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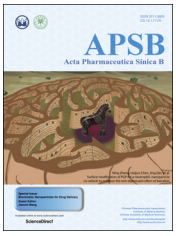

REVIEW

Biomacromolecules as carriers in drug delivery and tissue engineering

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KEY WORDS

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Abstract Natural biomacromolecules have attracted increased attention as carriers in biomedicine in recent years because of their inherent biochemical and biophysical properties including renewability, nontoxicity, biocompatibility, biodegradability, long blood circulation time and targeting ability. Recent advances in our understanding of the biological functions of natural-origin biomacromolecules and the progress in the study of biological drug carriers indicate that such carriers may have advantages over synthetic material-based carriers in terms of half-life, stability, safety and ease of manufacture. In this review, we give a brief introduction to the biochemical properties of the widely used biomacromolecule-based carriers such as albumin, lipoproteins and polysaccharides. Then examples from the clinic and in

Abbreviations: ABD, albumin binding domain; ACM, aclacinomycin; ACS, absorbable collagen sponge; ADH, adipic dihydrazide; ART, artemisinin; ASF, *Antheraea mylitta* silk fibroin; ATRA, all-trans retinoic acid; ATS, artesunate; BCEC, brain capillary endothelial cells; BMP-2, bone morphogenetic protein-2; BSA, bovine serum albumin; BSF, *Bombyx mori* silk fibroin; CC-HAM, core-crosslinked polymeric micelle based hyaluronic acid; CD, cyclodextrin; CD/BP, cyclodextrin–bisphosphonate complexes; CD-*g*-CS, chitosan grafted with β-cyclodextrin; CD-NPs, amphiphilic MMA–tBA β-CD star copolymers that are capable of forming nanoparticles; CIA, collagen-induced arthritis; CM, collagen matrices; CMD-ChNP, carboxylmethyl dextran chitosan nanoparticle; DHA, dihydroartesunate; DOXO-EMCH, (6-maleimidocaproyl)hydrazone derivative of doxorubicin; DOX–TRF, doxorubincin–transferrin conjugate; DTX-HPLGA, HA coated PLGA nanoparticulate docetaxel; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; EPR, enhanced permeability and retention; FcRn, neonatal Fc receptor; GAG, glycosaminoglycan; GC-DOX, glycol–chitosan–doxorubicin conjugate; Gd, gadolinium; GDNF, glial-derived neurotrophic factor; GO, grapheme oxide; GSH, glutathione; HA, hyaluronic acid; HA-CA, catechol-modified hyaluronic acid; HCF, heparin-conjugated fibrin; HDL, high density lipoprotein; HEK, human embryonic kidney; HSA, human serum albumin; IDL, intermediate density lipoprotein; INF, interferon; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; LDV, leucine–aspartic acid–valine; LMWH, low molecular weight heparin; MSA, mouse serum albumin; MTX–HSA, methotrexate–albumin conjugate; NIR, near-infrared; NSCLC, non-small cell lung cancer; OP-Gel-NS, oxidized pectin-gelatin-nanosliver; pDNA, plasmid DNA; PEC, polyelectrolyte; PTX, paclitaxel; RES, reticuloendothelial system; RGD, Arg–Gly–Asp peptide; rHDL, recombinant HDL; rhEGF-2/HA, recombinant human fibroblast growth factor type 2 in a hyaluronic acid carrier; SF, silk fibroin; SF-CSNP, silk fibroin modified chitosan nanoparticle; SFNP, silk fibroin nanoparticle; SPARC, secreted protein acidic and rich in cysteine; Tf, transferrin; TfR, transferrin receptor; TRAIL, tumor-necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor; VLDL, very low density lipoprotein

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recent laboratory development are summarized. Finally the current challenges and future prospects of present biological carriers are discussed.

1. Introduction

The development of carrier systems for effective delivery of therapeutic compounds or imaging agents is crucial in the battle against various diseases. The ideal carriers should be safe, efficient and have optimal bioavailability. In addition, stability, nontoxicity and non-immunogenicity, and targeting ability to a specific site are very important.

Miscellaneous drug carriers including liposomes, synthetic polymeric micelles, hydrogels, magnetic nanoparticles, microspheres and microcapsules have been developed in recent years for the diagnosis and treatment of disease[1–8]. However, it is very difficult to identify an ideal drug carrier system. The shortcomings of the above carrier systems are obvious: liposomes have poor stability, high cost, low drug loading content and undesired release of hydrophobic drugs[1,9]. The toxicity and nondegradable property of some nano-materials also limit their applications as drug carriers[10].

Natural-origin biomacromolecules perform a diverse set of functions in their native setting. For example, polysaccharides function in membranes, intracellular communication and as storage sites, whereas proteins function as structural materials, transport

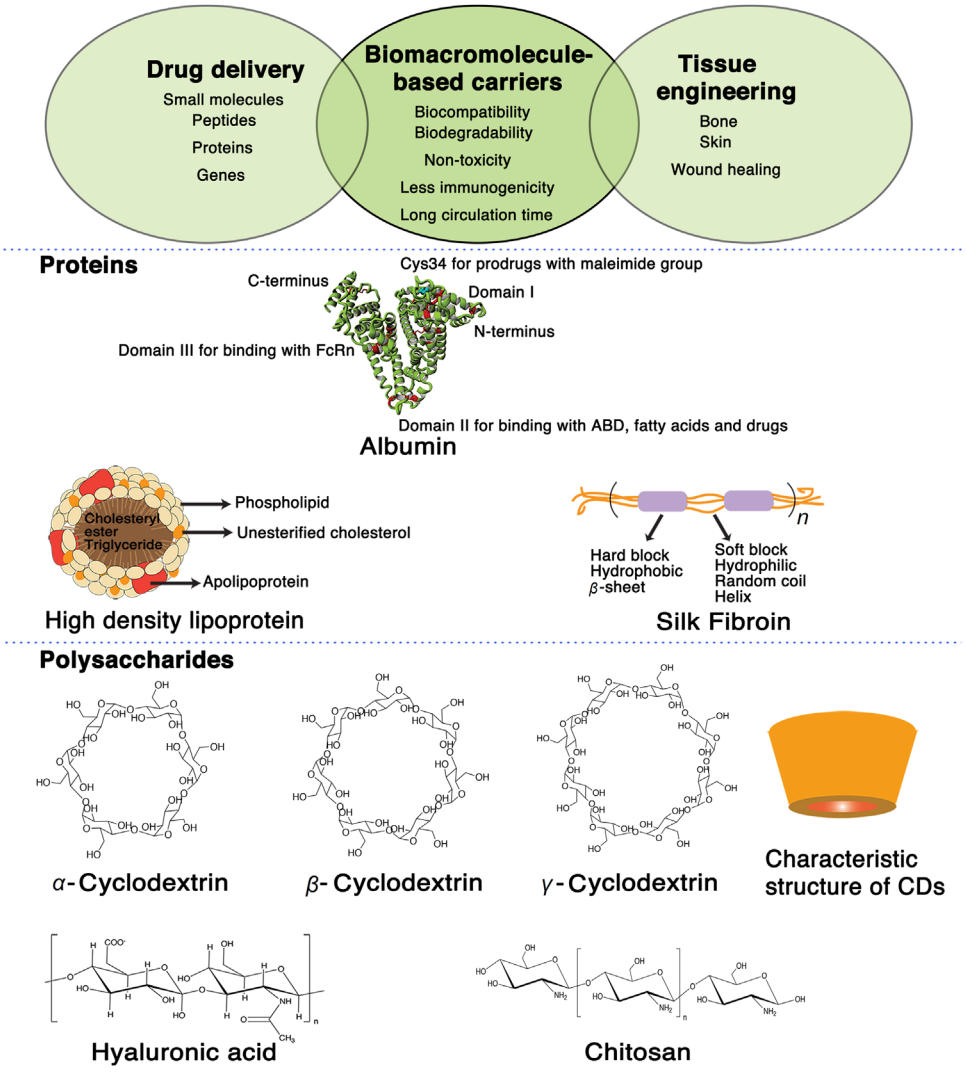

Figure 1 The properties and applications of biomacromolecule-based carriers and structures of representative proteins and polysaccharides.

