolecule itself having a constant conformation while a peptide is typically short oligomers of amino acid. Each natural amino acid of total 20 possesses an analogous code consisting three-letter and one-letter. Most significant properties of these amino acid are the polarity of their side chain which fundamentally commands the protein folding. The nonpolar side chains (i.e., $-CH_3$ group)

***Corresponding author: Amandeep Singh**, Department of Polymer Science and Technology, University of Calcutta, Kolkata, India, E-mail: adsingh.chemical@gmail.com. https://orcid.org/0000-0002-2465-3220
Kamlesh Kumari, Department of Chemical Engineering, SLIET Longowal, Punjab, India
Patit Paban Kundu, Department of Polymer Science and Technology, University of Calcutta, Kolkata, India; and Department of Chemical Engineering, Indian Institute of Technology, Roorkee, India

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are hydrophobic in nature and preferentially not interact with the water while polar side chains (i.e., –C(O)–, $–NH_2$, –OH, and –SH) are hydrophilic in nature and interacts. Amino acids have tendency to connect to form a long chain through amide linkage between –COOH group of one amino acid and $–NH_2$ group of adjacent amino acid. Generally, natural proteins have secondary structure in the form of α-helix, an α-helix secondary structure is a coil of right-handed spiral having backbone dihedral angles of $\varphi = –60°$ and $\psi = –45°$. Apart for this, another secondary structure is β-sheet comprise of two or more β-strands linked horizontally through hydrogen bonds having dihedral angles of $\varphi = –130°$ and $\psi = 120°$. The proteins accomplish various types of functional activities and distinguish several thousands of various molecules present in the cell. Such diverse as well as irregular three-dimensional protein structures designated as tertiary structures. The tertiary structures are ascertained from the primary structure as well as from elements of regular secondary structural.

Biopolymer-peptide and biopolymer-protein conjugates are of prodigious curiosity for several years. These conjugates are extensively explored due to their promising use in the medicine, nanotechnology, and biotechnology.

Conjugation of peptides and proteins with the natural polymers provides several benefits to the subsequent hybrid material. Bioconjugates combine the characteristics of their building constituents and overwhelmed the difficulties of each of them at the same time. For instance, the conjugation of proteins preserves or even raises the biological functions (i.e., enzymatic activity, receptor recognition, etc.) of the hybrid. Additionally, polymer subdues surface activity of protein in order to inhibit their degradation through proteolytic enzymes. Rise in the solubility as well as enhancement in protein's bio distribution are also anticipated features. PEGylation is a best example to be used to improve pharmacokinetics of the protein through its conjugation with polymer. This conjugation results to a substantial elongation of half-life of protein by prolonging the circulation inside the body.

In past few years, a substantial number of biopolymers are extensively used to deliver the small-molecule drugs, due to their exceptionally biocompatibility as well as biodegradability properties. In that direction, a collection of drug molecules were conjugated chemically to the biopolymers and these developed conjugates were supposed to be gradually release their load due to the breakage of biopolymer–drug bond under designed physiological parameters. Such conjugates were found to be offering various benefits as compared to their therapeutic precursors. Conjugates could improve the water solubility as well as bioavailability of drugs, and can also advance their pharmacokinetic and biodistribution aspects. Also, these bioconjugates can protect the drugs from their deactivation and further enables the transport of drugs to their specific targeted sites.

2 Synthesis of biopolymer-polypeptide conjugates

Application of the conjugates in the medicine domain involves great purity and distinct compound assemblies. Polymers in their form are highly required. The conjugates of the

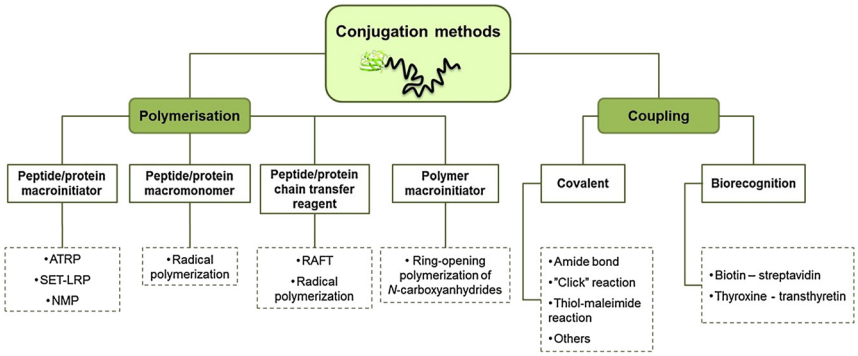

Figure 1: Methods of synthesis of polymer–peptide/protein conjugates.

biomolecules with various polymer chains can be produced using various approaches; mainly categorized into two sections of the reactions; the polymerization and the coupling. The conjugation of polypeptide with polymer through the polymerization is achieved using the peptide/protein macroinitiators/macromonomers/polymer macroinitiator method. Furthermore, the conjugation through the coupling method can further be divided into two parts; covalent coupling and coupling using bio-recognition groups. Various methods of synthesis of polymer–peptide/protein conjugates are shown in Figure 1.

2.1 Peptide conjugation by polymerization

The polymerization which leads to the conjugates is an approach of choice to develop conjugates having higher molar mass. Generally, polymerization technique generates one type of conjugate molecules devoid of the creation of unconjugated chains of polymers [1]. The conjugation by polymerization is accomplished either by peptide/protein macroinitiators, or peptide/protein chain transfer agents, or peptide/protein-based macromonomers, and several other polymer macroinitiators.

