Bioconjugate Chemistry

Review

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Polysaccharide-Based Conjugates for Biomedical Applications

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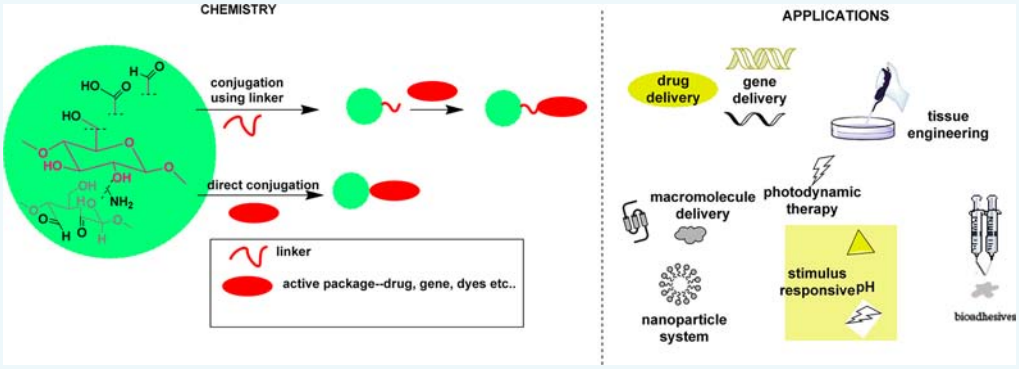
Arijit Basu,[†,‡,+] Konda Reddy Kunduru,[†,+] Ester Abtew,[†] and Abraham J. Domb*[,†]

[†]Institute for Drug Research, School of Pharmacy-Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel 91120

[‡]Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, 835215, India



ABSTRACT: Polysaccharides contain different functional groups (such as hydroxyl, amino, carboxylic acid, aldehydes) that make them ideal for conjugation. They are biodegradable, biocompatible, and hydrophilic. Polysaccharide conjugates have been used in drug, gene, and macromolecule delivery, tissue engineering, and other biomedical applications. Polysaccharide conjugates have also been used primarily for solubilization and controlled release of hydrophobic moieties. The advent of nanotechnology, gene therapy, and tissue engineering influenced the way these conjugates are now used. Modern day conjugates are modulated to be thermoresponsive, pH-responsive, photoresponsive, or target-specific (receptor mediated targeting). This Review briefly introduces different polysaccharides followed by different synthetic strategies used for conjugation; finally, recent applications were compiled.

1. INTRODUCTION

Covalently linking drug molecules to polymer carriers was reported more than 60 years ago. Since then, extensive reports on conjugation (or covalent linking) have appeared, mainly for improving the pharmacokinetics or pharmacodynamics of drugs. They have also been used as a carrier of macromolecules, tissue engineering, and as bioadhesives.[1,2]

Ringsdorf proposed a polymer conjugate model in 1975 consisting of a biocompatible polymer backbone, a solubilizer moiety, a covalently bound drug (with or without linker), and a targeting moiety.[3] In the case of polysaccharide conjugates the solubilizer moiety is redundant. We also observed substantial innovation due to the advent of nanotechnology, and stimulus responsive dosage forms.

Polysaccharides are natural compounds, nontoxic, and biocompatible. Therefore, they are widely used in drug delivery and other biomedical research.[4,5] A bioactive agent(s) is covalently bound to the polysaccharide backbone either by itself or via a linker. Polysaccharides are abundant in nature: produced by algae origin (alginate and carrageenan), plant origin (cellulose, pectin, and guar gum), microbial origin (dextran and chitin), and animal origin (hyaluronan, chondroitin, and heparin).[6] Polysaccharides possess a wide range of molecular weights and a significant number of functional groups for chemical modification.

Polysaccharides increase the aqueous solubility of the conjugated hydrophobic moiety for drug delivery or similar biomedical purposes. The release of the package may also be triggered in a desired manner: making it pH responsive or cleaving with specific enzymes.[7,8] Conjugation also enhances control over the release (sustained or targeted) and pharmokinetics (drug bioavailability, plasma half-life, degradation, biodistribution, accumulation, metabolism, and elimination). On the other hand, these altered properties may be detrimental, if conjugation is not fine-tuned to the requirement. Due to their higher molecular weight, polysaccharides accumulate in the body. They wait for biodegradation by enzymatic cleavages; once these huge polymeric units are degraded into smaller units, they undergo rapid renal clearance. The pharmacokinetics of polysaccharide conjugates are influenced by their charge, MW, extent of chemical modifications, polydispersity, and three-dimensional structures.

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Functional groups available on the drug and the polymer determine the mode of conjugation. Synthetic modification may be carried out to join the two. However, if such compatibility is not available, linkers are used. The linkers bridge together the polymer with the desired molecule (drugs or macromolecules).[9]

Polysaccharides adhere to biological tissues and mucosal surfaces, which renders them the material of choice for conjugated delivery system.[6] Biomedical applications of polymeric materials have increased significantly over the past three decades.[10−13] Net charge on polysaccharide is important for targeting; they can be either positively charged (chitosan) or negatively charged (alginate, heparin, hyaluronic acid, pectin, etc.).[14] They may form branched structures unlike proteins and nucleic acids.[15] Synthetically modified polysaccharides have potential applications in drug delivery, tissue engineering, gene delivery, and bioadhesion.[16−18]

2. CHEMICAL METHODS USED FOR CONJUGATION

The simplest polysaccharide molecules consist of monosaccharide repeating units with hydroxyl groups as the only functional group (cellulose, starch, pullulan, arabinogalactan). The amino sugars chitin/chitosan present opportunities to conjugate with the amine.[19] Polysaccharide with carboxylic acid groups (hyaluronic acid or alginate) also presents an opportunity for conjugation.[20,21] In this section, we briefly review the chemical modifications for polysaccharide conjugations.

2.1. Polysaccharide Used as Alcohol. Polysaccharides may undergo etherification and esterification. Earlier, these reactions were carried out using strong bases by activating the alcohol groups. Recent reports on conjugation employ activated esterification/etherification reagents.[22] Common methods to activate the hydroxy group are to employ carbonyldiimidazole (CDI),[23−25] using a carbamate mediated conjugation method,[26] or using aldehyde mediated acetal formation.[27]

2.2. Polysaccharide Used as Amine. Chitosan based polysaccharides have been widely used for synthesizing conjugates.[28,29] The amine is either alkylated or acylated. It also undergoes condensation followed by reductive amination. Other reactions involve amidation (EDC/NHS based activated esters), formation of azides (followed by copper catalyzed click reactions), and formation of urea with isocyanate or thiocyanates[30,31] (Figure 1).

2.3. Polysaccharide Used as Carboxylic Acid. Hyaluronic and alginic acids are active carboxylic acid functional groups. Schanté et al. have reviewed the popular methods of chemical functionalization of hyaluronic acid.[32] Yang et al. reviewed chemical modifications of alginates.[33] We compiled the chemical modifications for these polysaccharides. Esterification and amidation (with or without linker) remains the method of choice. Activation by EDC or DCC has been used for esterification. NHS or HOBT activated esters are used for amidation. Other methods, e.g., Ugi condensation and hydrazination, and reaction with epoxides, have also been reported (Figure 2).[34]

2.4. Partially Oxidized Polysaccharide. Partially oxidized dextran, arabinogalactan, and cyclodextrin are commonly used polysaccharides for this conjugation. The 2,3-diols can be oxidized to dialdehydes using $NaIO_4$ mediated,[35] enzymatic,[36] peroxide mediated[37] oxidation. Sixth ($-CH_2OH$) position oxidation using Dess−Martin periodinate or 2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) has also been reported (Figure 3).[38]

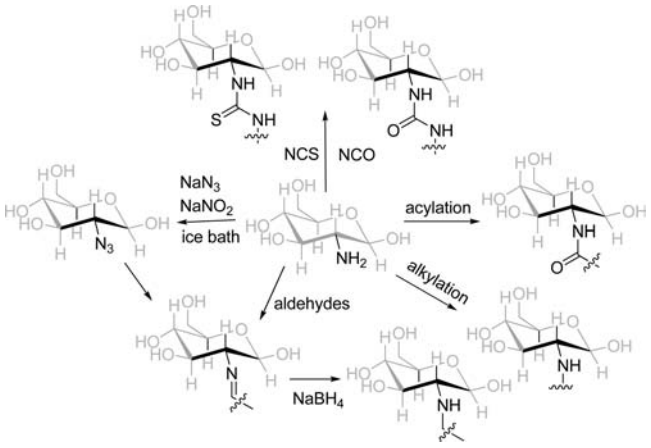

Figure 1. Chemical modifications where the polysaccharide is used as an amine (mainly chitosan).

