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REVIEW

Bioconjugates and Nanocarriers for Therapy, Imaging, Drug Design and Targeting

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Currently, bioconjugates and nanocarriers can be practically used in drug discovery, therapy, diagnosis, imaging, targeting, stability, *etc*. Also preparing nano drug carrier systems (NDCSs) is a common application of bio-conjugates. Current examples of bio-conjugates are nanoparticles, liposomes, micelles, dendrimers, quantum dots, carbon nanotubes, nano-fibers, but not limited to. As bioconjugates, nano drug carrier systems have important roles in the development of intelligent drugs such as tailor made drugs and drug targeting systems for early diagnosis steps and therapy. Currently, different drug carrier systems can be designed for improvement of the physico-chemical properties of drugs, controlling drug release, stability, *in vivo* performance and modification of the surface chemistry of drugs by using nanotechnology, bioconjugation and PEGylation. The anticancer efficacy of existing chemotherapeutic drugs can be improved by employing formulation, bioconjugation and nano-technological approaches. This current review focuses on the description of bioconjugates and nano drug carrier systems for diagnosis, imaging and therapy reported mainly in the last decade.

Keywords: Bioconjugates, Nano drug carrier systems, PEGylation, Drug targeting, Anticancer efficacy, Diagnosis, Imaging, Therapy.

INTRODUCTION

In the last three decades developments in nanotechnology, biotechnology, cell biology, pharmaceutical technology, medicinal chemistry and polymer chemistry have opened unexpected theranostic (diagnosis and therapy) opportunities for treatment of diseases. A vast number of natural, synthetic and semisynthetic drugs have been developed for imaging, diagnosis and therapy on diseases with poor prognoses such as cancer, fibrosis, viral infections, *etc*.[1,2].

It has been discovered that different drug carrier systems can be formed for alteration of the physicochemical properties of drugs, controlling of drug release and modification of surface chemistry of drugs by using nanotechnology, PEGylation and bioconjugation[1,2].

The anticancer efficacy and systemic toxicity of existing chemotherapeutic drugs can be improved by employing formulation, bio-conjugation and nanotechnological approaches. For this reason, drug delivery systems can be developed for more specifically targeted tumor tissue[3,4]. Cancer is a highly heterogeneous complex disease and it is well known that the malaise of cancer has ailed humans. Nanotechnology is an interdisciplinary research field developed with chemistry, engineering, material science, pharmaceutical technology, biology and medicine. It has various useful applications in cancer biology, such as early detection, discovery of cancer biomarkers and development novel treatment and drug carrier systems[5,6].This present review focuses on the description and recent applications on bioconjugates and nano drug carrier systems (NDCSs) for diagnosis, imaging and therapy.

Bioconjugates: Bioconjugation has enabled the production of new biomolecules. Each chemical modification or conjugation process comprises the reaction of one functional group with another, resulting in the formation of a covalent bond that involves the conjugation of antibodies, nucleic acids and other biologically active molecules with each other and with other molecular groups (Fig. 1)[1].

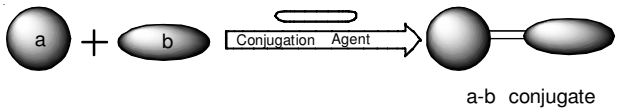


Fig. 1. Formation of a basic conjugate often involves the reaction of two molecules and a crosslinking agent

The major reactions used in bioconjugation along with the appropriate reaction partners, functional groups, activation agents or catalysts, the bonds formed as a result of the reactions and in some cases the reactive group intermediate or formed

new functional groups are listed in Table-1[1]. The ability to produce unique functional conjugates can make possible the creation of an actually unlimited selection of reagents having a wide variety properties. The use of these reagents can facilitate quantification, detection, purification, synthesis, imaging, therapy, diagnosis, *etc*.

Functional targets and current applications for bioconjugation examples are modification of amino acids, peptides and proteins, modification of sugars, polysaccharides and glycoconjugates, modification of nucleic acids and oligonucleotides, creating specific functionalities and blocking or protecting groups (Fig. 2)[1].