2.2 Peptide conjugation by coupling

However, conjugation by coupling is completed using biorecognition covalent connections. This technique suggests the development of tunable architectures having well characterized chains which are particularly beneficial for the bonding of peptides/proteins with the polymer of lower molar mass. It is important to mention that to acquire maximum coupling efficiency for the covalent linkage, the excess of polymer over protein/peptide amount is inevitable. Besides, a perfect purification phase is also essential next to the reaction completion [2]. The coupling using the biorecognition is described by

mild conjugation conditions and high selectivity, however, a slight excess of polymer component over the peptide/protein is also required to achieve an optimum yield of the conjugation.

2.2.1 Covalent coupling

Creation of the covalent linkages between a peptide/protein and polymer is accomplished using various reactions. Covalent coupling method is one of the widely used methods to synthesize the protein conjugates, for instance, conjugation of the PNIPAM to the anti-prostate specific antigen immunoglobulin G as described by Hoffman et al. [3]. Carboxylate-ended polymer chains were covalently linked to free amine groups present in the lysine. Polymer having carboxylic end-group with molecular weight 41,200 g/mol and dispersity index as 1.1 was produced using RAFT method of polymerization and thereafter was transformed to corresponding tetrafluorophenyl. Activated polymer gets reacted with protein to produce a conjugated product. Developed conjugate was employed as a sensor and used in the fabrication of a microfluidic immunoassay as shown in Figure 2.

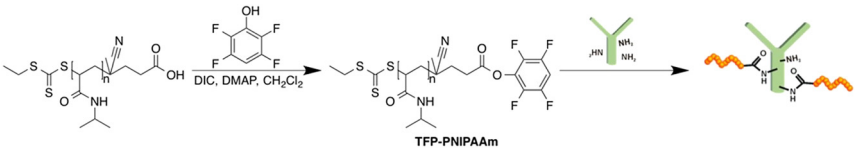

Figure 2: Synthesis of PNIPAM-antibody conjugate [3]. Reproduced with due permission.

2.2.2 Biorecognition coupling

Biorecognition coupling comprises the reaction of functionalized-polymer with protein. Biomolecule reacts with appropriate domain of protein as a ligand. The synthesis of bio-conjugates using biorecognition of thyroxine and biotin is conceivable because of strangely strong bonds between the reactive sites of the biomolecules. Streptavidin tetrameric assembly generates binding sites with in which the biotin remains through hydrogen bonding with the aspartic acid, tyrosine, asparagine, and serine [4]. Affinity of biotin towards the streptavidin is strong having the value of equilibrium constant (Ka) around $10^{13}\ M^{-1}$.

3 Therapeutic significances of BPCs

Applications of anti-PEG antibodies, as compared to PEGylated proteins and peptides, restricted the clinical relevance. IgM antibodies facilitate the permission of this

formulation over recurrence injection which leads to the toxicity issues and limited the efficacy of this therapeutic. In that direction, Sylvestre et al. [5] have perceived this occurrence in a virus-inspired polymeric system for the release of endosomal content that experiences pH-driven stimulus. However, peptide-polymer bioconjugate are considerably endured post single dose of the injection. The substitution of L-amino acid with another enantiomer (D-amino acid) peptide, the generation and toxicity of anti-PEG antibody was found to be reduced. In an experiment, mice were released from the L-melittin toxicity using prophylactic injection consisting platelet activating factor antagonist. However, the effect was observed to be very minimal which suggests that platelet activating factor is not a preliminary facilitator of the response of hypersensitivity. Consequently, it was established that D-amino acid peptide-polymer bioconjugates show an excellent tolerability *in vivo*.

Furthermore, the plasmid DNA transfection is said to be beneficial for the gene therapies demanding greater genetic elements containing CRISPR/Cas9 plasmids. However, it is restricted by the toxicity, humble intracellular release, and efficiency of transfection in the immune cell system. In that regard, Yi et al. [6] have proposed a synthetic non-viral gene delivery system consisting copolymer poly(ethylene glycol)-*b*-poly(propylene sulfide) bonded with cationic dendritic peptideas shown in Figure 3. Several self-assembled polymers were developed to ascertain the optimal formulations. However, the optimized formulation acts further in a most efficient way having slighter toxicity as compared to commercially available Lipo2K reagent. PPDP technology is

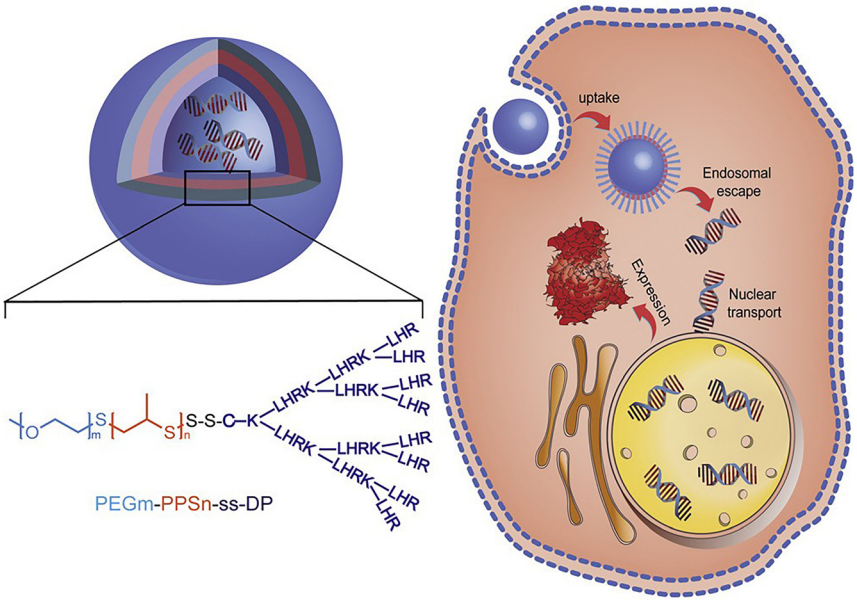

Figure 3: PEGm-*b*-PPSn-*ss*-DP (PPDP) gene delivery system [6]. Reproduced with permission.

defined as a stimuli-triggered polymer bioconjugate for the nano vector which is controllable to acquire miscellaneous difficulties in the domain of gene delivery.

