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Review

# Carbohydrate-Based Macromolecular Biomaterials

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**ABSTRACT:** Carbohydrates are the most abundant and one of the most important biomacromolecules in Nature. Except for energy-related compounds, carbohydrates can be roughly divided into two categories: Carbohydrates as matter and carbohydrates as information. As matter, carbohydrates are abundantly present in the extracellular matrix of animals and cell walls of various plants, bacteria, fungi, etc., serving as scaffolds. Some commonly found polysaccharides are featured as biocompatible materials with controllable rigidity and functionality, forming polymeric biomaterials which are widely used in drug delivery, tissue engineering, etc. As information, carbohydrates are usually referred to the glycans from glycoproteins, glycolipids, and proteoglycans, which bind to proteins or other carbohydrates, thereby meditating the cell−cell and cell−matrix interactions. These glycans could be simplified as synthetic glycopolymers, glycolipids, and glycoproteins, which could be afforded through polymerization, multistep synthesis, or a semisynthetic strategy. The information role of carbohydrates can be demonstrated not only as targeting reagents but also as immune antigens and adjuvants. The latter are also included in this review as they are always in a macromolecular formulation. In this review, we intend to provide a relatively comprehensive summary of carbohydrate-based macromolecular biomaterials since 2010 while emphasizing the fundamental understanding to guide the rational design of biomaterials. Carbohydrate-based macromolecules on the basis of their resources and chemical structures will be discussed, including naturally occurring polysaccharides, naturally derived synthetic polysaccharides, glycopolymers/glycodendrimers, supramolecular glycopolymers, and synthetic glycolipids/glycoproteins. Multiscale structure−function relationships in several major application areas, including delivery systems, tissue engineering, and immunology, will be detailed. We hope this review will provide valuable information for the development of carbohydrate-based macromolecular biomaterials and build a bridge between the carbohydrates as matter and the carbohydrates as information to promote new biomaterial design in the near future.


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## 1. INTRODUCTION

Biomaterials are generally regarded as substances other than food or drugs contained in therapeutic or diagnostic systems that are in contact with tissues or biological fluids.[1,2] The usage of biomaterials dates back to antiquity, when simple materials such as metal and wood were involved. Since the 1950s, synthetic polymers have been increasingly and successfully used in health care with the merit of biocompatibility, controllable mechanical properties, and "inert" nature. Although synthetic polymeric materials had huge success, great demand for revolutionary polymeric biomaterials is still urgent owing to the personalized and specialized requirements from patients and doctors. These requirements call for a new generation of polymeric biomaterials, which should be adaptive, complex, and intelligent. In other words, polymeric materials with functions resembling living matter are currently pursued by polymer scientists. To this goal, materials either made from nature or derived from nature would be promising as they not only inherit the adaptive and complex intrinsic properties from living matter but also increase the possibilities of self-organization, making multiscale hierarchical behaviors predictable.[3−6]

Considering such demands, a carbohydrate is the leaf that we should take out of Nature's book. Carbohydrate is a simple name covering a huge amount of biomacromolecules with larger diversity than one may expect. Besides the well-known glucose (Glc) and sucrose for energy, natural carbohydrates can be roughly divided into two categories, i.e., carbohydrates as matter and carbohydrates as information. The matter carbohydrates are the major structural components of living organisms, including plant, bacteria, fungi, etc. They exist mainly as polysaccharides and contribute to the abundance of carbohydrates on the planet. The long polysaccharide chains, either linear or branched with different conformations and the different orientations of the hydroxyl groups of stereoisomers of saccharides, control the microscopic and macroscopic properties of these polysaccharides. The information carbohydrates refer more to those in the field of glycobiology. The carbohydrates such as glycoproteins, glycolipids, and proteoglycans exist widely on cellular surfaces and in extracellular matrix (ECM), contributing significantly to cell−cell/cell−matrix communication. Here, different saccharides are "encoded" with information which could be read by either proteins or other saccharides. In some cases, these two categories can be unified in one macromolecule. In short, the abundance and diversity of carbohydrates exceed any other biomacromolecules in Nature, and they are major components on the cell surface, on the cell wall, and in ECM. These characters make them ideal building blocks besides protein, DNA/RNA, and synthetic polymers for macromolecular biomaterial design.[7] Different from other synthetic polymers, carbohydrate-based polymers present intrinsic bioactivities toward cells while allowing various acquired functionalities via chemical conversion of the abundant hydroxyl groups. However, the production of carbohydrate-based polymers has remained much less controllable than that of other synthetic polymers, thus hindering its widespread success in the field of biomaterials.

The structural diversity and complexity highlight the indispensable role of carbohydrates in biomaterial design, while difficulties in the preparation of the related material may also hinder the development of this type of material. Compared to synthetic polymers and peptides, successful examples based on carbohydrates as biomaterials are still limited. The few successful examples include the most abundant polysaccharide, cellulose, that is a structural component of the primary cell wall of green plants. Its derivative cellulose acetate was processed as bundles of hollow fibers for artificial kidney dialyzers, whereas cellulose acetate butyrate was used as a rigid oxygen-permeable contact lens material.[8] Glycosaminoglycans (GAGs), composed of alternating carbohydrate units of amino sugars and uronic acids or glucuronic acid in a linear chain, are ubiquitous in ECM, playing a significant role in shock absorbing, growth factor binding, cell attachment, and signaling.[9−11] Natural GAGs and


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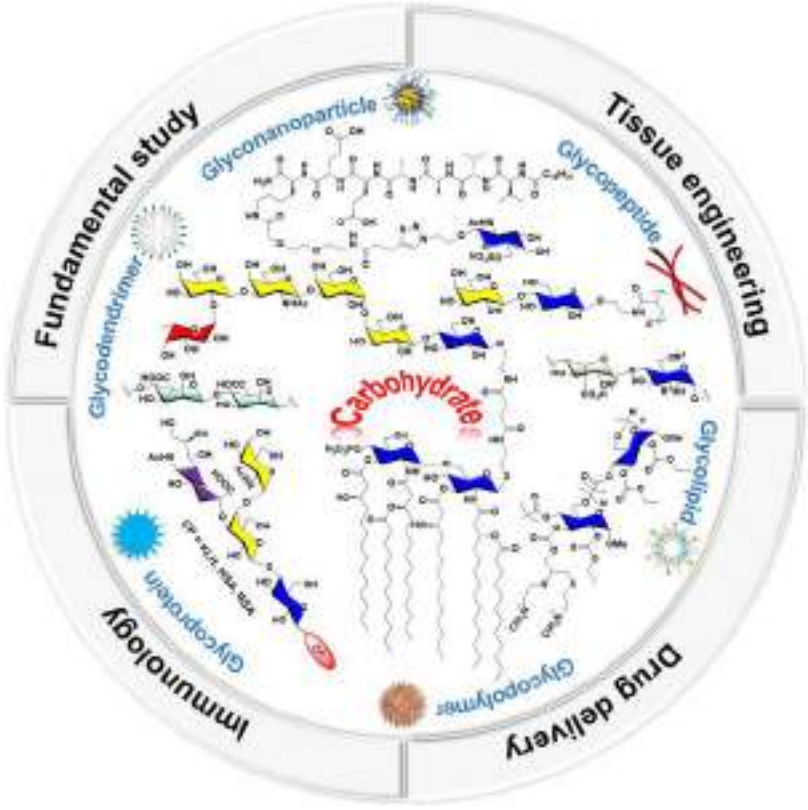


**Figure 1.** Illustrated overview of the carbohydrate-based macromolecular biomaterials with multiscale structure−function relationship in fundamental study, delivery system, tissue engineering, and immunology.

the corresponding mimics are widely employed in cell-based therapy and tissue engineering.[12,13]

In this review, we summarize the recent advances in the research field of carbohydrate-based macromolecular biomaterials. To demonstrate the role of carbohydrates from the viewpoint of macromolecular biomaterials, we will categorize the structural role and signaling role of carbohydrates together into three important applications, namely, delivery system, tissue engineering, and immunology (Figure 1). Before that, the fundamental structural features of carbohydrate-based macromolecules will be discussed, including naturally occurring polysaccharides, naturally derived synthetic polysaccharides, glycopolymers/glycodendrimers, supramolecular glycopolymers, as well as synthetic glycolipids/glycoproteins. In this review, we focus on the state of the art (within the past decade) with respect to a fundamental understanding to guide materials rational design. Thus, instead of clinical translation cases, the majority of the studies discussed here are in vitro or preclinical in vivo animal model systems or even in a conceptual state, which require extensive investigation to reach clinical use. Finally, we describe emerging and future trends in carbohydrate-based macromolecular biomaterials.

## 2. CHEMICAL STRUCTURES AND PROPERTIES

Carbohydrates in general refer to the molecules or their monomers (named monosaccharides) based on the general formula $C_x(H_2O)_n$ that possesses a carbonyl group, either an aldehyde or a ketone. In solution, monosaccharides mainly exist as cyclic forms of pyranose (Figure 2a) or furanose, which acquire an additional asymmetric center derived from the carbonyl carbon atom, termed the "anomeric carbon" (i.e., C-1 in the ring form of monosaccharides (Figure 2b)). The anomeric hydroxyl group participates in the formation of a glycosidic bond for a polysaccharide or a glycan chain in the $\alpha$-/$\beta$-configuration with the hydroxyl group at the other position from the neighboring saccharide. As shown in Figure 2c, cellulose consists of a linear chain with a $\beta$-1,4-linkage of D-Glc units. The typical chemical structures of monosaccharides and representative oligosaccharides and polysaccharides are listed in Figure 2. Due to the complexity of saccharides, the colored symbolic nomenclature (Figure 2) is widely used in carbohydrate chemistry and biology, which will also be employed in this review.

On the basis of the natural structures of carbohydrates and related synthetic molecules, the carbohydrate-based building blocks discussed in this review are categorized into five basic species according to their resources and chemical structures, which are (1) naturally occurring polysaccharides (with postmodification), (2) naturally derived synthetic polysaccharides (carbohydrate units incorporated into the main chain in a ring-closed configuration), (3) glycopolymers (carbohydrate units incorporated into the pendant groups in a ring-closed configuration) and glycodendrimers, (4) supramolecular glycopolymers, and (5) synthetic glycolipids and glycoproteins. The structure−property relationships, especially the mechanical and biological properties, will be addressed. Moreover, the fundamentals of the physiological processes—the multivalency effect—will be introduced in section 2.3.1 considering that the glycopolymers and glycodendrimers are primarily used for mimicking the natural glycans to investigate the underlying mechanism of the physiological processes. Meanwhile, carbohydrate−carbohydrate interactions (CCIs) and carbohydrate−protein interactions (CPIs) will also be addressed due


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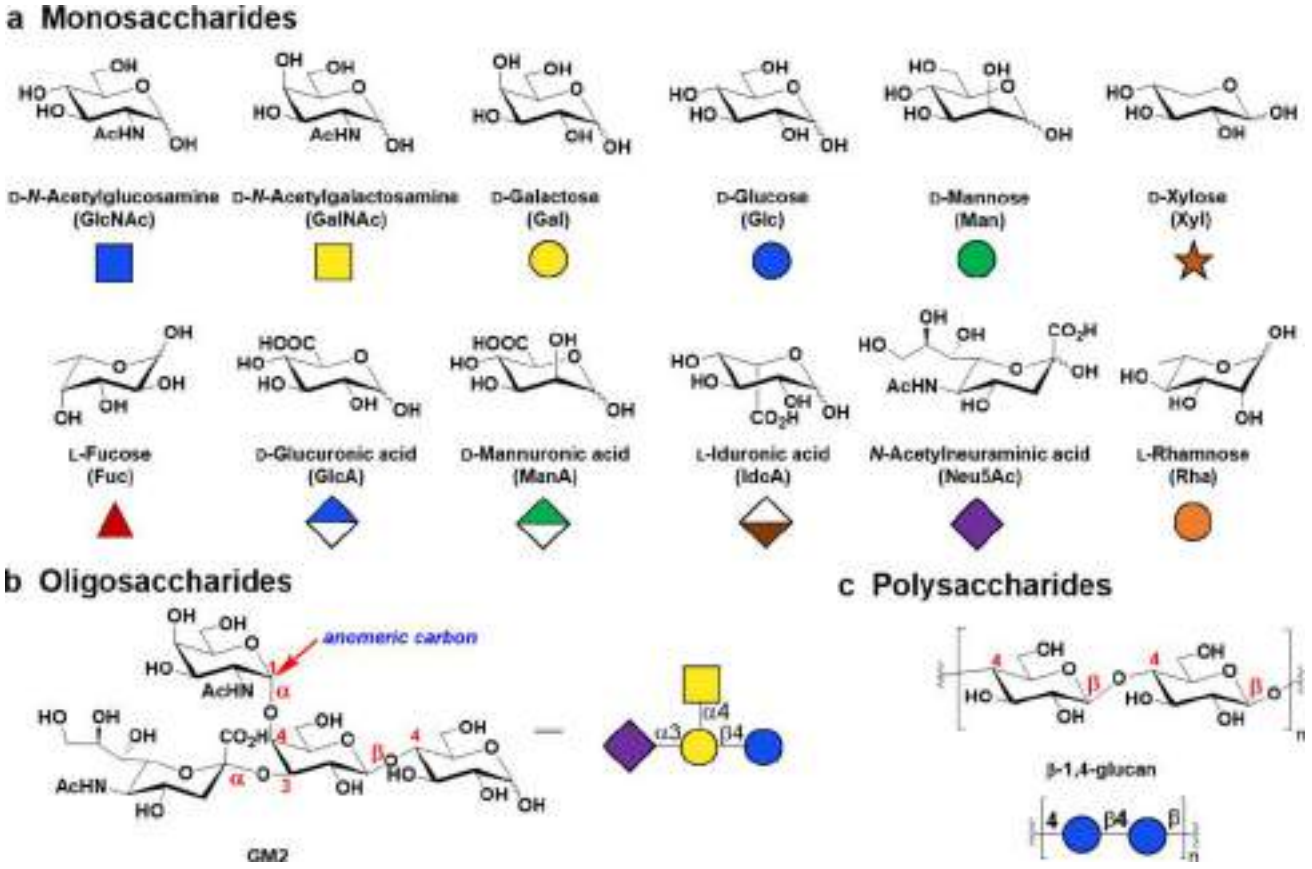


**Figure 2.** Chemical structures and their symbolic nomenclature of some of the common monosaccharides (a), oligosaccharides (b), and polysaccharides (c) with their anomeric linkage labeled in red.

to their fundamental functions mediating cell−cell communication and cell−matrix interactions.

### 2.1. Naturally Occurring Polysaccharides

Polysaccharides are ubiquitous in Nature, offering a great variety of chemical structures, from simple linear homopolymers to branched heteropolymers, and in the form of energy storage or structural materials. With the advances of extraction and purification technologies, scalable and relative homogeneous polysaccharides could be obtained with minimal batch to batch vibrations, allowing for generation of sustainable society-enhancing materials. Naturally occurring polysaccharides could be further modified to leverage desirable physical and chemical properties owing to the inherent tailorability from the hydroxyl and other functional groups.