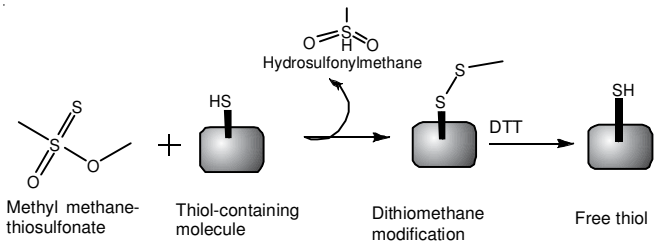


Fig. 2. 2,2′-Dipyridyl disulfide reacts with thiols to form an active pyridyl disulfide intermediate

Many other forms of homobifunctional reagents containing almost every conceivable chain length and reactivity are homobifunctional N-hydroxysuccinimide esters, homobifunctional imidoesters, homobifunctional sulfhydryl reactive crosslinkers, difluorobenzene derivatives, homobifunctional photoreactive crosslinkers, homobifunctional aldehydes, *bis*-epoxides, homobifunctional hydrazides, *bis*-diazonium derivatives and *bis*-alkylhalides[1].

Protein/peptide drugs are readily degraded at low pH (1-3) in the stomach and different digestive enzymes in the small intestine. Therefore, a pH-responsive drug delivery systems such as nanoparticles (NPs) and bioconjugates are prepared by using chithosan, poly(γ-glutamic acid, polyacrylic acid (PAA) and polyethylene glycol (PEG). Last researches show that bioconjugates and nano drug carrier systems may be suitable carrier for trans-mucosal delivery of protein/peptide drugs in the intestinal lumen and demonstrated the ability of the polymer to control the protein-ligand recognition and binding proses[7-13].

Nanoparticle bioconjugates are used for imaging, targeting and therapy of cancer. Anticancer drugs have severe side effects. The lack of tumor selectivity and recurrence of cancers with intrinsic/acquired drug resistance have also decreased the therapeutic efficacy of the drugs. Different nanovehicles such as nanoparticles, solid lipid nanoparticles, liposomes, polymeric micelles, quantom dots (QDs), dendrimer, *etc*. have been developed to solve such problems by using reduction-sensitive polymers, poly(amidoamine), polyvinylpyrrolidone, biodegradable and biocompatible poly(D,L-lactic-co-glycolic acid)-block polyethylene glycol copolymer, β-cyclodextrin, multiple PEGs. Intelligent nano carrier systems contain specific chains or ligands responsive to intrinsic or external stimuli such as changes in pH, temperature, ultrasound and enzymes[14-27]. The all actions of the stimuli-sensitive nanovehicles in the tumor are given in Fig. 3[18]. These systems show a long circulation time in blood due to their size and stability[18].

Polymeric and seramic nanoparticles have been widely employed as NDCSs, whereas metal and semiconductor nanoparticles act as probes for imaging and therapy. Among various nanoparticles, semiconductor quantum dots attracted much attention as probes for bioimaging. Also, bright emission, exceptional photostability, large surface area, large two photon absorption cross-section, availability in multicolor and with NIR photoluminescence are the most attractive properties of quantum dots for imaging and fluorescence imaging and photodynamic therapy of cancer[28,29].

PEGylation: PEG has been used for a long time as a modification and conjugation reagent for biological molecules to

TABLE-1
REACTIONS OF BIOCONJUGATION[1]

Bioconjugation reactions	Reactive group
Amine reactions: Reactive groups able to couple with amino-containing molecules are by far the most common functional groups present on crosslinking or modification reagents.	Isothiocyanates, isocyanates, acyl azides, N-hydroxysuccinimide esters, sulfonyl chlorides, tosylate esters, aldehydes and glyoxals, epoxides and oxiranes, carbonates, arylating agents, imidoesters, carbodiimides, anhydrides, fluorophenyl esters, hydroxymethyl phosphine derivatives and guanidination of amines
Thiol reactions: Reactive groups able to couple with sulfhydryl-containing molecules are perhaps the second most common functional groups present on cross linking or modification reagents.	Haloacetyl and alkyl halide derivatives, maleimides, aziridines, acryloyl derivatives, arylating agents, thiol–disulfide exchange reagents, vinyl sulfone derivatives, metal–thiol dative bonds, native chemical ligation and cisplatin modification of methionine and cysteine
Carboxylate reactions: They have been developed that allow conjugation through a carboxyl group.	Diazoalkanes and diazoacetyl compounds, N,N'-carbonyl diimidazoleand carbodiimides
Hydroxyl reactions: They are suitable for application in bioconjugate techniques for cross linking a hydroxyl-containing molecule with another substance, containing a nucleophile.	Epoxides and oxiranes, N,N'-carbonyl diimidazole,N,N'-disuccinimidyl carbonate or N-hydroxysuccinimidyl chloroformate, oxidation with periodate, enzymatic oxidation, alkyl halogens, isocyanates
Aldehyde/ketone reactions: These can be performed with aldehydes/ketones to modify or cross linked molecules containing them.	Hydrazine and hydrazide derivatives, schiff base formation, reductive amination, aminooxy derivatives and mannich condensation
Active hydrogen reactions: Cross linked molecules at active hydrogen are used in some modification reactions.	Diazonium derivatives, mannich condensation and iodination reactions
Photochemical reactions: Photoreactive groups can be induced with target molecules by exposure to UV light.	Aryl azides and halogenated aryl azides, benzophenones, anthraquinones, certain diazo compounds, diazirine derivatives, psoralen compounds
Cycloaddition reactions: They have highly specific reactant pairs which have a chemoselective nature.	Diels-Alder reaction, complex formation with boronic acid derivatives andclick chemistry: Cu^{1}-promoted azide-alkyne [3 + 2] cycloaddition