vehicles, nutrients and catalysts. Transport proteins as carriers for delivery of nutrients and other necessary molecules are of special interest. Inspired by such natural processes in organisms, scientists started to utilize natural and biological macromolecules, including proteins, polysaccharides, and lipoproteins, for the delivery of drugs and tissue engineering[11–13]. Biomacromolecule-based drug carriers are nontoxic, non-immunogenic and have high drug loading content, good biocompatibility and targeting ability[12]. Meanwhile, they are also capable of controlled and sustained drug release[13–16]. Many meaningful designs have been reported using biological carriers, some of which are already approved for clinical use. Biomacromolecules used as carriers include proteins (albumin, transferrin, lipoproteins, silk fibroin, collagen, keratin) and polysaccharides (chitosan, cyclodextrin, hyaluronic acid, heparin and pectin). The structural diagrams of these carriers are shown in Fig. 1. These biomacromolecules can be naturally obtained from animals and plants in abundant amounts and are renewable resources. They have good affinity to organisms and weak immune rejection, and can be degraded by *in vivo* enzymes; the metabolites also have low toxicity to organisms[17–19]. Biomacromolecule-based carriers have been reported in the form of prodrugs, drug conjugates, nanoparticles, microcapsules, hydrogels and tissue engineering scaffolds[3,11,12]. The use of biomacromolecule-based carriers has been shown to improve the pharmacokinetics of the payloads and to reduce systemic toxicity and immunogenicity[20–22]. Furthermore, the hydroxyl, amine and carboxyl groups on the chains of these biomacromolecules can be utilized for chemical modification, making them of great significance in biomedical field.

In this review, we focus on advances in development of natural biomacromolecule-based carriers for drug delivery. First, the biochemical and physiological properties of the carriers will be presented by class. Secondly, some representative examples of biomacromolecule-based carriers with applications in clinical use or in development will be summarized. Finally, the existing challenges and prospects of the natural biological carriers will be discussed.

2. Natural proteins as carriers for drug delivery and tissue engineering

2.1. Albumin

2.1.1. Properties of albumin

Albumin is the most abundant plasma protein (35–50 g/L human serum) with a molecular weight of 66.5 kDa. The average half-life of human serum albumin (HSA) is about 19 days[23]. HSA plays several essential biological roles[24,25]. It maintains colloid osmotic blood pressure, transports metal ions, insoluble small molecules and nutrients (including long chain fatty acids) to various organs. HSA binds many drugs and impacts the distribution, metabolism and therapeutic effects of such drugs. Finally, HSA degrades into amino acids, providing nutrition to surrounding tissues and cells. Since albumin has a molecular size of ~7.2 nm, which is above the renal clearance threshold, the blood circulation time of albumin is prolonged compared to other small molecules. In addition, albumin can be internalized by cells through caveolin-1-mediated gp60 receptor, can bind to the neonatal Fc receptor (FcRn) in circulating endosomes, and is known to enter the recycling pathway, consistent with the long half-life of this protein[26–31].

2.1.2. Accumulation of albumin in solid tumors and inflamed joints

It was first reported in 1954[32] that tumors were able to trap plasma proteins and utilize their degradation products for proliferation. During the next few years, the “EPR” (enhanced permeability and retention) effects of tumors were discovered[33–35], which provided a rationale for choosing suitable macromolecular carriers. Since 1990, many studies characterized the increased distribution of albumin in tumor and inflamed tissues. Stehle et al.[36] proposed albumin to be the major source of energy and nutrients for tumor growth as an explanation of the high albumin accumulation in tumor sites.

Similarly, studies reported since the late 1990s[37–46] found that SPARC (secreted protein acidic and rich in cysteine, known as osteonectin earlier) is overexpressed in stroma and various advanced cancer types including breast, lung, pancreatic cancers and melanoma. SPARC modulates cellular interactions with the extracellular matrix and is reported to have a high affinity for albumin, possibly contributing to the accumulation of albumin in tumors and inflamed tissues[47].

As a natural biomacromolecule, albumin posseses numerous positive characteristics of an ideal carrier for drug delivery[48,49]. These include good water-solubility, ready availability, biodegradability, lack of toxicity, minimal immunogenicity, and its preferential accumulation in tumor and inflamed tissues. Notably, both exogenous and endogenous albumin can act as carriers[48].

Generally, albumin can carry drugs through two main mechanisms: albumin–drug conjugation and drug encapsulation into albumin nanoparticles. Three forms of albumin–drug conjugates[50] include chemically-coupled exogenous albumin to drugs, endogenous albumin binding to prodrugs, and albumin-fusion proteins. The second albumin carrier methodology uses physical interactions to encapsulate drugs into albumin nanoparticles[51]. Both methods demonstrate positive drug delivery features. Whereas the chemically modified albumin can improve the pharmacokinetics of drugs, encapsulated formulations may improve stability and solubility.

2.1.3. Drug albumin conjugates and albumin binding prodrugs

The first drug albumin conjugate entering phases I/II clinical studies was a methotrexate–albumin conjugate (MTX–HSA)[52,53]. MTX was directly conjugated to the lysine residues of HSA through an amidation reaction. Stehle et al.[54,55] synthesized MTX–albumin conjugates bearing 1, 5, 7, 10 and 20 molecules of MTX on average and found that the loading rate determines tumor targeting properties of MTX–albumin conjugates in rats. Only loading rates of close to 1 mol of the cytostatic drug MTX/mol of albumin offered optimal conditions for targeting MTX–albumin conjugates into rodent tumors, which formed the basis for further preclinical and clinical research[52]. These conjugates enjoy the same favorable tumor targeting properties of albumin, *e.g.*, high tumor uptake rates, low liver uptake rates and a very long biological half-life. Since MTX is the most common drug used in the treatment of rheumatoid arthritis, MTX–HSA conjugates were also evaluated in a human model of rheumatoid arthritis using severe combined immunodeficient mice which were co-transplanted with human cartilage. Synovial fluid from patients with rheumatoid arthritis, synovial fibroblast invasion and cartilage degradation were reduced by MTX–HSA *in vivo*.

Encouraged by the previous results, Kratz et al.[56] focused their work on a prodrug concept that utilize endogenous albumin as a

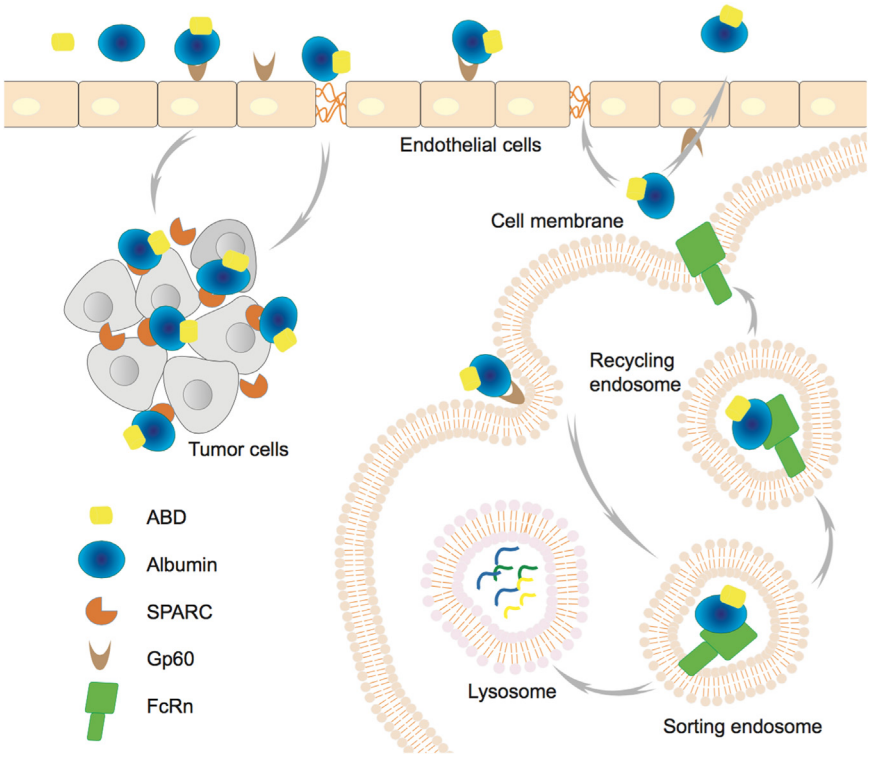

Figure 2 Schematic illustration of the *in vivo* process of ABD-based indirect targeting of FcRn in tumor tissues.

drug carrier. In this strategy, the prodrug, (6-maleimidocaproyl) hydrazone derivative of doxorubicin (DOXO-EMCH) was designed to bind rapidly and specifically to the cysteine-34 residue of circulating serum albumin after intravenous administration thereby forming a macromolecular drug complex in blood. DOXO-EMCH demonstrated dramatically improved therapeutic efficacy of doxorubicin in preclinical tumor models. The phase I/II study of DOXO-EMCH also gained favorable results in cancer patients[57,58].