Thereafter, Patrulea et al. [7] have proposed another chemical route for the coupling of derivatives of chitosan to the antimicrobial peptide dendrimers of various degrees of molecular masses using thiol-maleimide reactions strategy as shown in Figure 4. Research has concluded that the coupling of antimicrobial peptide dendrimers to the chitosan derivatives allow both the compounds to react in a good manner. The antimicrobial potential remains conserved during the incorporation of the antimicrobial peptide dendrimer conjugates in various bio-based polymers. In order to detect the exact route of reaction, electron and time-lapse microscopy was used. Results showed that the conjugates of antimicrobial peptide dendrimers with chitosan get affected after the complete demolition of the external as well as internal membrane of Gram-negative bacteria. These types of chemical-based strategies have potential to be used to prepare novel membrane disruptive therapeutics to eliminate the responsible pathogens present in the acute and chronic wounds.

Therapies in the combined form, i.e., photothermal therapy (PTT)-enhanced chemotherapy, are encouraging approach for cancer treatment. In that regard, Qin et al. [8] have proposed the redox-responsive polymer-based vesicles derived from amphiphilic triblock copolymer of PCL-*ss*-PEG-*ss*-PCL as shown in Figure 5. In order to evade the restricted healing consequences of the chemotherapeutic drugs derived from the systemic acquaintances, the redox-sensitive polymer-based vesicles were prepared and loaded with two different chemotherapeutics, named paclitaxel (PTX) and doxorubicin (DOX). Moreover, another one named indocyanine green (ICG) too was encapsulated and cell-penetrated. Outcomes of this research revealed that these polymer-based vesicles are capable enough to load various types of drugs with acceptable content of drug loading, and trigger the drug release in response of reductive environment and accomplish the greater cytotoxicity through combination therapy of chemo-photothermal.

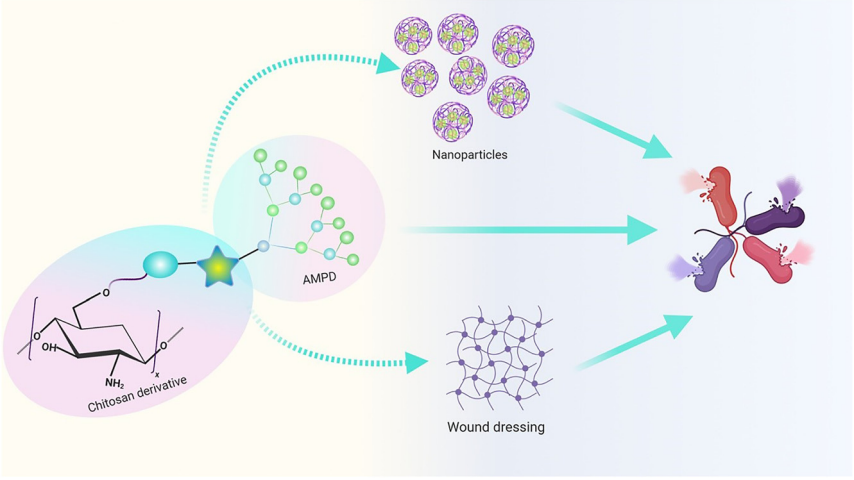

Figure 4: Antimicrobial peptide dendrimer-chitosan polymer conjugates [7]. Reproduced with permission.

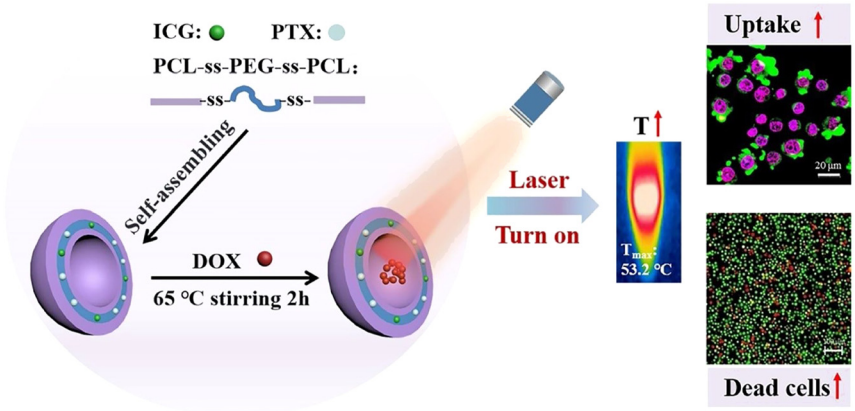

Figure 5: Dual peptides-polymer conjugates for PTT enhanced chemotherapy [8]. Reproduced with permission.

The strategy as well as the preparation of biodegradable, biocompatible, and established delivery methods for the drug as well as gene is important. In that domain, Charu Garg et al. [9] have proposed a method to prepare delivery method by integrating an amino acid into peptide backbone and conjugating with a low molecular mass cationic polymer named polyethylenimine (PEI). This approach lets authors to synthesize Boc-FΔF-AH-polyethylenimine conjugates miniseries using different concentrations of the Boc-FΔF-aminohexanoic acid. Such polymer conjugates get self-assembled within the medium and develop micelles (~144–205 nm) having core–shell conformation. The positive interface end of micelles enabled the linking of the plasmid DNA and transference within the cells. Hydrophobic core of these assemblies assisted in encapsulation process of drug molecule of hydrophobic nature. The DNA assemblies of these conjugates are nontoxic in nature and showed comparatively higher transfection efficacy than native polymer. Overall, these types of assemblies capable enough to deliver the drug as well as gene together *in vitro*, and thus, can be opted for the next-generation drug carriers.