Polysaccharides can come from plants, like starch and cellulose, from algael, like alginates and galactans, from mammalian tissues, like GAGs and hyaluronic acid (HA), and from microbial substances, like dextran and gellan gum. The most widely used polysaccharides as biomaterials will be briefly introduced here (Figure 3). Cellulose was comprehensively reviewed recently by others; thus, it will not be emphasized in this review.[14−16]

**2.1.1. Alginate.** Alginate is a family of linear copolymers with a 1,4-glycosidic linkage containing three types of block structures: M block (β-D-mannuronic acid), G block (α-L-guluronic acid), and MG block (alternating M and G polyuronic acids) (Figure 3). The M blocks are adopting the $^4C_1$ chair conformation that imparts flexibility to the chain, whereas G blocks are stiff structures of buckled shape due to

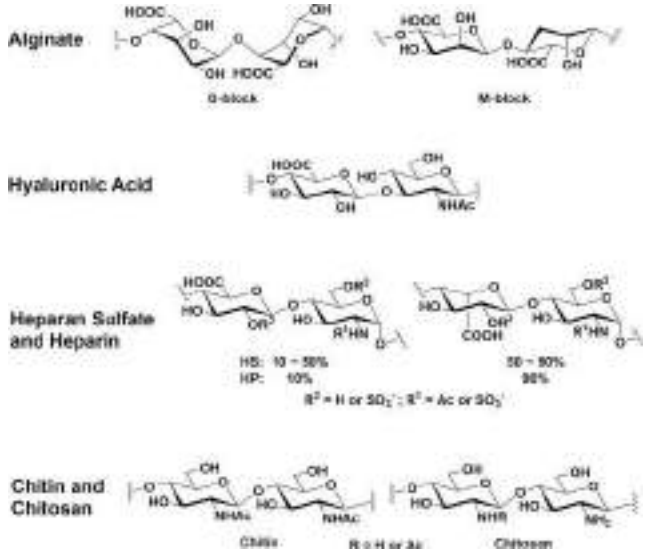


**Figure 3.** Selective chemical structures of naturally occurring polysaccharides discussed in this review.

the $^1C_4$ conformation of the guluronate residues. Moreover, G blocks are believed to participate in intermolecular cross-linking with divalent cations (e.g., $Ca^{2+}$). It has been reported that the composition (i.e., M/G ratio), G block length, and molecular weight are critical factors affecting the physical properties of alginate.[17] Due to the character in terms of safety, stability, solubility, viscosity, etc., alginates have been widely used in the pharmaceutical industry[18] and application in drug


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delivery, tissue engineering, wound healing therapy, and so on.[19] In addition, it has been reported that alginate can induce innate and adaptive responses.[20]

**2.1.2. Hyaluronic Acid.** Hyaluronan consists of repeating disaccharides composed of *N*-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA) with the repeating disaccharide motif (*β*-1,4-GlcNAc-*β*-1,3-GlcA) (Figure 3).[21] It is the largest polysaccharide found in vertebrates in the form of hydrated matrices. They are ubiquitous in ECM, playing a significant role in shock absorbing, growth factor binding, cell attachment, and signaling.[9−11] Commercially available HA is extracted from animal waste (e.g., rooster combs) or produced by genetic/metabolic engineering.[22] Nowadays, enzymatic, chemical, and chemoenzymatic synthesis of HA has also made remarkable progress.[18] Structurally, HA can be further modified through carboxylic acid, primary and secondary hydroxyl, as well as *N*-acetyl groups to advance the mechanical or binding property.[23] Functionally, HA could interact with chondrocytes, neuronal cells, etc., through surface receptors such as CD44 and hyaluronan-mediated motility receptor (CD168), making it the most advanced polysaccharide for clinical applications.[24,25] In addition, the interactions between HA and its receptors also regulate the activity of inflammation and cancer. Generally, high molecular weight HA is anti-inflammatory, whereas low molecular weight HA is pro-inflammatory and pro-cancerous. Yet, contradictory results were also reported due to the polydispersity, source, and impurity, etc., of HA.[26]

**2.1.3. Heparin/Heparan Sulfate.** Heparin (HP), a highly sulfated GAG with molecular weights ranging between 2 and 40 kDa, is one of the most important pharmaceuticals to inhibit formation of clot and thrombi, especially in surgery or trauma. HP consists of repeating units of *α*,*β*-1,4-linked uronic acids (90% of *α*-L-iduronic acid and 10% *β*-D-GlcA) and GlcNAc residues (Figure 3). Heparan sulfate (HS) is structurally related to HP with less sulfonic acid groups and a larger proportion of *β*-D-GlcA (10−50%). As an important component of most cell surfaces and ECMs, HS, in the form of HS proteoglycans, is involved in multiple biological processes, including development and homeostasis as well as progression of some diseases.[27,28]

**2.1.4. Chitin and Chitosan.** Chitin is the most abundant aminopolysaccharide in Nature, which is composed of a *β*-1,4-linkage of GlcNAc. It is the building material for the exoskeleton of crustaceans, insects, and the fungal cell wall.[29] The cationic chitosan (CS) is produced by the partial deacetylation of chitin, consisting of a randomly distributed *β*-1,4-linkage of GlcNAc and D-glucosamine units (Figure 3). The cationic property of CS is frequently utilized in pharmaceutical formulations and biomaterials. For instance, CS can complex with plasmid DNA, short interfering RNA (siRNA), etc., making it attractive in delivery systems.[30,31]

## 2.2. Naturally Derived Synthetic Polysaccharides

The complex structure of polysaccharides makes their related synthesis extremely challenging. To date, the only way to construct polysaccharides via a chemical or chemoenzymatic method is step-by-step synthesis. Although automated synthesis of oligosaccharides is developing, it is still far from making polysaccharide chains with similar regioselectivity and configuration control as Nature does. Thus, polymer chemists are keen to develop biomimetic artificial polysaccharides to reproduce either the structure or the function of natural polysaccharides. The following four categories of synthetic materials (sections 2.2−2.5) are designed and prepared for this propose with different emphasis on the mimicked aspects.

Naturally derived synthetic polysaccharides are defined as synthetic polymers with carbohydrate units incorporated into the main chain in a ring-closed configuration (Figure 4). Such

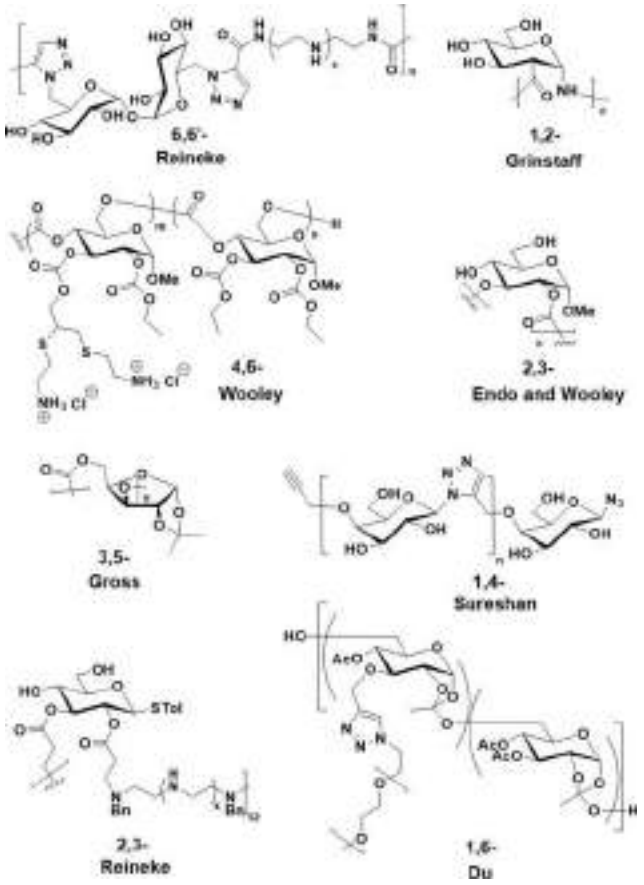


**Figure 4.** Selective chemical structures of naturally derived synthetic polysaccharides discussed in this review.

polysaccharides are derived from biobased feedstocks and designed to break down to regenerate biologically and environmentally resorbable natural products.[32] Compared to other types of biomimetic artificial polysaccharides, synthetic polysaccharides are characterized as the carbohydrate backbone with defined chemical structures and physical properties. Due to the structural complexity having different regio- and stereotypes of glycosidic linkages, the synthesis of naturally derived synthetic polysaccharides remains challenging. The most successful method was first reported in 1966 using cationic ring-opening polymerization (ROP) to afford a stereoregular *α*-1,6-linked poly(D-glucopyranose).[33] More recently, replacing the natural *O*-glycosidic bonds with an achiral linkage, e.g., amide, carbonate, etc., has enabled an expansion of the scope. A variety of naturally derived synthetic polysaccharides with ortho-ether, ester, amide, carbonate, triazolyl, or a combination linkages between the 1,4-,[34,35] the 1,6-,[33,36,37] the 1,2-,[38] the 2,3-,[39−41] the 2,6-,[42] the 4,6-,[43−45] and the 6,6′-[46] positions was obtained via ROP[47] or polycondensation reactions[42,48] (representative structures are listed in Figure 4). Noteworthy, ROP enables precise control over polymer molar mass, composition, architecture, and end group functionality, allowing for convenient manipulation of


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the resulting materials toward multiple applications. Herein, two representative species of ROP-afforded synthetic polysaccharides, poly(saccharide carbonate)s and poly-amido-saccharides (PASs), are detailed here as these materials have shown promising potential in a wide range of biomaterial applications.

**2.2.1. Poly(saccharide carbonate)s.** Polycarbonates derived from monosaccharides are particularly attractive as they originate from a renewable source. They are able to break down into $CO_2$ and their bioresorbable starting material. The pendant hydroxyl groups could be modified to tune the hydrophobic/hydrophilic ratio and to provide functionality for further tissue-engineering and biomedical applications.[49]

Polycarbonates based on various monosaccharide building blocks have been successfully synthesized with different backbone connecting structures. The structure−property relationship has been investigated.[50] For instance, the 2,3-linked poly(saccharide carbonate)s were first successfully prepared by Endo and co-workers with a five-membered bicyclic carbonate 4,6-*O*-benzylidene-2,3-*O*-carbonyl-*α*-D-glucopyranoside through anionic-initiated and -catalyzed ROPs without any elimination of $CO_2$ followed by deprotection of the benzylidene acetal group (Figure 4).[51] However, relatively broad dispersity and significant macrocyclic byproducts were observed and inevitable due to a backbiting reaction.[40] Wooley and co-workers optimized the polymerization conditions using 1,5,7-triazabicyclo[4.4.0]dec-5-ene as the organocatalyst, providing better control over the final properties of the polymers than the previous cases.[39] The glass transition temperature ($T_g$) was observed above 130 °C, arising from the main chain cyclic structures, making these materials attractive for tissue engineering and drug delivery. Another highly strained trans-configured bicyclic carbonate, 1,2-*O*-isopropylidene-D-xylofuranose-3,5-cyclic carbonate, was employed to generate 3,5-linked poly(saccharide carbonate)s homopolymer and copolymer (Figure 4) by Gross and co-workers via anionic ROPs, showing physicomechanical and biodegradable properties.[52]

In contrast, six-membered bicyclic carbonates could undergo thermodynamically favorable ROPs to generate well-defined structures with high tolerance of the functional groups in mild conditions. Wooley and co-workers developed a series of poly(D-glucose carbonate)s (PGCs) with varied alkyloxycarbonyl side chains showing a remarkable range of $T_g$ values (38−125 °C) with diversified physicochemical and thermal properties.[53] Moreover, the functional alkyne group could undergo copper-catalyzed azide−alkyne dipolar cycloaddition (CuAAC) or thiol−yne addition reactions for subsequent modification with functional molecules (e.g., dye) (Figure 4).[32,49] Buchard[44,54] and Gnanou[43,55] further enriched the scope of building blocks using $CO_2$ or $CS_2$ as the carbonylating reagent, making monomer synthesis more biofriendly.

**2.2.2. Poly-Amido-Saccharides.** Unlike most of the reported poly(saccharide carbonate)s which are completely insoluble in water, PASs closely resemble natural polysaccharides by possessing a backbone composed of pyranose rings through 1,2-amide linkage with *α*-stereochemistry. The PASs have advantages associated with synthetic polymers, such as great control over the structure and derivatization, while preserving features of natural polysaccharides, such as enantiopurity, biocompatibility, and hydrophilicity.

Grinstaff and co-workers were pioneers in developing various PASs through anionic ROP of different bicyclic mono- or disaccharide-based *β*-lactams (Figure 4) with well-defined structures and a high degree of polymerization ($DP_n$).[38,48,50,56−61] Examples, such as *α*-*N*-1,2-D-Glc,[38] *α*-*N*-1,2-D-Gal,[48] and *β*-*N*-1,2-D-altrose[57] PASs, were achieved via ring opening of the 4-membered *β*-lactam which are cis-fused to the *α*- or *β*-face of the pyranose rings, showing a left- or right-handed helical conformation. Functional groups, such as a carboxylated glucuronic[59] and amine,[61,56] were also imparted in the PASs at the molecular level to mimic natural alginic acid and chitosan, which largely expanded the current library of complex carbohydrate polymers with biological activities.

## 2.3. Glycopolymers and Glycodendrimers

Compared with the synthetic polysaccharides, which more structurally mimic the naturally occurring polysaccharides, glycopolymers and glycodendrimers more functionally mimic natural polysaccharides (i.e., GAGs), glycoproteins, and mucins. Glycopolymers are normally referred to as synthetic polymers with carbohydrates as pendent groups. With a synthetic polypeptide backbone, the term glycopolypeptide is employed. Early examples of glycopolymers were in the 1940s−1960s.[62] This research field became a hot topic mainly promoted by two historical findings. One came at the end of the 1980s as the development of glycobiology found the multivalent binding of carbohydrates and proteins. Then the multiple presentation of saccharides as pendent groups nicely mimick the multiple end saccharides of glycans, giving the opportunity of glycopolymers to be investigated as models for CPIs. The research was pioneered by Kiessling and co-workers with significant contributions from Bertozzi and co-workers as well as other research groups.[63] The second finding came from the remarkable development of highly efficient click reactions and controlled radical polymerization, such as atom transfer radical polymerization (ATRP), reversible addition−fragmentation chain transfer polymerization (RAFT), ring-opening metathesis polymerization (ROMP), and ROP, etc., providing precise structures.[64,65] On the basis of these controlled radical polymerization methods, various glycomonomers were designed and subsequently polymerized with overwhelming production of related polymers and materials, which have been nicely summarized in various reviews.[62,66−68]

The resulting glycopolymers and glycodendrimers can be altered systematically to dissect the structural features that strengthen their activities. Considering that the development of glycopolymers and glycodendrimers is driven by the demands in biorelated investigations and applications, where a multivalent interaction is the key principle, in this part, multivalency will be introduced followed by the structure−property relationship of glycopolymers and glycodendrimers.

**2.3.1. Multivalency of Carbohydrate−Protein Interactions.** Glycan-binding proteins (GBPs) (which exclude glycan-specific antibodies) are found in all living organisms and fall into two overarching groups—lectins and sulfated GAG-binding proteins. These proteins bind to carbohydrates, resulting in the CPIs. Lectins bind to carbohydrates via their carbohydrate-recognition domains (CRDs). Since many GBPs are oligomeric with each subunit typically having a single CRD, many GBPs show multivalent interactions with glycan ligands. The monovalent interactions of the carbohydrate and its CRD are relatively weak (i.e., $K_a = 10^3−10^4\ M^{-1}$). The functions of lectins are often dependent on the multivalency, which endows lectins with enhanced binding affinity (stronger affinity than the sum of the single contributions from each unit) and the ability to cross-link glycans. Multivalent interactions are


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employed throughout biological processes, such as cell differentiation, intercellular signal transduction, inflammation, and the immune response.[69,70] It is well appreciated that multivalent binding can not only enhance the functional affinity of cell surface CPIs[71,72] but also improve CPIs specificity (Figure 5).[63,73] Examples include human immunodeficiency viruses (HIV) infection toward dendritic cells (DCs). On one hand, DC-SIGNs (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin) in the DC membrane tend to form tetramers followed by further clustering into a DC-SIGN lipid raft. On the other hand, HIV envelope glycoprotein gp120 exhibits multiple carbohydrates (high mannose) together engaging in multivalent interactions with DC-SIGN to enhance the avidity and specificity.[74,75] In addition, the low-affinity multivalent interactions are kinetically labile, allowing for reversibility of a robust biological system.