Exploiting endogenous albumin as a carrier would have several advantages over exogenous albumin drug conjugates. The former would avoid the use of commercial (possibly pathogenic) albumin. In addition, the albumin-binding prodrugs could be chemically well-defined and based on straightforward organic chemistry. Manufacturing processes would be inexpensive and applicable to a wide range of drugs. Regulatory analytical requirements would be comparable to any other low-molecular weight drug candidate.

Besides low-molecular weight drugs, peptides and proteins can also be conjugated to albumin. Byeon et al.[59] presented a HSA conjugate linked to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) *via* a bifunctional PEG derivative. The prepared HSA–TRAIL had a size of 15.4 nm and exhibited good bioactivity in MiaPaca-2 cells and mouse splenocytes. In collagen-induced arthritis (CIA) mice, HSA–TRAIL showed superior targeting to inflamed tissues compared with naive TRAIL. The circulating half-life for HAS–TRAIL was more than 26 times longer than that of TRAIL. Furthermore, HSA–TRAIL showed superior anti-inflammatory efficacy in CIA mice.

2.1.4. *Albumin fusion proteins*

As an alternative to chemically coupling albumin to drugs, the application of genetic engineering allows the DNA of albumin and the therapeutic proteins or peptides to be expressed as one continuous open reading frame typically in yeast or mammalian cells as albumin fusion proteins[60]. Albumin-fusion technology represents a simple and flexible alternative platform for the productions of proteins with extended circulatory half-lives. Over the last 20 years, various peptides and proteins with diverse functions as well as biochemical/biophysical properties have been genetically fused to albumin including small bioactive peptide hormones (such as glucagon-like peptide-1 and β-natriuretic peptide), growth factors (erythropoietin and granulocyte colony-stimulating factor), coagulation factors (FVIIa, FIX, and von Willebrand factor), anticoagulants (hirudin, infestin and barbourin) cytokines (IL-2, interferon (INF)-α-2b, INF-β), hormones (growth hormone and insulin), enzymes (human betyrylcholinesterrase), redox modulators (thioredoxin) and a variety of antibody fragments and alternative antibody scaffolds[61].

Albinterferon-α-2b, a recombinant polypeptide composed of INF-α-2b genetically fused to human albumin, has an extended half-life and early evidence indicates that it is efficacious and well tolerated. Albinterferon-α-2b has been evaluated in phase III clinical trial and its safety and efficacy are thus far confirmed. Thus, albinterferon-α-2b has the potential to become an important therapy for chronic hepatitis C and other diseases[60]. This platform could potentially be used to produce a broad spectrum of bioactive molecules and this approach is not restricted by molecular size or biological activity.

Noncovalent association of macromolecules with endogenous albumin has been explored as an alternative to direct fusion with albumin. It was reported that albumin binding domain (ABD) derived from streptococcal protein G is composed of 46 amino acids forming a left-handed three-helix bundle and shows very strong affinity to HSA, bovine serum albumin (BSA) and mouse serum albumin (MSA)[62,63]. The use of endogenous albumin as a

carrier for ABD-fused proteins is attracting more and more attention. Andersen et al.[64] designed ABD-$(Z_{HER2:342})_2$ and $(Z_{HER2:342})_2$-ABD fusion proteins and found that protein genetically fused to ABD does not interfere with shFcRn binding of HSA. Thus, the ABD fusion technology is a widely applicable strategy for extending circulatory half-life and improving bioavailability of protein and peptide drugs. Li et al.[65] used ABD to modify the N-terminal of hTRAIL and got ABD-hTRAIL fusion protein, which could quickly and specifically bind to plasma albumin once administrated. The ABD-hTRAIL fusion protein utilized endogenous albumin as a carrier to extend the circulatory half-life of hTRAIL, and the half-life of ABD-hTRAIL was 40–50-fold greater than that of hTRAIL. Tumor uptake of ABD-hTRAIL was also significantly increased. Thus the use of endogenous albumin as a drug carrier is an attractive and efficient strategy for tumor therapy. Fig. 2 illustrates the *in vivo* process of ABD-based indirect targeting of FcRn in tumor tissues.

Another ABD consisting of 52 amino acid residues from protein Zag was also reported to bind human, rat, mouse, horse and dog serum albumin[66]. Cantante et al.[67] has developed a Zag ABD fused with an anti-TNFα VHH camelid derived sdAb. The fusion protein showed specific binding to human, rat and mouse serum albumins and exhibited a strong increase in circulating half-life in mice to approximately 39-fold compared with the parental sdAb.

In conclusion, ABD-fused proteins utilizing endogenous albumin as a carrier can be potentially used as a universal method to improve the pharmacokinetics properties and therapeutic effects of protein and protein-derived drugs.

2.1.5. *Albumin microspheres and nanoparticles*

Albumin microspheres are generally prepared by chemical cross-linking or by addition of an organic solvent and stabilization at elevated temperatures. ^{99m}Tc macroaggregated albumin has been developed for clinical diagnosis for various disease including sentinel node detection in breast cancer and other solid tumors, leg edema, protein-losing enteropathy and rheumatoid arthritis[68].

Abraxane®, one of the commercialized nanoparticle drug delivery systems, is an albumin-bound form of paclitaxel (PTX) which uses nab-technology developed by American Bioscience, Inc.[69]. This formulation, consisting of water-soluble nanoparticles with a diameter of ~130 nm, is the first albumin-based delivery system approved by the US FDA for the treatment of metastatic breast cancer (2005), metastatic non-small cell lung cancer (NSCLC) (2012), and metastatic pancreatic cancer (2013).

Inspired by the success of Abraxane®, albumin-based nanoparticles as a carrier for drug delivery have stimulated great interest. Choi et al.[70] fabricated inhalable TRAIL/Dox HSA nanoparticles with a diameter of ~340 nm by conjugating Dox onto albumin and adsorbed with apoptotic TRAIL protein. The TRAIL/Dox HSA-NP displayed synergistic cytotoxicity and apoptotic activity in H226 lung cancer cells. Later, they developed a new nanoparticle formulation of TRAIL/Dox HSA NPs with a diameter of 60–120 nm by using the nab™ technology. The TRAIL 1.0%/Dox HSA NPs had markedly greater apoptotic activity than Dox HSA NPs in HCT116 tumor-bearing BALB/c *nu/nu* mice[71].

Recently our group has developed a Wpep-conjugated cross-linked HSA nanoparticle loaded with PTX for efficiently targeting therapy to metastatic breast cancer[72], the cross-linked biomimetic HSA nanoparticle is considerably stable under physiological conditions while it realizes redox-responsive drug release in intracellular environment with high concentration of glutathione (GSH) (~10 mmol/L). The Wpep–HSA NP showed significant accumulation at tumor site and exhibited stronger antitumor efficacy.

As the most widely studied biological carrier, albumin has achieved successful application in the clinic. The albumin–drug conjugates, albumin binding prodrugs, albumin nanoparticles and albumin fusion proteins or peptides in clinical studies are summarized in Table 1.

2.2. *Transferrin*

2.2.1. *Properties of transferrin*

Human transferrin (Tf) is an iron-binding protein containing 679 amino acids and has a molecular weight of 79.57 kD. Tf carries iron into cells expressing Tf receptor (TfR). Tf is biodegradable, nontoxic and non-immunogenic and can achieve site-specific targeting *via* TfR expressed on cell surface[73]. As a result, Tf is usually used as a targeting ligand in drug delivery systems. Many Tf/TfR-mediated drug delivery systems to target tumors have been explored due to the overexpression of transferrin receptors on malignant tumor cells.

