EXPERT OPINION

Therapeutic potential of carbohydrate-based polymeric and nanoparticle systems

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Introduction: Carbohydrates are key participants in many biological processes including reproduction, inflammation, signal transmission and infection. Their biocompatibility and ability to be recognized by cell-surface receptors illustrate their potential therapeutic applications. αYet, they are not ideal candidates because they are complex and tedious to synthesize. However, recent advances in the field of polymer science and nanotechnology have led to the design of biologically relevant carbohydrate mimics for therapeutic uses. This review focuses mainly on the therapeutic potential of glycopolymers and glyconanoparticles (GNPs).

Areas covered: The significance of engineered glycopolymers and GNPs as nanomedicine is highlighted in areas such as targeted drug delivery, gene therapy, signal transduction, vaccine development, protein stabilization and anti-adhesion therapy.

Expert opinion: Major effort should be focused towards the design and synthesis of more complex and biologically relevant carbohydrate mimics in order to have a better understanding of the carbohydrate–carbohydrate and carbohydrate–protein interactions. The full therapeutic potential of these carbohydrate-based polymeric and nanoparticles systems can be achieved once the pivotal participation of the carbohydrates in biological systems is clarified.

Keywords: drug and gene delivery, glyconanoparticles, glycopolymers, signal transduction, therapeutics, vaccine

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1. Introduction

Carbohydrates play a key role in a myriad of biological processes [1-3]. Pivotal participation of the carbohydrates in several cellular processes including cell growth regulation, differentiation, adhesion, cancer cell metastasis, cellular trafficking, inflammation by bacteria and viruses and immune response makes them very useful in the development of synthetic carbohydrate-based systems for therapeutic needs. With recent advances in polymerization techniques and glycoscience, the design and synthesis of carbohydrate-based materials, particularly, glycopolymers and glyconanoparticles (GNPs) have become the subject of intense research in the last few decades [3]. Synthetic glycopolymers can function as mimics of naturally occurring polysaccharides, hence displaying, anticoagulant, anti-inflammatory and anti-tumor properties. With the advent of nanotechnology, GNPs having sugar residues on the surface have gained a lot of attention; ranging from studies of carbohydrate–protein and carbohydrate–carbohydrate interactions as well as in the fields of targeted drug and gene delivery and vaccine development. The emerging field of so-called *glyco-nanotechnology* has started to show some promises for future clinical applications. This review highlights the importance of carbohydrate-based

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Article highlights.

- Glycopolymers and glyconanoparticles (GNPs) as valuable carbohydrate mimics with great therapeutic potentials.
- Glycopolymers and GNPs as non-viral gene delivery agents have gained substantial importance.
- The emerging roles of glycopolymers in signal transduction and protein stabilization.
- Initial studies have shown that GNPs are promising platforms for future vaccine development.
- The biological evaluation of these carbohydrate-based polymeric and nanoparticles systems need to be addressed in greater details and mechanistic studies need to be carried out both *in vitro* and *in vivo* to understand the pivotal participation of the carbohydrates.

This box summarizes key points contained in the article.

polymeric and nanoparticulate systems towards the development of highly promising therapeutic drugs.

2. Glycopolymers

Glycopolymers have played significant roles in the study of carbohydrate-based biological processes with therapeutic potentials [3-6]. The saccharide units in glycopolymers are capable of molecular recognition and interact with specific carbohydrate receptors. Hence, glycopolymers, with their high biocompatibility and multivalency, are interesting materials for biomimetic models of glycoconjugates such as glycoproteins, polysaccharides, mucins, glycans and viral particles [7,8]. Studies in the past decades have revealed that parameters such as the size and shape of glycopolymers as well as the type of ligand and ligand density need to be considered in order to achieve the desired biological functions. The advent of powerful polymerization techniques and recent advances in controlled polymerization reactions provided a means to easily access diverse glycopolymer topologies. Commonly employed radical polymerization reactions are atom-transfer radical polymerization and reversible addition-fragmentation chain-transfer polymerization (RAFT), whereas a nonradical approach involves the ring-opening metathesis polymerization (ROMP) of glycomonomers [9-12]. These robust-tailored glycopolymers have found emerging applications in the biomedical fields such as drug and gene delivery carriers and vaccine development and in the signal transduction processes.

2.1 Glycopolymers as therapeutics and drug-delivery systems

The typical features of glycopolymers such as biocompatibility, target specificity, solubility and ability to facilitate receptor-mediated uptake through cell-surface lectins make them attractive materials as carriers for therapeutics or as therapeutics themselves. As such, glycopolymers have been explored in applications such as macromolecular drugs [13-18] and drug-delivery systems [19-22] for targeting diseases such as influenza, cancers and Alzheimer's disease (AD). In 1990, Bovin and co-workers disclosed the first example of a glycopolymeric influenza hemagglutinin inhibitor derived from polymeric sialosides **1** (**Figure 1**) of varying carbohydrate densities [23]. Monovalent sialosides, β-sialosides or polymers with low quantities of α-sialosides showed little or no inhibition, whereas a maximum level of inhibition was observed at 20% when the sialoside density was varied from 10 to 30%. Whitesides and co-workers pioneered this area by thoroughly studying the inhibition activity of polymeric sialosides **2** and **3** (**Figure 1**) [13,24-28]. After several studies, it was shown that incorporation of sialic acid (SA) into the side chains of polyacrylamide strongly enhanced the ability to inhibit hemagglutination mediated by influenza A X-31. These glycopolymers were prepared using poly[*N*-(acryoyloxy)succinimide], a polymer preactivated by incorporation of active ester groups. The most effective inhibitor had 10% benzylamine and 20% SA on the polyacrylamide backbone [25]. Further studies showed that combining C-sialoside-acrylamide copolymers and low–molecular-weight monomeric neuraminidase inhibitors displayed greater inhibition of hemagglutination [29]. However, the mechanism of this synergistic effect is still unclear. Although polyacrylamides of high molecular weight are more effective inhibitors of hemagglutination, they are, however, more tedious to synthesize, toxic and harder to clear by the kidney [7]. Due to these concerns, glycodendrimers have emerged as attractive targets [30,31]. A *C*-linked aromatic glycopolymer **4** (**Figure 1**) was prepared by enzymatic polymerization to act as inhibitors of neuraminidases, which are also targets for influenza treatment [32]. Recently, a new class of linear glycopolymers **5** (**Figure 1**) containing thiosialoside residues was synthesized by radical copolymerization, and preliminary studies indicated that these glycopolymers displayed potent inhibitory activity against the neuraminidases [33].

The treatment of the HIV infection continues to be a challenge, and glycopolymers have been investigated as therapeutics in this area. Sulfated maltoheptaose-derived methacrylate glycopolymers were synthesized by radical polymerization technique followed by deacetylation and sulfation [15]. The anti-HIV activity of these maltoheptaose methacrylate glycopolymers was found to increase with increase in degree of sulfation. It was also observed that the distance between branched maltoheptaose units in the polymethacrylate main chain was crucial for high anti-HIV activities. Furthermore, these sulfated glycopolymers have low cytotoxicity and as such are promising therapeutic materials.