As discussed earlier, the covalent bonding of polyethylene glycol chains is a very general method to enhance the properties of drug. Bonding of the polyethylene glycol chains increases pharmacokinetic characteristics of the therapeutic drug by enhancing the hydrodynamic radius. Chemical formulation as well as structural properties of the polyethylene glycol-biomolecule conjugates should be properly characterized using any quick and facial technique to utilize the efficiency of therapy. The mass spectrometry is used for this purpose since it is a finest analytical method to investigate the biopharmaceuticals existing in very minute amount. Furthermore, stoichiometric ratio of the components consisting conjugates of biomolecule and polyethylene glycol are simply analyzed through the data attained from the mass spectrometry. Binding sites of the polyethylene glycol chains of biomolecules are analyzed using data of tandem mass

spectrometry. In the study, angiotensin II peptide was PEGylated by using PEG chains with Mw = 600 containing modified acid halide end-groups [10].

Furthermore, a thermo- and pH sensitive linear l,d-octapeptide-poly(dimethylamino ethyl methacrylate) ((l-Val-d-Val)4-PDMAEMA) conjugate system was synthesized by Santis et al. [11]. Hydrophobic uncharged (l-Val-d-Val)4 octapeptide linear conjugate was prepared to self-assemble in the form of nanotubes using the tubular self-assembling characteristics. Thermo- and pH sensitive poly(dimethylamino ethyl methacrylate) was synthesized using atom transfer radical polymerization method. Thereafter, conjugates were developed using by click-chemistry onto a solid phase synthetized peptide. Due to the significantly strong interactions among peptide moieties, a long channel of nanotubes is made. At the basic pH and 25 °C temperature, it was observed that the size of nanotube channel is not considerably change. However, in the presence of basic pH and temperature greater than lower critical solution temperature of poly(dimethylamino ethyl methacrylate), a substantial rise in the length of nanotubes is perceived. Size of nanotube was found to be reserved up to several days post cooling. However, the sonication considerably diminishes the length of nanotube by developing low polydisperse nanotubes. Elongation of prepared nanotube found to be completely changeable on reinstating the pH in acidic range. These prepared thermo-responsive peptide-polymer conjugates having tunable length flexible between several micrometers and hundreds of nanometers. These tunable conjugated nanotubes and their mechanism is shown in Figure 6.

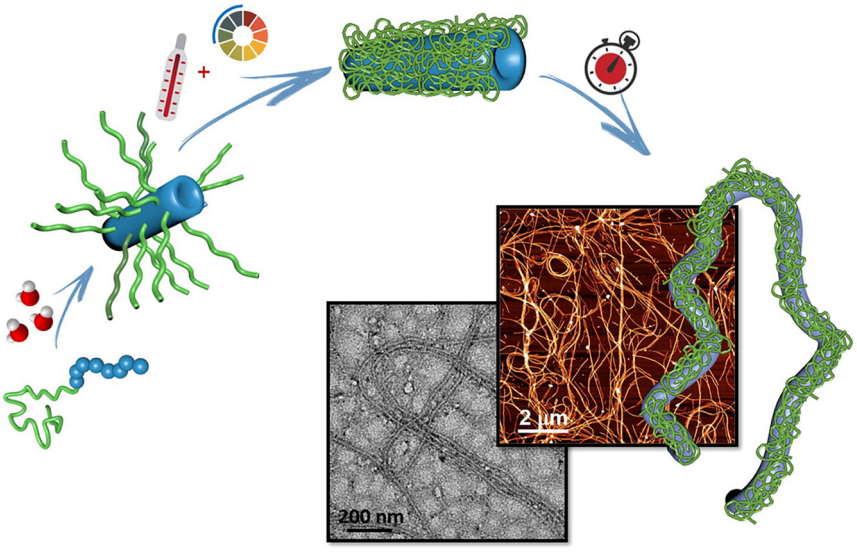

Figure 6: Switchable length nanotubes from a self-assembling pH and thermosensitive linear l,d-peptide-polymer conjugate [11]. Reproduced with permission.

Furthermore, the glucagon-like peptide-1 is an important moiety to treat type 2 diabetes mellitus because it prompts the secretion of insulin in glucose-dependent pattern and possesses the ability to expedite the control over the weight. Native glucagon-like peptide-1basically is a small incretin peptide and is vulnerable towards the firm proteolytic inactivation. Various glucagon-like peptide-1 analogs as well as their bio-conjugation are widely used to address such problems. Since the modifications are commonly supplemented through the detriment of the potency as well as through the induction of the immunogenicity. In that regard, Tsao et al. [12] have presented that using a conjugation of zwitterionic polymer with poly-(carboxybetaine), pharmacokinetic characteristics of the native glucagon-like peptide-1 can be significantly increased without any side consequences on the potency as shown in Figure 7. Furthermore, the poly-(carboxybetaine) conjugated with the glucagon-like peptide-1 delivered an adequate control over the glycemic up to the as high as 6 days in rat mode. These outcomes evidenced that such poly-(carboxybetaine)-based conjugations can be used along with the native glucagon-like peptide-1 in the treatment of glycemic control.