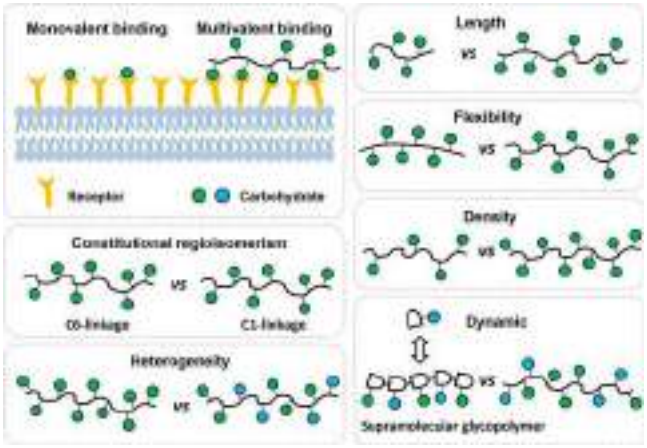


**Figure 5.** Multivalency effect on CPIs with some influencing factors that have been investigated through well-defined glycopolymers.

In synthetic systems, due to advances of organic chemistry and polymerization strategies, glycopolymers and glycodendrimers with precise structures and topologies have been achieved, providing a fertile approach to investigate and mediate the effect of glycan structures in various carbohydrate-mediated interactions. CPIs are typically characterized from a thermodynamic perspective in which the favorable binding enthalpy compensates for an unfavorable entropic contribution.[70,76] Thus, multivalency could be affected by multiple factors, including the flexibility and length of the polymer or glycosidic linkages, the density of the carbohydrate ligands, and the overall architecture of the glycopolymers.[63,70] There are two extreme examples: one with the glycopolymer containing a highly flexible backbone that allows one to easily overcome the conformational entropy loss, and another with the glycopolymer consisting of a stiff linker, well-defined architecture, and orientation that perfectly fits the binding ligand geometry.[77] Despite the wealth of choices of glycopolymers and glycodendrimers, these two examples are yet challenging to achieve, although theoretically supramolecular glycopolymers could provide the opportunity for super flexibility due to the fast monomer−polymer exchange rate.

**2.3.2. Structure−Property Relationship.** As shown in Figure 6, different glycopolymers have been achieved with vinyl, norbornene, carbonate, or peptide as backbone linkages, providing varied yet well-controlled lengths, architectures, and flexibilities. The tunable backbone and linkages between the carbohydrate ring and the backbone enriched the aggregation behavior of the corresponding glycopolymers.[78,79] Moreover, multicomponent copolymerization and postmodification allow for further manipulation of the species and density of the carbohydrate epitopes. Glycodendrimers, on the other hand, already have a well-defined architecture and full surface functionalization. However, the chemistry of the core scaffold and periphery linker should be adjusted to regulate the property.[80]

The flexibility of the backbone/glycosidic linker contributes to the binding affinities toward proteins or lectins through control over the conformational entropy penalty. Besides the extreme example with a rigid and well-defined geometry,

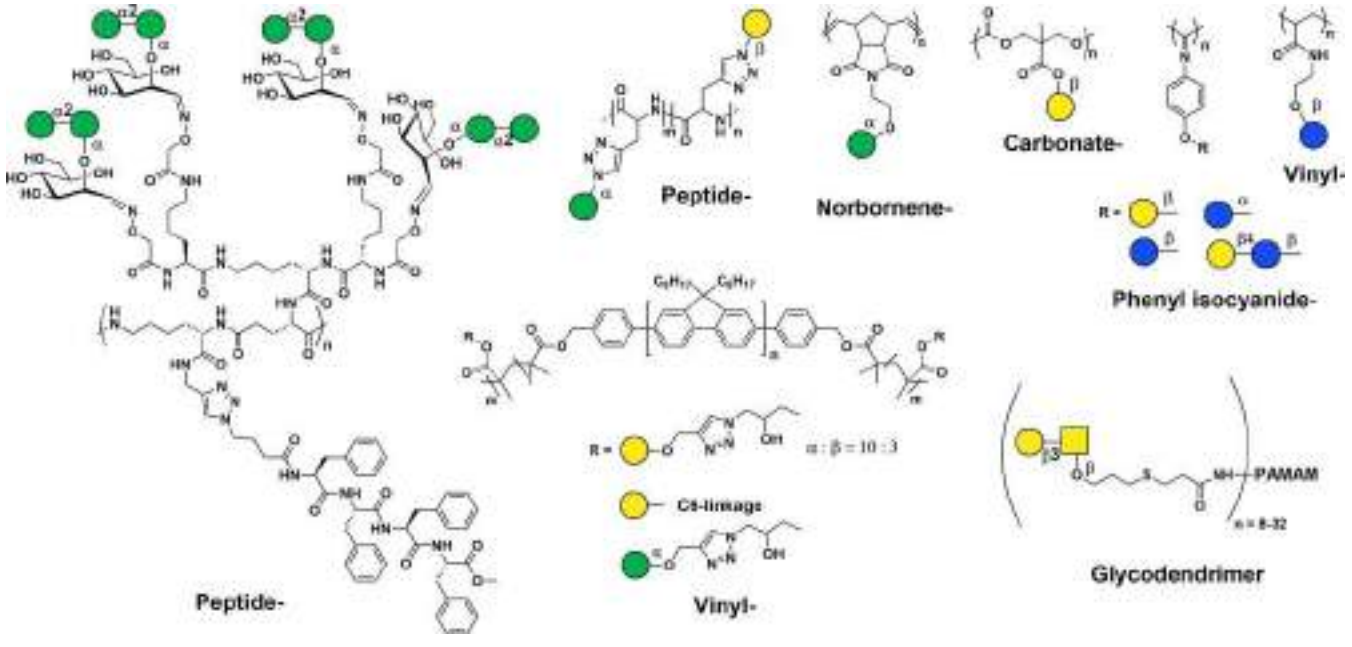


**Figure 6.** Examples of glycopolymers and glycodendrimers discussed in this review.


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moderate flexibility facilitates glycopolymers and glycodendrimers to overcome the entropy penalty, thereby adopting a spatial arrangement that complements the binding protein or lectin. Kobayashi and co-workers reported a series of rigid cylindrical phenyl isocyanide glycopolymers that exhibited little specific interactions with lectins, indicating that the flexibility of the saccharide linker and the polymeric backbone is essential for specific molecular recognition by lectin (Figure 6).[81] However, it is also noted by Becer and co-workers that a flexible poly(methacrylate)-based glycopolymer with a random coil configuration showed faster binding kinetics yet less binding affinity and higher $IC_{50}$ toward DC-SIGN in comparison with the polypeptide-based glycopolymer counterparts in which carbohydrates might be displayed on the polymer backbone surface potentially due to the rather rigid configuration (Figure 6).[82] Thus, the balance between the flexibility of the backbone/glycosidic linker and the origination of the carbohydrate epitopes should be well appreciated.

The length of the glycopolymers has also been extensively investigated toward the protein/lectin binding. It is observed that long glycopolymers tend to show higher binding affinities than short ones owing to the enthalpy gain with multiple binding. In addition, long glycopolymers provide more opportunities to induce supramolecular polymerization/organization of the receptors anchored on the supported lipid bilayer or cell membrane, in other words, to increase the local concentration of CRDs, which could also contribute to a higher binding affinity.[83] The Kiessling group systematically studied the affinities of a series of norbornene-based glycopolymers (carrying Man) with different $DP_n$s toward concanavalin A (ConA). The higher of the $DP_n$, the higher the binding affinity (Figure 6).[84] Haddleton and co-workers also revealed a similar trend with the binding behavior of their methacrylic glycopolymer and ConA.[85] Besides the plant lectin, they also investigated the length effect toward binding with DC-SIGN in which a long glycopolymer showed a higher affinity than short ones.[82,86,87]

The density and heterogeneity of the carbohydrates could be tuned to regulate the binding processes.[88] The Kiessling group produced a family of norbornene-based glycopolymers with similar length, polarity, and steric properties that differ only in their carbohydrate densities (Figure 6). The influence of the epitope density on the formation of Con A clusters was examined, which suggested higher binding affinities with higher carbohydrate densities, although an extremely high sugar density was unfavorable probably due to the high conformational penalty.[89,90] Heterogeneous glycopolymers/dendrimers bearing variable densities of different carbohydrates provide multicomponent presentations, allowing for enhanced affinity and selectivity toward specific receptors. Gibson and co-workers reported that heterogeneous glycopolymers with optimized ratios displayed increased inhibitory activity compared to homogeneous glycopolymers against a RCA120 and the cholera toxin.[91] Another example also showed advances of the heterogeneity: heterogeneous glycopolymers, poly(Man-$\beta$Glc) and poly(Man-$\beta$Gal) with both the binding unit $\alpha$-Man and the nonbinding unit $\beta$-Glc or $\beta$-Gal toward ConA, show higher binding affinities (ca. 5-fold increase) as compared to the counterpart without a nonbinding unit poly(Man-alkyne).[92]

The constitutional regioisomerism effect in the presence of multivalency, including binding ability to lectins and an internalization pathway after cell uptake, was studied in both linear and brush glycopolymer systems.[93,94] Amphiphilic triblock copolymers were prepared consisting of conjugated polyfluorene as the middle block and glycopolymer as the side blocks with Gal-linked through C1 and C6 glycosidic linkages, respectively (Figures 5 and 6). Nanoparticles made by the C1-Gal polymer exhibited a high binding affinity toward peanut agglutinin (PNA) and *Erythrina cristagalli* agglutinin lectins, while nanoparticles made by the C6-Gal polymer did not. Interestingly, although both regioisomers showed a similar binding behavior toward asialoglycoprotein receptor (ASGPR) expressed in Hep G2 cell lines, different internalization pathways of the two nanoparticles were observed. Nanoparticles of C1-Gal went through early endosome, late endosome, and lysosome, while nanoparticles of C6-Gal were only observed at early endosome.

Besides the aforementioned factors, glycomonomer sequences, overall architecture, and self-assembled hierarchical structures could also significantly influence the intrinsic properties of the glycopolymers/glycodendrimers, such as hydrophilicity and binding affinity.

**2.3.3. Carbohydrate−Carbohydrate Interactions.** Carbohydrates can also bind with complementary carbohydrates via CCIs. CCIs show specific biological roles in cell−cell and cell−matrix interactions. A dramatic example is the species interaction between marine sponges, mediated via homotypic binding of glycans on a large cell−surface glycoprotein. Another example is the compaction of the mouse embryo at the morula stage, which seems to be facilitated by a Lewis$^x$−Lewis$^x$ interaction (Figure 7a).[7] The single-site affinity of such

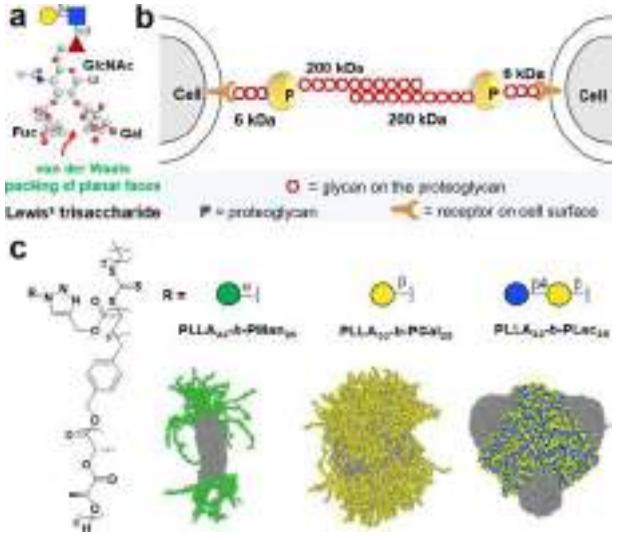


**Figure 7.** (a) Structure of Lewis$^x$ trisaccharide. (b) Illustration of cell−cell recognition through CCIs. (c) Self-assembled structures formed by glycopolymers with different carbohydrates proposed by Brownian Dynamics simulation. Reproduced with permission from ref 99. Copyright 2019 American Chemical Society.

interaction is not strong and is sometimes difficult to measure. However, the interaction can be enhanced by multivalency. If the molecules are present in very high copy numbers on the cell surface, a large number of relatively low-affinity interactions can collaborate to produce a high-avidity "Velcro" effect that is sufficient to mediate biologically relevant interactions (Figure 7b).[95] CCIs are mainly based on van der Waals contacts between the corresponding polyamphi-


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philic surfaces involved in the process. The van der Waals contacts typically include dipole−dipole interactions, London dispersion forces, and hydrogen bonding, which is always stronger than the two former types of interactions. There are many interesting open questions as to how carbohydrates are recognized by complementary carbohydrates.[96] Although current examples are more concentrated on the relatively complex oligosaccharide cases, CCIs have also been found to play important roles in the cases of self-assembled glycopolymers and other related polymers.[97,98] For example, two glycopolymers with a similar postmodification ratio of Man and Gal from the same polylactide backbone showed different self-assembled morphologies. Different CCIs between the Man-containing and the Gal-containing polymer chains were observed through Brownian dynamics simulation (Figure 7c).[99] A similar phenomenon was observed when Lac modification was introduced on the same backbone with a comparable saccharide modification ratio. Moreover, Man/Gal modification on the polycaprolactone (PCL)-containing block copolymer backbone resulted in different crystallization properties, further attributed to the phase separation of the two glycopolymer chains on the mixed-shell micelle surfaces.[98] Besides the above polyester backbones, which may amplify the CCIs with their inherited crystallization properties, strong glycopolymer association in solution was also observed when a trisaccharide with a branched structure was employed. A higher degree of aggregation was induced at elevated temperature owing to the stronger CCIs in comparison to the counterparts with protecting groups on saccharides.[100]

### 2.4. Supramolecular Glycopolymers

Supramolecular materials are emerging as next-generation biomaterials due to the directional, tunable, reversible, noncovalent molecular interactions that leverage the properties originating from the dynamic nature of their constituents.[101] Owing to the dynamic and modular properties of supramolecular assemblies, multiple functional epitopes can be facilely integrated to achieve high complexity and improved biological properties. For instance, the adaptability of the supramolecular polymers, originating from constant monomer−polymer exchange, provides satisfactory binding affinity (Figure 5). However, the dynamic property is a double-edged sword that could also dramatically decrease the mechanical property of the resulting materials. One strategy is to tune the strength of supramolecular interactions and manipulate monomer−polymer exchange kinetics to enhance the mechanical property.

Carbohydrates in supramolecular chemistry were well documented by Seeberger and co-workers focusing on illustrating the role of carbohydrates as stabilizers of complex architectures.[102] We, on the other hand, are more interested in well-defined nanofiber structure (or termed supramolecular glycopolymers) with their dynamic and bioapplication addressed. The discussed supramolecular glycopolymers in this review could be classified into three categories according to the hydrophobic scaffold, namely, peptide, C3-benzene-1,3,5-tricarboxamide (BTA), and aromatic crystalline (Figure 8).