AD is a progressive disease, the most common form of which is dementia for which the cause and prevention are not well understood. Research on this area has mainly focused towards the search for an effective inhibitor of AD. AD is characterized neuropathologically by extracellular deposition of amyloid senile plaques, which are primarily composed of fibrils of the amyloid-β (Aβ) peptide, a small peptide

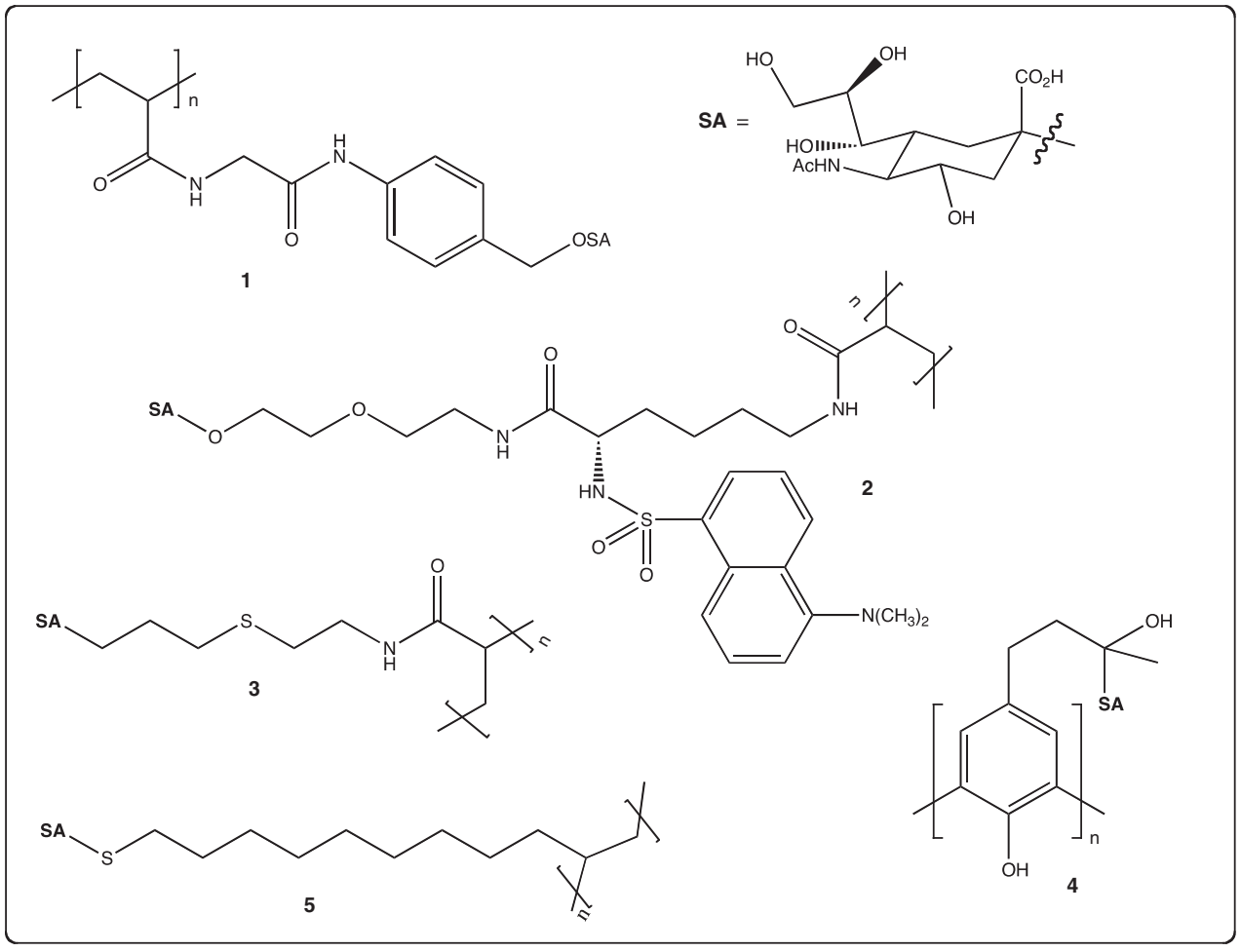

Figure 1. Chemical structures of glycopolymeric sialosides designed for the treatment of influenza.

composed of 39–43 amino acids [34]. Thus, several investigations look at the development of medicinal compounds that inhibits Aβ aggregation. In the glycopolymer field, sulfated glucosamine- [35] and tetrahalose-based [36] glycopolymers were synthesized and investigated as novel inhibitors of Aβ aggregation. The inhibitory effect was dependent on the type of saccharide, sulfate group and amphiphilicity. Using a cyanoxyl-mediated free-radical polymerization technique, glycopolymers bearing sulfated lactose groups that are capable of mimicking heparin were synthesized and their anticoagulant activities were investigated [37-39]. Heparin, a sulfated polysaccharide, is currently used as an anticoagulant drug because it can bind and inhibit thrombin. The sulfated glycopolymer displayed anticoagulant activity but was not as active as heparin.

The glycopolymer was also found to promote a bFGF-specific proliferative cell response. Overall, the studies showed that sulfated glycopolymers could potentially be used as therapeutic agents. In a recent study, glycopolymer-based dithiocarbamates (DTC) conjugates were prepared by RAFT polymerization and subsequently modified with gold(I) phosphine [40]. The resulting polymer-DTC derivatives and their gold compounds were tested for their *in vitro* toxicity in both normal and cancer cell lines. It was shown that the cationic glycopolymer DTC derivatives and their gold conjugates displayed higher accumulation as well as cytotoxicity to cancer cells under hypoxic conditions in comparison to the normoxic ones. Overall, the polymeric gold glycoconjugate has the potential to serve as an anticancer agent for the treatment of cancer, without significantly affecting normal tissues.

Other than being used as therapeutics, glycopolymers have also been explored as potential drug-delivery vehicles. The need to maximize therapeutic activity while minimizing negative side effects has fueled the search for targeted and controlled polymeric drug-delivery systems [41]. Synthetic glycopolymers, with the presentation of multiple pendant carbohydrates, represent an interesting platform for the design of targeted polymeric drug-delivery systems because carbohydrate moieties play an important role in molecular recognition processes [3]. Generally, the drug is incorporated in a