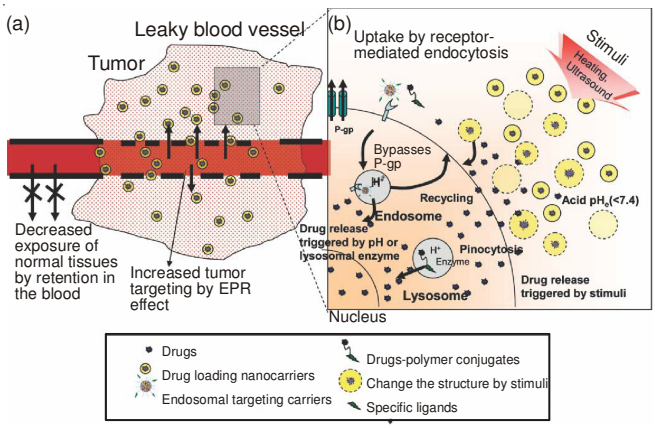


Fig. 3. Schematic illustration of actions of the stimuli-sensitive nanovehicles in the tumor site. (a) Nanovehicles administered intravenously (b) On arrival in the tumor, the drug release from nanovehicles can be triggered by several stimuli such as pH, temperature and ultrasound

increase in the water solubility, to decrease in immunogenicity, to protect modified protein and peptides from digestion. For these reasons, many drug candidates using PEGylation are currently in clinical trials and on the market. PEG is non-toxic, non-immunogenic, non-antigenic, highly soluble in water and FDA approved. PEGylation is the process of covalent attachment of PEG polymer chains to another drug or therapeutic protein. PEGylation or covalent attachment of PEG improves the pharmacokinetic properties of protein drugs. *In vivo* circulation lifetimes are increased and dosages are decreased, resulting in improved patient quality of life. PEG may be attached to proteins using a variety of different chemical reactions. The PEG-drug conjugates have several advantages such as a prolonged residence in body, a reduction of protein immunogenicity and decreasing degradation by enzymes. The PEGylation has a major role in drug delivery, stability, increasing the potentials of proteins and peptides as therapeutic agents (Fig. 4)[30,31]. As shown in Fig. 4[31], PEG is shielding the protein surface from degrading agent by steric hindrance. There is one advantage of this technique for stability. The conjugation of PEG to thiol, hydroxyl or amino groups can be done by using chemical and enzymatic methods. Most popular PEG derivatives and their properties are given in Table-2. Approved PEG conjugates can be used different diseases such as; acute lymphoblastic leukemia, immunodeficiency disease, hepatitis C, acromegaly and treating

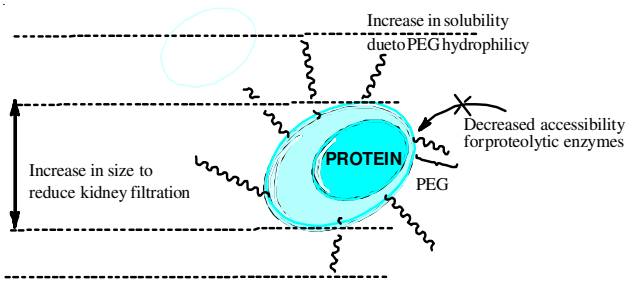


Fig. 4. Main advatages of PEGylated protein

of neutropenia during chemotherapy[31]. PEG-based new drug carrier systems namely dendrimers, NPs and drug conjugates could also open the way for stability, solubility, preparing of pro-drugs, drug targeting and changing release properties of drugs[31-37].