2.2.2. *Transferrin drug conjugates*

Chemical conjugation has been a frequent approach for transferrin to delivery drugs. Gong et al.[74] synthesized two kinds of transferrin conjugates: Tf conjugates with monomeric artemisinin (ART) and an ART dimer, respectively. Both the ART–Tf and dimer–Tf conjugates maintained the hydrophobic ART in solution and showed better targeting efficacy against cancer cells compared to commonly used chemotherapeutic anticancer drugs. Szwed et al.[75] developed a doxorubicin–transferrin (DOX–TRF) conjugate and investigated its toxicity in human leukemia cells. The DOX–TRF conjugate exhibits higher toxicity in comparison to free drugs and induces significant changes in the GSH antioxidant system in human leukemia cell lines. In addition, Tf–cisplatin, Tf–chlorambucil, Tf–mitomycin C, Tf–daunorubicin, and Tf–gemcitabine have also been developed, all of which displayed enhanced cytotoxicity to tumor cells and reduced toxicity to normal tissues compared to the free drugs[76,77]. However, it has been reported that the half-life of the internalized Tf is only ~8 min and Tf is rapidly cycled back to the cell surface and released[78]. If the chemical bond between Tf and drug molecules is not cleaved in the intracellular environment during this 8 min, the loaded drugs will be exported along with Tf, leading to low intracellular free drug concentration and reduced cytotoxicity.

2.2.3. *Transferrin drug adducts*

An alternative way for transferrin to function as a drug carrier is to form Tf/drug adducts. Tf/drug adducts can be fabricated by simply adding the drug solution to Tf solution followed by vortexing and co-incubation.

Yang et al.[79] developed ART, dihydroartesunate (DHA) and artesunate (ATS) adducts with Tf, and the resulting ART–Tf, DHA–Tf and ATS–Tf adducts showed significant anticancer effects on human liver hepatocellular carcinoma (HepG2) and lung adenocarcinoma (A549) cells with minimal side effects on normal human liver cells (HL-7702).

Table 1 Summary of albumin-based drug carriers in clinical studies.

Mode of drug delivery	Drug name	Active pharmaceutical ingredient (API)	Indication	Clinical status	Notes
Albumin–drug conjugates	MTX–HSA	Methotrexate	Metastatic renal carcinoma	Phase II	No further clinical assessment
	CJC-1134-PC	Exendin-4	Type II diabetes	Phase II	A third phase II terminated
Albumin-binding drugs/ prodrugs	DOXO-EMCH	Doxorubicin	Small cell lung cancer	Phase II	Renamed as INNO-206
	Victoza	GLP-1 (7-37)	Type II diabetes	Approved	2009 in Europe and 2010 in the USA
	CJC-1008	Dynorphin A (1-13) peptide	Postherapetic neuralgia	Phase II	No further clinical assessment
	CJC-1131	GLP-1 (7-36)	Type II diabetes	Phase I/II	
bumin-based nanoparticles	Abraxane	Paclitaxel	Metastatic breast cancer; Locally advanced or metastatic NSCLC; Metastatic pancreatic adenocarcinoma	Approved	
Fusion proteins and peptides	Albiglutide™	GLP-1 (7-36)	Type II diabetes	Approved	Marketed as Tanzeum (USA) and Eperzan (Europe)
	Balugrastim	G-CSF	Chemotherapy-induced neutropenia	MAA submitted (Europe)	Formerly known as albugranin™ and Neugranin™
	rIX-FP	Factor IX	Haemophilia B	Phase III	
	MM-111	Anti-HER2 scFv and anti-HER3 scFv	Gastric and breast cancer	Phase II	
	Albuferon	IFN-α-2b	Chronic hypertitis C	BLA and MAA withdrawn	
	AlbuBChE	Butyrylcholinesterase	Cocaine addiction	Phase II	
	rFVIIa-FP	Factor VIIa	Haemophilia A and Haemophilia B	Phase I	

2.2.4. *Tf/TfR mediated carriers for drug and gene delivery*

Since TfR is overexpressed in a variety of tumor cells and brain capillary endothelial cells, Tf/TfR-mediated cellular events have been exploited in carrier systems that deliver therapeutic drugs and genes into malignant cells and brains[80]. A Tf-conjugated liposome complex carrying a BCL-2-specific anti-sense ODN showed improved targeting and internalization into K562 cancer cells *in vitro* and *in vivo*, extending the survival time, and improving tumor growth inhibition compared to the antisense ODN alone[81,82]. Wagner et al.[83] conjugated Tf to protamine or polylysine *via* disulfide bridges for the delivery of a plasmid DNA containing the *luciferase* gene to eukaryotic cells, and achieved high-level expression of luciferase. This delivery system was termed as "transferrinfection". Studies carried out by Liu et al.[84] also suggested that Tf-modified nanoparticles loaded with doxorubicin is a promising cytotoxic agent in glioma therapy.

A previous study of our group demonstrated that using a Tf-conjugated polyethyleneglycol-modified polyamidoamine dendrimer could realize efficient, noninvasive and brain-targeting gene delivery[85].

Nam et al.[86] designed a lauric acid-*O*-carboxymethyl chitosan-Tf micellar system for hydrophobic drug delivery and site-specific targeted delivery. The results show that this drug carrier exhibits low cytotoxicity, high cellular uptake, sustained release and site-specific targeting properties.

2.3. *Lipoproteins*

2.3.1. *Properties of lipoproteins*

Lipoproteins are particles formed by the aggregation of lipids such as triglycerides, phospholipids and cholesterol esters. As endogenous nanoparticles that transport cholesterol and other lipids through the blood to various cell types, lipoproteins are immune-free, not absorbed by the reticuloendothelial system (RES) and are regarded as excellent candidates for the targeted delivery of therapeutic drugs, imaging agents and nucleic acids to various tissues[87].

There are five classes of lipoproteins with different structure and function, including chylomicron (75–1200 nm), very low density lipoprotein (VLDL, 30–80 nm), intermediate density lipoprotein (IDL, 25–35 nm), low density lipoprotein (LDL, 18–25 nm) and high density lipoprotein (HDL, 8–12 nm), among which LDL and HDL are the most widely studied lipoproteins as carriers[88].

Three strategies have been developed to utilize lipoproteins as carriers for drug or imaging agents, including surface loading through noncovalent interaction on the phospholipid shell, covalent modification of the phospholipid or protein, and encapsulation in the nanoparticle core through reconstitution techniques. Examples of each strategy will be illustrated below.

2.3.2. *Low density lipoprotein-based carriers for drug delivery*

LDL is a spherical BNP with a particle size of 18–25 nm and is composed of a hydrophobic core consisting of esterified cholesterol and triacylglycerol and surface coat of phospholipids surrounded by a single apoB-100 protein. ApoB-100 accounts for over 95% of the LDL apoprotein and is exposed at the surface, allowing for receptor recognition with nine amino acids at residues 3359–3367 serving as the binding domain for the LDL receptors (LDLRs)[89]. It was reported that LDLRs are overexpressed on various tumor cells because large quantities of cholesterol and fatty acids are required for supporting rapid proliferation of tumor cells[90]. Therefore, LDL could also target tumor cells as carriers.

In the past few decades, LDL has been investigated for its ability to deliver drugs to cells expressing the receptors. Crich et al.[91] developed a gadolinium (Gd)–AAZTAC17/LDL adduct using surface loading strategy and found that Gd–AAZTAC17/LDL adduct is an efficient probe in the magnetic resonance visualization of subcutaneous tumors in B16 melanoma-bearing adult C57BL/6 mice. The method is easy to manufacture but prone to probe/drug leakage because the surface probe will transfer to the outer phospholipid layer of cell membrane to maintain thermodynamic stability. Zheng et al.[92] used core loading strategy to bind PDT agents in the lipid core of LDL in the reassembling process. In addition, cholesterol conjugates mimicking the native cholesterol esters can be loaded into LDL by core loading. Pietzsch et al.[93] attached ^{18}F-containing ligands to the lysine-ε-amino groups on apoB-100 for imaging and Sobal et al.[94] attempted radio-iodination of tyrosine side chains for SPECT detection but leads to a change in LDL's transport properties. Therefore, although chemical modification is more stable, it may influence the LDL's intrinsic functions.

2.3.3. *High density lipoprotein-based carriers for drug delivery*

HDL is the smallest of the lipoproteins with a diameter of 8–12 nm. It is well known as the "good" cholesterol because it not only removes excess cholesterol from atherosclerotic plaques but also plays an important role in anti-inflammatory and anti-oxidative activities to protect the cardiovascular system. As an endogenous nanocarrier, circulating HDL transports endogenous proteins, vitamins, hormones, and microRNA to various organs. Compared with other synthetic nanocarriers (*e.g.*, liposomes, micelles, inorganic and polymeric nanoparticles), natural-origin HDL has unique features that allow it to deliver cargo to specific targets more efficiently. Many types of cancer cells have been reported to overexpress SR-BI that mediates cholesterol delivery by HDL[95].