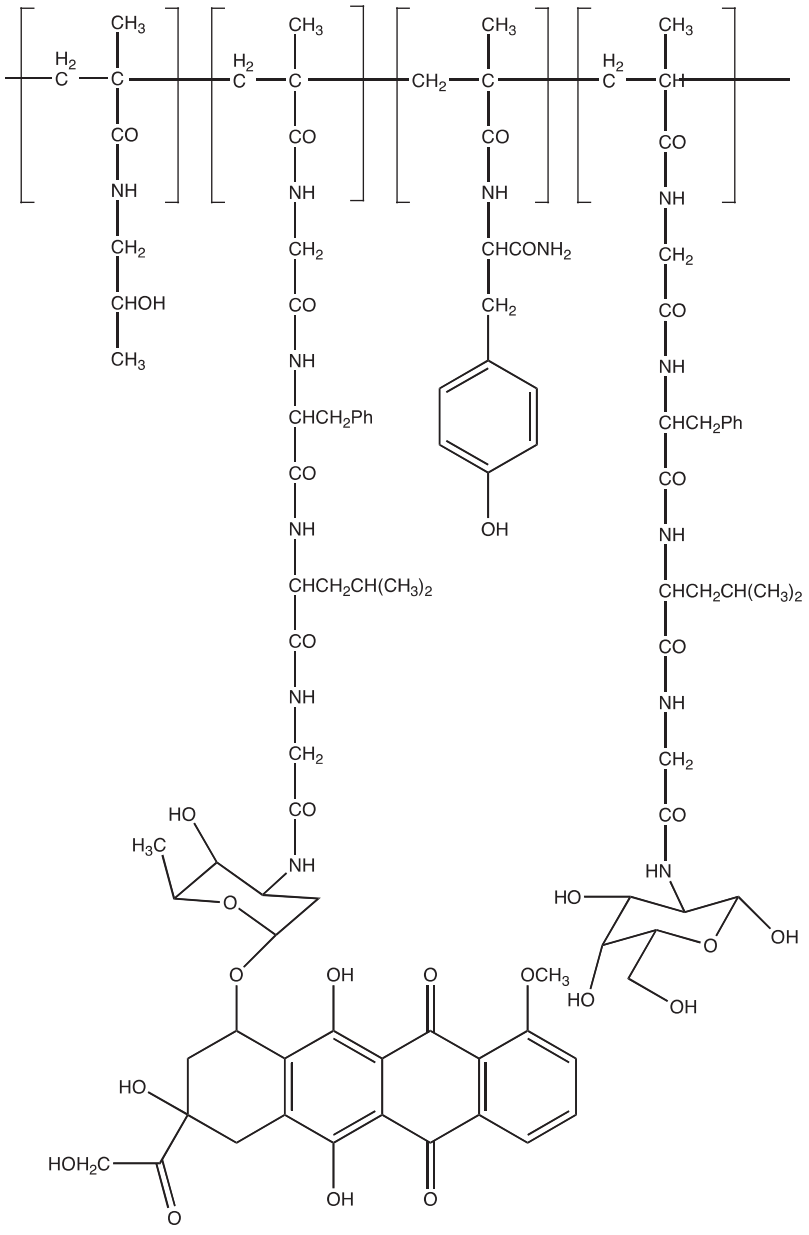

Figure 2. Chemical structure of *N*-(2-Hydroxypropyl)methacrylamide copolymer-doxorubicin-galactosamine conjugate (PK2) for hepatoma.

polymer matrix and is released in the human body in a controlled manner. Some typical requirements for glycopolymeric drug-delivery agents include biocompatibility, water solubility, target specificity, stability, controlled release, sufficient rate of biodegradation and easy clearance from the body. A remarkable polymeric drug known as PK2 (FCE28069), an *N*-(2-Hydroxypropyl)methacrylamide copolymer-doxorubicin (DOX)-galactosamine conjugate (**Figure 2**), has been assessed in a Phase I clinical trial for hepatoma [42-44]. PK2 was designed based on a similar structure of the first cancer drug conjugated with a polymer (PK1) to be tested clinically [45]. PK2 incorporates an additional targeting ligand, namely galactosamine, which specifically targets liver tissues. This is the only targeted polymer conjugate containing galactosamine moiety to enter clinical trials. Preclinical studies using a rat model have shown that PK2 displays a ~ fivefold reduction in cardiotoxicity when compared with free DOX following administration at various doses [46]. During the Phase I clinical study, patients having primary or secondary solid hepatic tumors were administered with PK2. HPLC and ^{123}I-based imaging indicated the rapid clearance of PK2 from the plasma and ~ 30% of the administered drug accumulated in the liver at 24 h [42]. PK2 was retained in the liver and mostly concentrated in normal liver tissue rather than in the tumors. This

indicates that the galactosamine-targeted polymer is mainly delivered to normal regions of the liver, which was rationalized by an increased asialoglycoprotein receptor (ASGP-R) expression in the normal liver and the phagocytosis by Kupffer cells with 'galactose particle' receptor expression [47]. Although the receptor of the galactosamine ligand is expressed on healthy hepatocytes, the drug concentration in tumor tissue was still significantly higher than it would have been with the administration of free DOX alone.

Another application of polymers as drug-delivery vehicles is found in pH-sensitive glycopolymers. These pH-sensitive glycopolymers were prepared by free-radical polymerization of methacrylic acid and 6-acryloyl-glucose-1,2,3,4-tetraacetate, using 1,6-hexandiol diacrylate and 1,6-hexandiol propoxylate diacrylate as crosslinking agents. These glycopolymers were designed for colon-specific drug delivery [48]. A model drug, olsalazine [3,3′-azobis (6-hydroxy benzoic acid)] as an azo derivative of 5-aminosalicylic acid, was entrapped in these hydrogels. The drug-release profiles (at pH 1 and pH 7.4 at 37°C) showed that the amount of drug released is dependent on the degree of swelling. The pH-dependent swelling and drug-release profile of these glycopolymers are potentially useful for the development of oral drug-delivery carriers.

Recently, Hedrick and co-workers reported the synthesis of amphiphilic glycopolymers via a metal-free organocatalyzed ring-opening polymerization of functionalized cyclic carbonates for targeted drug delivery [49]. The resulting glycopolymers self-assembled into micelles in aqueous solutions with a mean size < 100 nm and narrow size distribution. The micelles were loaded with DOX, and it was shown that the galactose-containing micelles could deliver DOX more efficiently into ASGP-R-positive Hep G2 cells than in ASGP-R-negative HEK293 cells. Furthermore, the enhanced uptake of DOX-loaded galactose-containing micelles by Hep G2 cells greatly increases cytotoxicity of DOX as compared with HEK293. This new family of amphiphilic block glycopolymers is a promising carrier for targeted drug delivery to liver tissues/cells. Wang *et al.* synthesized glycohydrogels for the treatment of norovirus infection, which is an important cause of acute gastroenteritis [50]. The hydrogel resulting from the polymerization of 1-acrylamido-3,6-dioxa-8-octyl-*O*-(α-L-fucopyranosyl)-(12)-*O*-(α-D-galactopyranosyl)-(13)-β-D-galactoside and acrylamide was effective in absorbing the virus with high affinity. The entrapped virus in the glycolhydrogel would be rendered harmless as it is cleared from the body. This approach could be potentially useful in the worldwide fight against viral gastroenteritis outbreaks. Macrophages are promising targets for carbohydrate-based therapeutics because they express carbohydrate-binding receptors, which internalize bound material via receptor-mediated endocytosis [51]. As such, well-defined glycopolymers were synthesized by RAFT polymerization and were fluorescently labeled in order to determine macrophage-specific targeting both *in vitro* and *in vivo*.

Mannose- and *N*-acetylglucosamine-based glycopolymers were shown to specifically target mouse bone marrow-derived macrophages *in vitro* in a dose-dependent manner as compared with a galactose-containing glycopolymer (30- and 19-fold higher uptake, respectively). Furthermore, mannose glycopolymer displayed enhanced uptake in M2-polarized macrophages, which was retained *in vivo*, as alveolar macrophages showed sixfold higher internalization of mannose glycopolymer (vs galactose) following intratracheal administration in mice [52]. The glycopolymers also possess a reactive pyridyl disulphide functionality that could potentially be explored for bioconjugation with other biomolecules for the design of targeted drug-delivery systems. Using a polymerization-induced self-assembly method, a range of novel glycopolymer-decorated block copolymer nano-objects was recently prepared directly in concentrated aqueous solution [53]. The resulting nanospheres, worm-like micelles or vesicles were found to interact strongly *in vitro* with galactose-specific lectins. Furthermore, the galactosylated vesicles were highly biocompatible and thus can allow intracellular delivery of an encapsulated molecular cargo. These initial results could eventually lead to new platforms for targeted drug delivery.

2.2 Glycopolymers as nonviral gene delivery agents

Gene delivery is a type of therapy for treating and controlling diseases in which a viral or non-viral vector is used to transport foreign genes into somatic cells to increment defective genes there or provide supplementary biological functions [54-56]. Gene therapy continues to emerge as a powerful strategy for the treatment of genetic diseases, cancers, cardiovascular diseases and infectious diseases. With the recent advancements in the field of nanotechnology, polymer-mediated gene delivery systems have recently developed as an alternative to viral-based delivery systems because polymers induce less immune and inflammatory responses, easy scale-up procedures and possess a larger nucleic acid storage capacity [57]. Carbohydrate-based polymeric vehicles have significantly contributed to progress in the field of non-viral DNA delivery with some successes in preclinical models, both *in vitro* and *in vivo*. Typical polymeric scaffolds based on the natural polysaccharides are chitosan, dextran, hyaluronan, pullulan and schizophyllan with each having not only unique properties but also certain limitations [58]. With the advent of sophisticated polymerization techniques, in particular, controlled living radical polymerization and ROMP, well-defined cationic glycopolymers were easily synthesized and investigated as potential non-viral gene delivery vehicles.