The nanosized conjugates of polymer and peptides consisting of functional peptides along with the synthetic polymer eradicate the obstacles of solo peptides. In a current research by Qiao et al. [13], authors have presented a comparison between U87 cells proteome modified using KLAK, mere polymer, and the conjugates of polymer and KLAK using the quantitative proteomics approach. Outcomes of this research have showed that the protein which are present in the oxidative stress response as well as in Nrf2/ARE route were found to be considerably advanced after the treatment using P–KLAK. However, the extra expression of the sequestosome 1 is not explored well for the KLAK toxicity.

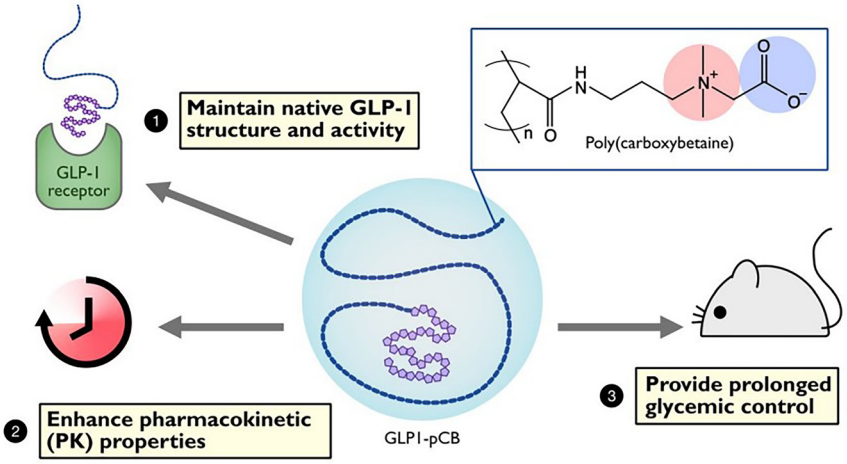

Figure 7: Conjugation of zwitterionic poly(carboxybatine) polymer onto 96 native glucagon-like peptide-1 [12]. Reproduced with permission.

The development of the conjugates of polymer and drug conjugate has potential to control the sustainable release of the drugs in the treatment of gastric cancer. In another research, conjugate of arginine-glycine-aspartic acid (RGD) with polyethylene glycol (PEG)-paclitaxel (PTX) consisting of disulfide bond were prepared. Amphiphilic nature of the conjugate assists the assembling of the micelles (RGD@Micelles) that can be disintegrated in reduction of the glutathione. The produced micelles were found to be spherical in shape having a hydrodynamic diameter about 50 nm. These nanoparticles were significantly stable in the physiological conditions. Release of paclitaxel against the glutathione from micelles was studied by Shi et al. [14] as illustrated in Figure 8. The *in vitro* cell study showed that the micelles hybrid of arginine-glycine-aspartic acid has capability to aim the cells of gastric cancer and can constrain the proliferation of cells through inducing apoptosis activity. The *in vivo* study showed that these micelles can deliver to the site of tumor and can prevent the growth efficiently of tumor through releasing the paclitaxel within the tumor cells. These kinds of the micelles showed higher therapeutic efficacy along with minimum side effects, which indicates the ability of these material for the gastric cancer treatment.

Furthermore, the immunotherapy is an utmost auspicious approach for cancer therapy. Generally, the human papillomavirus (HPV) is said to be responsible for maximum cases of the cervical cancer. A chief persistence of a therapeutic human papillomavirus vaccine includes the stimulation of CD8 + cytotoxic T lymphocytes (CTLs) and these stimulates has capacity to destroy the human papillomavirus contaminated cells. In that direction, Hussein et al. [15] have developed multi-antigenic systems based on the polymer consisting of E6 as well as E7 epitopes. Researchers have prepared epitope

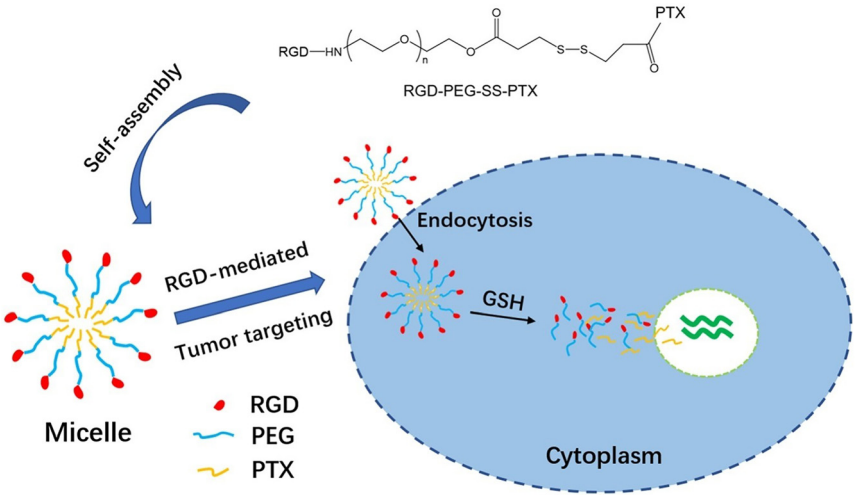

Figure 8: RGD peptide-decorated micelles assembled from polymer–paclitaxel conjugates [14]. Reproduced with permission.

conjugates of N-terminus for binding the unprotected peptides. This technique permits the inclusion of two different antigens into polymeric dendrimer.