Lou and co-workers[96] reported a delivery system composed of recombinant complex of HDL and aclacinomycin (rHDL–ACM). The rHDL–ACM complex has the same basic physical and biological binding properties of native HDL and showed a preferential cytotoxicity for SMMC-7721 hepatoma to normal l02 hepatocytes.

Recent studies have shown that HDL is a promising delivery system for siRNA, as HDL could overcome the barriers mentioned above with mechanisms of action distinct from those of other conventional nanocarriers. Notably, endogenous HDL has been reported to be involved in the transport of microRNA *in vivo*, suggesting the potential of using HDL as a natural delivery carrier for nucleic acids[95]. Modification of siRNA with lipophilic groups, such as cholesterol, offers a convenient method of loading siRNA in HDL. Soutschek et al.[97] conjugated cholesterol to ApoB siRNA that was chemically stabilized with a phosphorothioate backbone at the 3′ end of the sense and antisense strands and two 2′-*O*-methyl nucleotides at the 3′ end of the antisense strand. ApoB siRNA conjugated with cholesterol (Cho–ApoB–siRNA) displayed increased stability and better gene silence effect in human serum than the unconjugated form. Except for tumor cells, HDL was also used to deliver cholesterol-conjugated siRNA for organic anion transporter 3 (Chol–siOAT3) into brain capillary endothelial cells (BCECs)[98]. The results showed that HDL–Chol–siOAT3 significantly decreased OAT3 mRNA levels in BCECs after intravenous injection, while free Chol–siOAT3 failed to achieve this.

2.4. Silk fibroin

2.4.1. Properties of silk fibroin

Silk fibroin (SF) is an insoluble protein with bulky hydrophobic domains secreted by silkworms and spiders or other insects, and can be easily purified as sericin-free silk-based biomaterials[99]. Silk fibroin has been used as an exemplary scaffolding material because of its highly adaptable material properties, excellent biocompatibility and mild foreign body response *in vivo*. Moreover, silk fibroin can self-assemble into mechanically robust material structures that are also biodegradable and non-cytotoxic, indicating utility for gene delivery[100].

2.4.2. Silk Fibroin as a drug carrier

In the past decades, SF has been widely investigated in biomedical and pharmaceutical fields because of its remarkable mechanical properties, good biocompatibility, controllable biodegradability and low immunogenicity. Silk fibroin modified chitosan nanoparticle (SF–CSNP), a biocompatible material, has been widely used as a potential drug delivery system. Yang et al.[101] developed such a SF–CSNP for treatment of hepatic cancer and achieved improved cell responses. Numata et al.[102] synthesized silk-based block copolymers that were bioengineered with poly(L-lysine) domains for plasmid DNA (pDNA) delivery to human embryonic kidney (HEK) cells.

Wang et al.[103] designed new silk fibroin nanoparticles (SFNPs) coated with four different cationic polymers, GCS, TMC, PEI and PEG–PEI. The cationic polymer coatings significantly enhanced the colloidal stability of SFNPs in biological media. Furthermore, doxorubicin-loaded SFNP@GCS and SFNP@(PEG–PEI) showed higher cytotoxicity against HeLa cells.

2.4.3. Silk fibroin as scaffolds for tissue engineering

Stable, spherical, negatively charged and low toxic silk nanoparticles (150–170 nm) have been prepared from silk fibroin solutions of domesticated *Bombyx mori* and tropical tasar *silkworm Antheraea mylitta*[104]. Recently *Bombyx mori* silk fibroin (BSF) has been widely applied as a tissue engineering scaffold for the generation of blood vessels, skin, bone, ligaments and nerves[105]. *Antheraea mylitta* silk fibroin (ASF) is structurally different from BSF. ASF contains fewer glycine residues and more alanine, aspartic acid and arginine residues and contains Arg–Gly–Asp (RGD) sequences[106]. Since the RGD sequence binds to integrin receptors on cell surface, utilizing ASF could achieve targeting and benefit cell attachment. Ma et al.[104] utilized ASF in conjugation with PEI to create a gene carrier. The ASF/PEI/pDNA complex has significantly increased transfection efficiency and reduced cytotoxicity to mouse fibroblast cells (L929).

Farokhi et al.[107] constructed a bio-hybrid silk fibroin/calcium phosphate/PLFA nanocomposite scaffold as vascular endothelial growth factor (VEGF) delivery system with sustained release profile by using freeze-drying and electrospinning. The histology analysis showed that after ten weeks of implantation, new bone tissue formation happened in the defected site, suggesting that SF could be considered as an effective scaffold for bone tissue engineering applications.

2.5. Collagen

2.5.1. Properties of collagen

Collagen, the major structural protein component of extracellular matrix, accounts for ~30% of the total proteins of mammals and provides support to connective tissues such as skin, tendons, bones, cartilage, blood vessels, and ligaments[108]. Collagen is responsible for signal transduction in the regulation of cell adsorption, migration, proliferation, differentiation and survival[109]. There are 27 types of collagens identified to date, among which collagen I is the most abundant and the most investigated for biomedical applications. Collagen is biocompatible, biodegradable, non-immunogenic[110] and can be reconstituted into fibrous structures simulating the native extracellular matrix in tissues. Collagen has been used in a variety of applications, including but not limited to sponges for wound healing, mini-pellets, hydrogels, patches and nanoparticles for drug delivery and tissue engineering.

2.5.2. Collagen as a drug carrier

Several studies have already been carried out using collagen as a carrier in drug delivery systems in earlier years. Wahlig et al.[111] reported on sustained-release preparations for antibiotics such as gentamycin using collagen as a carrier. Fujioka et al.[112] developed a minipellet using collagen as a carrier for protein delivery. The model protein drug interferon was constantly released from the minipellet and sustained serum TNF concentrations were observed. Bettini et al.[113] prepared a porous collagen-based hydrogel scaffold in the presence of iron oxide nanoparticles to retain water-soluble molecules and then activate their release under an external magnetic field. Controlled release of the loaded fluorescein from the NPs-collagen gel was realized by means of a very simple, economic and safe application of an external and weak static magnetic field. Helary et al.[114] evaluated highly dense collagen matrices (CM) as novel medicated wound dressings for the treatment of chronic wounds. The CM40 loading 200 mg/mL ampicillin showed an effective release of payloads over 3 days and the antibacterial effect was continued over four days, whereas collagen sponges demonstrate full antibiotic release within 16 h. Furthermore, the collagen matrices showed almost no cytotoxicity to fibroblasts.

2.5.3. Collagen scaffolds for tissue engineering

Collagen-based scaffolds can be considered as ideal biomaterials for tissue engineering applications due to the good properties of collagen discussed above.

Bayrak and co-workers[110] utilized porcine and bovine collagen type I and elastin for tissue engineering scaffolds and validated the absence of immune responses with xenogeneic collagen and elastin, suggesting that they are suitable constituents of tissue engineered matrices. Inzana et al.[115] designed 3D printing of composite calcium phosphate and collagen scaffolds with high resolution for bone regeneration for the first time and hypothesized that collagen–CaP composites will improve the scaffold's mechanical strength, cytocompatibility and bone regeneration. Lopez-Noriega et al.[116] designed a collagen-based scaffold modified with thermoresponsive liposomes carrying PTHrP 107–111 peptide that has pro-osteogenic and anticatabolic effects on bone cells, this novel system can be regarded as a platform with high promising application in the field of tissue engineering as it can deliver various therapeutic drugs.

Except for tissue engineering, collagen is also used as a controlled proliferation technology for mass production of therapeutic proteins. Wong HL developed a 3D collagen microsphere culture system that encapsulates GDNF (glial-derived neurotrophic factor)-secreting HEK293 cells[117]. This system provides a physiologically relevant 3D environment for cell proliferation and the

production rate of GDNF was significantly enhanced in the 3D system compared to monolayer culture.