Glycopolymers are hydrophilic and biocompatible molecules that are bioactive towards specific biomolecules, depending on the nature of the carbohydrate moieties. Cationic glycopolymers have the ability to complex with anionic DNA via electrostatic interactions and form viral-like nanoparticles (termed polyplexes). Several factors such as molecular architecture, molecular weight, surface charge,

charge density and hydrophilicity highly impact the gene transfection efficiency of cationic polymers. As such, research in the past few years has focused on the optimization of cationic glycopolymers in order to improve their gene transfection efficiency [57,59]. Reineke's laboratory led the design of a novel class of glycopolymer-based delivery vehicles known as poly(glycoamidoamine)s (PGAAs) [60]. These glycopolymers were prepared by polymerizing the methyl ester or lactone derivatives of various carbohydrates (D-glucarate, meso-galactarate, D-mannarate and L-tartarate) with a series of oligoethyleneamine monomers containing 1 – 4 ethyleneamines. They were able to condense DNA into polyplexes [61]. The effect of hydroxyl number, stereochemistry and amine content on the biological activity was extensively studied. Both the number of hydroxyl groups and amine units significantly affected the transfection efficiencies of the polyplexes [62]. As expected, an increase in amine content increases the gene expression level regardless of the type of carbohydrate unit in the glycopolymer segment. The stereochemistry of the hydroxyl groups also affects the transfection efficiency by altering the stability of the polyplex structures [63]. Further studies probed many aspects of polymer structure to find the structural elements that lead to efficient delivery [64-67]. Recently, the same group disclosed for the first time the use of nontoxic and degradable PGAAs *in vivo* and showed their unparalleled, virus-like efficacy for delivering oligonucleotides (ODN) to the nucleus of primary cells *in vitro* and *in vivo* [68]. These glycopolymers bound with ODN decoys and formed stable polyplexes that have the ability to achieve therapeutic potency of ODN decoys directed against NF-κB into primary cardiomyocytes and the *in vivo* murine heart. A small library of cationic glycopolymers (linear trehalose-oligoethyleneamine 'click' copolymers) was synthesized and the delivery of plasmid DNA (pDNA) to human dermal fibroblasts and rat mesenchymal stem cells were examined [69]. By varying the number of secondary amines within the polymer repeat unit, the endgroup functionalities and the molecular weight, the biological efficacy of these delivery vehicles was compared with Lipofectamine 2000, JetPEI, and Glycofect controls. The trehalose-oligoethyleneamine glycopolymers displayed high pDNA binding affinity and gene efficiency to primary progenitor cell types in the presence of serum, antibiotics and growth factors.

Narain and co-workers synthesized a library of well-defined cationic glycopolymers via RAFT polymerization without the use of protecting group chemistry [70]. A number of parameters such as molecular architectures (*block* vs *random*), molecular weights and monomer ratios (carbohydrate to cationic segment) were varied and studied in detail for their cytotoxicities and ability to act as non-viral gene delivery agents. The results indicated that linear statistical copolymers with high degree of polymerization were the ideal vectors for gene delivery purposes. They display lower toxicity and higher gene expression in the presence and absence of serum, as compared with the corresponding diblock copolymers. However, the efficacy and stability of these statistical copolymers *in vivo* have not yet been investigated. Cationic hyperbranched glycopolymers with varying molecular weights and compositions were also synthesized by RAFT polymerization for DNA condensation and gene expression *in vitro* [71]. Hyperbranched glycopolymers (30 kDa or lower) with high galactose content showed improved gene expression at varying polymer/plasmid ratios in the presence and absence of serum. Cellular uptake and gene expression were studied in two different cell lines (Hep G2 and HEK293 cells) in the presence of lectins and was found that polyplexes-lectin conjugates displayed enhanced cellular uptake *in vitro*; however, their gene expression was cell-line and lectin-type dependent. The role of lectins on gene expression in different cell lines warrants further investigations.

Branched poly(ethylenimine) (bPEI) is a commercially available cationic polymer and is well-studied due to its superior gene transfection efficacies. It is also often used as a standard to compare gene delivery efficacies of other cationic polymers. However, its toxicity has been a major drawback for clinical applications. The formulation of ternary complexes (coating anionic polymers on cationic complexes) is a good alternative approach for reducing its toxicity. To this end, pH-sensitive glycopolymers of linear and hyperbranched architecture were synthesized by RAFT and the resulting anionic glycopolymers were complexed with bPEI at varying weight by weight (w/w) ratios to reduce the toxicity of PEI *in vitro* [72]. The study showed that the cellular uptake and gene expression of PEI polyplexes were greatly improved in both Hep G2 and HEK 293T cells, in the presence of the anionic glycopolymers. This was directly related to the interactions of the anionic glycopolymers with Hep G2 and HEK 293T cells. Future *in vivo* studies will look at the role of pH-sensitive glycopolymers in improving the gene expression of PEI polyplexes.

Although cationic glycopolymers were shown to serve as excellent gene vectors, the interactions of these carriers with blood cells and plasma components are not well explored. Recently, the role of carbohydrate-based carriers for blood biocompatibility was studied for the first time [73]. Hyperbranched glycopolymers with varying molecular weights were synthesized by RAFT, and their biological evaluation using blood coagulation assays, hemolysis assays, platelet and complement activation analysis and *in vitro* cytotoxicity assays confirm their biocompatibility. Hyperbranched glycopolymers (15 – 38 kDa) were hemocompatible *in vitro*, and their cytotoxicity was found to be dependent on the type of cell line used and the polymer concentration.

2.3 Glycopolymers in vaccine development

The tremendous progress in the fields of glycochemistry, glycobiology, glycomedicine and immunology in the last decades has seen an enormous acceleration of the pace of vaccine development. Vaccination is considered to be the most effective method of preventing, controlling and eradicating many

diseases that affect human population. Carbohydrate-based vaccine design has received great attention, and several promising carbohydrate-based vaccine candidates including antibacterial, anticancer, antiparasite and antivirus have been prepared in recent years [74]. However, the non-immunogenicity of carbohydrates remains one of the main challenges in the development of carbohydrate-based vaccines. Although many multivalent scaffolds have been exploited in carbohydrate-based vaccine development, only a few reports disclosed the role of synthetic glycopolymers in the design of a vaccine [3]. For instance, by engineering the surface of biodegradable poly(D,L-lactic-co-glycolic acid) microparticles with a glycopolymer, the delivery of synthetic leishmania antigen-promoting T-cell-mediated specific immune responses was shown to be promising [75]. However, although leishmaniasis is transmitted by sandflies and affects an estimated of 12 million people worldwide with more than a million of new cases reported annually, studies in an animal model remain challenging.

Shiga toxin (Stx), which is produced by *Shigella dysenteriae* and Shiga toxigenic *Escherichia coli* (STEC), leads to serious gastrointestinal diseases in humans (diarrhea, hemorrhagic colitis and life-threatening systemic sequelae such as neurological damage and hemolytic-uremic syndrome [HUS]) [76]. Research has been mainly focused towards the development of an effective Stx neutralizer that specifically binds to and inhibits Stx in the circulation. A polymer-based heterobifunctional inhibitor, termed as (*S*)-PolyBait, was designed in such a way that ligands were precisely arranged onto the scaffold to allow optimal binding of the toxins and neutralization of immune proteins [77]. (*S*)-PolyBait was found to promote formation of a ternary stable complex with Stx 1 and human serum amyloid P component (HuSAP), and the complexes were directed to the reticular endothelial system for disposal. *In-vivo* studies indicated that (*S*)-PolyBait protected mice expressing HuSAP from intoxication by Stx1. Further studies looked at the impact of the nature, length of linker (short, long and extra-long), size of the polymeric backbone (different molecular weights) and pendant ligand type (fused or separate) on the activities of the heterobifunctional ligands [78]. Overall, the studies showed that inhibition does not depend on molecular weight and the polymer density of the glycans. However, it was slightly influenced by the structure of the pendant ligand and the length of the spacer.