Peptide-based supramolecular glycopolymers have been widely investigated to mimic glycosylated protein function.[102,103] The primary structure of the peptide subunits enables the capability to assemble into defined supramolecular domains, while the carbohydrate periphery provides hydrophilicity and functionality. One of the most remarkable examples of peptide-based supramolecular glycopolymer was developed by the Stupp group.[104] Glycopeptide supramolecular nanofibers with (non)sulfated saccharides displaying on the surface with a high density showed a rather good binding ability and bioactivity toward multiple proteins in vivo. At the molecular level, as shown in Figure 8, the short spacer separating the monosaccharide from the peptide sequence allows for the display of a functional monosaccharide on the nanostructure surface. The surface display together with the intrinsic dynamic property of the supramolecular polymer enable the adaptive configurations, providing good accessibility toward different proteins. Various biologically important heparin-binding growth factors could efficiently bind with these nanofibers as measured by surface plasmon resonance spectroscopy. Meanwhile, the filamentous shape was maintained, as confirmed by confocal fluorescence imaging, showing a strong colocalization of the protein along the fibrous nanostructures. Their biological properties were studied in detail and will be introduced in section 4. Noteworthy, such glycopeptide supramolecular nanofibers have shown potential scalability and great promise for the clinical translation.

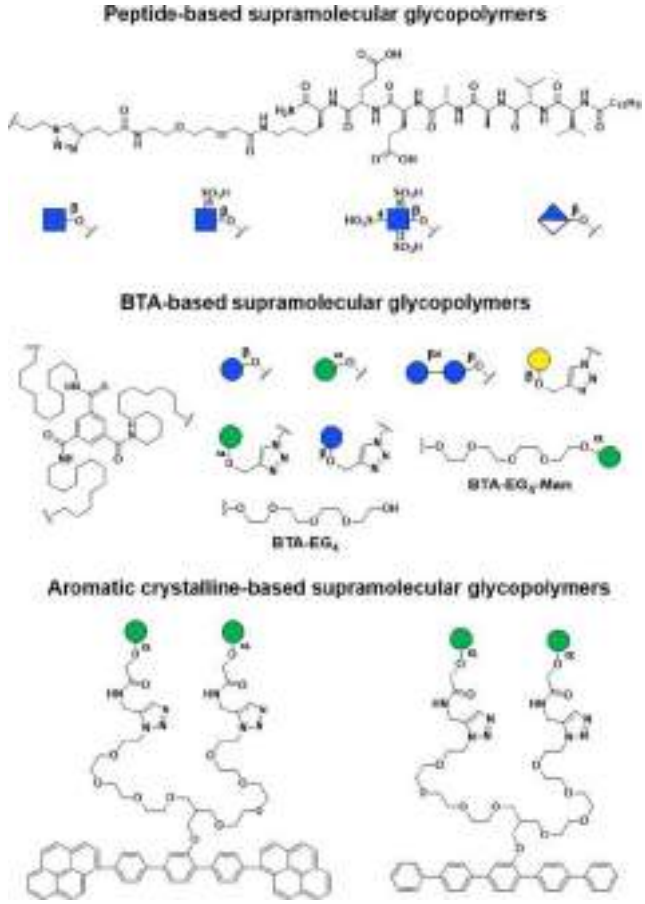


**Figure 8.** Examples of supramolecular glycopolymers discussed in this review.

The Meijer group systematically explored the BTA system, revealing the structure−dynamic−property relationship on supramolecular (co)polymerization in water.[105−108] The BTA molecule normally consists of a benzene core, three amide groups that could form 3-fold intermolecular hydrogen bonding, aliphatic chains, and a hydrophilic periphery (a carbohydrate with or without tetraethylene glycol to tune the


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hydrophobic/hydrophilic ratio). Unlike the peptide-based glycopolymer, BTA glycopolymers preferentially show higher dynamic properties yet also allow for control over supramolecular copolymerization.[109] BTA-$EG_4$-Man could only form small micelles, while BTA-$EG_4$ could polymerize into micrometer-long 1D nanofibers (Figure 8). Upon copolymerization of BTA-$EG_4$-Man with BTA-$EG_4$, a much slower monomer−polymer exchange was observed in comparison with the polymerization of individual monomers, suggesting a stabilization effect of copolymerization. In comparison, monomer−polymer exchange of an aromatic crystalline-based glycopolymer was significantly slowed down due to the strong $\pi-\pi$ stacking and hydrophobic interaction (Figure 8). Along with the dynamic property of the hydrophobic core, the multiple hydroxyl groups of the carbohydrates allow for extensive hydrogen-bonding interactions, providing supramolecular interactions to fabricate dynamic and self-healing materials.[109]

## 2.5. Synthetic Glycolipids and Glycoproteins

Besides the typical carbohydrate-based polymeric structures, in this review, we also include synthetic glycolipids and glycoproteins. Glycolipids and glycoproteins are important glycoconjugates in Nature, which provide the blueprint for the design and synthesis of glycopolymers and other related polymeric structures. They also fall into the macromolecular scale with high molecular weights. Sometimes, homopolymeric or heteropolymeric saccharide chains could be found in these structures. Moreover, the glycolipid assemblies are widely investigated as immune adjuvants and antigens, which even fit the requirements of "carbohydrate-based macromolecular materials" better than the glycolipid molecule itself. In this review, we expand the "traditional" scope of carbohydrate biomaterials. Inclusion of the synthetic glycolipids and glycoproteins as information materials, mainly with immunological functions, will be important for this extension. Currently, the synthesis of glycolipids and glycoproteins is still developing quite rapidly with significant achievements in automated synthesis of saccharides through enzymatic, chemical, and chemoenzymatic methods, which is the general trend for preparation and further research of carbohydrates.[110−113]

**2.5.1. Glycolipids.** Glycolipids, one of the most popular biosurfactants, widely exist in plants and cell membranes. As amphiphilic compounds, they can self-assemble into different architectures.[114] CCIs and hydrophobic interactions of lipid chains serve as driving forces for glycolipid self-assembly, while other factors, including the length and number of alkyl chains, degree of unsaturation, position and number of double chain, introduction of additional functional groups (e.g., −COOH), as well as the linker connecting the carbohydrate moiety and the lipid chain, show essential effects on determining the morphologies of the assemblies.[102] Thus, systematic investigation on the self-assembly of glycolipids not only contributes to in-depth understanding of naturally occurring phenomena but also promotes their potential in many applications.

Due to the limited sources and microheterogeneity of naturally afforded glycolipids, synthetic glycolipids are pursued for investigation in multiple fields. First, glycolipids could be used to mimic a biological membrane.[115] Brea and co-workers reported Gal-derived single-chain glycolipid oleoyl $\beta$-D-1-thiogalactopyarnose, which can self-assemble into highly stable artificial cells (Figure 9a).[116] Notably, the artificial cells are

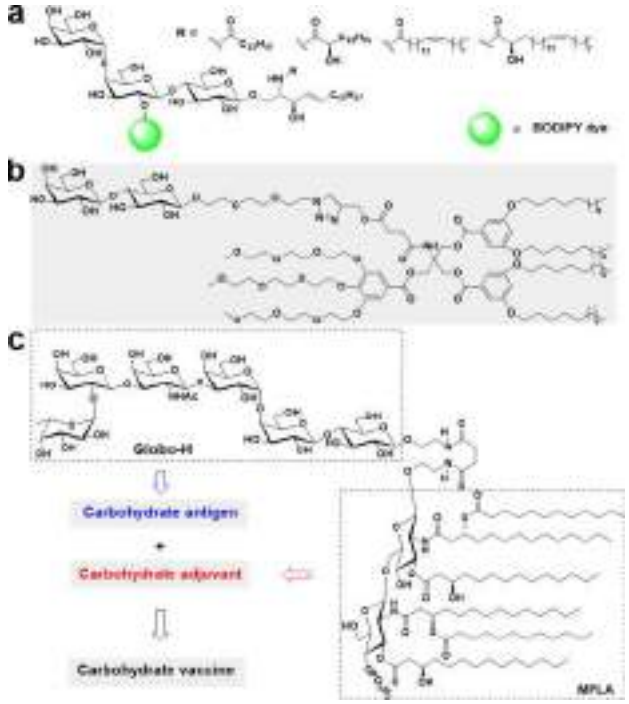


**Figure 9.** Representative structures of glycolipids. (a) Chemical structure of Gal-derived single-chain glycolipid Oleoyl $\beta$-D-1-thiogalactopyarnose. (b) Representative amphiphilic Janus glycodendrimer. (c) MPLA-Globo-H conjugate.

compatible with biomolecules. They not only allow for incorporation of functional transmembrane proteins but also function as microreactors to realize rolling circle amplification of DNA.

Furthermore, multivalent glycoliposomes and micelles have been widely used to investigate CPIs and CCIs.[117] Amphiphilic Janus glycodendrimers (Figure 9b) have been explored by the Percec group with the self-assembled vesicles serving as a superior platform for lectin recognition. Related works have been detailed in a previous review.[115] Considering that the structure of the alkyl chain may largely affect the membrane organization and protein internalization, Sibold et al. prepared various $Gb_3$ glycosphingolipids labeled with a BODIPY fluorophore.[118] Liquid-ordered ($l_o$) and liquid-disordered ($l_d$) giant unilamellar vesicles (GUVs) were afforded when the resulting glycosphingolipids were coassembled with other components. $Gb_3$ with a saturated $C_{24:0}$ fatty acid mostly distributes in the $l_o$ phase, while the unsaturated $C_{24:1}$ fatty acid mainly locates in the $l_d$ phase, and the Shiga toxin B subunits exclusively bind to $Gb_3$ in the $l_o$ phase of the GUVs. Moreover, utilizing the interaction between glycolipids and some cells and tissues, colloids (such as liposomes or micelles) formed by glycolipids, and other components can be also used in targeted drug delivery.[119]

Moreover, glycolipids play a central role in immunology. Some naturally derived and synthetic glycolipids, such as monophosphoryl lipid A, i.e., MPLA (Figure 9c), have been used as adjuvants in immunological research. Meanwhile, some tumor-associated carbohydrate antigens (TACAs), such as Globo-H (Figure 9c), are presented in glycolipid form. For this reason, fully synthetic self-adjuvanting TACAs and the mixture of carbohydrate antigen and adjuvant part in glycolipid form have also been prepared into glycoliposomes and used in


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vaccine development. Detailed information will be introduced in section 5.

**2.5.2. Glycoproteins.** Glycoproteins produced by post-translational modification of proteins play an important role in various biological processes. Molecules such as monoclonal antibodies, lectins, immunoglobulins, some enzymes, and hormones exert their function in the form of glycoprotein. Generally, protein glycosylation contributes not only to the improved protein stability featuring a longer serum half-life and better resistance to proteolysis but also to the biological functions of proteins.[120,121]

Natural glycoproteins can be divided into *O*-linked glycoproteins and *N*-linked glycoproteins. In the former type, glycans are conjugated to hydroxyl groups of amino acid (serine, threonine, or tyrosine) residues, while the latter are glycoproteins with glycans attached to amide side chain of asparagine.[122] For example, mucins are highly glycosylated proteins that are abundant in the mucus of humans and animals. They play important physiological roles, such as cell adhesion and signaling, immune response, and so on.[123] The overexpression of mucins and aberrant glycosylation are related with the development and progression of cancer. Among the mucin family, MUC1, a type I transmembrane glycoprotein, has been most investigated. It could be used as the biomarker of breast, prostate, and lung cancers. Therefore, MUC1 is an attractive target for the investigation and development of a cancer vaccine.

Similar to all of the other glycoconjugates, the natural glycoproteins are also heterogeneous. To better understand the functions of glycoproteins, artificial glycoproteins have been prepared through different methods, including the use of engineered cell lines or recombinant enzymes, solid-phase peptide synthesis (SPPS), as well as native chemical ligation.[121] Glycoproteins have been explored as scaffolds for targeted drug therapies. Due to the high expression of some lectins on diseased organs or cancer cells, therapeutic drugs which are covalently linked on the glycoprotein through a biodegradable linker are delivered to target cells or tissues, taking advantage of the specific recognition between lectins and glycans.[120]

In addition, artificial glycoproteins have been used to modulate immune response. For example, P-selectin glycoprotein ligand 1 (PSGL-1) could be chemoenzymatically synthesized. Binding of PSGL-1 and its analogues to P-selectin may interrupt the immune cascade induced by interaction between P-selectin and sialyl Lewis$^X$ tetrasaccharide (SLe$^X$).[124] Moreover, artificial glycoproteins could be used as vaccines in which the carrier protein with Th-cells epitopes could convert T-cell-independent immune response induced only by carbohydrate antigens to a T-cell-dependent pathway.

## 3. THERAPEUTIC AND DIAGNOSTIC DELIVERY SYSTEMS

Carbohydrate-based macromolecular materials have been widely used as promising carriers for safe and efficient delivery of drugs, genes, proteins, and imaging agents, etc., in diagnostic and therapeutic systems. Biomaterials made from polysaccharides and other carbohydrate-based macromolecules have been reviewed frequently;[30,67,68,125−127] thus, we will only focus on the recent developments with emphasis on the functions achieved by the materials. Because of the matter and information roles of carbohydrates, the biomaterials included in this section are divided into three categories according to the different roles of the carbohydrate-based macromolecules, i.e., as a delivery vehicle or hydrogel, as a stabilizing shell of the vehicle, and as targeting agents for protein binding.

### 3.1. Delivery Biomaterials with Carbohydrate-Based Macromolecules as the Skeleton

Polysaccharides and other carbohydrate-based macromolecules have been widely used as delivery nanoobjects and materials because of their biocompatibility and tailorability. Some naturally occurring polysaccharides, such as chitosan, alginate, and cellulose, have been widely used as model polymers to make such biomaterials. The advantages of employing carbohydrate-based macromolecules as scaffolds include but are not limited to their ease of modification, stiffness of the relative rigid backbone, degradability, etc. Moreover, owing to the inherent stiffness and tailorability from the hydroxyl and other functional groups, the steric properties of (synthetic)-polysaccharides could be tuned for modulating the pharmacokinetics of guest drug molecules. Many reviews on this topic can be found in journals and books.[30,128−130] In this section, we will summarize some representative examples focusing on the properties brought about by the carbohydrate polymer backbones, which provide beneficial interactions with guest molecules.