2.6. Keratin

2.6.1. Properties of keratin

Keratins are the major structural fibrous proteins providing outer covering such as hair, wool, feathers, nails, and horns. Keratins are cysteine-rich proteins and have a large number of inter- and intra-molecular disulfide bonds since the cysteine residues are easily oxidized, which are relevant to many of the mechanical, thermal, and chemical properties of wool fibers[118]. The amino acid sequence of various human hair keratins reveals that many of the keratin proteins contain a cell adhesion motif leucine–aspartic acid–valine (LDV) which is recognized by the integrin family of protein $\alpha_4\beta_1$[119]. Therefore human keratin might be suitable for tissue-engineering scaffolds.

2.6.2. Keratin as scaffolds for tissue engineering

The biomedical application of keratins is based on chemical reductive degradation of the interlinked disulfide bonds of keratinous materials *via* oxidative or reductive extraction. Xu et al.[120] constructed keratin scaffolds for skin wound repair and regeneration by freeze-drying reductive solutions with varying keratin concentration. These well-interconnected scaffolds are hydrophilic and have good cytocompatibility. Keratin scaffolds achieved earlier vascularization and better skin repair compared with the self-healing process of full-thickness wounds. Park et al.[121] prepared keratin-based hydrogels that were shown to augment the process of excision wound healing by increasing collagen synthesis during the wound healing process *in vivo*. Scaffolds of human hair proteins were fabricated by Verma et al.[122] and studies show that these scaffolds have the capability to enhance cell–cell contacts with LDV-mediated cell-matrix contacts and support long-term cell culture. Apel et al.[123] used a keratin-based hydrogel for peripheral nerve recovery and regeneration in a mouse tibial nerve model. Results show that keratin hydrogels significantly improve electrophysiological recovery compared with empty conduits and sensory nerve autografts at an early time point of regenerations and keratin hydrogels also produce long-term functional and histological outcomes.

3. Polysaccharides as carriers for drug delivery and tissue engineering

3.1. Chitosan

3.1.1. Properties of chitosan

Chitosan, composed of glucosamine and *N*-acetyl-glucosamine, is a type of natural-origin polysaccharides produced by deacetylation of chitin. The amine groups in the glucosidic residue make chitosan a positively charged material with reactive sites for conjugation. Chitosan is insoluble in water at neutral pH while it becomes a water-soluble cationic polyelectrolyte in relatively acidic condition when the amino groups are protonated. Chitosan can self-assemble into nanostructures through electrostatic interactions, hydrophobic interactions, hydrogen bonds and van der Waals forces together[124]. Moreover, the physical chemical properties such as solubility and viscosity of chitosan depend on the degree of deacetylation and molecular weight[125,126]. A lower degree of deacetylation can increase the solubility and viscosity and a higher molecular weight can decrease the solubility and increase the viscosity of chitosan. In addition, chitosan has a unique feature of adhering to mucosal surfaces and penetrating to tight junctions between endothelial cells. With non-toxic, biodegradable and biocompatible properties, chitosan has already been approved by FDA for use in wound dressings[127]. Chitosan-based drug delivery systems have aroused great interest since the early 1990s. A variety of work has been reported on chitosan and its potential application in biomedical fields including wound dressing, tissue engineering and therapeutic drug delivery.

3.1.2. Chitosan-drug conjugates

The concept of polymer–drug conjugates was first proposed in 1975[128]. Since then several polymer–drug conjugates have entered clinical trial stages because of their unique therapeutic properties including improved pharmacokinetics, reduced side effects and enhanced therapeutic efficacy[129]. Natural-origin chitosan has obvious advantages over synthetic polymers including good biocompatibility, biodegradability and non-immunogenicity. Chitosan–drug conjugates have been widely developed owing to its abundant availability and reactive amino groups which can be directly or indirectly linked to drug molecules.

Son et al.[130] first synthesized a glycol-chitosan–doxorubicin conjugate (GC–DOX) which could self-assemble into nanoaggregates with sizes of 250–300 nm and passively accumulate to tumor tissue due to the EPR effect. They used *cis*-aconityl spacers with pH-sensitive properties which could facilitate controlled drug release in the slightly acidic endosomes/lysosomes. Subsequently Lee et al.[131] based a similar CS–PTX conjugate using a biodegradable succinate linker that remains intact in gastric acid while being cleaved in the physiological environment. Lee et al.[132] also constructed a low molecular weight chitosan (6 kD) conjugated insulin delivery system with a disulfide linkage which is responsible for the intracellular GSH at high concentration resulting in improved bioavailability of insulin. Yang et al.[133,134] synthesized a chitosan-*O*-isopropyl-5′-*O*-d4T monophosphate conjugate through phosphoramidate linkage for the treatment of HIV infection. The mild sustained release of the chitosan–d4T conjugate and its nanoparticle form resulted in enhanced anti-HIV selectivity compared to free d4T.

3.1.3. Chitosan-based nanocarriers

Chitosan self-assembled nanomaterials also have good compatibility, biodegradability and low toxicity like chitosan[124]. They have emerged as potential nanocarriers for therapeutics and tissue engineering.

Since chitosan is positively charged under acidic conditions, it could complex with negatively charged nucleic acids, including DNA, mRNA and siRNA through electrostatic interaction and form nanostructures. There is a commercially available chitosan-based transfection reagent named Novafect[135]. However, the complex nanostructures may be not stable in neutral or alkaline conditions because the amine groups of chitosan will be less protonated. Recently, Sadreddini et al.[136] designed carboxylmethyl dextran chitosan nanoparticles (CMD–ChNPs) to encapsulate snail siRNA and anti-cancer drug doxorubicin. The co-delivery system exhibited significant changes of epithelial mesenchymal transition (EMT)-releated gene expression including down regulation of MMP-9 and vimentin and up regulation of E-cadherin in human colorectal cancer (HCT-116) cells.

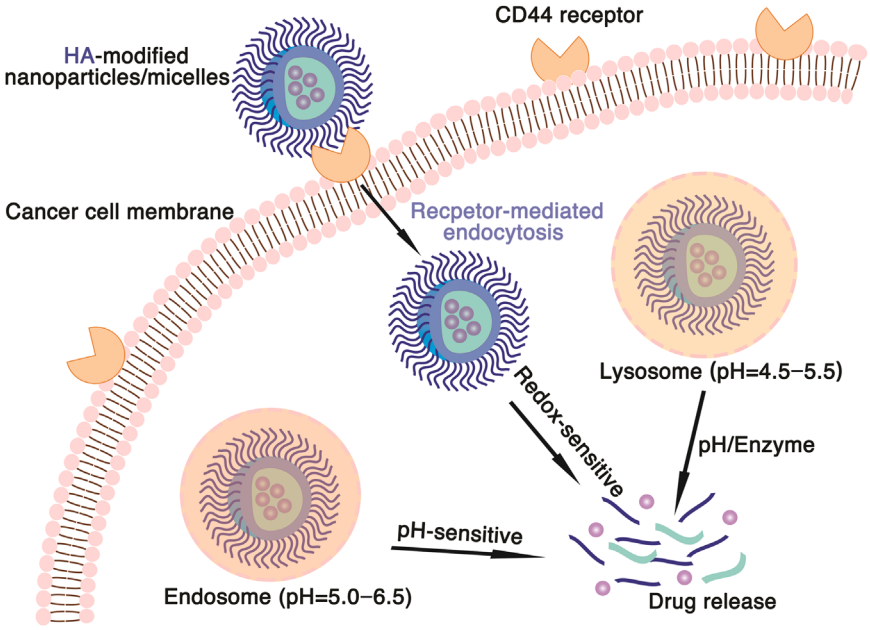

Figure 3 Schematic illustration of HA-modified nanoparticles or micelles targeting CD44-overexpressing cancer cells.

Ionically crosslinked chitosan nanoparticles could also conjugate fluorescent probes for imaging. Bor et al.[137] developed BODIPY-conjugated chitosan nanoparticles with a diameter of 70.25 ± 11.99 nm in spherical shape. The BODIPY-conjugated chitosan nanoparticles show significantly reduced cytotoxicity to human lung adenocarcinoma (A549) cells and normal human bronchial epithelial (BEAS 2B) cells. Therefore, this fluorescent-conjugated chitosan nanoparticle might be a promising platform for bio-imaging application.

Significant efforts have been made to explore an ideal scaffold for tissue engineering for years. In addition to the protein-based scaffolds in the previous introduction, chitosan has also been studied as a useful biomaterial in diverse tissue engineering applications due to its hydrophilic surface that promotes cell adhesion, proliferation and differentiation, good biocompatibility and biodegradability[138].