Recently, a glycopolymer consisting of linear polyacrylamide bearing pendant oligosaccharides (about 33 trisaccharide haptens) was prepared to act as a carrier for oligosaccharide haptens [79]. The glycopolymer was further modified in order to introduce a reactive azide endgroup for subsequent conjugation to a propargylated immunogenic carrier protein, chicken serum albumin, via the *Azide-Alkyne* Huisgen cycloaddition process. The resulting glycopolymer-protein conjugate was shown to be highly immunogenic in mice and could induce a more robust immune response than haptenated tetanus toxoid conjugate vaccine. Although this initial study is promising, a detailed investigation still remains to elucidate whether the improved immunogenicity of the glycopolymer vaccine stems from the higher payload of sugar in the construct or the better presentation of haptens onto the long polymeric backbone. Furthermore, the replacement of the polyacrylamide backbone with other polymeric systems needs to be taken into consideration because the incidence of high levels of acrylamide-specific antibodies in human subjects has been observed in patients with severe fibromyalgia [80].

2.4 Glycopolymers in carbohydrate-mediated signal transduction

Glycans play a crucial role in biological processes such as reproduction, cell–cell interactions and infection. Elucidating the intrinsic molecular features involved in glycan recognition and function remains a challenging area; however, with rapid advancement in chemical synthesis, isolation and characterization techniques, a number of glycan derivatives or mimics can be synthesized. Glycopolymers are such glycan mimics that can be easily accessed by polymerization techniques whereby their structural features can be tuned accordingly for optimum activity. The ability of glycopolymers to cluster carbohydrate receptors, hence activating signaling, has led to their investigations as possible tools to understand the molecular mechanisms underlying carbohydrate-mediated signal transduction [4]. Signal transduction plays a vital role in activating cellular functions, cell differentiation and cell proliferation. The structural features of the glycopolymers can influence protein recognition and hence signal transduction. Several studies have looked at how to maximize protein recognition of glycopolymers, and key parameters include glycopolymer length, density of carbohydrate ligands, flexibility of polymer backbone and glycopolymer architecture. An excellent recent review by Kiessling and Grim discusses in detail how each of these parameters can affect cell signaling [4].

Glycopolymers can also mimic mucins and as such have been investigated in selectin-mediated inflammation [14,81,82]. L-Selectin is an important mediator in the inflammatory response. Sulfated galactose-derived neoglycopolymers (**Figure 3A**) were prepared by ROMP, and studies indicated that they not only bound to L-selectin but also promoted the proteolytic release of L-selectin from the cell surface [14,81]. Further work in this area investigated the role of carbohydrate spacing in promoting L-selectin shedding [83]. Dendritic-cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN) is a C-type lectin expressed on the surface of DCs of great medical interest as it binds to carbohydrates on the surfaces of pathogens (HIV-1) [84]. Synthetic glycopolymers with high affinity for DC-SIGN are attractive materials that could offer important antimicrobial adhesion properties and thus provide novel therapeutic strategies for HIV treatment. A library of mannose-substituted glycopolymers (**Figure 3B**) was synthesized via a post-polymerization modification approach

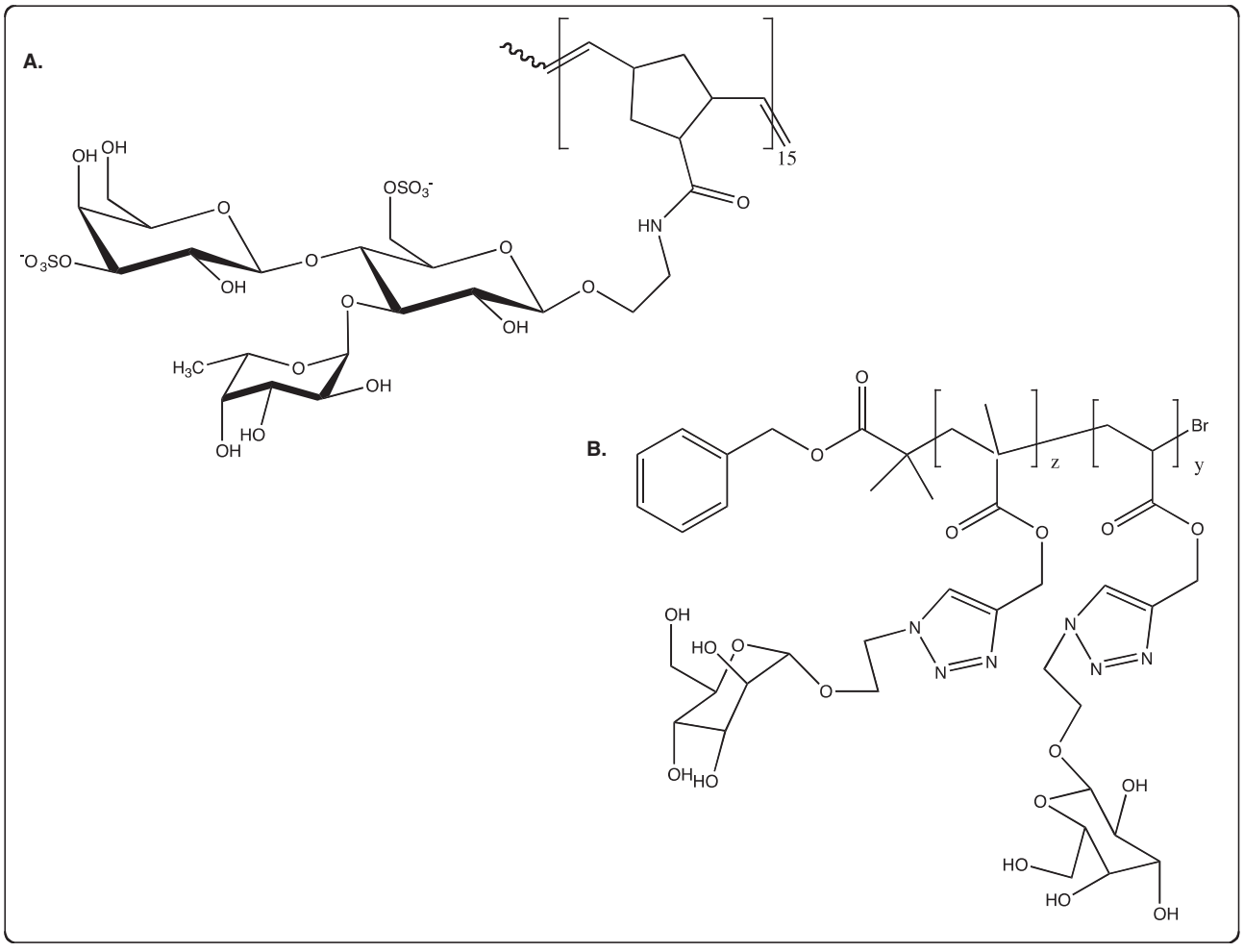

Figure 3. Chemical structures of (**A**) sulfated galactose-substituted neoglycopolymers and (**B**) mannose-substituted glycopolymers.