Polymultivalent is a novel type of ligands which combines different ligand clusters together. In that way, a biocompatible and water-soluble polymer is developed by Duret et al. [16] as shown in Figure 9. Developed conjugates show two types of multivalency. These conjugates were developed using the covalent coupling of a certain amount of tetrameric cRGD peptide along with a pre-determined copolymer. Presence of the multiple sets of the clusters onto similar backbone ended up to a higher potency. Due to the polymultivalency of such conjugates, the improvement was stretched to a modest cell adhesion assay. Additionally, the confocal microscopy as well as the flow cytometry showed that the fluorescent polymultivalent conjugates that emit the far-red/near-infrared region were found to able to the specifically as well as selectively label cells.

Furthermore, an amphiphilic and water-soluble peptide-poly (1-vinylimidazole) bioconjugate was developed using 'grafting from' method which is based on the radical polymerization (thiol-mediated) by Dule et al. [17] as shown in Figure 10. It has been observed that the linkage of fluorescein isothiocyanate on peptide's N-terminus during bioconjugation leads to the formation of fluorescent bioconjugate. Due to the possessing

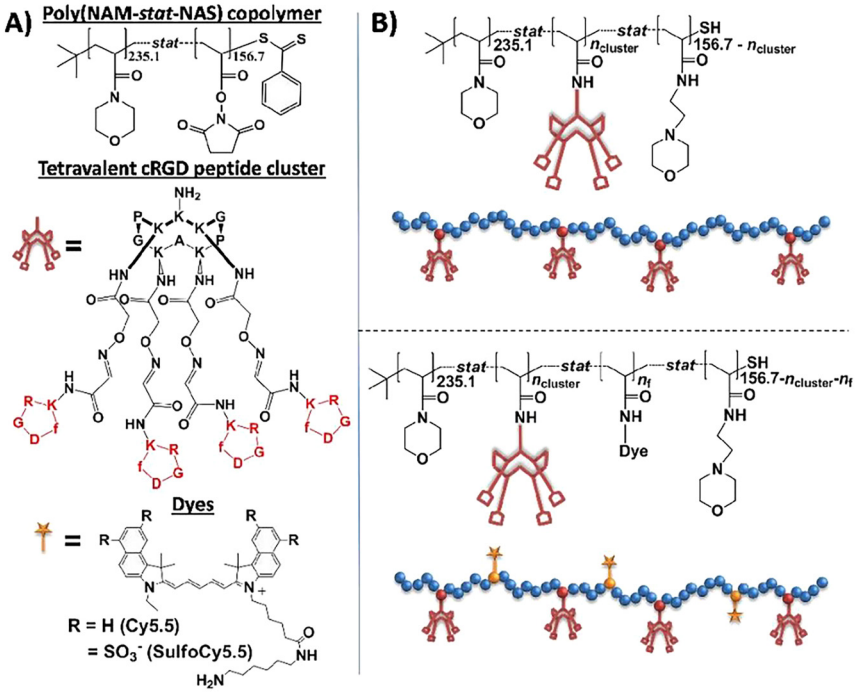

Figure 9: Water-soluble conjugate. (A) Structure of reactive copolymer, tetravalent cRGD peptide-cluster and cyanine 5.5 dyes. (B) Structure and schematic representation of polymultivalent conjugates [16]. Reproduced with permission.

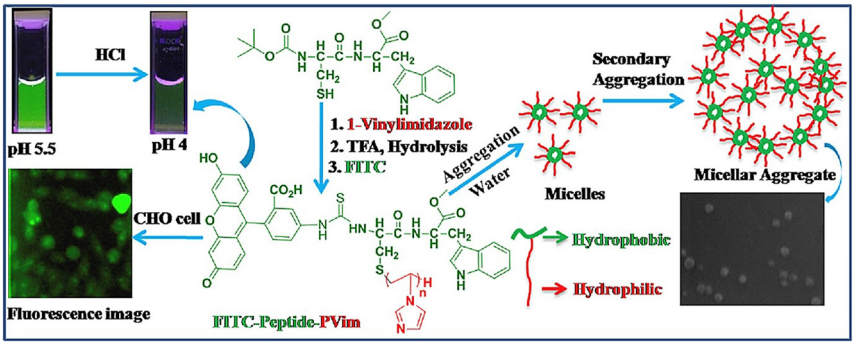

Figure 10: Cysteine-based amphiphilic peptide-polymer conjugates via thiol-mediated radical polymerization [17]. Reproduced with permission.

of amphiphilic nature, such bioconjugate experiences impulsive self-assembly into the preliminary form of micelles which further leads to the formation of bigger aggregations. Aqueous fluorescein isothiocyanate-Cys (PVim)-Trp-OMe conjugate shows green emission and consequently revelated fluorescence nature at 520 nm. Furthermore, amphiphilicity of such fluorescent bioconjuagte systems also initiates the self-assembly phenomenon with hydrophobic natured FITC-tagged core and hydrophilic natured PVim. Non-toxic as well as highly stable fluorescent conjugates are being applicable in domain of imaging of Chinese hamster ovary (CHO) cells.

Furthermore, the self-assembly as well as the bioactivity of a peptide–polymer conjugate (denoted as DGRFFF–PEG3000) consisting of RGD cell adhesion motif were examined in the aqueous solution by Castelletto et al. [18]. These conjugates were designed to be amphiphilic through incorporating of three different hydrophobic phenylalanine residues, RGD unit, and poly(ethylene glycol) (Mw 3000 kg mol^{-1}). Signals of the β-sheet structure were determined beyond the critical aggregation concentration using spectroscopic measurements and X-ray diffraction. Fibrils were found to be appeared in spite of the crystallization of PEG. Results recommend that the DGRFFF possesses the tendency of aggregation and it is appropriately strong not to be prohibited by the crystallization of PEG. Furthermore, the viability, adhesion, as well as the proliferation study of the human corneal fibroblasts was conducted. On the TCP, DGRFFF–PEG3000 films were developed at sufficiently minor concentration and cell viable as well as cell proliferation was seen. Wherever, a surface with low attachment experiences neither cell adhesion nor proliferation which indicates the unavailability of RGD motif in order to motivate the cell adhesion.