2.5. Click Chemistry in Polysaccharide Biomaterials. Polysaccharides may be propagylated and/or azidated (introduction of N_3) for copper mediated "click-chemistry". Chitosan may be azidated through diazotization followed by reacting with sodium azide[31] or trifluoromethanesulfonylazide (TfN_3).[39] Sixth position hydroxyl is most reactive toward azidation. Azido-derivatives are synthesized, first introducing a good leaving group, followed by nucleophilic substitution using sodium azide.[40] Once they are prefunctionalized, Huigen 1,3-dipolar cycloaddition reaction between azides and terminal alkynes[41] may be performed (Figure 4).

A common challenge in polysaccharide chemical modifications is solubility. Most of the polysaccharides have crystalline structure, with strong intramolecular hydrogen bonding. This makes them soluble in warm aqueous medium. Solubility is also strongly pH dependent (chitosan in acidic medium, HA in basic). Conjugation reactions like carbodiimide coupling, esterification, amide coupling, oxidation, and reduction should be performed in aqueous media. Solubility makes purification difficult, especially for multistep reactions. Reagents have evolved over the past few years, specifically for polysaccharide chemistry. Use of microwave irradiation significantly reduces the use of toxic solvents as well as the reaction time. Normally they are greener, cleaner, and proceed with higher yields.[42]

3. APPLICATIONS OF POLYSACCHARIDE CONJUGATES

Polysaccharide conjugates have been known for many years, though few have been approved or are undergoing clinical trials.[8] Polysaccharides with amine, carboxylic acid, and aldehyde functionality have been used widely for the conjugations. Most of the bioactive substances (drugs, proteins, and nucleic acids) are easier to conjugate by using standard reaction conditions or through "click chemistry". Table 1 shows polysaccharide conjugates undergoing clinical trials.

3.1. Delivery and Imaging. Polysaccharide conjugates have been explored for delivery of bioactive substances (drug, dyes, proteins, and nuclear materials). Various conjugation methods have been reported for controlled release, stimulus responsive release, targeted delivery, and nanohybrids. A few examples of different polysaccharide conjugates in delivery are described in the following sections.

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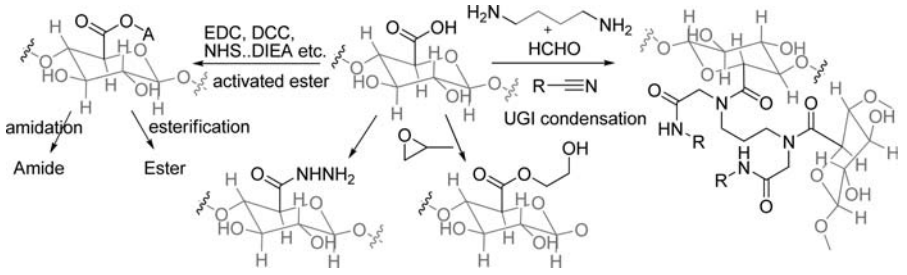

Figure 2. Chemical modifications where the polysaccharides are treated as carboxylic acids (hyaluronic acid, alginic acid, and chondroitin).

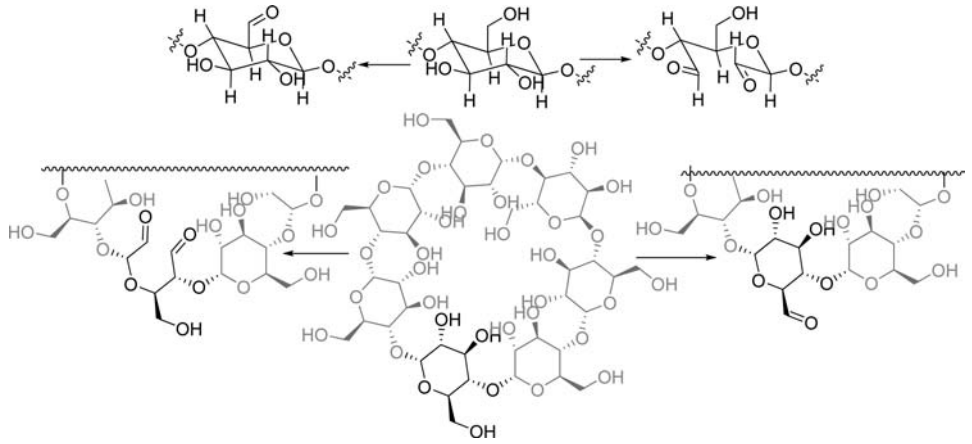

Figure 3. Chemical modifications for partially oxidized polysaccharides (Dextran, arabinogalacton, HA, chitosan, cyclodextrin).

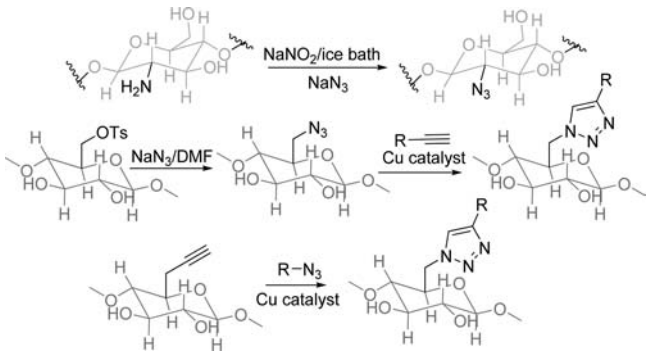

Figure 4. Copper catalyzed click chemistry used for conjugation of prefunctionalized polysaccharides [Huigen 1,3-dipolar cycloaddition reaction].

3.1.1. Chitosan Based Conjugates. The amine group of chitosan is generally used to synthesize the conjugates. Following are a few recent examples of chitosan based conjugates.

Chitosan based conjugates are used to trigger release photosensitive drug chlorin e6(ce6), an example of photodynamic therapy. pH-sensitive tertiary amine (3-diethylaminopropyl isothiocyanate) assists in adhering to the cancer cells (since the pH around cancer cell is acidic). This protonation generates the required singlet oxygen species from the drug and helps destroy the cancer cells.[30] In a similar study, glycol chitosan was conjugated with an iodinated derivative (3,5-bis(acetamido)-2,4,6-triiodobenzoic acid) and photosensitive drug ce6. The iodinated conjugate showed enhanced singlet oxygen generation (Figure 5).[48]

Chitosan conjugate bearing the Shiga toxin (Stx) ligand (globotriose) successfully inhibited the Stx-producing *Escherichia coli*. In this example, the glycol-conjugate is used as an active agent. (Figure 6).[49] Table 2 shows some of the examples of chitosan conjugates.

An innovative controlled copolymerization with chitosan and *N*-(2-hydroxyethyl)prop-2-enamide was reported under γ-ray

Table 1. Examples of Polysaccharide Conjugates That Are Under Clinical Trials

polysaccharide−drug conjugate	brief description	ref
HA-paclitaxel	Conjugate is administered through intravesicle instillation; significant response has been observed in 9 out of 16 patients. It has shown good bladder efficiency, minimal toxicity, and no systemic absorption.	43
β-Cyclodextrin, PEG copolymer-camptothecin	Conjugate administered through intravenous infusion; 6 out of 12 patients were stable. Three patients survived more than 10 months without disease progression.	44
Carboxymethyldextran-camptothecin derivative delimotecan	Conjugate administered through intravenous infusion. Two patients partially responded with anal cancer and head/neck cancer. Adverse effects were also reported (leucocytopenia, neutropenia, skin rash, fatigue, and diarrhea).	45
Carboxymethyl dextran-camptothecin derivative	Conjugate administered through intravenous infusion. Disease in 14 out of 27 patients was stabilized. Dose limiting toxicities include thrombocytopenia, neutropenia, and reversible hepatotoxicity.	46
Oxidized dextran-doxorubicin	Conjugate administered through intravenous infusion. In 12 of 13 patients, disease was stabilized for 4 months; toxicity reported (reversible thrombocytopenia and hepatotoxicity).	47