employing 'click' chemistry [85]. The ability of these well-defined glycopolymers to inhibit the interactions between DC-SIGN and the HIV envelope glycoprotein gp120 was studied. Studies indicated that as the mannose density increased, the ability of the glycopolymers to disrupt the gp120-DC-SIGN interaction also increased. This work may provide more insights into the mechanisms of HIV infection and hence potential new therapeutics. A recent study showed that glycopolymers that bind to DC-SIGN do elicit key cellular signaling responses [86]. Chondroitin sulfate (CS) glycosaminoglycans are involved in various physiological processes such as cell division, inflammation and spinal cord injury [87]. The synthesis of CS mimetics has recently been sought in order to better relate CS structure to function and find defined molecular tools for manipulating CS activity. To this end, CS glycopolymers with defined sulfation sequence and tunable chemical and biological properties were prepared by ROMP. The glycopolymers had key features of glycosaminoglycans and mimicked the biological activities of natural CS polysaccharides [88]. It was later shown that the CS-E (GlcA-4S, 6SGalNAc) disaccharide sulfation motif is crucial for the inhibitory activity of CS proteoglycans. Furthermore, the CS-E glycopolymers could promote signaling [89]. Overall, these studies illustrate the power of glycopolymers to mimic CS proteoglycans for a better understanding of their structure–activity relationship.

2.5 Glycopolymers for protein stabilization

Proteins are commonly used as reagents in research laboratories and as therapeutics for many diseases, particularly, insulin for diabetics [90]. However, the low effectiveness as protein drugs has been mainly attributed to the inherent instability of proteins. Proteins can degrade easily under 'environmental stresses' such as light, desiccation and fluctuations in pH and temperature. As such, the addition of additives, such as sugars, polymers, salts, amino acids, and osmolytes, help to prevent denaturation of proteins. Nevertheless, protein instability remains a major concern despite the addition of additives. Thus, the design of new and nontoxic additives for protein stabilization remains an area of active research. In this respect,

Maynard and co-workers recently disclosed the use of a novel trehalose glycopolymer for protein stabilization in response to various environmental stresses including heat and lyophilization [91]. The glycopolymer prepared by RAFT polymerization consists of a polystyrene backbone with pendant trehalose units. The natural nonreducing disaccharide trehalose is generally regarded as safe by the US FDA and is widely used in the food and cosmetic industries [92]. The trehalose glycopolymer possessed a pyridyl disulfide endgroup that allowed covalent conjugation to free cysteines. When the polymer was conjugated to lysozyme, the stability of the protein against heat and lyophilization stress was significantly increased. Further studies investigated the ability of a small library of trehalose glycopolymers as excipients for protein stabilization [93]. The trehalose was modified with a styrenyl acetal, methacrylate acetal, styrenyl ether or methacrylate moiety, and the glycopolymers with different molecular weights were synthesized by free-radical polymerization. The resulting glycopolymers were found to stabilize proteins significantly better than no additive. They were also noncytotoxic to at least two mouse and two human cell lines. These trehalose glycopolymers are promising candidates to stabilize proteins; however, more work remains to be done to elucidate the mechanism of how the polymer stabilizes the proteins and the *in vivo* stability of this glycoconjugate.

3. Glyconanoparticles

The therapeutic potential of GNPs and their importance in the biological mediation were realized when Penadés and coworkers first developed GNPs in 2001 to study carbohydrate–carbohydrate interactions in water between oligosaccharide epitopes [94]. Since the discovery of the multivalent nature of carbohydrate-mediated interactions and the specific tumor-associated carbohydrate antigens (TACAs) (glycosphingolipids and glycoproteins) involved in the initial step of tumor spreading, GNPs have been extensively used for the multivalent presentation of carbohydrates in biomedical applications. The globular shapes and diversified nanometer sizes of GNPs, some of which are comparable to those of biomacromolecules, make them efficient scaffolds for the syntheses of 'glycocalyx-like' building blocks exposing multiple copies of oligosaccharide conjugates [94]. Gold, silver, semiconductor quantum dots and magnetic (iron oxide) GNPs are the four different types of nanoparticles functionalized with carbohydrates so far, whereas most of the applications have been reported using gold GNPs probably due to the ease of preparation and greater stability of the resulting functionalized nanoparticles. The glyconanomaterials reported for biomedical applications make use of structural polysaccharides as biocompatible coatings of metallic nanoparticles or as carriers in drug-delivery systems. Typical examples are dextran- and carboxydextran-coated magnetic nanoparticles such as Resovist® (Cliavist™) [95], Feridex® (Endorem™) [96] and Sinerem® [97], which have been, or still are, in clinical use as contrast agents, or polysaccharide-based nanomaterials such as alginate [98] and chitosan nanoparticles [99], which are in preclinical development as carriers for drug delivery in therapy against cancer. Excellent reviews have already been published focusing on the preparation and application of polysaccharide nanoparticles as carriers [100,101] and multifunctional nanomaterials for biomedical applications [102]. Polysaccharides act as the bioadhesive material in these nanoparticles (increasing drug loading, prolonging residence time and enhancing biocompatibility).

In another study, the accessibility of a wide variety of these structured nanosystems, in terms of shape, size and organization around stable nanoparticles, has readily contributed to their development and application in nanomedicine. In this context, glycosylated gold nanoparticles, glycosylated quantum dots [103-105], fullerenes [106,107], single-wall nanotubes [108,109] and self-assembled GNPs using amphiphilic glycopolymers [110] or glycodendrimers [111,112] have received considerable attention for their applications in powerful imaging, therapeutic and biodiagnostic devices. A study using mouse melanoma model to test GNPs as possible inhibitors of experimental lung metastasis has shown that carbohydrate–carbohydrate interaction was the first recognition step for this process. In this work, GNPs presenting lactose (lacto-GNPs) was successfully used to significantly reduce the progression of experimental metastasis [113] showing a clear biological effect of lacto-GNPs. This demonstrates the potential application of glyconanotechnology in biological processes. Hitherto, a number of publications on the synthesis and applications of GNPs in biomedicine [114], glycoscience and material science were reported [115]. Several reports have demonstrated that these GNPs represent a good bio-mimetic model of carbohydrate representation at the cell surface. Despite the wide coverage of GNPs and their applications in biomedicine, this section of this review focuses only on the therapeutic potential of GNPs. One of the main challenges in this field is the development of new therapeutic systems that may ensure high stability, specificity and low toxicity. An ideal therapeutic system should be effective at low doses, selective and specific.

The major obstacle of cancer drugs has been the resistance to cancer cells in limiting the therapeutic efficacy of chemotherapeutic agents. Among several mechanisms of drug resistance, *P*-glycoprotein is the best known and most extensively studied to be mediator of drug resistance to diseased cells [116]. Studies have shown that nanoparticles may be able to circumvent *P*-glycoprotein-mediated resistance because it may avoid recognition by the *P*-glycoprotein efflux pump by means of being enveloped in an endosome when entering the cell, leading to high intracellular drug concentrations [117]. Ligand-targeted strategies, especially those using receptor-targeting ligands, may have particular potential for overcoming drug resistance because these ligands are usually internalized via receptor-mediated endocytosis and drugs released at the target site [117]. In another development,

a nanoparticle formulation of paclitaxel, in which serum albumin is conjugated as a carrier (nanometer-sized albumin-bound paclitaxel [Abraxane]), has been applied in the clinic for the treatment of metastatic breast cancer [118]. These and many more reports are clearly a step ahead to prove the therapeutic efficacy of GNPs in biomedicine. The subsequent sections of this review discuss other areas in which GNPs have been successfully utilized as therapeutic agents and the way forward.