In a further research, Collins et al. [19] have carried-out *in situ* one-pot preparation of peptide-polymer conjugates as shown in Figure 11. The conjugation gets formed proficiently without any further purification of dithiophenol maleimide functionalized polymer as a disulfide bridging agent for therapeutic oxytocin. These conjugations of the

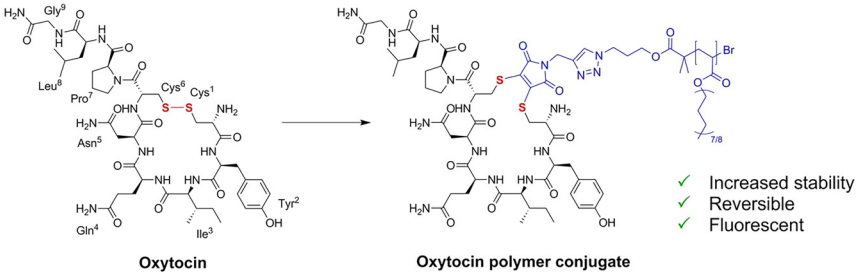

Figure 11: Structure of therapeutic peptide oxytocin and conjugation reaction with maleimide functional polymer [19]. Reproduced with permission.

polymers were found to be reversible in nature and suggestively improve the stability of the solution.

Further, Wilke et al. [20] have developed peptide–polymer bioconjugates consisting of a poly(ethylene oxide) block along with a precursor segment from the mussel foot protein-1 enzymatically oxidized by tyrosinase as shown in Figure 12. A functional transition from the weak/reversible binders to the strong/irreversible adsorption onto an aluminum oxide surface was detected. A set of mussel foot protein-1-*block*-PEO conjugates along with various lengths of PEO-blocks were developed. Different molar masses (850, 3200, and 5200 g/mol) of PEO were prepared and the effects of block length onto enzyme activable formation was analyzed. It was found that the variation in the PEO-block length scientifically affects the activation kinetics of conjugates. Further, the

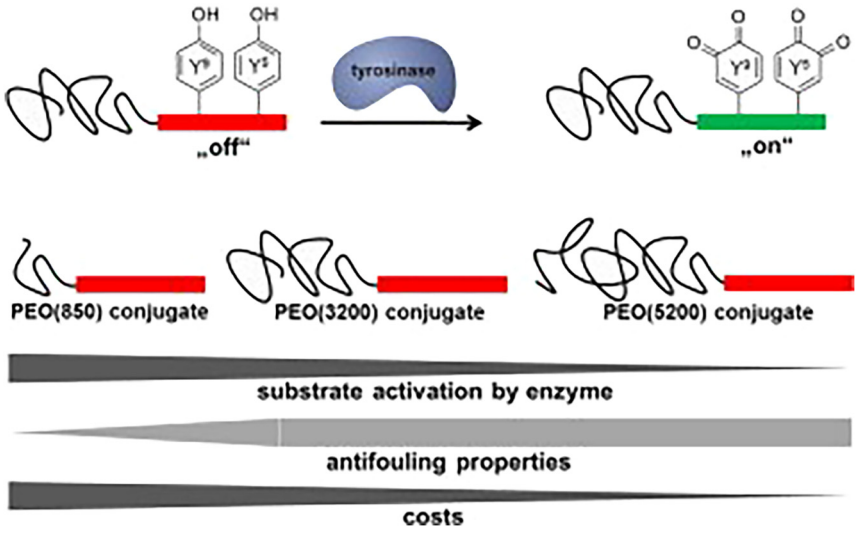

Figure 12: Poly(ethylene oxide) blocks on enzyme activable coatings [20]. Reproduced with permission.

adhesion behavior of such activated bioconjugates and stability of resulting Al_2O_3 coatings as well as the antifouling features of coated Al_2O_3 surfaces get affected. Mussel foot protein-1-*block*-PEO3200 shows the superior cooperation since enzyme activation as well as adhesive features exhibited better results. The stable coatings onto the Al_2O_3 were developed within the activated state that further reduces the interactions between the albumin proteins. Coating was found to be appropriately denser as well as stable to reach comparable antifouling characteristics as covalently PEGylated Al surface.

Also, the catheter-originated infections are bigger problems in the modern medicine and this result to a substantial financial load along with increasing the risk of morbidity. Henceforth, an instant requirement to remove such problems was experienced. A functionalized substrate consisting of characteristically antibacterial features as well as biocompatibility may prevent the bacterial colonization. In that direction, Zhang et al. [21] have prepared a method to develop polymer brushes having hierarchical construction onto the polyurethane through surface-initiated atom-transfer radical polymerization (SI-ATRP). The surface-functionalized polyurethane consisting of poly(3-[dimethyl-[2-(2-methylprop-2-enoyloxy)-ethyl]-azaniumyl]-propane-1-sulfonate) brushes at the bottom surface whereas antimicrobial peptide-conjugated poly(methacrylic acid) (PMAA) brushes onto the exposed surface. Polyurethane-DMH layer exhibited good bactericidal capacity towards the Gram-positive as well as Gram-negative bacteria. It avoids the accretion of the bacterial wreckage over the surface. Instantaneously, polyurethane-DMH system was found to be possessing excellent hemocompatibility along with low cytotoxicity. Moreover, integrated antifouling as well as bactericidal features of the polyurethane-DMH under the hydrodynamic environment were established using *in vitro* circulating model. Also, functionalized surface consists insistent antifouling as well as bactericidal capacity under the static and hydrodynamic circumstances. Furthermore, microbiological as well as histological outcomes of animal model study revealed about the existence of *in vivo* anti-infection property.