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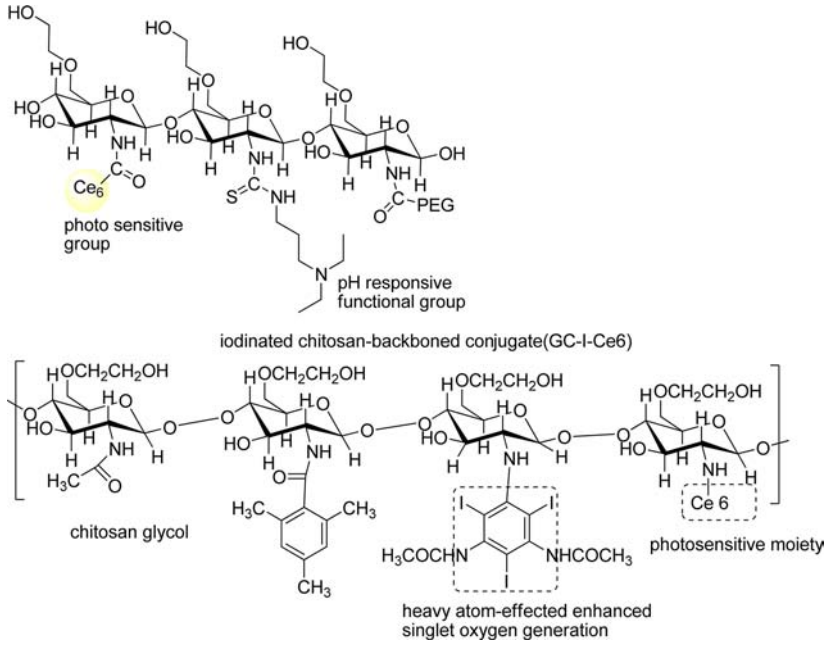

Figure 5. Chitosan-conjugate is used for photodynamic therapy. In this conjugate, Ce 6 is the photoactive package with a pH sensitive tertiary amine for recognition and targeting. In the second example, iodinated phenyl ring generated singlet oxygen species is described.

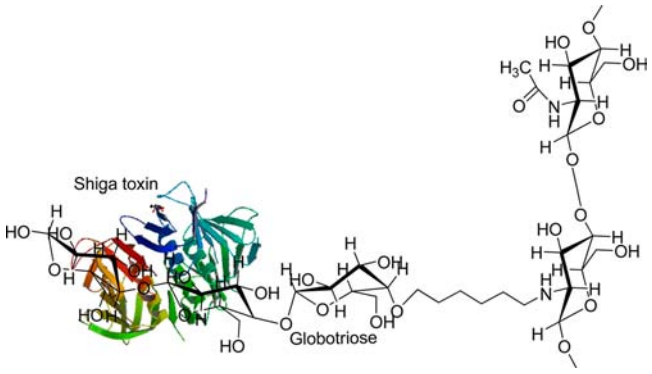

Figure 6. Example of chitosan being used as protein carrier, Shiga toxin (*E. coli*).

irradiation. The researchers also conjugated chromone-3-carboxaldehyde, and observed a pH-sensitive release (Figure 7).[61]

Chitosan-VitE-acetylcysteine conjugate was synthesized—a smart approach for overcoming the problem of drug absorption from gastric mucosa. Acetyl cysteine moiety improved the mucosal bioadhesion. The inner core is made with Vit-E, which made it hydrophobic. The particles self-assemble with hydrophobic inner core and hydrophilic mucosa adhering the cysteine moiety outer surface. The inner core is hydrophobically loaded drugs like paclitaxel (Figure 8).[62] Roy et al. provide an in-depth review on paclitaxel and docetaxel based polysaccharide conjugates.[63]

Dextran and chitosan conjugates have been used for making hydrogels through unusual thia-Michael addition reaction. The hydrogel was used for topical of loading vancomycin on the wound surface (Figure 9).[64]

3.1.2. Hyaluronic Acid Based Conjugates. The carboxylic acid functional group in HA[65] and alginate[33] have been used to make conjugates. In this section, a few examples of carboxylic acid based polysaccharide conjugates are presented (Table 3).

Adamantane terminated gold nanoparticles and β-cyclodextrin conjugated hyaluronic acid were coordinated.[77] This gold nanoparticle-cyclodextrin-HA hybrid encapsulates hydrophobic anticancer drugs (Figure 10). Citric acid and PEG diamine were used to synthesize amine functionalized carbon dots. These carbon dots were then conjugated with HA. These carbon dots may be used for real time imaging of HA receptors (Figure 11).[78]

HA-retinoic acid conjugate self-assembled nano particles were used to deliver paclitaxel (Figure 12).[79]

Cystamine-conjugated oxidized HA was used to couple with gold nanoparticles. These modified gold nanoparticles were then coupled with interferon-α for treating hepatitis C. The nanohybrid was targeted for the hepatocytes that express HA receptor. Once the package (interferon-α) reaches the liver, it is recognized by its corresponding receptor. The package is released on site (Figure 13).[80]

3.1.3. Alginate Conjugates. Alginate salt is converted into alginic acid by treatment with dilute HCl. Most of the reported conjugates are synthesized either via DCC/DMAP or EDC/NHS chemistry, either esterification or amidation. Conjugation followed by reductive amination to oxidized alginate and Ugi condensation has also been reported.[33] Ampiphilic alginates are synthesized by reacting with hydrophobic scaffolds (e.g., alkyl chains, hydrophobic polymers). They self-assemble as micro/nanoparticles and gels in aqueous media.[81,82] Many alginate-conjugates have been reported. A few recent examples are as follows:

Alginate-curcumin: The conjugate enhances the aqueous solubility of curcumin. Conjugated curcumin shows enhanced anticancer activity.[83]

Alginate-cisplatin: Sodium alginate cisplatin conjugate was synthesized and incorporated into a liposomal system. The liposomes were surface activated with epidermal growth factor for targeted delivery.[84]

Alginate-oligonecleotide: Couvreur reported an innovative alginate-nucleotide based conjugate. The device may be

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Table 2. Examples of a Few Reported Chitosan Conjugates

chitosan−drug conjugate	brief description	ref
Chitosan−5-fluorouridine	The conjugate microspheres were prepared using chitosan-5-fuorouridine and compared to unconjugated microspheres of chitosan and drug. Both microspheres showed high retention. The conjugate swells quickly in buffers pH 7.4; it disintegrates gradually for 24 h after incubation. Conjugate showed a gradual drug release of 50% drug in 61 h. Unconjugated chitosan-drug system rapidly released the drug.	50
Chitosan−doxorubicin	Conjugates prepared via cis-aconityl linkage. Loading of the nanoparticles was high (to 38 wt %). Conjugates accumulated into the tumor tissue due to the enhanced permeability and retention effect. The cis-aconityl spacer showed pH-sensitive behavior, resulting in hydrolysis in acidic environment of endosomes/lysosomes and the release of the drug to cytoplasm.	51
Chitosan−paclitaxel	Conjugates prepared via succinate linker. It remained unaltered at acidic pH in stomach. It enters the bloodstream and cleaves at physiological pH. The conjugates were suitable for oral administration with comparable IC_{50} values to paclitaxel. About 42% of PTX were bioavailable after oral administration of 5 mg paclitaxel/kg of the conjugate.	52
Chitosan−insulin	Chitosan−insulin conjugates administered orally to diabetic rats. This system controls blood glucose levels effectively for several hours. The conjugate releases the drug via glutathione with higher in concentration inside cells and tumors. The in vivo study demonstrated 43% of insulin was bioavailable after oral administration, indicating intestinal absorption of insulin is significantly enhanced.	53
Carboxymethylchitosan−6-mercaptopurine	Conjugate prepared via an α,β-unsaturated linker for intracellular delivery. Linker was fairly sensitive to the glutathione concentration. Concentration of glutathione in cancer cells is almost 4 times higher than that in normal cells. Due to this, the conjugates with the disulfide linker prefer release of the drug in the tumor tissue.	54
Chitosan−stavudine (d4T)	Conjugates prepared via phosphoramidate linkage. Stavudine is nucleoside reverse transcriptase inhibitor for human immunodeficiency virus (HIV) infection. Adverse effects and poor cell uptake efficiency limit clinical application. In vitro drug release studies at pH 1.1 and pH 7.4 suggest that both the conjugate and its nanoparticles release the drug for prolonged periods.	55−57
Chitosan−doxorubicin	Conjugate prepared via succinate linker. It has a spherical shape and smooth surface with a narrow size distribution and core−shell structure. The nanosystem was further conjugated to $Her2^+$antibody, to discriminate from and $Her2^-$ cells. It has potential to be used in targeted drug delivery with no side effects in Her 2+ breast and ovarian cancers.	58
Chitosan−salicylic acid	In vitro antiplatelet activity of conjugates reveal at low concentrations antiplatelet aggregation capability of conjugate is better than that of low-dose aspirin. The platelet adhesion test shows significant difference between the effect of conjugate and that of the control group.	59
Chitosan−atorvastatin	Bioavailable and stable conjugates were prepared for atorvastatin. Nanoconjugates are nearly 100-fold more soluble than pure atorvastatin. In vitro drug release studies in simulated gastric fluid and simulated intestinal fluid suggest sustained release of atorvastatin from the conjugate. Oral administration of nanoconjugate to rat exhibits nearly 5-fold increase in bioavailability compared to atorvastatin.	60

replenished with drug from time to time. Complementary nucleotide sequences-drug conjugate finds the alginate device to refill the drug depot.[85,86]