3.1 GlycoGNP in gene delivery

An attractive target for receptor-mediated interaction is carbohydrates and, in particular glycoconjugates, which play important roles in cancer development and metastasis [119-122]. Indirectly, the potential of GNPs as drug-delivery agent has been demonstrated by Chitosan nano- and microparticles; having a rich history as drug-delivery systems [123-125], including their use as tumor-selective delivery and therapy. This includes tumor-targeting chitosan nanoparticles for optical/MR dual imaging that was based on polymeric nanoparticulate technology and molecular imaging [124]. Hydrophobically modified glycol chitosan (HGC) nanoparticles , a novel nanosized drug carrier in cancer treatment that can self-assemble in aqueous solution to form stable nanoparticles, were developed and used as drug-delivery agents by Hwang and co-workers [126,127]. In their work, selective accumulation of HGC nanoparticles in the tumor interstitium by the EPR effect was observed when systemically administered to deliver various types of therapeutic agents.

In another study, Narain and co-workers have extensively studied the mechanism of uptake of cationic gold GNPs as a novel gene delivery vehicle. In this study, they found that cationic GNPs underwent receptor-mediated endocytosis following vesicular escape, owing to the net cationic character of the nanoparticles [128]. The results suggested that the biocompatibility of nanoparticles and their easy uptake by cells followed by vesicular escape and nuclear entry, in the absence of external impulse, proved successful in the fabrication of a novel type of gene delivery vehicle.

3.2 GNPs in anti-adhesion therapy

The discovery of the important role that carbohydrates play in recognition processes has suggested a new antimicrobial therapy based on their anti-adhesion potential. It is noted that glycosphingolipids expressed on the tumoral and endothelial cell surfaces seem to be involved in the critical adhesion step. An interaction between the glycosphingolipids GM3, expressed in a murine melanoma cell line (B16), and Gg3 or lactosylceramide of endothelium cells has been proposed to be involved in the first adhesion step of tumoral cells to the endothelium before transmigration [129]. However, the inhibition of this step by GNPs that present carbohydrate antigens expressed either in the tumor or the endothelium cells might provide effective anti-adhesion therapy. The first demonstration that gold GNPs behaved as anti-adhesion agents against progression of lung metastasis in mice was reported in 2004 [130]. Lactose GNPs were tested as a potential inhibitor of the binding of melanoma cells to the endothelium. An *ex vivo* experiment was designed for the evaluation of the antimetastasis potential of the GNPs. In this study, 70% tumor inhibition was reported as compared with the group inoculated only with melanoma cells. This method of treatment was used to develop antimicrobial adhesion agents using carbohydrate-biofunctionalized nanoparticles, including polystyrene nanospheres. Through a PEG tether, these polymeric nanoparticles (150 nm in diameter) were covalently biofunctionalized with multiple mannose or galactose moieties [131,132]. After incubating *E. coli* strains with the polymeric nanoparticles carrying two carbohydrate functionalities, electron microscopy revealed that carbohydrate-biofunctionalized nanoparticles mediated aggregation of bacterial cells. The binding of carbohydrate nanoparticles to bacterial cells was via adhesin-receptor (carbohydrate–protein) interaction as demonstrated by the strong binding of mannosylated nanoparticles with a mannose-specific *E. coli* ORN178 strain and binding of galactosylated nanoparticles with a galactose-specific *E. coli* O157:H7 strain.

Another demonstration of antiadhesive application of GNPs was illustrated by Svarovsky and co-workers [131], whereby they reported the synthesis of three-dimensional self-assembled monolayers of gold particles functionalized with the Thomsen–Friedenreich (TF) moiety. According to preliminary data, *in vitro* and *in vivo* antitumor and antimetastatic bioassays revealed the TF-gold nanoparticles ability to inhibit tumor growth and lung metastasis formation against an implanted metastatic breast cancer cell line. In 2003, Franklin and co-workers showed that carbohydrate nanoparticles also reduced *Campylobacter jejuni* populations about 70% in turkey poults when the birds were gavaged with mannose nanoparticles [133]. No apparent cytotoxicity was observed when these polymeric nanoparticles were tested *in vitro* and *in vivo* [134,135], indicating the clinical potential of these carbohydrate-biofunctionalized nanoparticles for antimicrobial adhesion treatment. Penadés and co-workers illustrated that gold manno-GNPs displaying different densities of linear and branched mannose oligosaccharides acted as antiadhesive inhibitors of DC-SIGN-mediated HIV transinfection of human-activated peripheral blood mononuclear cells. The authors demonstrated that presentation of simple linear di-, tri- and tetra-oligosaccharides on the nanoclusters resulted in efficiencies similar to those observed for complex branched penta- and hepta-mannosides [136]. It is now clear that the high-mannose glycan clusters of HIV envelope glycoprotein gp120 promote HIV infection by their interaction with the C-type lectin DC-SIGN [97]. However, mimicking the cluster presentation of the oligomannosides on the surface of the virus has become a strategy for designing carbohydrate-based antiviral agents. Recently, gold GNPs coated with selected oligomannoside motifs (manno-GNPs) has been designed to mimic the presentation of the high mannose

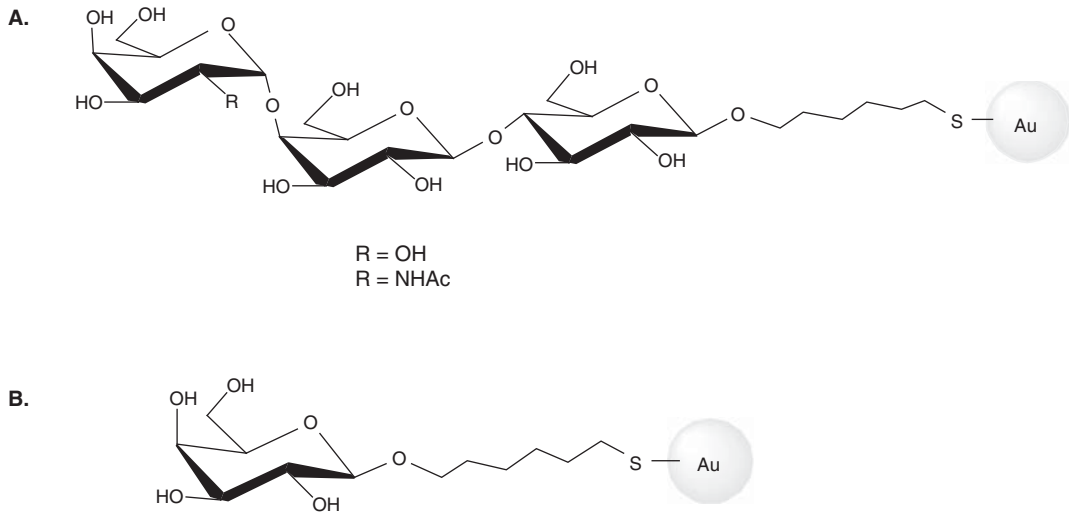

Figure 4. Glycan encapsulated gold nanoparticles (**A** and **B**) that selectively inhibit Shiga toxins 1 and 2.

glycans of gp120 [137]. In this research, manno-GNPs were able to inhibit the DC-SIGN-mediated HIV trans-infection of human-activated peripheral blood mononuclear cells at nanomolar concentrations in an experimental setting, which was designed to mimic the natural route of virus transmission from DCs to T lymphocytes. This result proves that synthetic manno-GNPs could function as an antiadhesive barrier at an early stage of HIV infection.