Since chitosan is fragile and has poor mechanical properties, Saber-Samandari et al.[139] synthesized a copolymer grafted chitosan scaffold for bone tissue engineering with drug delivery capacity. Poly(acrylic acid-*co*-acrylamide) was used as the grafted copolymer, hydroxyapatite was investigated as bone substitute and celecoxib was selected as a model drug in this scaffold carrier. This chitosan-based scaffold demonstrated good compatibility without any cytotoxicity and the drug release from the scaffold displayed a biphasic pattern with a low initial burst and a sustained release of up to 14 days. The results suggest that this nanocomposite scaffolds might be efficient drug carriers in bone tissue engineering.

3.2. *Cyclodextrin*

3.2.1. *Properties of cyclodextrin*

Cyclodextrins (CDs) are cone-shaped α-1,4-linked macrocyclic oligosaccharides with a hydrophilic exterior and a hydrophobic cavity that allow the formation of inclusion complexes with hydrophobic compounds[140]. CDs are natural products formed during the digestion of cellulose by bacteria. The most common CDs are α-CD, β-CD, and γ-CD that composed of six, seven and eight D-glucopyranose units (Fig. 1), respectively.

CDs are biocompatible, biodegradable and non-toxic materials and the central empty cavity of CDs (host) is capable of loading hydrophobic molecules (guest) through van der Waals force and hydrogen bonds[141]. Because of this unique structure, the physicochemical properties of the guest, such as poor solubility, instability and undesired side effects can be masked[142,143]. Moreover, the hydroxyl groups of CDs are chemically reactive to modify functional molecules. Therefore, multifarious CD-based supramolecules and nanoparticles have been explored for drug delivery and medical imaging.

3.2.2. *Cyclodextrin-based delivery systems*

CDs are usually served as carriers in the form of conjugates, supramolecules or nanoparticulate systems. Since α-CD has a relatively small cavity which can only entrap small molecules and γ-CD has a high production cost, β-CD with moderate cavity and low production cost is the most widely applied CD in pharmaceutical research.

Mizusako et al.[144] developed a CD-based novel carrier-drug conjugate with active drug targeting function by folate modification and controlled drug release property by using a pH-cleavable spacer. Recently cationic β-cyclodextrin–chitosan conjugates as potential carrier for gene delivery has been reported by Eslaminejad et al.[145]. In the study pmCherry-C1 gene is successfully delivered to glioma cells with high transfection efficiency.

Monteil et al.[146] developed a kind of cyclodextrins–bisphosphonate complexes (CD/BP). A series of characterizations including NMR spectroscopy, UV–vis and ITC analysis indicated cyclodextrins and bisphosphonates successfully formed 1:1

inclusion complexes and only the side chain of bisphosphonate was involved in the inclusion phenomenon. However, the *in vivo* stability of the host-guest supramolecule remains to be established.

Nafee et al.[147] synthesized amphiphilic MMA–tBA β-CD star copolymers that are capable of forming nanoparticles (CD-NPs) smaller than 200 nm in diameter and CD-NPs loaded with anticancer idarubicin show sustained release over 48 h. Yuan et al.[148] explored a nanocarrier system formed by chitosan grafted with β-cyclodextrin (CD-*g*-CS) for poorly water-soluble drugs. The CD-*g*-CS nanoparticles were prepared by an ionic gelatin method with the controlled size of 202.0–589.0 nm and zeta potential of +23.0 to +43.0 mV. Moreover, the CD-*g*-CS carrier realized controlled release of the payloads.

3.3. Hyaluronic acid

3.3.1. Properties of hyaluronic acid

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan (GAG) in the extracellular matrix (ECM) of many soft connective tissues, composed of alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine that linked together *via* alternating β-1,4 and β-1,3 glycosidic bonds[149]. Due to its abundant negative charges, HA exhibits excellent swelling property. In the ECM of most tissues, the high molecular weight HA, along with other structural macromolecules, contributes to the mechanical integrity of the network. Many researchers have reported that HA has targeting ability to specific cells by binding with cell surface receptors such as CD44 and RHAMM and can be utilized for tumor-targeted drug delivery[150–152]. Since HA is biocompatible, biodegradable, bioactive, non-immunogenic and non-thrombogenic with complex biological functions ranging from matrix organization, cell adhesion and migration, angiogenesis and morphogenesis, wound healing and inflammatory responses to cancer metastasis[153,154], it can also be regarded as an attractive carrier for tissue engineering.

3.3.2. HA-based carriers for drug delivery and tissue engineering

Similarly to chitosan and cyclodextrin, HA was chemically modified with the 5β-cholanic acid to form self-assembled nanoparticles (200–400 nm) that combine both passive tumor targeting based on the EPR effect and a more specific or active targeting exploiting the affinity of HA towards CD44[155].

HA-based or functionalized nanoparticles have received tremendous attention for CD44 targeted drug and protein delivery in recent years (Fig. 3). Liang et al.[156] designed a multifunctional nanoparticle based on activatable HA conjugating two near-infrared (NIR) dyes of Cy5.5 and IR825 as a targeted theranostic agent for enhanced fluorescence/CT/photoacoustic imaging guided photothermal therapy. Han et al.[157] engineered a bioreducible core-crosslinked polymeric micelle based hyaluronic acid (CC-HAM) by simple method using D,L-DTT in aqueous conditions. The CC-HAM exhibited enhanced structural stability under diluted conditions with PBS containing FBS or sodium dodecyl sulfate. DOX was encapsulated in the micelle core with high drug loading efficiency (>80%) and robust drug release of DOX from CC-HAMs was observed in the presence of glutathione. Overall, bioreducible CC-HAM can be applied as a potent doxorubicin delivery carrier with improved stability for targeted cancer therapy. Lee et al.[158] developed hollow particles using a silica core and catechol-modified hyaluronic acid (HA–CA) shell for an anticancer drug carrier. The DOX-loaded HA–CA particles demonstrate pH-triggered release behavior and dramatic *in vitro* anti-tumor-effect, suggesting that they are promising novel drug carrier. Zhong group[159] has done many studies on HA-based carriers for targeted cancer therapy and they have recently reported a HA coated PLGA nanoparticulate docetaxel (DTX–HPLGA) formulation which showed efficient targeting ability to $CD44^+$ A549 cells through CD44-mediated pathway and achieved effective tumor inhibition. To realize controlled drug release, they introduced a reductively cleavable surfactant into the nanostructure to form the reduction-responsive HA-coated PLGA nanoparticle[160]. In another study from this group, a GSH-sensitive HA–SS–mertansine prodrug with high drug loading capacity was synthesized for targeted breast cancer therapy[161]. Moreover, HA engineered nanomicelles loading with 3,4-difluorobenzylidene curcumin were explored for targeted killing of $CD44^+$ stem-like pancreatic cancer cells[162] and HA-shelled pH-sensitive paclitaxel prodrug micelles were developed for targeted therapy of CD44-overexpressing breast cancer[163]. HA-based nanogels were developed for targeted imaging and cancer therapy as well[164–166].

Sheu et al.[167] fabricated an injectable oxidized hyaluronic acid/resveratrol (Oxi-HA/Res) hydrogel for future application in cartilage tissue engineering. It was investigated that Oxi-HA/Res hydrogel was able to maintain chondrocyte phenotype and allow for ECM synthesis. Additionally, the Oxi-HA/Res hydrogel has no toxicity to chondrocyte cells and allows the promotion of gene expression of aggrecan and type II collagen, which are major ECM components of chondrocytes.

A biological hydrogel of recombinant human fibroblast growth factor type 2 in a hyaluronic acid carrier (rhEGF-2/HA) has entered clinical trial in periodontal intrabony defects. Patients treated with rhFGF/HA exhibited significantly more probe depth reduction, probing attachment level and probing bone level gains than the control group[168]. The clinical parameters of periodontal wound healing were greatly improved one year after treatment.

3.4. Heparin

3.4.1. Unique properties of Heparin

Heparin is a water-soluble and negatively-charged polysaccharide with important biological functions including anticoagulant activity, strong binding to growth factors such as VEGF, basic FGF and bone morphogenetic protein-2 (BMP-2)[169,170]. Therefore heparin has been widely studied as an anticoagulant drug as well as antitumor drug delivery carriers due to its multi-targeting capability and anti-angiogenesis activity[171].