Alginate-rhodamine: Saha et al. report a self-indicating alginate based (Hg^{2+} and Cr^{3+}) scavenger. Ca^{2+}alginate beads conjugated with rhodamine recognize and also scavenge these toxic metal ions.[87]

3.1.4. Chondroitin Conjugates. Chondroitin sulfate is seldom used as a delivery vehicle. Possibly its anionic sulfated structure makes it too specific. Being a part of cartilage, it is quickly recognized and integrated. Here are a few recent examples of CS used as conjugated carrier molecule.

Ampiphilic CS−histamine conjugate was synthesized. It self-assembles into nanoparticles in aqueous media. Due to the pH-sensitive structure of imidazole, the nanoparticles show pH-responsive behavior. The nanoparticles were used to deliver DOX. They exhibited on−off drug release behavior: releasing DOX in acidic surroundings and sealing off in neutral surroundings. These pH-responsive flexible micelles may accurately deliver other hydrophobic anticancer drugs.[88]

Aceclofenac loaded CS conjugates (CS-SLN) were reported for the effective management of osteoarthritis. In vivo experiments show enhanced uptake of SLNs by the knee joint. Enhanced accumulation is due to interactions with CD44, annexin, and leptin receptors with CS conjugated SLNs. Therefore, CS-SLNs are potential vectors for carrying drug molecules useful for the treatment and management of osteoarthritis.[89]

3.1.5. Conjugates Using Arabinogalactan, Dextran, and Pullulan. The alcoholic groups of saccharides may be oxidized to aldehydes by methods already described above. These aldehydes may conjugate with amine containing substrates. Polysaccharides may also be used as alcohols without any chemical modifications (Table 4).[22] Also illustrated are a few recent examples for these polysaccharide conjugates.

Oxidized dextran conjugated with hematin has been used to enhance the bioavailability of hydrophobic graphene oxide doxorubicin nanohybrid (Figure 14).[110]

Hollow nanoporous Ag−Au nanoparticles were synthesized and coated with dextran. The nanoporous silver can oxidize the surface dextran. The oxidized dextran was then used to conjugate with doxorubicin through a Schiff's base formation. The release was also observed to be pH responsive (Figure 15).[111]

Amphotericin B is a hydrophobic antifungal drug with poor bioavailability. In 1999 this group synthesized amphotericin B-oxidized arabinogalactan conjugate to improve its aqueous solubility.[112] The conjugate significantly improves the pharmacokinetics and reduces toxicity of amphotericin B. It also retains its antifungal property.[113] However, we observed that free aldehyde groups may cause some toxicity if kept uncoupled or not reduced.[114] Our group also reported conjugation by tosylation.[115] Detailed pharmacokinetic studies on this conjugate reveal that the pharmacokinetics are mainly dictated by the macromolecular moiety and show a significant molecular weight dependency.[116,117] Recently, we reported a scaleup procedure for arabinogalactan amphotericin B conjugate.[118] The conjugate was found to be active against leishmania,[119,120] and others parasites.[121,122]

Folic acid and methotrexate (MTX) were conjugated to AG (AG-folic acid-MTX). This conjugate can differentially deliver a cytotoxic cargo to cells overexpressing folate receptors. Linking of methotrexate via an endosomally cleavable peptide

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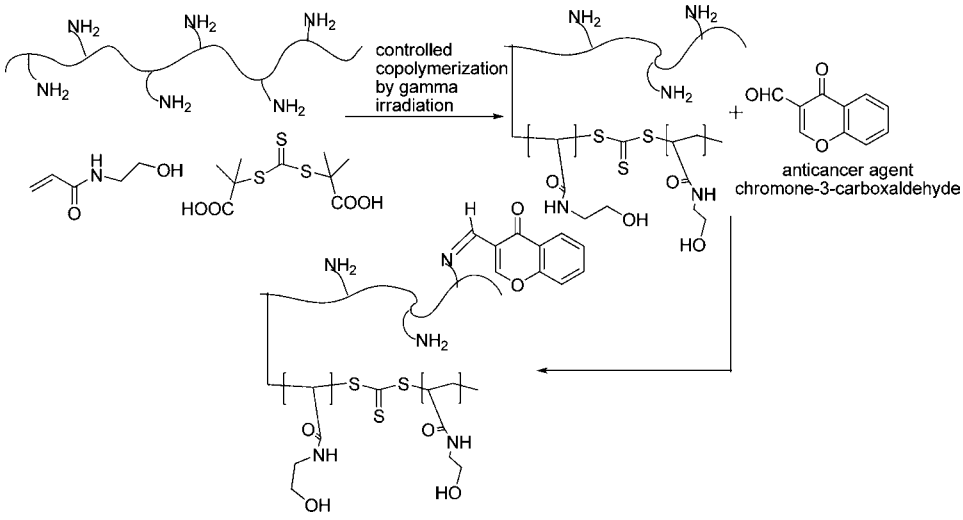

Figure 7. Innovative chitosan acrylate copolymerization used to deliver chromone-3-carboxaldehyde in a pH responsive manner.

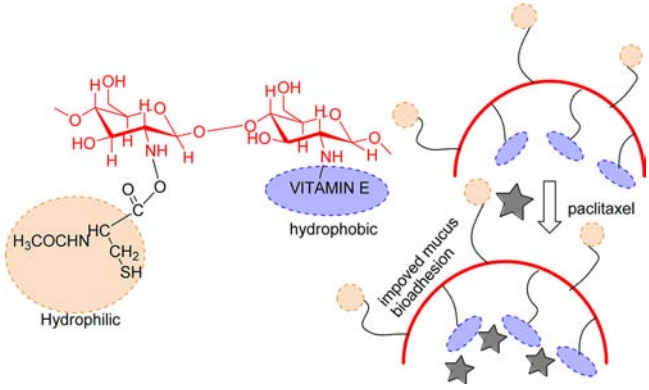

Figure 8. Vitamin-E and acetyl cysteine conjugated chitosan have been used for improved bioadhesion to gastric mucosa.

demonstrates a target-activated release mechanism. This FA-AG-GFLG-MTX drug conjugate displays increased cytotoxicity to cells overexpressing folate receptor.[123] An innovative Cathepsin K-sensitive tetrapeptide spacer has been reported for conjugating Paclitaxel (PTX) to pullulan (Figure 16).[124]

Partially oxidized polysaccharide conjugates have been used in gene delivery. Gene delivery requires a cationic core for carrying the genetic material.[125−128] Generally, this cationic core consists of quarternized amine.[129,130] The outer shell is hydrophilic to enhance bioavailability.[131] Oxidized dextran (or other polysaccharides) with aldehyde functional groups is ideal for making this kind of system. The aldehyde conjugates with poly/oligo amines like spermine or polyethylene imine, followed by reductive amination. The resultant system self-assembles in aqueous solution with inner cationic core.[132] Our group optimized the amine-polysaccharide combination for complexation of plasmids. The group reported arabinogalactan, dextran, and pullulan at various degrees of oxidation conjugating with 10 different amines—all together 300 conjugates. Most of the conjugates formed stable complexes with various plasmids. The structure of the polycation plays a significant role in the transfection activity of polycations.[133] Oxidized dextran spermine conjugate nanoparticle system successfully delivered genetic materials[134−136] such as pCMV-GFP plasmid[137] and pSV-LacZ.[138,139] Dextran-spermine and lipoplexes were compared. It was demonstrated that dense positive charges and sufficient incorporation of secondary amines determine gene expression in vivo.[140]