Surface-modification of drug delivery vehicles with PEGhas shown promise as a method to improve the stability and *in vivo* performance of various drugs (doxorubicin, non-viral drug) and gene vectors. In addition, PEGylation has recently been shown to dramatically improve nanoparticle and polymeric nanoparticles transport through biological obstacles (Fig. 5[43]). PEGylation of therapeutic colloids may also improve their cytoplasmic transport by minimizing attractive forces to cytoskeletal elements and reducing non-specific adhesionto cytoskeletal elements.This situation may be important in allowing drug/gene vectors, which have escaped endosomes, to efficiently reach the nucleus[38-43].

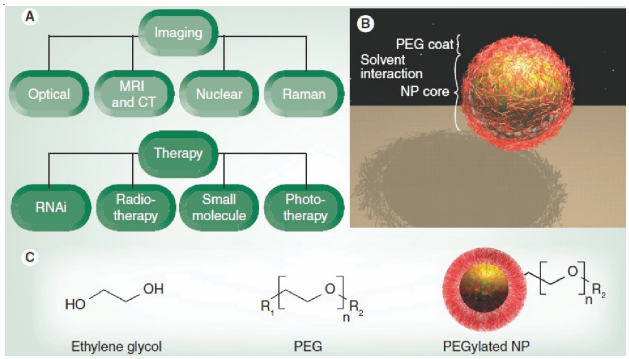
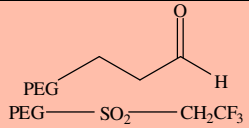
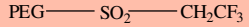
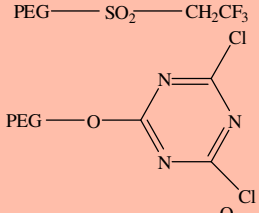
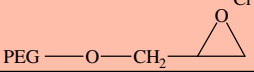


Fig. 5. Nanoparticle applications of PEG. (A) Use of nanoparticles in imaging involves different modalities, including optical and radionuclide techniques. (B) PEGylated nanoparticle. (C) Monomers of ethylene glycol are polymerized into PEG for nanoparticle coating. PEG contains the linkage group (R_1) and a terminus that interacts with solvent (R_2)

TABLE-2
PEG DERIVATIVES THAT MAINTAIN THE CHARGE OF THE NATIVE PROTEIN IN THE FINAL CONJUGATE[31]

Structure	Alkylating PEGs	Properties
	PEG-Aldehyde (also in the form of more stable acetal)	A two steps reaction; the first product (a Schiff base) is reduced by $NaCNBH_3$. When the coupling reaction is carried out at relatively low pH (4,5-5), it labels only the α-amino group.
	PEG-Tresyl or tosyl	Not much used because the chemistry leads to a mixture of products.
	PEG-Dichlorotriazine or chlorotriazine	Now they are abandoned for therapeutic application because of their toxicity.
	PEG-3-epoxide	Slowly reactive, rarely used.

Nanocarriers: Nano drug carrier systems, carbon nanotubes, dendrimers, liposomes, nanospheres, nanocapsules, polymeric micelles, polymeric nanoparticles, quantum dots, solid lipid nanoparticles, are able to increase the selectivity, efficacy and stability of therapeutic agents. These systems are used for delivery of therapeutic and diagnostic agent. However cytotoxicity, drug leakage, hemolytic toxicity, hydrophobicity, immunogenicity, reticuloendothelial system uptake limit the use of these systems. These short comings are overcome by using some changes such as surface modification, bio-conjugation, encapsulation, pegylation, *etc*. These applications are often used to control nano drug carrier systems' properties such as increasing longevity and stability of the carrier and drug in the circulation, favorably changed bio-distribution, targeting effect, stimuli (*e.g.* pH, temperature) sensitivity and contrast properties. Soluble synthetic polymers, specific ligands (antibodies, peptides, folate, transferrin and sugar moieties for targeting), pH or temperature sensitive lipids or polymers, chelating compounds can be used to be surface modifiers[3,44-47].

Great progress has been made in the treatment of a variety of diseases by improving nano drug carrier systems. They are increasing in significance as alternative drug carriers according to classical dosage forms. Controlled drug delivery, enhancement bioavailability entrapped drugs, changing the pharmacokinetics and the toxicity of drugs, improvement of tissue distribution and targeting of drugs have been improved. Nano drug carrier systems' properties are given in Table-3.