3.4.2. Heparin–drug conjugates

Heparin–drug conjugates are currently investigated as excellent candidates for drug delivery vehicles and combination therapy. She et al.[172] reported a dendronized heparin–DOX conjugate with pH-sensitive property by combination of the features of dendrimer and heparin. The dendronized heparin–DOX conjugate self-assembled into compact nanoparticles with negatively charged surface and showed high antitumor efficacy both *in vitro* and *in vivo*. A heparin–indomethacin conjugate with an ester linkage for sustained and esterase-sensitive drug release was synthesized by Li et al.[173]. The conjugate could self-assemble into spherical nanoparticles with a diameter <200 nm in aqueous solution due to its amphiphilic property.

Choi et al.[174] reported a conjugate of low molecular weight heparin (LMWH) and four bis-deoxycholates named LHbisD4 as a

potent anti-angiogenic drug that can be administrated orally with less toxicity for anti-lymphangiogenic therapy. Further studies revealed that LHbisD4 could also suppress the formation of new lymphatic vessels and inhibit metastasis by blocking VEGF-C pathway.

3.4.3. *Heparin-based nanocarriers for drug delivery and tissue engineering*

When conjugated to hydrophobic molecules, heparin has the potential to assemble into nanoparticles. Zhang et al.[175] designed an amphiphilic conjugate of low molecular weight heparin (LMWH) and all-trans retinoic acid (ATRA) that can self-assemble into nanoparticles and encapsulate the anticancer drug DOX. The DOX–LMWH–ATRA nanoparticles demonstrate good compatibility and accumulation in tumors *via* the EPR effect and LMWH-based endocytosis. Co-delivery of the three components in one nanoparticle system has achieved an enhanced antitumor effect compared to monotherapy. A novel nanocarrier of heparin modified grapheme oxide (GO) was synthesized by using a pH-sensitive linker (adipic dihydrazide, ADH) to deliver DOX and facilitate controlled release for anticancer therapy[176]. The GO–ADH–Hep/DOX nanosystem displayed effective cytotoxicity to human breast cancer cells (MCF-7) and human hepatocellular carcinoma cells (HepG2) with reduced cardiotoxicity and pulmonary toxicity compared to free DOX and unmodified GO.

Lee et al.[177] designed a heparin-conjugated fibrin (HCF) carrier system to deliver recombinant human BMP-2 (rhBMP-2) for bone tissue engineering. Previous studies showed that HCF carriers exhibited a slower and more controlled release of rhBMP-2 compared to fibrin and traditional carrier absorbable collagen sponge (ACS). The HCF carrier system loaded with rhBMP-2 shows reduced adipose tissue formation and enhanced mineralized tissue formation, but the lack of space-maintaining properties remains an obstacle with this carrier system.

Subsequently, a heparin-based polyelectrolyte (PEC) carrier for the delivery of BMP-2 was also developed by Wang et al.[178] to enhance the posterolateral fusion in porcine model. The radiological fusion score of PEC groups is higher and the newly formed bone integrated better into the native bone compared to ACS.

3.5. *Pectin*

3.5.1. *Properties of pectin*

Pectin is a natural polymer existing in the fruits, roots, stems and leaves as a component of the cell walls of most plants. Pectin acts as an accompaniment of fibrin and both of them are constituents of the intermediate joint of the adjacent cells and make the plant tissues cells tightly bound together[179].

As an ionic branched macromolecule with high molecular weight, pectin can be converted into hydrogels, intended as flexible network of polymer chains that can swell but do not dissolve in water[180]. In addition to good biocompatibility, biodegradability and non-toxicity, pectin is a natural hydrocolloid and is suitable for drug delivery and tissue engineering.

3.5.2. *Pectin as carriers for drug delivery and tissue engineering*

Among natural polymers, pectin has unique features for drug delivery, such as muco-adhesiveness and ease of dissolution in basic environments and the ability to form gels in acidic environments.

Given the negatively charged property, pectin was found to be suitable for coating b-PEI polyplexes and showed decreased transfection with a concomitant lower cytotoxicity and higher stability[181].

With a different approach, Katav et al.[182] suggested a chemical modification to pectin to make the anionic polymer applicable for DNA delivery: the pectin structure was modified with amine groups, and the modified compound formed complexes with plasmid DNA while exhibiting high drug stability. Other studies have investigated the formation of pectin nanoparticles with different cations to entrap the DNA for transfection.

Coimbra et al.[183] prepared porous scaffolds obtained from the freeze-drying of pectin/chitosan polyelectrolyte complexes. The study found that cells adhered to this pectin/chitosan complex scaffold and proliferated and the scaffold is nontoxic to human osteoblast cells. The pectin/chitosan couples may act as potential scaffold for bone tissue engineering. Tummalapalli et al.[184] developed a novel oxidized pectin-gelatin-nanosliver (OP-Gel-NS) flower like nanohydrocolloids and ciprofloxacin hydrochloride incorporated into the OP-Gel to generate OP-Gel-Cipro dressings. The histological examination demonstrated that OP-Gel-NS and OP-Gel-Cipro dressings exhibit good hydrophilicity and sustained antimicrobial nature, promote cell growth and proliferation, and lead to rapid wound healing effect.

Glucans are polysaccharides of glucose monomers linked by glycosidic bonds and β-glucan particles are most widely studied glucan-based drug carriers. Since the glucan particles are often used for oral drug delivery and would be included in other reviews, herein we would not give detailed examples and discussion about it.

4. Conclusions and perspectives

Natural biological carriers including proteins and polysaccharides have the advantage of good compatibility, biodegradability, long blood circulation time, non-toxicity and non-immunogenicity. Thus they are regarded as ideal carriers for the delivery of therapeutic drug, protein, gene and imaging probe as well as tissue engineering. Researchers have been developing versatile and multifunctional biological carriers for decades by using different strategies including covalent linkage and physical encapsulation, and some have achieved encouraging progress. There is an increasing interest in combined application of two or more kinds of biomacromolecules or combined use of biomacromolecules and synthetic polymers or inorganic nanoparticles in one carrier system to realize multiple functions.

Although native biological carriers have versatile advantages, utilizing them for drug delivery may not preserve the same *in vivo* properties. Chemical conjugation may affect the carrier's intrinsic physiochemical properties and non-covalent interaction of the carrier and its payloads may be not stable *in vivo*. For example, drug conjugation to the 34-Cys of albumin changes its endocytosis mechanism[23]. Furthermore, the circulating half-lives of most biological carrier-based therapeutics are extended as compared to free drugs, but significantly shorter than the native biomacromolecules. There are still many challenges related to production, quality control, storage, safety and selectivity of natural protein- and polysaccharide-based drug carriers. Oral drug delivery of large-protein based drugs may have poor stability due to proteolysis and poor absorption leading to low bioavailability. Researchers have adopted encapsulation methods, cross-linkers and

protease inhibitors to enhance the stability of protein-based carriers while using targeting ligands and permeability enhancers to promote drug absorption. Although there are many preclinical studies of HA-based carriers in drug delivery and tissue engineering, very few HA-based drug delivery systems have entered into clinical trials. In addition, conflicting results have been reported probably because that the effect of molecular weight (MW) on HA functions *in vivo* was rarely evaluated in many studies related to HA[185]. It is reported that MW is also related to the immune-response of HA and HA with low MW seems to be immunogenic while HA at high MW does not induce immune response[185]. On the other hand, it is known that HA containing 10–100 disaccharide units could promote tumor growth, whereas HA containing more than 100 units might inhibit tumor growth[186]. And similar to albumin, chemical modification of HA may also influence the receptor-mediated uptake by cancer cells[151]. As a result, the properties and functions of HA still need to be fully clarified.

Therefore, future research should be aimed at developing therapies that can utilize the advantages of biological carrier in full measure to gain best results. Selective tissue distribution, cell type-specific targeting of the biomacromolecule-based carriers should be explored and technologies that permit the easy production and good quality of the protein- and polysaccharide- carriers should be developed[187].

Up to now, only several biological carrier-based therapeutics have been approved for clinical use. Thus more effort should be made to accelerate the translation of research findings from laboratory to clinic , which requires close cooperation of pharmacists , materials scientists and clinical doctors. Biological carrier systems that can be administrated in multiple routes should be designed, which is intended to meet the clinical demand and ensure patient compliance. In summary , there is a promising future for the clinical application of natural biological drug carriers.

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