Gene expression in mesenchymal stem cells was enhanced through dextran−spermine conjugate gene carrier on a three-dimensional tissue engineered scaffold.[141] Hydrophobic oxidized dextran−spermine conjugate was synthesized by reacting NHS-fatty acid esters with dextran−spermine conjugate.[142] This system was used for delivery of the following genetic materials: pCMV-GFP encoding green fluorescence protein, pSV-hGal encoding h-galactosidase, and pLNC-luc encoding luciferase.[143] We also reported that PEGylated−dextran−spermine (PEG-D-SPM) based gene carrier shows modest transfection efficiency in the leukemic cell.[144]

Saccharides may also be used as alcohols owing to low reactivity compared to carboxylic acids, aldehydes, and amines. They are seldom used directly for conjugation. Cellulose conjugation using carbodiimide coupling of functional molecules (aminofluoresceinas a model compound) on cellulose surfaces has been reported.[145] Use of spacers/linkers, however, is common; they provide appropriate control over conjugation. Polyethylenimine (PEI)-conjugated pullulan have been investigated for gene delivery. Pullulan-PEI conjugate is hemocompatible and safe, and does not compromise transfection efficacy

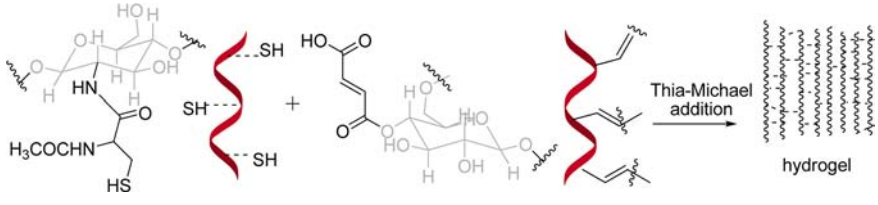

Figure 9. Dextran−chitosan conjugate-based hybrid material used to make hydrogel through thia-Michael addition.

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Table 3. Examples of a Few Hyaluronic Acid Conjugates

HA-conjugate	brief description	ref
HA-doxorubicin	Conjugates prepared via adipic dihydrazide linker. The nanoconjugate exhibits sustained release of DOX in vitro and in vivo. In vivo study performed on the breast tissues of rodents bearing human breast cancer xenografts. Toxicities were lower compared to doxorubicin. The conjugate significantly inhibits breast cancer progression, leading to an increased survival rate.	66
N-(2-hydroxypropyl) methacrylamide and HA copolymer–doxorubicin	Conjugate prepared by succinate/adipatedihydrazide linker. Cytotoxicity of *N*-(2-hydroxypropyl)methacrylamide-HA-DOX bioconjugate was higher against human breast cancer (HBL-100), ovarian cancer (SKOV-3), and colon cancer (HCT-116) cells. The conjugates show minimal cytotoxicity toward mouse fibroblasts NIH 3T3.	67
HA-paclitaxel	Conjugate prepared through succinate/adipatedihydrazide linker. In vitro cytotoxicity of the conjugate against the CD44 (+) human ovarian carcinoma cell lines SKOV-3ip. NMP-1 can be blocked by preincubation with a molar excess of free HA. HA-based prodrugs administered regionally have antitumor activity in vivo.	68
HA-Taxol	Conjugate prepared via succinate ester and adipic dihydrazide linker. Conjugates showed selective toxicity toward the breast, colon, and ovarian cancer cell lines that are known to overexpress HA receptors. No toxicity was observed in mouse fibroblasts at the same concentrations used with the cancer cells. Conjugate is also nontoxic under described experimental conditions. The targeted cytotoxicity of bioconjugates is receptor-mediated cellular uptake of the bioconjugate followed by hydrolytic release of Taxol.	69,70
HA-paclitaxel	Conjugate prepared via 4-hydroxybutanoic acid derivative. Much stronger inhibitory effect observed compared to paclitaxel against RT-4 and RT-112/84 bladder carcinoma cells. In vivo studies show conjugate is more effective than paclitaxel against RT-112/84, i.p. cell lines. Clinical trials have been reported for this conjugate.	43,71
HA-paclitaxel	Conjugate prepared via esterification. The conjugate exhibits pronounced cytotoxic effect for HA receptor overexpressing cancer cells.	72
HA-sodium butyrate	Conjugate prepared via esterification. Conjugate was evaluated on MCF7 cell line. Compared to sodium butyrate, an improvement of antiproliferative activity was observed.	73
HA-curcumin	Conjugate prepared via esterification. Cytotoxicity was improved through enhanced solubility and cell internalization ability of the conjugate against L929 fibroblasts.	74
HA-methotrexate	Conjugate prepared via peptide linkage has potential for treating osteoarthritis.	75,76

Figure 10. Design of gold nanoparticle-cyclodextrin-HA hybrid for delivery of hydrophobic chemotherapeutic agents.

Figure 11. HA-conjugated PEG amine was synthesized to load carbon dots for imaging.

Figure 12. Example of self-assembled particles made through HA (hydrophilic)-retinoic acid (lipophilic) conjugates. Used as drug carrier for hydrophobic drugs.

of PEI.[146] Xu et al. report hydroxypropyl cellulose modified with cationic poly((2-dimethyl amino)ethyl methacrylate) as gene vectors. This system efficiently delivers plasmid DNA and shows low cytotoxicity in HEK293 cells.[147]

The 3,4-hydroxyl group of saccharide is modified through acetal formation. Acetal bonds hydrolyze in a pH-dependent manner; various linkers and diamines have been explored for release of the SiRNA at desired pH (Figure 17).[148]

Colistin, a peptide antibiotic, was conjugated with succinylated dextran. Colistin−dextran conjugate prolongs the release of the drug without affecting the antimicrobial activity (Figure 18).[106]

A dual gene and chemotherapy delivery system has been reported. Poly(β-amino)ester (PBAE) for gene delivery and pullulan conjugated methotrexate as a chemotherapeutic delivery vehicle. The genetic material forms the inner core complexes with cationic carrier. The outer shell is made with pullulan-conjugated methotrexate (Figure 19).[149]

Polysaccharide conjugates have also been reported as a delivery vehicle for proteins.[150] Acid labile cholesteryl-modified pullulan conjugate was synthesized through Huisgen 1, 3-dipolar cycloaddition reaction. The nanoparticles swell under acidic pH to release the protein present in the inner core. At physiological pH, the formulation remains stable (Figure 20).

Carbon nanotubes (carboxyl single-walled) coated with modified chitosan enhances water solubility and biocompatibility. They are further coated with HA to target CD44 receptors for selectively target cancer cells. The resultant

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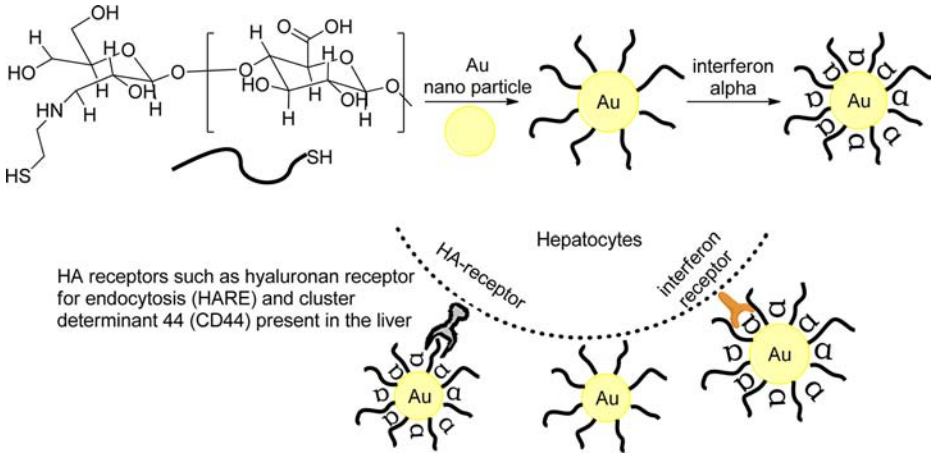

Figure 13. Cystamine-oxidized dextran−HA conjugate used to couple with gold nanoparticles. This nanohybrid was used as carrier for interferon. It was targeted to release in the liver through HA receptor recognition.

delivery system has been loaded with doxorubicin (Figure 21).[151]