Cationic chitosan has been widely used in gene delivery systems, which could benefit from the bulky and tailorability properties of the polysaccharides. Gene therapy is a technique that has started to deliver results in clinical trials.[131,132] The recent development of CRISPR-Cas9 technology, allowing for genome editing to target disease-related genes in patients, further opened a broad avenue for gene therapy.[133] Cationic chitosan is capable of forming rather stable polyelectrolyte complexes with anionic genetic material (plasmid DNA, miRNA, and siRNA, etc.) through electrostatic interactions, making it an attractive nonviral vector, given its biocompatibility, biodegradability, reduced immunogenicity, and immunotoxicity.[30,31,134] The chitosan is conceived to stabilize the genetic materials, protect it from degradation, promote cellular uptake, and unpack the genetic material. However, compared with the viral vector, low transfection efficacy is the main obstacle. Therefore, systematic studies have been performed to investigate the effects of the structural characteristics, e.g., the degree of acetylation and molecular weight, toward the physicochemical/biophysical properties of resulting vehicles in MCF-7 breast cancer cells, providing a golden rule for rational design.[135] After screening, an ideal complex formulation with a molecular weight of 40 kDa, a degree of acetylation of 12%, a (±) charge ratio of 1.5, and an averaged diameter of less than 190 nm, showed identical transfection efficiency with the commercial positive controls (DharmaFECT and Novafect O 25). These nanocomplexes could successfully downregulate the target mRNA expression in MCF-7 cells, offering a promising nonviral vehicle for breast cancer gene therapy. Apart from tuning the molecular weight and acetylation degree, functionalization could also provide better transfection efficacy.[136−138] For instance, as shown in Figure 10, enhanced stiffness and bulkiness of chitosan were induced by a high acetylation degree (80%). An acid-responsive siRNA delivery system was achieved by conjugating flexible, aqueous-soluble aminoethoxy branches to the modified chitosan via acid-cleavable ketal linkages.[138] The resulting acid-transforming chitosan showed greatly enhanced aqueous solubility and improved siRNA complexation. Notably, the mildly acidic endosome/lysosome environment


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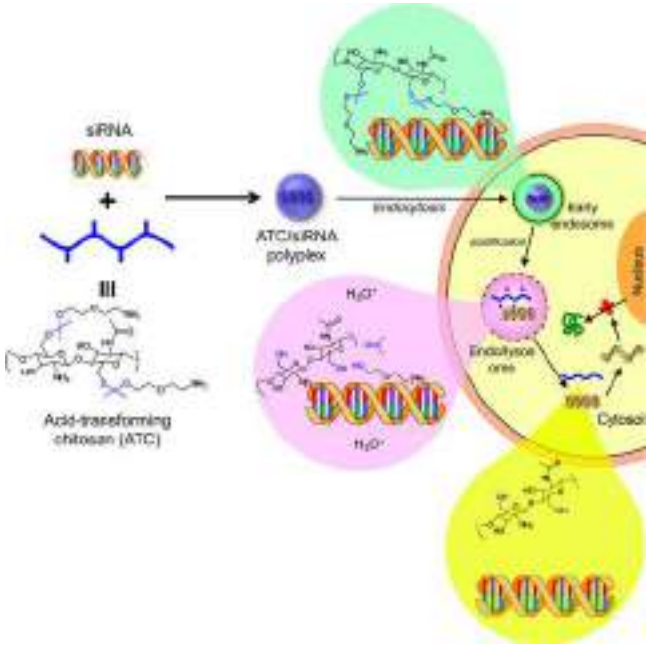


**Figure 10.** Acid-transforming chitosan/siRNA polyplex preparation, cellular uptake, acid transformation, siRNA release into the cytoplasm, and gene silencing. Adapted with permission from ref 138. Copyright 2018 American Chemical Society.

could effectively trigger the hydrolysis of ketal linkages, resulting in the formation of chitosan polymers with low solubility and a bulky structure, thus reducing molecular interaction with siRNA and cooperatively promoting the cytosolic release of siRNA.

Cationic chitosan, modified with stearic acid and blood−brain barrier (BBB) crossing peptide (TGN), can also be employed in drug delivery systems. Such modified chitosan can be self-assembled into micelles, which showed great potential for enhancing drug delivery performance crossing the BBB.[139] The physicochemical property remained almost unchanged upon loading of model drug (curcumin). Sustainable release was observed owing to the steric backbone structure. Following a burst release of curcumin in the first 16 h, a steady and controlled release over the next 56 h was observed. Further in vivo study revealed the TGN peptide-mediated entry into the rat brain within 1 h post injection, demonstrating the system as a potential therapeutic cargo for crossing the BBB.

Synthetic PGCs developed by the Wooley group were also employed as drug delivery nanocarriers owing to their biocompatibility, degradability, functionality, and especially the backbone steric bulky property, allowing for the finely tuned drug release kinetics (Figure 11).[49] Cation- and PEG-modified PGCs were coassembled with a redox-responsive prodrug (diPTX), yielding nanocarriers that show preferential drug release in a cancer cell line (SJSA-1, with an $IC_{50}$ value of ca. 0.14 $\mu$M) with high GSH concentrations in comparison with the healthy cell line (MC3T3, with an $IC_{50}$ value of ca. 5 $\mu$M). The enhanced selectivity and the following sustained release were attributed to the bulky property of the hydrophobic saccharide backbone, enabling increased containment of the prodrug and sustained release of the free drug PTX with high GSH concentrations. The lung bioluminescence signal of osteosarcoma (OS) lung metastases mice with

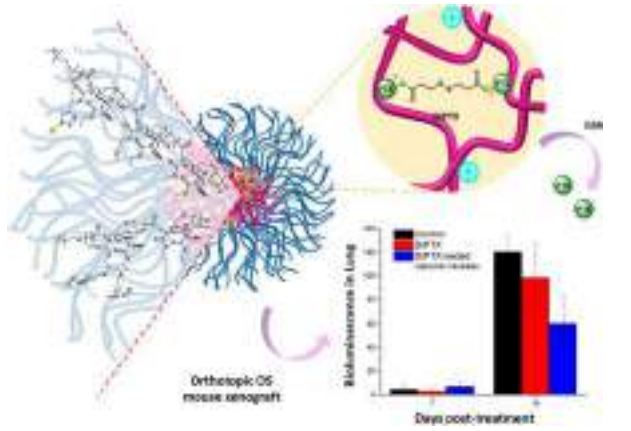


**Figure 11.** Structure of the coassembled PGC-based nanocarrier with diPTX. Redox-responsive drug release enables treatment of orthotopic OS mouse xenograft with a significant reduction in tumor progression in the mice lungs treated with diPTX@CPGC but not with the free diPTX prodrug. Adapted with permission from ref 49. Copyright 2018 American Chemical Society.

nanocarriers treatment was significantly lower than that of the PBS controls, showing their promise as optimized anticancer therapeutic agents in treating OS lung metastases.

Inorganic nanoparticles/imaging agents could also be loaded into the polysaccharides-based systems due to specific tailorability. Jia and co-workers simultaneously loaded both a silver nanoparticle (AgNP) and $^{99m}$Tc into a HA platform for X-ray computed tomography and single-photon emission computed tomography (SPECT) imaging.[140] HA, possessing a large number of negatively charged carboxyl groups, could interact with $Ag^+$ ions and serve as a stabilizer for AgNP complexes upon addition of the reducing agent ($NaBH_4$). The ultrasmall AgNP ($D_h$ = 13.5 nm as measured by dynamic light scattering) showed linear X-ray attenuation as a function of concentration, indicating promise for CT imaging. After conjugating the chelator 6-hydrazinonicotinyl and radiolabeling with $^{99m}$Tc, the resulting $^{99m}$Tc-HA-AgNPs could be used as a radiotracer for SPECT imaging. Given the negligible cytotoxicity and prolonged stability at the tested concentration as well as the ultrasmall size, allowing for the nonspecific accumulation in tumors through the enhanced permeability and retention effect,[141] the $^{99m}$Tc-HA-AgNPs could serve to evaluate the biodistribution in vivo of a tumor model and to develop imaging guided therapeutic system.

Carbohydrate-based macromolecules contribute to the scaffold of not only delivery vehicles but also macroscopic materials, such as hydrogels.[130,142] As depicted in Figure 12, gastric resident drug delivery systems based on triggerable tough hydrogels (TTHs) were fabricated with the alginate scaffold given the well-recognized biocompatibility and excellent mechanical and stimuli-responsive properties.[143] TTHs consisted of orthogonal double networks of alginate and polyacrylamide, cross-linked by ionic $Ca^{2+}$ and disulfide bonds, respectively. Noteworthy, alginate was cross-linked by divalent $Ca^{2+}$ cations through associating with carboxylic groups from different alginate chains, affording an ionically cross-linked network. Thus, TTHs can be de-cross-linked and eventually dissolved into solution by biocompatible chelators and reducing agents. The resulting TTH showed exciting properties, including significant dehydration and rehydration, long-term residence in the stomach of a large animal model


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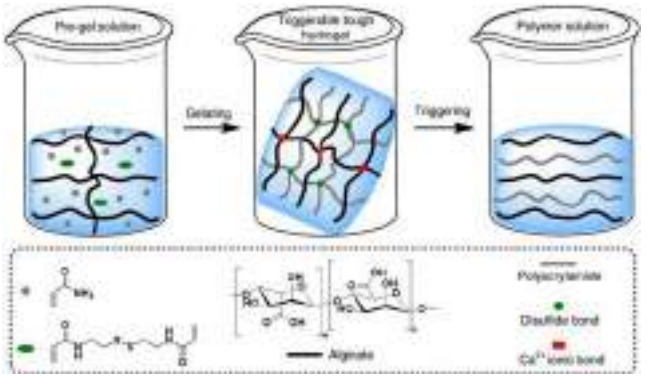

**Figure 12.** TTH dosage form concept and synthesis design. Adapted with permission from ref 143. Copyright 2017 Springer Nature under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

(pig), remarkable triggerable properties with on-demand dissolution, and controlled drug release profiles. With further safety studies and stress tests, not limited to drug delivery, more applications could be applied to, for instance, bariatric interventions and tissue engineering.

### 3.2. Delivery Nanocarriers with Carbohydrate-Based Macromolecules as the Hydrophilic Shell

Polysaccharides are hydrophilic and thus could be used as stabilizers for delivery nanocarriers in biological systems. For instance, in a human trial, HA was used as a stabilizer for generating micronutrient particles, which showed high heat stability and rapid gastric acid-stimulated dissolution for oral micronutrient delivery.[144] In addition, some polysaccharides/glycopolymers provide prolonged in vivo circulation duration, as it has been reported that glycosylated nanoparticles showed excellent stability in fetal bovine serum and minimal interactions with the serum proteins.[145] More examples of delivery system for drug, gene, imaging agents, etc., will be given. Compared with section 3.1, in which the interactions between delivery carriers and guest molecules are more important, sections 3.2 and 3.3 will address the nonspecific or specific interactions between the delivery carrier and cells to gain much insight into the structure−property relationships. Moreover, some strategies will be introduced, like polymerization-induced self-assembly (PISA), which significantly simplified the fabrication process of nanocarriers, allowing for facile scale-up in clinical use.[146,147]

The hydrophilic property of glycopolymers/polysaccharides was mostly utilized in delivery systems. Along with other responsive or functional motifs, a smart delivery system could be achieved. Degradable and biocompatible Gal-modified amphiphilic block copolymer was conjugated with doxorubicin via acid-labile Schiff base linkages, showing pH-triggered drug release.[148] Glc-modified photoresponsive amphiphilic copolymer could coassemble with hydrophobic Nile red dye into micelles, showing UV-stimulated controlled release.[149] Poly-D-glucosamine was used to stabilize magnetite nanoparticles for hyperthermia treatment.[150] D-Glucosamine could also be modified onto the miniemulsion periphery through post-polymerization reaction to stabilize the nanoparticle.[151] The fabricated NPs consisting of disulfide bonds showed glutathione-responsive release of the encapsulated drug (gemcitabine). In vitro study confirmed an enhanced cytotoxicity by 2-fold in pancreatic cancer cells (AsPC-1) owing to sustained cellular release of gemcitabine.

Most of the aforementioned materials were employed for in vitro studies. However, for in vivo and clinical cases, the protein absorption in blood circulation should be circumvented to gain prolonged blood circulation duration and less nonspecific accumulation. Although some of the glycopolymers/polysaccharides feature excellent antifouling behavior, there are still plenty of parameters to play with, e.g., geometry, size, molecular orientation on the cell surface, roughness, and topography.[152−154] To date, not all of the factors have been well understood, even with some conflicting examples, like the geometry effect. Stenzel and co-workers explored the roughness and surface topography effect toward protein absorption in a serum.[145,155] As shown in Figure 13, compartmentalized

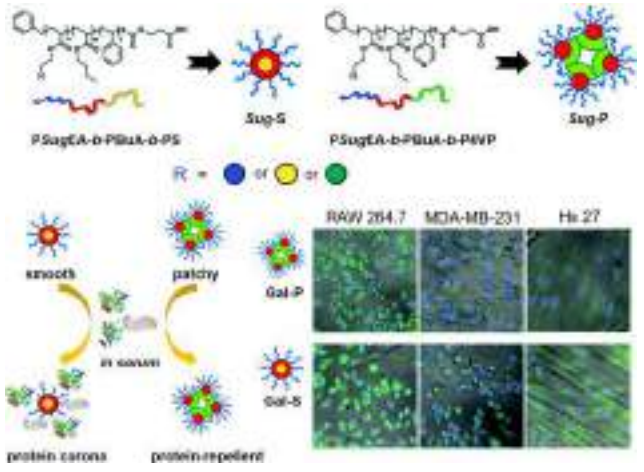

**Figure 13.** Schematic illustration of the formation of smooth and patchy nanocarriers (above) with representative confocal images showing internalization of Gal-based nanoparticles after 24 h of incubation (below). Reproduced with permission from ref 145. Copyright 2019 Royal Society of Chemistry.

spherical nanoparticles were prepared from a set of linear ABC triblock glycopolymers with Glc, Man, or Gal patches, allowing for combined evaluation of the surface topography and functionality. As controls, spherical smooth nanoparticles with the same glycosylated surface were obtained. The patchy nanoparticles displayed significantly reduced serum protein absorption therefore lowered nonspecific uptake by different cell lines, including macrophages (RAW264.7), breast cancer cells (MDA-MB-231), and fibroblasts (Hs27). Moreover, the carbohydrate type influenced the relative protein abundance on the corona of the smooth nanoparticles, with Man and Gal showing more profound absorption.

Gene delivery requires similar parameter optimization. The Reineke group made a great contribution in the fundamental study to advance carbohydrate-based polymeric gene delivery and therapy. A very recent review by Reineke et al. is highly recommended for detailed information.[156] Briefly, two species of cationic glyco-copolymers, synthesized by step growth or radical polymerization with carbohydrates in the backbone or side chain, respectively, were used for complexation with nucleic acids. The storage, gene transfection efficacy, and biodistribution were investigated to find the effects of polymer length,[157] sequence,[158] surface charge,[41,159,160] carbohydrate size,[157] end group functionalization,[161] cell type,[160,162] etc., in great detail. The well-established structure−property relationships provide golden rules for the development of polymer-based gene delivery vehicles that can also be used for nonviral


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gene therapies. For instance, trehalose-based cationic copolymers facilitated colloidal stability and biocompatibility. The yielded plasmid/siRNA polyplexes that resist aggregation upon lyophilization and reconstitution in water together with enhanced transfection promoted high gene expression following lyophilization and reconstitution.[156]

Alongside the insight of the fundamental study of the structure−property relationship, advanced nanotechnology to engineer the desired delivery vehicles is required. Examples include 3D printing, an emulsion template, and PISA. Among them, PISA allows for facile scale-up in clinical use. Taking advantages of the chain-end reactivity of solvophilic macromolecules for the polymerization of a second monomer, PISA advances the formation of the nanosized structures, allowing for a much higher nano-objects concentration (up to 50 wt %).[146,147] Carbohydrate-based nano-objects (spherical micelles, wormlike micelles, and vesicles, etc.) could also be generated through PISA with glycopolymer/polysaccharides serving as either a hydrophilic linear macroinitiator[163] or a bottlebrush macroinitiator[164,165] and eventually as a stabilizer. Armes and co-workers developed a family of novel Gal-modified diblock copolymer nano-objects through RAFT using PISA in a concentrated aqueous solution (Figure 14).[163]

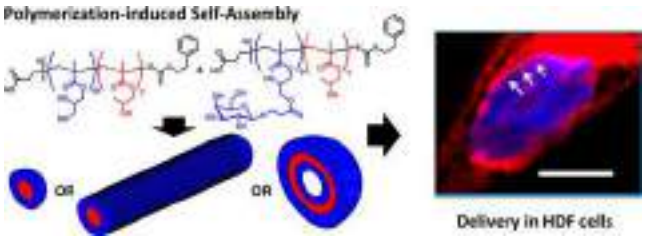


**Figure 14.** PISA with polyGal serving as the hydrophilic stabilizer, and confocal microscopy image showing effective intracellular delivery of RhB, which is selectively staining the nuclear membrane. Adapted with permission from ref 163. Copyright 2013 American Chemical Society.