Furthermore, the nanoparticles (NPs) have gained substantial attention in order to develop the drug-delivery vehicles owing to the merits of NPs, i.e., lengthy circulation time as well as inactive directing to the tumor site. The polymer–peptide conjugates (PPCs) have capacity to get self-assembled into the NPs in an aqueous solution. These resulting NPs as drug vehicles get joined with the virtues. In that aspect, Cheng et al. [22] have presented a facile synthetic technique based on the thiol–acrylate Michael addition reaction as one-pot synthesis for amphiphilic hyperbranched poly(β-thioester)s conjugated with the cytotoxic peptide $(KLAKLAK)_2$ and poly(ethylene glycol) as demonstration in Figure 13. The amphiphilic hyperbranched poly(β-thioester)s get self-assembled into the NPs in aqueous media and hyperbranched poly(β-thioester)s (PPHD) consisting acid-labile β-thiopropionate group acted as interior of NPs. Amphiphilic hyperbranched poly(β-thioester)s NPs exhibited improved cellular uptake and also indulge the antitumor activity. The antimicrobial properties may be attributed to the spherical structure along with superficial positive charges and mitochondria-regulated apoptosis of $(KLAKLAK)_2$. As compared with the linear polymer–peptide conjugates, the stability of NPs as well as the loading efficiency of amphiphilic

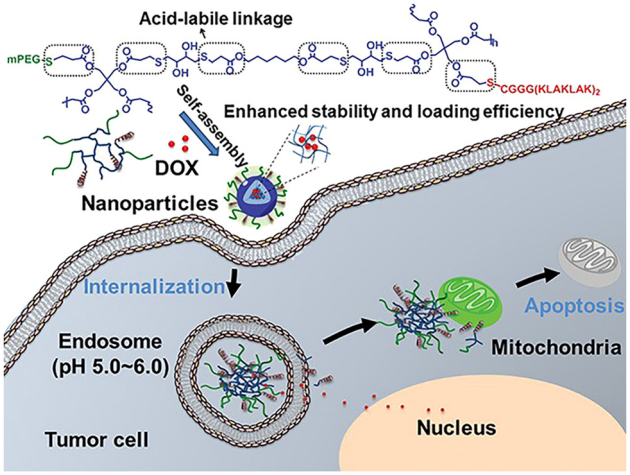

Figure 13: pH-responsive hyperbranched polymer–peptide conjugates. Adapted from [22]. Reproduced with permission.

hyperbranched poly(β-thioester)s for the drug was found to be suggestively enhanced. It suggests the presence of a stronger intermolecular interaction caused by the intertwisting of highly branched networks in NPs core. Generally, the doxorubicin (a typical chemotherapeutic drug) is readily released from the amphiphilic hyperbranched poly(β-thioester)s NPs under acidic pH of lysosome which leads to the effective nuclear drug translocation as well as resultant potent drug efficacy. Additionally, the DOX-loaded amphiphilic hyperbranched poly(β-thioester)s NPs exhibited greater cytotoxic activity as compared to the DOX-loaded PPHD-P as well as the blank amphiphilic hyperbranched poly(β-thioester)s NPs. Consequently, the DOX-loaded amphiphilic hyperbranched poly(β-thioester)s NPs with improved stability as well as loading efficiency showed good potential as antitumor nanodrugs.

In recent research, a combined therapy consisting of thermal ablation as well as polymer conjugated heat shock protein 90 chemotherapy was developed and analyzed for the tumor abolition of hepatocellular carcinoma. Heat shock protein 90 inhibition is important to prevent the reappearance of hepatocellular carcinoma, and combination of ablation with targeted therapy seems excessive possibilities to advance the prognosis as well as survival of hepatocellular carcinoma patients [23]. Elastin-like polypeptide (ELP) is a macromolecular transporter with temperature responsive features that can be accumulated within the solid tumors and may aggregated in the tumor tissue over expose to the hyperthermia. ELP was conjugated with anticancer drug doxorubicin (DOXO) as well as three various cell penetrating peptides (CPP) to constrain the growth of tumor in the mice as compared to the free doxorubicin [24].

4 Conclusions

This chapter highlighted the substantial usefulness of biopolymer-polypeptide conjugates and their potential therapeutic interests. The chemically conjugated therapeutics represents their ability to work either as active pharmaceutical ingredient (API) itself or as drug vehicle. Conjugation mechanism fascinates the consideration of concerned investigators since it advances the previously present drug product features and also trains entirely new properties. Thus, conjugated peptides or proteins, as chemically associated moieties, exhibits significant properties such as variation of pharmacokinetics, development in permissibility and safety, diffusion into difficult-to-enter segments; for example, blood–brain barrier (BBB) transcytosis, intracellular supply, etc. Additionally, the influence of chemically-functionalized peptide/protein medicines on the therapeutic area is enormous due to their applications in different fields such as oncology, ophthalmology, neurology, immunology, muscle disorders, endocrine, etc.

5 Challenges and future perspectives

A most common contest related to the biopolymer-polypeptide conjugates is their complexity and this difficulty can be resolved using a cautious selection, proper optimization of the peptide/protein, linker, and also polymer payload. Furthermore, the computational as well as the theoretical studies need to be conducted to support the mechanisms of peptide or protein interactions with different polymers to explore their stability and biological activity. New experimental and computational methods shall explore the structural properties of biological as well as synthetic components of a bioconjugate that shall further simplify these structure function relations required to tune the bioconjugates to any specific applications.

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