3.1.6. Other Saccharide Based Conjugates. Heparin is an anticoagulant, but its conjugates have been explored as a drug (mainly cytotoxic) carrier. Heparin inhibits cancer cell adhesion, deactivates heparinase, and activates NK cells. It also interferes with growth factors such as bFGF and VEGF and prevents tumor angiogenesis and metastasis.[152] However, anticoagulant activity limits its use. Paclitaxel−heparin conjugate shows reduced anticoagulant activity and significant hemocompatibility.[8,153]

Cyclodextrin Conjugates. Cyclodextrins are cyclic oligosaccharides with hydrophilic exterior and hydrophobic interior. Cyclodextrin conjugated nanoformulation (CRLX101, previously called as IT-101) was synthesized by covalently linking camptothecin to β-cyclodextrin-PEG copolymer.[154] It is currently undergoing Phase II clinical trials for ovarian cancer.[44]

Preclinical and clinical data confirm improvement over problems associated with camptothecin (solubility, formulation, toxicity, and pharmacokinetics). Moreover, it enhances pharmacodynamics and efficacy of camptothecin.[155]

3.1.7. Insights as Carrier Molecule. Polysaccharide conjugates are mainly used for drug and gene delivery applications. We observed that amino (chitosan) and carboxylic acid (HA) containing polysaccharides are mostly used to make drug conjugates. They are easier to synthesize, and have reactive functional groups. They complement most of the drug molecules. Since chitosan is cationic, it has selective applications like antimicrobial coating, cell adhesion, and carrier of genetic or macromolecules. Ampiphilic chitosan-based polymers are easier to synthesize, so they are fabricated easily as self-assembled nanosystems.

HA is anionic. It binds to specific receptors (CD44), overexpressed in cancer cells. HA−receptor interactions are crucial in cell adhesion, growth, and migration. HA is one of the signaling molecules in cell motility, inflammation, wound healing, and cancer metastasis.[156] Therefore, HA conjugates are chosen for target specific drug release. Both Chitosan and HA, if directly conjugated, can be cleaved in a pH responsive manner. Linkers/spacers have also been used.

Another class of modified polysaccharide is the oxidized saccharide system. Polysaccharide, like pullulan, arabinogalactan, and dextran, can be easily oxidized to dialdehydes. These aldehydes can be conjugated with different amines, and they may be rendered cationic. Such systems are ideal as gene delivery vehicles with cationic inner core and hydrophilic outer surface.

Other polysaccharides like chondroitin are mainly used for targeting osteocytes. Heparin is mainly used as an anticancer drug carrier; the primary use of β-cyclodextrin is for encapsulation and pharmacokinetics enhancement. In general, the following were observed: aminopolysaccharide (chitosan) as nanosystems, HA as targeted and stimulus response, and oxidized polysaccharide in gene delivery.

3.2. Tissue Engineering and Support. Polysaccharide-based materials are biocompatible, and promote cell adhesion, proliferation, and differentiation. For decades they have been used with little or no fibrous encapsulation to create various tissue analogs.[157] Tissue engineering hydrogels are synthesized by cross-linking polysaccharides or polysaccharide hybrids. Detailing numerous examples of hydrogels for tissue engineering is not within the scope of this Review. A few examples of innovative scaffold synthesis by covalent cross-linking are listed.

Chitosan and chondroitin based materials have been used extensively for bone tissue engineering.[158] Chondroitin sulfate is one of the materials of choice for tissue scaffolds, as it is a part of cartilage. CS based hydrogels have been extensively explored.[159] CS helps in cartilage regeneration by promoting synthesis of proteoglycans.[160−164]

Alginate hydrogels have been covalently modified with RGD-containing peptides to control cell behavior and bone formation.[165,166] HA with aldehyde functional groups and conjugated to glycidyl methacrylate, 2-aminoethyl methacrylate, and peptides form stable cross-linked tissue scaffold networks. Hydrogel scaffolds are used for the controlled delivery of osteoinductive and angiogenic growth factors with tunable degradation properties.[167−171] Other polysaccharide materials have also been investigated for bone, cartilage, and in skin tissue engineering applications.[172]

Peptide−polysaccharide conjugates have been prepared by the ene thiol mediated conjugation method. Pentenoate was conjugated to HA, followed by reaction with thiol terminated peptides. Finally, a dithiol peptide was used to construct a hydrogel that may be used as a support for proteoblast for tissue engineering (Figure 22).[173]

Gels can also be formed by covalently cross-linking alginate with adipic hydrazide and PEG using standard carbodiimide chemistry.[174]

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Table 4. Examples of Dextran and Related Polysaccharides Used for Making Conjugates

polysaccharide-conjugate	description	ref
Carboxymethyl dextran-paclitaxel	Conjugate prepared via esterification. In vivo results show significant antitumor activity against colon 26, MX-1, LX-1, HT-29, cancer cell lines.	90,91
Carboxymethyl dextran-7-ethyl-10-aminopropyloxy-camptothecin (CPT)-(T-2513)	Conjugate prepared via amide linkage. Conjugate was ∼1000-fold less potent than T-2513 in human cancer cell lines (WiDr, SK-BR-3, HeLaS3). In vivo it was ∼10-fold superior to T-2513 against Walker-256 carcinoma. Significant antitumor activity was observed against human tumor xenografts.	92−95
Carboxymethyl dextran-exatecan (DX-8951, camptothecin analogue)	Conjugate prepared via amide linkage. Conjugate (DE-310) exhibited similar or greater antitumor activity than multiple administrations of DX-8951f against various human tumor xenografts and murine solid tumors.	96,97
Oxidized dextran-doxorubicin	Conjugate prepared via glycine linkage. The conjugate (AD-70) show enhanced activity, higher plasma concentration, lower acute toxicity, and low accumulation in heart of Walker256 rats.	47
Oxidized dextran-cytarabine	Conjugate prepared via amide linkage; it improved the life span of leukemic mice.	98
Oxidized dextran-methotrexate	Conjugate prepared via amide linkage. The cytotoxicity of the conjugate against H80 was equivalent to methotrexate (H80 brain tumors). In vivo studies indicate modest, but significant increases in survival after intracranial polymeric delivery of methotrexate or conjugate in rats.	99
Dextran-methotrexate	Conjugate prepared via esterification. Antiproliferative effects were 4- to 10-fold lower compared to free drug against A549, SW707, and P388 cell lines. In vivo studies indicate greater cytotoxicity in comparison with the parent drug, but anti-leukemic effect does not improve against P388 mouse leukemia model.	100,101
Carboxymethyldextran-methotrexate	Conjugate prepared via amide linkage and conjugate showed 2 times lower potency compared to free drug. In vivo studies indicate no significant difference in drug accumulation at the tumor site between the MMP-sensitive and the MMP-insensitive conjugates, which shows that the tumor targeting via EPR effect (HT-1080 bearing mice, overexpresses MMP, i.p.).	102−104
Dextran-rosuvastatin	In vitro release studies indicate that the formulations containing conjugates show a slow-release behavior. Among the four kinds of selected microparticles, the one with network structure show the most rapid dissolution. The spherical microparticles show the lowest release rate.	105
Dextrin-colistin	Colistin release from the conjugate was studied at physiological concentrations of amylase. Dextrin with ∼1 mol % succinylation had ∼80% drug release within 48 h. Compared to ∼33% from sodium colistin methane sulfonate. Conjugates exhibited comparable antimicrobial activity to sodium colistin methane sulfonate against a range of Gram-negative pathogens but with significantly reduced in vitro toxicity toward kidney cells.	106
Pullulan-doxorubicin	Conjugate nanoparticles were prepared as carriers for codelivery of pyrrolidine dithiocarbamate and doxorubicin. Both drugs exhibited controlled released from the nanoparticles. In vitro tests including cell viability and folate receptor-mediated endocytosis were conducted against A2780 and A2780/DOX cells. Compared to free doxorubicin, the nanoconjugates were effective but less potent. For A2780/DOX cells, they showed enhanced cellular uptake, increased targeting capacity, and cytotoxicity.	107
Methylcellulose/2-hydroxyethylcellulose-indomethacin	Methylcellulose/2-hydroxyethylcellulose-indomethacin conjugates prepared by esterification. Biodegradation in colonic fermentation depends on the cellulose ether and the amount of indomethacin conjugated. In vitro release experiments show that hydroxyethylcellulose-based conjugates with fewer indomethacin residues exhibit sustained drug release. Release was triggered in colonic fermentation, but not in simulated media of the stomach and small intestine.	108
Polyrotaxane-3′-azido-3′-deoxythymidine (AZT)	Conjugates of 3′-azido-3′-deoxythymidine (AZT) and tocopherol and their nanoparticles were prepared. In vitro anti-HIV activity for the conjugates and their nanoparticles were more potent against HIV-1 and HIV-2 strains than free AZT. Reduced toxicity was observed against uninfected MT-4 cells.	109