Among them, Gal-modified vesicles could be rapidly taken up by primary human dermal fibroblasts with negligible cytotoxicity. Preliminary investigation with encapsulated rhodamine B (RhB) octadecyl ester indicated intracellular delivery ability, leading to new opportunities for (targeted) drug delivery. Polysaccharides could also be employed as initiators. Starting from dextran—a natural hydrophilic steric stabilizer backbone—photoinitiated PISA was performed, resulting in a broad set of glyco-nanostructures that could be used for drug/gene delivery or medical imaging.[164]

### 3.3. Delivery Vehicles with Carbohydrates as Targeting Agent

**3.3.1. GLUT5 and Breast Cancer.** Glucose transporter-5 (GLUT5) is a fructose (Fru) transporter allowing for Fru to be transported. GLUT5 is revealed to be highly expressed in human breast cancer but absent in normal human breast tissue. This phenomenon enables early diagnosis and treatment of breast cancer using Fru as the targeting ligand.[166] Due to the multivalency effect, Fru-based glycopolymers are intensively employed. Gottschaldt and co-workers modified the D-Fru moiety onto a high molar mass linear poly(ethylene imine) for targeted delivery of genetic material, which displayed increased specific uptake for triple-negative MDA-MB-231 breast cancer cells.[167] In comparison, the Man-, Glc-, and Gal-based glycopolymer counterparts showed much less uptake.[168]

Although the presence of the bioactive group, i.e., Fru, is sufficient to achieve bioactivity, the eventual in vitro uptake and in vivo circulation of nanocarriers are significantly affected by a number of parameters, such as nanocarrier size, morphology, stiffness, surface chemistry (glycopolymer length and orientation), and presence of drug, etc. Stenzel and co-workers developed a series of Fru-based nanocarriers and exploited the effects of the following factors in great detail.[169−180] (a) The length of the glycopolymer: Three block copolymers, with different lengths of Fru chain were synthesized and self-assembled into micelles with relatively similar sizes (128−160 nm). The polymers with a longer Fru chain length always provided higher cellular uptake and better penetration in multicellular tumor spheroids. SAXS analyses revealed a large water content on the outer shell in the long glycopolymer micelles, providing better mobility to facilitate interaction with receptors. Moreover, the in vivo study revealed that micelles with a long Fru chain length showed reduced clearance by a mononuclear phagocyte system (Figure 15).[171] (b) Fru concentration and surface arrangement:

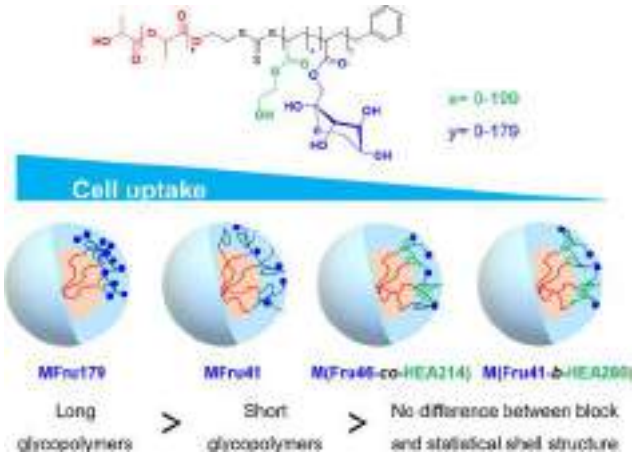


**Figure 15.** Illustration of the effects including the length of glycopolymer, saccharides concentration, and surface arrangement toward cell uptake. Reproduced with permission from ref 178. Copyright 2019 American Chemical Society.

Polymers with a high density of Fru in addition to a long Fru-containing hydrophilic block promoted high uptake by breast cancer cells. However, the blocky or statistical arrangement did not show a significant difference toward uptake efficacy (Figure 15).[178] (c) Morphology and aspect ratio: There is so far no clear trend on the morphology, especially the spherical and nonspherical nanoparticles. However, some preliminary result on aspect ratio of rod-like micelles revealed the following trend: short rods (ca. 500 nm) were more easily internalized by cells than the medium (ca. 1000 nm) and long ones (ca. 2000 nm). Yet, no significant difference was observed between medium and long ones, regardless of correlated length discrepancy.[170] (d) Stiffness: With a specific aspect ratio, the stiff rod-like micelles were more easily uptaken by cells with much deeper penetration in comparison with their soft counterparts. The hypothesized cellular uptake mechanism was proposed as the balance between the membrane bending energy and the stretching energy. With the stiff rods, the membrane bending energy


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dominates and nanoparticles will be internalized at a perpendicular entry angle, whereas in the case of flexible rods, the membrane stretching energy dominates and nanoparticles eventually adhere to the membrane surface.[174] (e) Presence of drug: The drug-loading process combined with its location can significantly influence the physicochemical property and the resulting biological activity. Encapsulation of a hydrophobic drug changes the hydrophilic−hydrophobic balance, resulting in a size increase or even morphology switches.[172,177,179,180] Upon loading curcumin, the morphology of poly(1-*O*-MAFru)$_{36}$-*b*-PMMA$_{192}$ was transformed from cylindrical micelles to vesicles and the cellular uptake decreased with a larger loading amount. SANS/SAXS revealed that the drug was located in the shell, leading to dehydration of the shell and subsequently reducing the cellular uptake. More influencing factors and the collaborative effects toward physiological activity need to be further investigated.

**3.3.2. GLUT1 for Crossing BBB or Tumor Targeting.** Glucose transporter-1 (GLUT1), highly expressed in brain capillary endothelial cells, could be potentially employed for enhancing the delivery of the carriers across the BBB to achieve accurate brain tumor diagnosis and satisfactory therapeutic effects.[181] Moreover, GLUT1 is also abundantly expressed in most of the tumors and present on vascular endothelial cells, enabling the targeting delivery with improved delivery efficiency and therapeutic efficacy.[182]

GLUT1 facilitates bidirectional diffusion of Glc molecules along the concentration gradient, promoting Glc-modified carriers to overcome the BBB. Glc-integrated liposome formulations have been reported to target GLUT1 for brain delivery yet with a low transportation level.[184] Kataoka et al. achieved boosted BBB crossing and brain accumulation of Glc-modified nanocarriers through rapid glycemic control.[183] The size and Glc density of the nanocarriers were precisely controlled by multimolecular association of oppositely charged pairs of PEG-based block ionomers (Figure 16). Noteworthy, the C6 linkage of the Glc ensured the specific interaction with GLUT1. The resulting nanocarrier Glc(6)/m was ca. 30 nm, and the Glc density was tuned as 0%, 10%, 25%, and 50%. In vivo study with glycemic control showed a 56-fold higher brain accumulation of a 25% Glc(6)/m formulation (up to 6% dose/g-brain) compared to the free-feeding mice, suggesting that BBB crossing was dependent on blood Glc concentration and promoted by GLUT1.

Besides Glc, GLUT1 also transports substances with similar structures, such as 2-deoxyglucose, 3-*O*-methyl Glc, Gal, Man, and other Glc analogues.[185] Dual-targeting daunorubicin liposomes with both *p*-aminophenyl-*α*-D-mannopyranoside and transferrin conjugations successfully crossed the BBB for targeting brain glioma with improved therapeutic efficacy.[186] Doxorubicin-loaded cationic albumin was modified with Man and showed greater accumulation in brain glioma compared with the bare albumin.[187] Paclitaxel-loaded liposomes with both Man−vitamin E derivative and dequalinium modification was developed, providing an efficient strategy for treating invasive brain glioma.[188]

Due to the overexpression of GLUT1 in most of the tumors, Kataoka and co-workers developed Glc-modified nanocarriers for targeting the cancer stem cells (CSCs).[182,189] CSCs represent a small subset of tumor cells, which are highly involved in drug resistance, distant metastasis, due to their self-renewal ability and multilineage differentiation capacity.[190] Due to the active Glc metabolism in CSCs, GLUT1 is

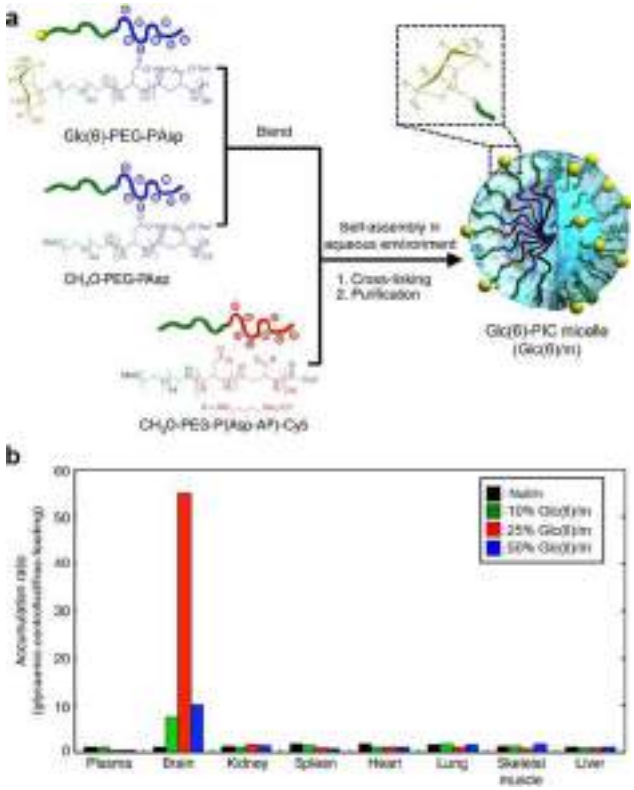


**Figure 16.** (a) Scheme of Glc(6)-conjugated PIC micelle (Glc(6)/m) preparation via the assembly of oppositely charged block copolymers. (b) Accumulation ratio in mice (glycemic-controlled/free feeding) of each micelle at 48 h calculated from the biodistribution values. Adapted with permission from ref 183. Copyright 2017 Springer Nature under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

promising for CSC-targeted treatments with Glc-based nanomedicine.[191] As shown in Figure 17, Glc installed PEG-*block*-cationic polymer complexed with siRNA followed by decoration onto a 20 nm AuNP through Au−S coordination to afford monodispersed Glc-NP. The Glc-NPs displayed enhanced gene silencing activity and improved antitumor efficacy in the MDA-MB-231 orthotopic tumor via intravenous administration compared with the control NPs without Glc modification. It is particularly noted that the Glc-NPs were able to efficiently reduce the CSC proportion in the orthotopic tumors. With the similar strategy, the Kataoka group constructed Glc-modified nanocarriers loaded with cisplatin, which showed selective accumulation in tumors through targeting the GLUT1 on the tumor vasculature, enhancing antitumor activity.[189]

**3.3.3. ASGPR and Liver Cancer.** ASGPR, abundantly and predominantly expressed at the surface of hepatic (liver) cells, is a hetero-oligomer consisting of two homologous transmembrane proteins with a $Ca^{2+}$-dependent CRD that interacts with Gal, GalNAc, and related galactosides.[193,194] Once ASGPR binds the targeted ligands, the complexes can be internalized by ASGPR-mediated endocytosis; thus, Gal moieties can be used as targeted ligands for diagnosis and therapy with, e.g., hepatocellular carcinoma (HepG2), hepatitis, and malaria.[195−199] To regulate the delivery system, parameters such as nanocarrier size, surface carbohydrate density, patterning, and material flexibility were systematically explored.[199]


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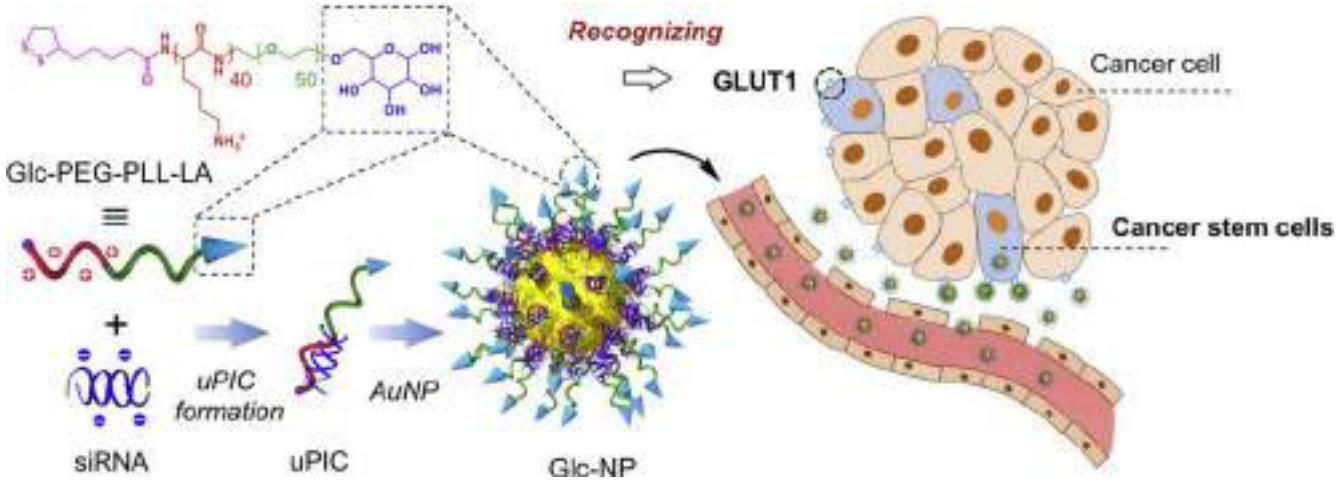


**Figure 17.** Schematic illustration of Glc-NP preparation from Glc-PEG-PLL-LA, siRNA, and AuNP combined with the selective recognition by the cancer stem cells through GLUT1 receptor. Adapted with permission from ref 182. Copyright 2019 Elsevier.