3.3 GNPs in vaccine/disease development

In 1931, it was demonstrated for the first time that polysaccharides could be rendered antigenicaly by adsorption on colloidal carriers (adjuvant effect). However, organic colloidal particles were already used as carriers for bacterial polysaccharides [138]. Nanoscopic systems incorporating therapeutic agents with molecular-targeting and diagnostic imaging capabilities are emerging as the next generation of functional nanomedicines to improve the outcome of therapeutics. The development of compounds that enhance immune responses to recombinant or synthetic epitopes is of considerable importance in vaccine research. The proteins on the surface of breast cancer cells for example, present mainly linear, truncated core 1 mucin-type glycans such as α-*N*-acetyl-D-galactosamine (αGalNAc, the Tn-antigen glycan), with complete or near-complete absence of core 2 residues [139,140]. These differences have been targeted as a strategy for cancer immunotherapy [141-143]. Accordingly, multivalent glycoconjugates have been prepared in which mucin glycans are presented on a variety of scaffolds, including peptides [144-146], lipopeptides [147-150], dendrimers [151] and proteins [152]. Some of these approaches have developed as far as clinical trials [153-158]. Snippe and co-workers designed and prepared multifunctional GNPs to induce an anti-carbohydrate immune response by using 'direct' synthesis [159]. In their study, they obtained the first fully synthetic carbohydrate vaccine based on gold GNPs. Gold nanoparticles (ca. 2 nm in diameter) coated with the synthetic tetrasaccharide Gal(β1-4) Glc(β1-6)[Gal(β1-4)]GlcNAc(β1-(TetraPn), which corresponds to the single repeating unit of the *Streptococcus pneumoniae* type 14 capsular polysaccharide (Pn14PS), a conjugate of the T-helper ovalbumin peptide ($OVA_{323-339}$), and 5-mercaptopentyl β-D-glucopyranoside (Glc) in variable ratios were prepared and used to immunize mice. Significant titers of specific and functional IgG antibodies against Pn14PS were induced only by the GNPs coated with TetraPn/Glc/$OVA_{323-339}$ in 45/50/5 proportions. Barchi and collaborators in 2012 [160] developed several nanoparticles carrying glycopeptides bearing TACAs, and their immune responses were evaluated after mice immunization. In this work, particles were encrusted with both the tumor-associated glycopeptides antigens containing the cell-surface mucin MUC4 with TF antigen attached at diverse sites and a 28-residue peptide from the complement-derived protein C_3d to act as a B-cell-activating 'molecular adjuvant'. A small but significant IgG and IgM production was generated against each glycopeptide antigen linked to the nanoparticles, indicating how useful these GNPs may be. Recently, Cameron and co-workers described peptide-free multivalent glycosylated nanoscale constructs as potential synthetic cancer vaccines that generated significant titers of antibodies selective for aberrant mucin glycans [161]. A polymerizable version of the Tn-antigen glycan was prepared and converted into well-defined glycopolymers by RAFT polymerization. The polymers were then conjugated to gold nanoparticles, yielding 'multicopy-multivalent' nanoscale glycoconjugates. Immunological studies indicated that these nanomaterials generated strong and long-lasting production of antibodies that are selective in the Tn-antigen glycan and crossreactive towards mucin proteins displaying Tn [161]. Stx is the major pathogenic determinant of several Gram negative bacteria including *E. coli* O157:H7 (STEC) and *S. dysenteriae*. Treatment of this disease is mostly supportive, as post-diarrheal

antibiotic treatment enhances toxin production and progression towards HUS [162], which makes it difficult in treating STEC (O157:H7). Research has been mainly focused towards the development of an effective Stx neutralizer that specifically binds to and inhibits Stx in the circulation. In 2010, Iyer and co-workers developed gold GNPs (**Figure 4**) that present a multivalent display similar to the glycolipids on the cell surface to compete for these toxins [163]. They found that the highly soluble GNPs were nontoxic to the Vero monkey kidney cell line and protected Vero cells from Stx-mediated toxicity in a dose-dependent manner. The inhibition is highly dependent on the structure and density of the glycans; selective inhibition of Stx1 and the more clinically relevant Stx2 was achieved. Interestingly, natural variants of Stx2, Stx2c and Stx2d possessing minimal amino-acid variation in the receptor binding site of the B-subunit or changes in the A-subunit were not neutralized by either the Stx1- or Stx2-specific gold GNPs. According to the researchers, the results indicated that tailored GNPs that mimic the natural display of glycans in lipid rafts could serve as potential therapeutics for Stx1 and Stx2 (**Figure 4**) [163]. The production of fully synthetic carbohydrate vaccines based on gold nanoparticles as carriers is still in its infancy; however, the above examples show that this strategy is very promising due to the versatility of these systems for introducing a controlled amount of a variety of different ligands on the same platform.

4. Conclusion

This review has focused mainly on the therapeutic potential of glycopolymers and GNPs. The advent of new powerful polymerization methods has afforded glycopolymers of diverse architectures that have been well exploited for various applications ranging from therapeutics to vaccine development, signal transduction processes and drug and gene delivery. Despite the progress made to date, several issues still need to be addressed before the application of glycopolymers in clinical use. Toxicity issues of these materials remain, perhaps, the most crucial one that needs to be addressed. GNPs with their nanometric size, high water solubility and good stability in biological systems provide outstanding potential for future biomedical applications. They can be considered as molecular carriers for the targeting, intracellular trafficking and delivery of biomolecules (proteins, peptides, drugs, genes, DNA). However, a better knowledge of how these nanoparticles interact with biological systems is required.

5. Expert opinion

It is widely recognized that developing carbohydrate-based polymers and nanomaterials offers huge potentials in the development of promising therapeutic drugs. However, in reality, the development of such therapeutics can be highly challenging due to the complexity associated with the derivatization of biologically relevant carbohydrate molecules. Despite the tremendous progress in the field of carbohydrate chemistries, only a handful of approaches exist for the direct or one-step functionalization. Most of the chemical modification methods of simple carbohydrate molecules require multistep approaches, which can limit their large-scale exploitation and cause a significant increase in the cost of production. In addition, biologically relevant carbohydrate ligands/receptors are much more complex and hence there is a need to find better synthetic strategies for the development of carbohydrate mimics having similar or greater affinity as the naturally occurring carbohydrate ligands. Despite the complexity of carbohydrates, significant progress in the field of polymer science and nanotechnology has somehow helped in the development of biologically relevant polymeric and nanoparticles carbohydrate mimics for therapeutic uses. The preparation of advanced polymeric materials with predetermined compositions, molecular weights and architectures has been a major player in such developments. In addition, the preparation of 'smart materials' that respond to temperature, pH, light and magnetic field has also contributed significantly in the development of novel therapeutics. Therefore, there should be more investigations in the fabrication of 'smart carbohydrate materials' for the development of novel therapeutics. In addition, the role of glycopolymers in signal transduction and protein stabilization is still in its infancy and remains an exciting future area of research. The role of glycopolymer topology on signaling and the mechanism of glycopolymer-mediated protein stabilization have not yet been explored. This notwithstanding, glyconanotechnology has become a major field with significant developments of carbohydrate polymeric and metallic-based nanoparticles. However, their full exploitation as therapeutic drugs is yet to be achieved due to several limitations. Although initials results have shown GNPs to be very promising as platforms for future vaccine development, more research is required to better understand how they manipulate the immune system. Most biological studies so far have been limited to *in vitro*, but *in vivo* studies are extremely important to fully understand the true potential of these carbohydrate-based therapeutics. Also, biocompatibility studies has been carried out only with a handful of materials, but in reality, all synthetic carbohydrate materials should be carefully evaluated for their biocompatibility rather than just assuming they are biocompatible due to the presence of carbohydrate residues in the materials.

To realize the full potential of carbohydrate-based polymers and nanoparticles, major effort should be focused towards the development of more complex and biologically relevant carbohydrate materials. More studies should be directed towards the biological evaluation of these materials *in vitro* and *in vivo*. Therefore, it is now time for chemists to team up with pharmacologists, clinicians and biologists to explore the therapeutic potential of such materials even further.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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