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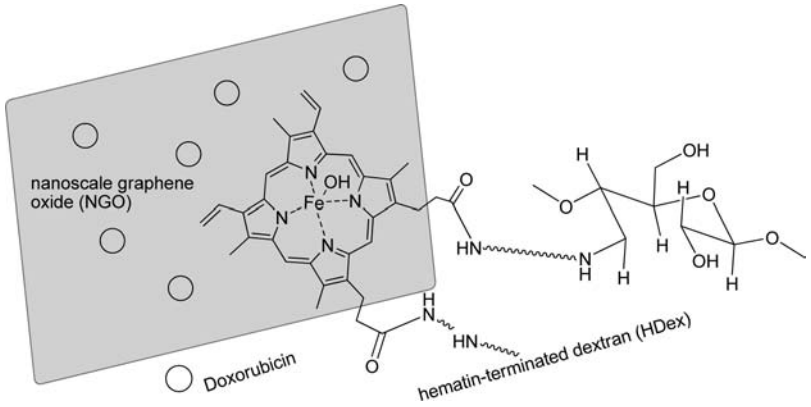

Figure 14. Oxidized dextran-hematin conjugate used to load graphene oxide-doxorubicin nanohybrid.

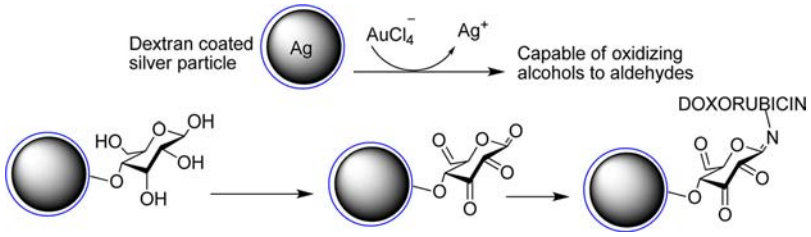

Figure 15. Ag−Au hollow nanospheres coated with dextran. The Au−Ag system oxidizes dextran, enabling doxorubicin to be conjugated.

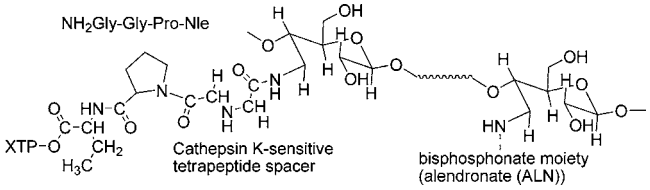

Figure 16. Oxidized pullulan used to conjugate PTX and alendronate, cleavable in a stimulus responsive manner.

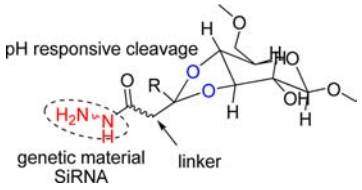

Figure 17. Diols were converted to acetals. The acetal bonds are hydrolyzed in a pH dependent manner.

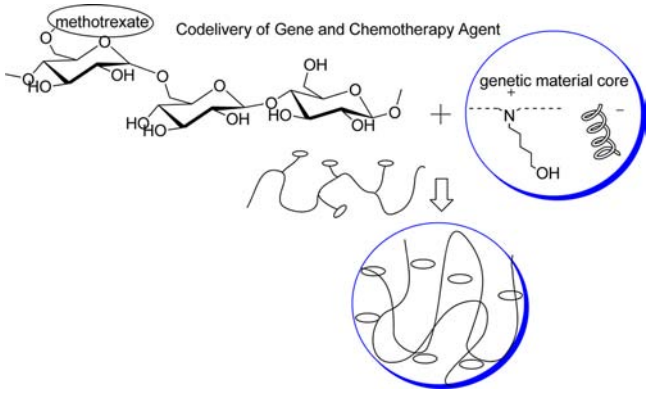

Figure 19. Polysaccharide-conjugate in codelivery of gene and chemotherapy using self-assembled nanoparticles.

3.3. Bioadhesives Based on Polysaccharide Conjugate. Tissue adhesives are used in wound closure and healing, drug delivery, implantation of medical devices, tissue engineering, and bone applications. Two types of substances are normally used to seal the wound: surface adhesives and sealants. They can be both natural and/or synthetic substances in the form of monomers, prepolymers, or linear polymers, which undergo polymerization or cross-linking reaction. Sealants typically contain two or more substances that undergo chemical reaction upon mixing, thereby forming an insoluble adhesive bond on the affected area. On the other hand, adhesives create covalent/secondary bonds with biological

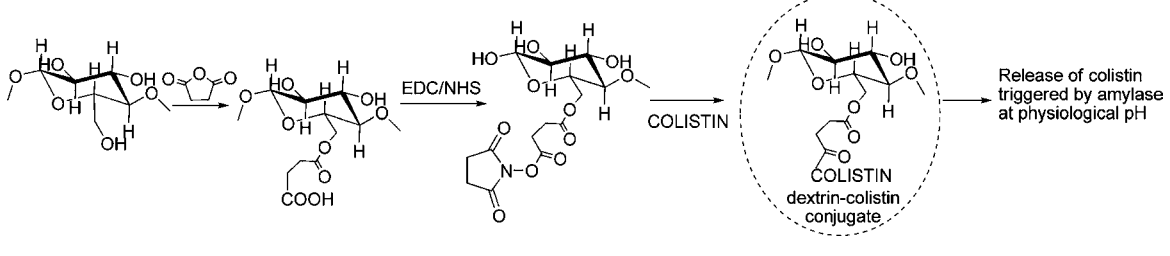

Figure 18. Amylase catalyzed release of colistin from dextran−colistin conjugate.

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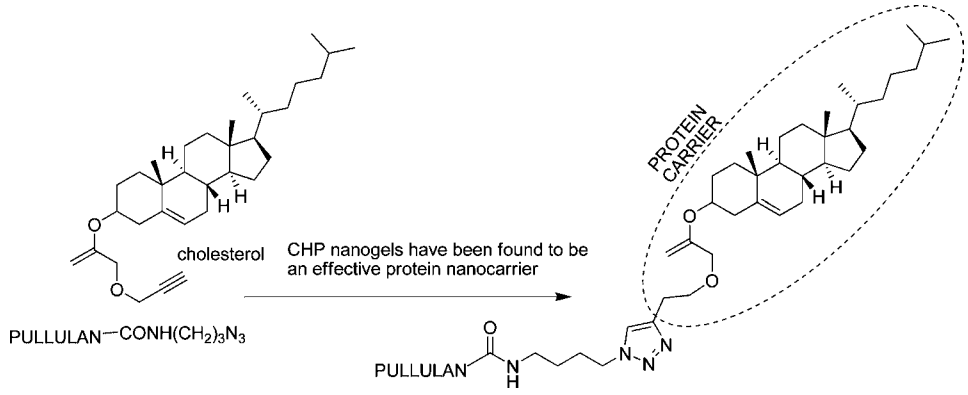

Figure 20. Cholesterol-pullulan (CHP) conjugated protein nanocarrier was developed through Huigen 1,3-dipolar cycloaddition reaction between azides and terminal alkynes.

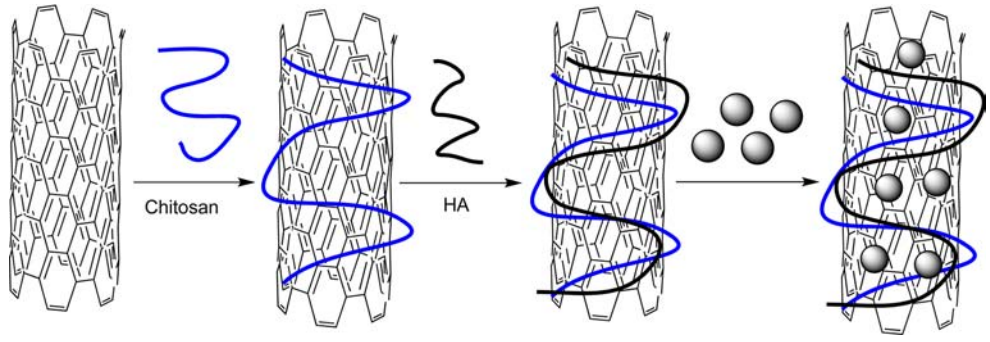

Figure 21. Single-walled carbon nanotubes modified with chitosan. Further, they were coated with HA as an anticancer drug carrier.