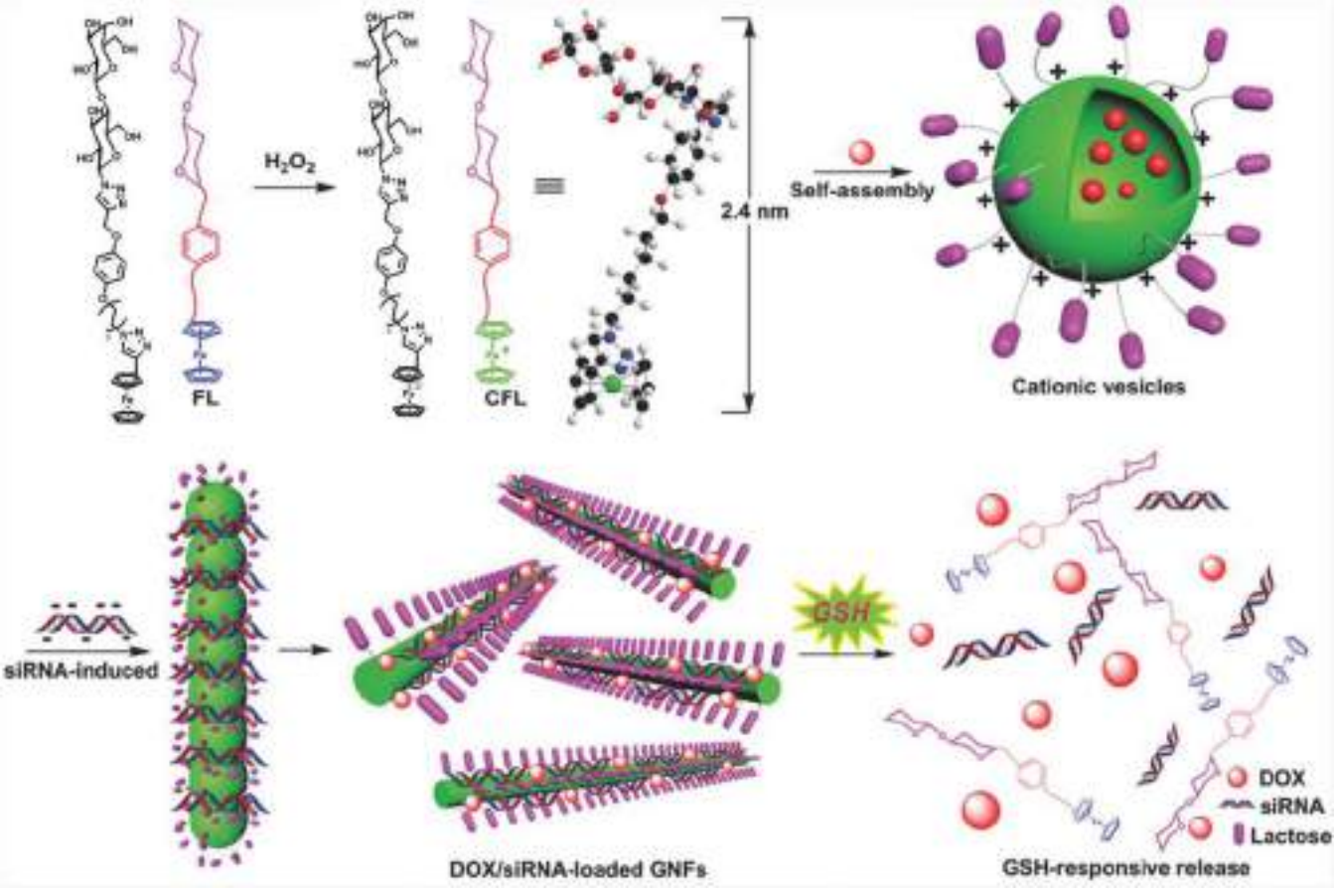


**Figure 18.** Schematic illustration of the molecular structure of CFL, formation of DOX/siRNA-loaded GNFs via self-assembly of CFL in the presence of DOX and siRNA induction, and disassembly of the GNFs upon GSH stimulus. Adapted with permission from ref 192. Copyright 2017 Wiley-VCH.

Reineke and co-workers evaluated a family of 3-guanidinopropyl methacrylamide (GPMA)-based polymeric gene delivery vehicles toward HepG2.[200] The hydrophilic polyGalNAc block was introduced not only as a stabilizer for plasmid−polymer complexes but also to target hepatocytes. Therefore, the resulting polyplex formulations with polyGalNAc block showed less cytotoxicity and improved gene delivery efficiency. In contrast, the complex without polyGalNAc was too toxic and eventually triggered cell apoptosis. Manoharan and co-workers also reported conjugation of siRNA to GalNAc derivatives, which further enhanced the targeted delivery to hepatocytes.[201] Drug delivery studies based on ASPGR targeting were also reported.[202] A miktoarm star copolymer with hydrophobic polycaprolactone and hydrophilic polyGal arms was coassembled with DOX.[203] The nanocarrier was efficiently uptaken through ASGPR-mediated endocytosis.


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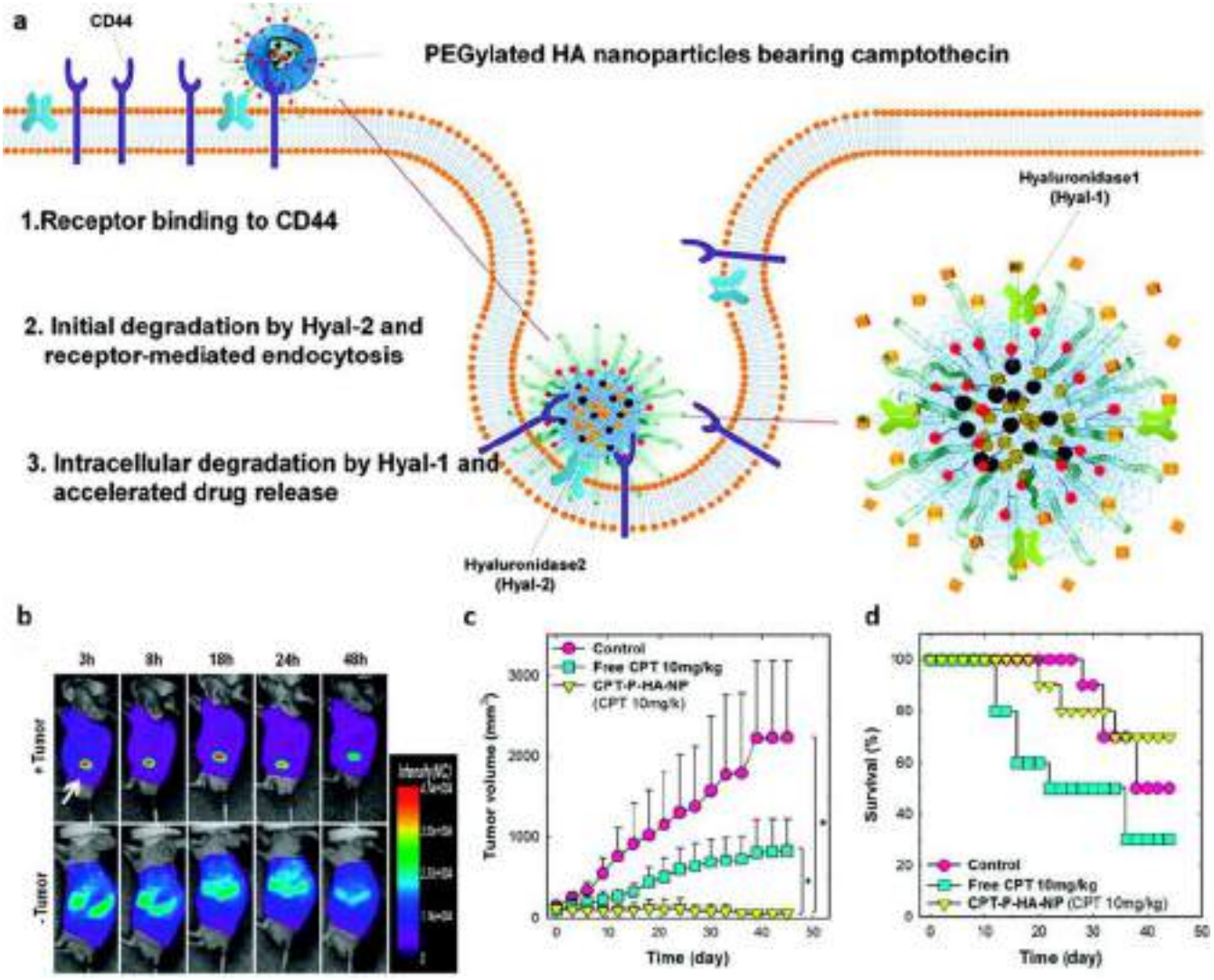


**Figure 19.** Drug-loaded PEG-conjugated HA NPs (Drug-P-HA-NPs) for effective cancer therapy. (a) Schematic illustrations of hypothetical cellular uptake pathways and subcellular drug-release behaviors of Drug-P-HA-NPs. (b) In vivo noninvasive fluorescence images of CPT-P-HA-NPs in tumor-bearing mice and normal mice. (c) Tumor growth and (d) survival rates of MDA-MB231 human breast cancer xenografts treated with saline, free CPT, and CPT-loaded P-HA-NPs at a CPT dose of 10 mg $kg^{-1}$. Adapted with permission from ref 209. Copyright 2011 American Chemical Society.

Hepatic-targeted codelivery with controlled release of drug/siRNA was realized both in vitro and in vivo.[192] As depicted in Figure 18, amphiphilic cationic ferrocenium-modified lactose derivatives (CFLs) underwent supramolecular coassembly with DOX, fabricating drug-loaded cationic vesicles, which further complexed with polyanionic siRNA and triggered the formation of nanofibers (GNFs). These GNFs displayed excellent biocompatibility, enhanced cell-penetrating ability, and hepatoma targetability owing to the presence of multivalent Lac units. In vivo study in both HepG2 and HepG2/ADR subcutaneous tumor-bearing nude mice showed excellent tumor targeting delivery, enhanced therapeutic efficacy, and reduced systemic toxicity to organs due to the redox (GSH)-responsive property of the ferrocenium units. This proof-of-concept work broadened the scope of nanocarriers in targeted drug/siRNA codelivery to overcome drug resistance and reduce adverse side effects in cancer chemotherapies.

**3.3.4. CD44-Mediated Targeting.** CD44 is a cell−surface glycoprotein, which is strongly expressed on chondrocytes and some tumor cells (e.g., MDA-MB-231 breast cancer cells), plays crucial roles in cell−cell interaction, cell adhesion, and migration, etc.[204] HA could specifically bind with CD44, making it a nice ligand for CD44-mediated targeting.[205] Since the first report of CD44-targeted HA liposomes in 2001,[206] HA-mediated CD44 targeting has been extensively investigated, particularly in the fields of imaging and drug delivery.[205,207,208]

HA could serve as a polymeric scaffold as well as a functional cue for self-assembled nanocarriers. As shown in Figure 19, Park and co-workers developed hyaluronidases-responsive nanocarriers for cancer therapeutics consisting of amphiphilic PEGylated-HA conjugates (P-HA-NPs) and hydrophobic anticancer drug camptothecin (CPT).[209] The P-HA-NPs could be internalized into cancer cells through receptor-mediated endocytosis but not by normal fibroblasts, showing cancer cell-specific uptake. In vivo noninvasive fluorescence images indicated the specific accumulation of CPT−P-HA-NPs in tumor tissue owing to the prolonged circulation and HA-mediated CD44-targeting. MDA-MB-231 mouse tumor models showed no significant increases in tumor size for at


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least 35 days with CPT-P-HA-NPs formulation, implying a high antitumor activity with minimal adverse side effects (Figure 19c and 19d). The group further established a theranostic system for early tumor detection and targeted tumor therapy using P-HA-NPs.[210] A near-infrared fluorescent dye was conjugated onto the NPs, allowing for visualization of small-sized colon tumors and liver-implanted colon tumors through the near-infrared fluorescence imaging technique. With the encapsulation of the anticancer drug irinotecan (IRT), IRT-P-HA-NPs showed high antitumor efficacy with minimal systemic toxicity due to the HA-mediated CD44 targeting.

## 4. TISSUE ENGINEERING

### 4.1. Overview

The concept of tissue engineering was brought up as a novel therapy in 1993, which utilizes both biology and engineering for the development of functional substitutes for damaged tissues, providing patients with renewed health and quality of life.[211] Throughout the past three decades of development, numerous questions have been answered, yet the still unmet challenges have to be overcome. The general strategy of tissue engineering is to use biomaterial scaffolds as artificial ECM, which can host living cells and support their survival, proliferation, differentiation, and functions, thus enabling the formation of new tissues.

As the blueprint for tissue-engineering scaffolds, natural ECM is composed of a complex biomacromolecular network surrounding cells. The ECM is not only important for stabilizing the tissue structures and mechanics but also crucial for providing many bioactive cues to the surrounded cells. On one hand, ECM has a complex macromolecular composition. The variations in the relative amounts of different macromolecules and macromolecular organization are the fundamental basis for the amazing diversity of ECM properties and the broad range of tissue types. In particular, some ECM could be as hard as those of bones or teeth tissues, some can form a transparent and soft matrix like that of a cornea, while others could have enormous tensile strength such as that of tendons. On the other hand, at the interface between cells and their surrounding ECM, there are highly dynamic interactions mediated by different cell surface receptors with precise spatiotemporal distributions. The mechanical and biochemical properties of ECM play an important role in regulating various cell behavior, ranging from cell adhesion, proliferation, migration, to differentiation. Meanwhile, cells are constantly remodeling the ECM in order to regulate their own behavior.[212,213]

An ideal ECM-mimicking biomaterial scaffold for tissue-engineering applications should provide proper mechanical and biochemical properties for the encapsulated cells in order to regulate relevant cell behavior and promote tissue regeneration.[214,215] As one key form of natural ECM component, carbohydrate-based macromolecules are particularly attractive for tissue-engineering scaffolds. They can provide not only the structural components with adjustable mechanical properties but also the beneficial biochemical cues for mediating cell−scaffold interactions.[212] In addition, carbohydrate-based macromolecules can be facilely functionalized to introduce bioadhesiveness, which can improve tissue integration of the corresponding biomaterials and thereafter in situ tissue repair.[216,217] In this section, we will present and discuss the fundamental studies that have advanced the tissue-engineering applications of carbohydrate-based macromolecular biomaterials in the past decade.

#### 4.1.1. Mechanical and Biochemical Cues from Carbohydrate-Based Macromolecular Scaffolds.

As scaffolding materials, carbohydrate-based macromolecules can influence cell behavior and tissue-engineering outcomes through regulating both the mechanical and the biochemical properties of the scaffolds (Figure 20). On one hand,

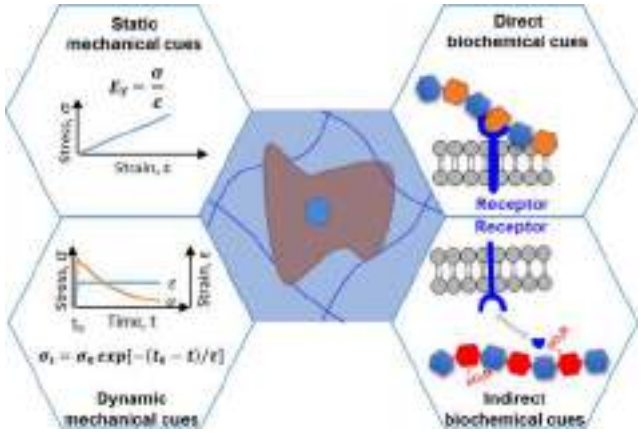


**Figure 20.** Overview of the mechanical and biochemical cues from carbohydrate-based macromolecular scaffolds for regulating cell behavior, including static mechanical cues, e.g., elastic modulus ($E_Y$), dynamic mechanical cues, e.g., stress−relaxation arising from reversible cross-links, direct cell−carbohydrate interactions, e.g., HA-CD44 binding, and indirect cell−carbohydrate interactions, e.g., interactions between the sulfated polysaccharide-sequestered growth factors (e.g., transforming growth factor $\beta$, TGF-$\beta$) and cell surface receptors.

carbohydrate-based macromolecular scaffolds can affect the cell behavior in mechanotransductive ways. For this purpose, the mechanical properties of the scaffolds are usually adjusted to match the tissues to be regenerated or repaired. Static mechanical properties can be tailored in many ways, including changing the molecular weights or polymer concentrations, blending carbohydrate-based macromolecules to customize the network structures, controlling the cross-linking densities and manufacturing process of the carbohydrate-based macromolecular scaffolds, and so on. In the past few years, the design of tissue-engineering scaffolds has moved from static properties to dynamic properties, thus providing adaptable scaffolds with time-dependent properties closely mimicking those of living tissues (Figure 20). Such dynamic mechanical properties of carbohydrate-based macromolecular scaffolds, including the responsiveness,[218−221] self-healing properties,[222−225] viscoelasticity, and stress−relaxation,[225−229] mainly originate from reversible interactions between carbohydrate-based macromolecules.