Conclusion

Recent advances in modern medicinal chemistry, pharmaceutical technology and pharmacology point out the need for development of new carrier systems for active drug delivery, which may enhance their therapeutic value. It is now state of the art and strategy to prepare tailor-made targetable drug carriers such as intelligent drugs. They are the assembly of molecules prepared with bioconjugation, PEGylation and nanotechnology with diverse physicochemical and biological properties. Cyclodextrins, PEG, polymers and other suitable molecules can be used to produce a derivative characterized by low molecular weight, high solubility, drug complexation properties and specific recognition.

As a result, it is currenly important to prepare a wide array of bioconjugates with different physicochemical, biological and biopharmaceutical properties by obtaining by proper selection of the cyclo-polysaccharide unit, PEG molecular

TABLE-3
NDCSS AND THEIR PROPERTIES

Nano drug carrier systems	Properties
Carbon nanotubes	New alternative and efficient tool for transporting and *trans* locating active molecules. Possesses two different structures, single and multiwall. Possess a high tensile strength, are excellent conductors of electricity and are both chemically and thermally stable. Suitable potential for drug delivery, biomaterials and biosensor applications due to their high aspect ratio and high surface area. Useful to increase transdermal penetration for hydrophobic drugs[3, 48-51].
Dendrimers	Potentially relevant to drug delivery applications and diagnostic imaging. Have three components: an initiator core, branches and terminal functional groups. The initiator core is in the heart of the molecule and branches extend outward from it. Terminal groups can be modified to obtain both a charged and hydrophilic or lipophilic function for the desired biological and drug delivery applications. Have tremendous potential in the biomedical applications involving multifunctional nanoparticle systems combining targeting, imaging, diagnostics and therapy[3, 52-55].
Liposomes	Classified into three categories: multi-lamellar vesicles, large uni-lamellar vesicles and small-lamellar vesicles. Classified in terms of composition and mechanism of intracellular delivery into five types; conventional, pH-sensitive, cationic, immuno-liposomes and long-circulating liposomes. Improve pharmacokinetics properties and drug release, to enhance intracellular penetration. To provide for convenience vehicles for poorly soluble drugs. To improve the efficacy and reduce the side effects of anticancer drugs. To explore for the ocular, nasal, transdermal, rectal, parenteral and oral drug delivery applications. To improve the drug bioavailability by controlled/targeted delivery[3, 56-61].
Polymeric micelles	Applied chemotherapy of cancer, drug delivery to the brain, formulations of antifungal agents, delivery of imaging agents and polynucleotides. To use for delivery of the gene, diagnostic and therapeutic agents[3, 62, 63].
Polymeric nanoparticles	To provide a better penetration of the particles inside the body as their size allow delivery *via* intravenous injection or other routes such as ocular, pulmonal. Used to be adjuvants and carriers for vaccine, peptide and protein. Suitable for gene delivery and drug targeting[3, 64-70].
Quantum dots	Adapted to the desired application by conjugation to a recognition moiety, *e.g.* antibodies, peptides, oligonucleotides. Applicable for imaging, proteomic and genomic applications, *in vitro* nano diagnostics and therapeutic applications. Suitable for immunolabeling, cell motility assays, *in situ* hybridization and as live cell markers[3, 71-78].
Solid lipid nanoparticles	Administered at different routes such as oral, rectal, topical, ophtalmic, parenteral, pulmonal and as a vaccine adjuvant. Have good biocompatibility, biodegradability, high bioavailability, low toxicity, good storage capability (stability), protecting chemically labile drugs from degradation, drug targeting, mucoadhesiveness and offering sustained release. Most of the protein and peptide drugs, poorly water-soluble drugs, anticancer drugs, vaccine adjuvants and colloidal drug carrier used in therapotics can be successfully formulated into solid lipid NPs according to lipid microemultions, liposomes and polymeric nanoparticles. To change the pharmacokinetics and the toxicity of drugs[3, 79-91].

weight and targeting moiety. PEGylation is known to enhance circulation time by inhibition of nonspecific protein adsorption and to improve their cytoplasmic transport rates, possibly by reducing non-specific adhesion to cytoskeletal elements. Multifunctional nano drug carrier systems exhibiting interesting surface properties could be obtained by using different polymers. All these strategies however, may be more important in diagnosis, imaging, targeting and therapy, today, tomorrow and future. Thus, bioconjugation is an important hot-topic which offers accelerated development with high promises in pharmaceutics and related fields.

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