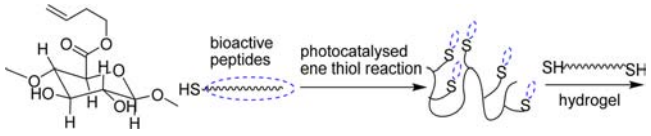

Figure 22. Design of HA based peptide carrier rendered through ene thiol reaction, followed by dithiol mediated cross-linking to form a hydrogel.

surfaces. The biodegradability and compatibility of polysaccharides enables them to be used as bioadhesives. Chitosan and oxidized polysaccharides have been widely utilized as bioadhesives.[175]

Polysaccharide based bioadhesives are chemically modified to have reactive groups in their polymeric framework. These reactive groups are designed to react under physiological conditions or through external stimulus to cross-link instanta-

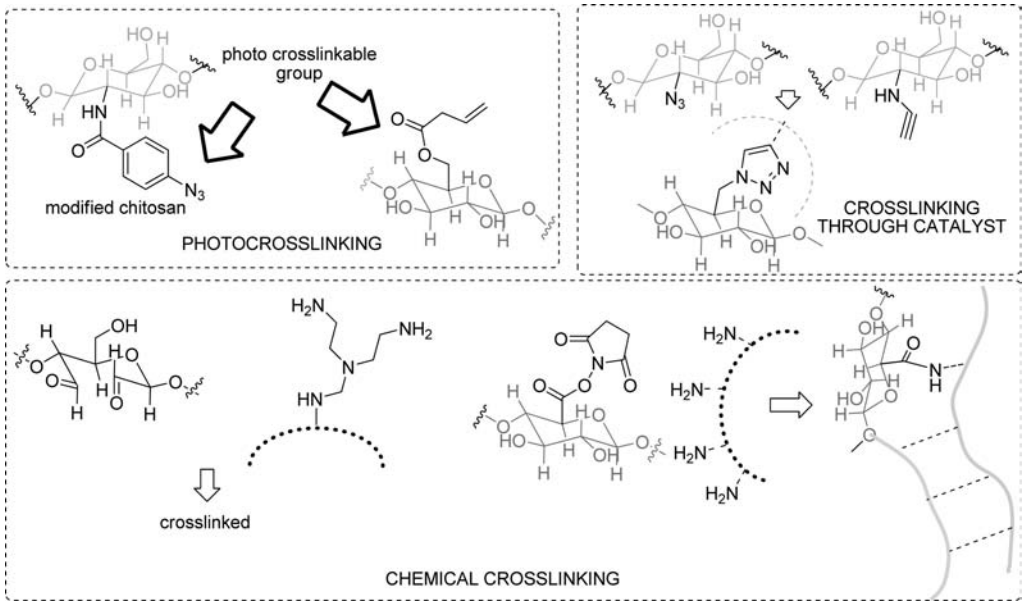

Figure 23. Different modes of cross-linking observed for bioadhesives using polysaccharide-conjugates.

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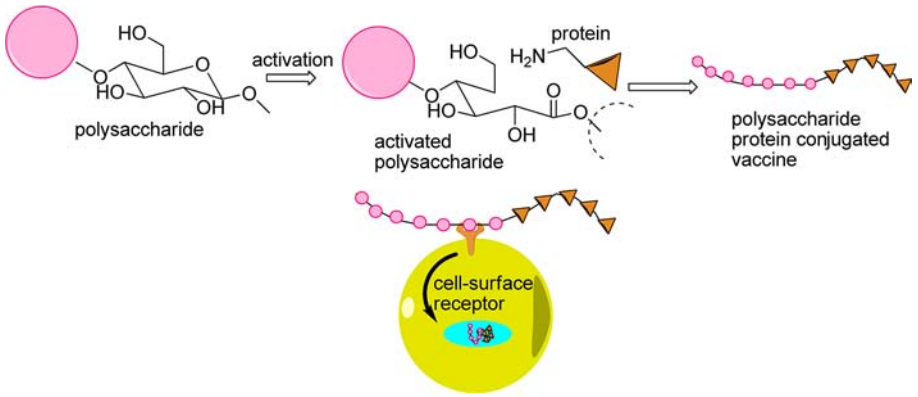

Figure 24. Example of polysaccharide conjugate vaccine. Capsular polysaccharide induces production of antibodies when conjugated to proteins.

neously.[176] They may be photo-cross-linkable; mostly azido[177] or acrylate[159] conjugate have been reported as photo-cross-linkable moiety.[178]

Other bioadhesives involve chemical cross-linking. This normally involves separate packages of two mutually reactive materials, mixed just before they are applied. They form chemical conjugates only when mixed. Oxidized dextran[179−181] or HA/chondroitin[182,183] activated esters (NHS esters) with amines are commonly used.[184] An overview on the mechanism of polysaccharide-conjugate bioadhesives is given in Figure 23.

3.4. Conjugated Vaccine. Rappuoli and Gregorio describe protein−polysaccharide conjugates as one of the miracles of immunology. The bacterial capsular polysaccharide as such is non-immunogenic and does not induce the production of antibodies. However, it becomes a powerful immunogen when conjugated to a protein.[185] Conjugate vaccines have been extremely successful over the years. In most cases, they eliminate diseases against which they are targeted. Polysaccharide protein conjugates have been synthesized by standard glycopeptide conjugation methods.[186] The chemical modification method depends on the structure of the saccharide. A brief description of conjugated vaccine is given in Figure 24.

■ CONCLUSIONS

Polysaccharides have been used as carrier molecules designed to deliver the appropriate package. Release may be modified by fine-tuning the chemistry and may be made stimulus responsive.[187−189] These materials have been useful as drug delivery vehicles, nanohybrids, magnetic nanoparticles, photodynamic therapy, gene and macromolecule delivery, and imaging.

Making the polysaccharide-conjugate system uniform, reproducible, and scalable is challenging. The polysaccharide structure (MW, functional groups, purity) varies from batch to batch and source to source. Therefore, it is difficult to fabricate a reproducible system with appropriate properties. The next challenge is more regulatory than technical. Are polysaccharide-conjugates simple carriers or new chemical entities? In many cases, we observed significant changes in pharmacodynamics (like enhanced efficacy, safety, and dose reduction) of the conjugate. Regulatory authorities treat polymer−drug conjugates as new chemical entities. Kim et al. state, "Polymer conjugation creates new chemical drugs, which need additional FDA approval although the used drug is already approved".[190]

Polysaccharide-conjugates have been used as an active moiety in the case of bioadhesives and vaccines. Bioadhesives use chemically modified polysaccharides that react instantaneously on specific stimuli. In vaccines, the conjugated polysaccharide is used as an antigen for recognition.

We witnessed significant use of polysaccharide conjugates due to demand for newer and smarter materials. The advent of "click chemistry" made a positive impact on the chemistry of conjugation. Most of the utilized chemistry is validated and has been used for many years. Innovations have been observed in the use of linkers, and the use of stimulus sensitive groups has increased. Overall, polysaccharide-conjugates provide a safe, biodegradable, and tunable option for making newer and smarter materials for biomedical use.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: avid@ekmd.huji.ac.il. Tel: 972-2-6757573. Fax: 972-2-6757076.

Author Contributions

[+]Arijit Basu and Konda Reddy Kunduru contributed equally.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AG, arabinogalactan; AmB, amphotericin B; AZT, 3′-azido-3′-deoxythymidine; CDI, carbonyldiimidazole; CPT, camptothecin; DCC, dicyclohexylcarbodimide; DOX, doxorubicin; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; EPR, enhanced permeability and retention; GAGs, glycosaminoglycans; HA, hyaluronic acid; HOBT, hydroxybenzotriazole; MTX, methotrexate; NHS, *n*-hydroxysuccinimide; PEG, polyethylene glycol; PEI, polyethylenimine; PTX, paclitaxel; Stx, Shiga toxin; TEMPO, 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl; TfN3, trifluoromethanesulfonylazide; β-CD, β-cyclodextrin

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