On the other hand, the scaffolding carbohydrate-based macromolecules can also affect the cell behavior through diverse biochemical cues, which can induce either direct or indirect interactions between carbohydrate structures and their encountered cells (Figure 20). While the direct interactions are mediated by the binding of carbohydrate structures with their cell surface receptors, the indirect interactions could be mediated by the functional peptides conjugated to carbohy-


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drate structures or by the growth factors sequestered by carbohydrate-based macromolecules (Figure 20).

**4.1.2. Scope of Carbohydrate-Based Macromolecular Scaffolds for Tissue Engineering.** As we have pointed out two aspects of the scaffold properties to be emphasized in this review, herein we also provide an overview on the five types of carbohydrate-based macromolecules listed in section 2 in order to set a clear outline for the following detailed discussions on their tissue-engineering applications.

Among them, naturally occurring polysaccharides have been predominantly used in tissue engineering and hence will be emphasized in the following discussions. As naturally derived synthetic polysaccharides, PASs are now coming close to tissue-engineering applications thanks to their structural features closely resembling natural polysaccharides (section 2.2.2). Very recently, Grinstaff and co-workers successfully synthesized a new type of cationic PASs with controlled molecular weight and low polydispersity, which can mimic the chitosan structure and bind to mucin in solution and on ex vivo tissue samples. This example implicates that such PASs have great potential in tissue-engineering applications.[56,61] A recent advance in PASs has been reviewed by Grinstaff's group.[230] It is noteworthy that, to the best of our knowledge, the other subtype of naturally derived synthetic polysaccharides, namely, poly(saccharide carbonate)s, is more distant from tissue-engineering applications than PASs. This could be partially due to the fact that the development of such polymers is still in its infancy. Meanwhile, the poor water solubility of most poly(saccharide carbonate)s may have hindered their applications in tissue-engineering scaffolds. As the third type of carbohydrate-based macromolecules discussed in this review, glycopolymers have been used in tissue engineering in a few cases. Their capability in mimicking cell−matrix interactions via multivalency is attractive for cell culturing. For example, Akaike and co-workers developed a Gal-derived polystyrene, poly(*N*-*p*-vinylbenzyl-4-*O*-*β*-D-galactopyranosyl-D-gluconamide) (PVLA), to mimic the ASGPR−carbohydrate interaction for regulating hepatocyte cell adhesion.[231] However, it has not been successfully used for 3D cell-culturing scaffolds yet. This may be due to their synthetic polymer backbones, which are usually difficult for cellular degradation. In addition, the synthetic skill needed for preparing glycopolymers may also hinder the widespread applications. Similarly, regardless of their precise structures and attractive bioactivities, glycodendrimers remain unexplored in tissue-engineering scaffolds mainly due to their complicated and tedious synthesis. With future advances of standardized and scale-up synthetic methods, the bright future of glycopolymers and glycodendrimers in tissue engineering can be foreseen. Supramolecular glycopolymers, self-assembled from carbohydrate amphiphiles or the glycopeptides, have been demonstrated as promising biomimetic hydrogel scaffolds for tissue engineering due to their ECM-mimicking nanofibrous morphologies and carbohydrate bioactivities.[232−241] As for the carbohydrate-based macromolecules discussed in this review, synthetic glycolipids and glycoproteins have been used as carbohydrate biomaterials mainly for immunological applications (section 5). However, there are many opportunities in tissue engineering where such carbohydrate-based macromolecular biomaterials could serve as nonscaffolding components to improve the efficiency of bioactive proteinaceous or small molecular drugs to promote stem cell differentiation and tissue regeneration.

Therefore, extended from this overview, discussions will be mainly given to naturally occurring polysaccharides (section 4.2), glycopolymers (section 4.3), and peptide-based supramolecular glycopolymers (section 4.4). Along with the fundamental studies where cell−biomaterials interactions were studied and relevant biological or physicochemical mechanism were underlined, those with in vivo validations will be highlighted. In addition, we will summarize other applications of carbohydrate-based macromolecular biomaterials in tissue-engineering-relevant fields (section 4.5), including their use in organoid development (section 4.5.1) and cancer spheroids (section 4.5.2).

## 4.2. Naturally Occurring Polysaccharides in Tissue Engineering

As discussed in the overview, among the five types of carbohydrate-based macromolecules, naturally occurring polysaccharides have been predominantly used in tissue engineering. This is mainly due to their abundance, biocompatibility, biodegradation properties, and similarity to ECM components. In addition, their structural features (as discussed in section 2) allow for various chemical modifications and facile control over the mechanical and biochemical properties of the resulting scaffolds. This is of key importance for proper cell−scaffold interactions, cell behavior regulation, and tissue regeneration.[242,243]

**4.2.1. Cell Regulation by Mechanical Properties.** Proper mechanical properties of the scaffolds are of crucial importance for the encapsulated cells. However, often the mechanical properties of polysaccharide scaffolds have been characterized and presented in reports but leave their connections to cell behavior and their influence on tissue-engineering outcomes unclear.[244] This may due to the different scopes of studies, indicating the gap between materials science and the cell biology of mechanotransduction.[245] Herein, only studies that have observed remarkable results regarding mechanical regulation of cell behavior in carbohydrate-based macromolecular scaffolds, thus providing connections between certain mechanical properties and cell behaviors or tissue-engineering outcomes, will be presented. Without a clear correlation, other studies are out of our scope in this review, with exceptions given to the scaffolds that were validated with in vivo (pre)clinical results.

*4.2.1.1. Modulation of the Scaffold Mechanical Properties.* Compared to end-functionalized poly(ethylene glycol) (PEG) that has been widely used for synthetic hydrogel scaffolds, a polysaccharide with abundant hydroxyl groups allows for not only complex noncovalent interactions but also tailored extent of chemical cross-linking, thus offering a broader range of scaffold mechanical properties covering that of almost all tissue types, from soft tissues, such as brain, up to hard tissues, such as bone. Such a structural feature of carbohydrate macromolecular biomaterials ensures their application in studying the mechanical regulation of cell behaviors and tissue-engineering outcomes. Herein, we will first discuss the modulation of the hydrogel scaffold mechanical properties before reviewing their influence on cell behavior.

To make polysaccharide hydrogel scaffolds, there are four common ways for cross-linking the network structure and modulating the polysaccharide scaffold mechanical properties (Figure 21). Covalent cross-linking and polymer blending (e.g., forming double-network or interpenetrating-network struc-


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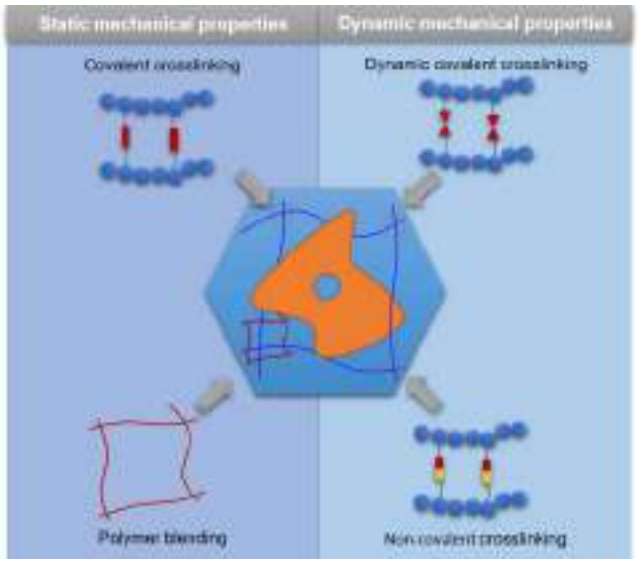


**Figure 21.** Schematic illustration of four ways for modulating the polysaccharide scaffold mechanical properties. Covalent cross-linking and polymer blending (e.g., forming double-network or interpenetrating-network structures) are effective in modulating the static mechanical properties (e.g., stiffness), while dynamic covalent cross-linking and noncovalent cross-linking are effective in modulating the dynamic mechanical properties (e.g., stress−relaxation).

tures) are effective in modulating the static mechanical properties (e.g., stiffness), while dynamic covalent cross-linking and noncovalent cross-linking are effective in modulating the dynamic mechanical properties (e.g., stress−relaxation).

Among the main naturally occurring polysaccharides emphasized in this review (i.e., alginate, HA, heparin/heparan sulfate, chitin/chitosan), alginate and chitosan can be cross-linked directly by noncovalent interactions to yield tissue-engineering scaffolds. Therefore, albeit useful, chemical modification of these two types of polysaccharides is not necessary for hydrogel scaffold preparation. This makes them easy to be used by the vast majority of tissue-engineering researchers, including those who do not have access to polymer functionalization, purification, and characterization platforms. Consequently, alginate and chitosan are the two most frequently used polysaccharides in tissue engineering.[244] Specifically, alginate hydrogels can be easily prepared by mixing the polymer solutions with divalent cations (e.g., $Ca^{2+}$).[244] It has been reported that the composition (i.e., M/G ratio), G-block length, and molecular weight are critical factors affecting the mechanical properties of alginate−$Ca^{2+}$ hydrogels. As cationic polysaccharides, chitosan can be used in tissue-engineering scaffolds based on the formation of polyelectrolyte complexes via layer-by-layer deposition, making it particularly useful for tissues with multilayered structures.[244] It was also reported that chitosan can be directly cross-linked by metal ions. For instance, Lu and co-workers reported that at appropriate pH values, chitosan can be cross-linked into stable hydrogels by a variety of transition metal ions, specifically $Ag^{+}$, $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Pd^{2+}$.[221] For the other naturally occurring polysaccharides, chemical modification is usually needed to impart functional motifs that can enable noncovalent cross-linking. Although this additional functionalization could be challenging, it can provide more flexible and modular design of the cross-linked polysaccharide networks. Many functional groups have been utilized in noncovalently cross-linked polysaccharide hydrogel scaffolds, including macrocyclic host−guest moieties,[219,220,225,246−249] metal ion-binding ligands,[226] dock-and-lock (DnL) structural binding peptide motifs,[223,250] hydrophobic groups,[224] and so on.

Regardless of the above-mentioned advantages of noncovalent cross-linking, it is often challenging to achieve proper mechanical strength and long-term stability of the resultant scaffolds. Therefore, covalent cross-linking, used alone or combined with noncovalent cross-linking, represents a more common method for preparing polysaccharide hydrogel scaffolds. It offers more possibilities in tailoring the mechanical properties and stability of the resulting polysaccharide scaffolds. To enable biocompatible chemical cross-linking of polysaccharides, many functional groups can be conjugated, including thiols, acrylates, methacrylates, tyramides, maleimides, adipic dihydrazides, tetrazines, norbornenes, and so on.[251,252]

Between noncovalent and covalent cross-linking, dynamic covalent cross-linking has emerged in recent years for adaptable polysaccharide scaffolds. It provides special covalent bonds for cross-linking, which can be broken and reformed reversibly. The resulting adaptable hydrogels have biomimetic viscoelastic properties and can be locally permissive to complex cellular functions while maintaining their long-term mechanical strength. Promising dynamic covalent chemistry that can establish adaptable polysaccharide hydrogels under biocompatible conditions includes Schiff base reactions between amine and aldehyde groups, hydrazone formation between aldehyde and hydrazine, oxime formation between hydroxylamine and aldehyde/ketone, disulfide formation between thiols, reversible Diels−Alder reactions, and so on.[253]

In addition to materials selection (e.g., molecular weight and content of selected polysaccharides), the mechanical properties can be customized by selection of the above-mentioned cross-linking chemistries and the cross-linking densities. Moreover, blending of polysaccharides with other biocompatible polymers is also an effective strategy for modulating the mechanical properties. For example, as a special form of blending system, interpenetrating polymer network (IPN) hydrogels are considered as promising scaffolds for load-bearing tissues due to their superior mechanical properties.[255] Qiu and co-workers developed a conjoined-network hydrogel from the blending of chitosan and gelatin, where sodium phytate was used to electrostatically cross-link the biopolymers (Figure 22).[254] The resulting conjoined-network hydrogels exhibited a high compressive modulus and toughness with mechanical properties adjustable by changing the chitosan content in the conjoined network.

*4.2.1.2. Cell Regulation by the Hydrogel Static Mechanical Properties.* As the main aspect of the static mechanical properties regulating cell behavior, the stiffness of the biomaterial substrates and 3D matrix has been intensively investigated using synthetic polymeric biomaterials.[256−259] In an early example of polysaccharide scaffolds, Shoichet and co-workers used photopolymerizable methacrylamide chitosan for preparing scaffolds with variable Young's modulus ($E_Y$) ranging from less than 1 kPa to greater than 30 kPa to investigate the effect of substrate stiffness on adult neural stem/progenitor cells (NSPCs).[260] It was revealed that NSPCs preferred to proliferate on a stiff substrate with $E_Y$ of 3.5 kPa. Thanks to the tunable stiffness of such scaffolds, a detailed differentiation profile of NSPCs correlated to stiffness was established: neuronal differentiation of NSPCs was favored on soft


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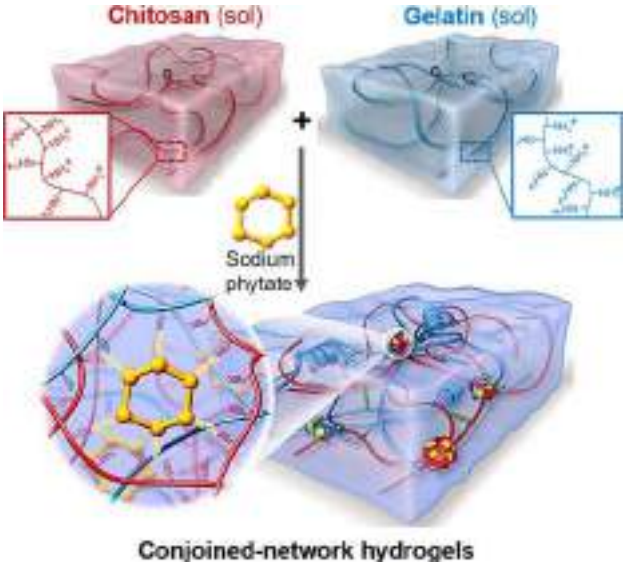


**Figure 22.** Chitosan-blended conjoined-network hydrogel. Reproduced with permission from ref 254. Copyright 2019 The American Association for the Advancement of Science under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/legalcode).

substrates with $E_Y$ less than 1 kPa, oligodendrocyte differentiation was favored on stiffer scaffolds (> 7 kPa), while oligodendrocyte maturation and myelination were best on scaffolds with $E_Y$ < 1 kPa. Astrocyte differentiation was only observed from a small portion of cells (< 2%) seeded on soft hydrogel substrates, including hydrogels with $E_Y$ < 1 kPa and those with $E_Y$ = 3.5 kPa.

To establish a clear connection between scaffold stiffness and cell behavior, it is a prerequisite to tailor stiffness independent of the other materials properties, such as compositions, architectures, and presentation of bioactive ligands. This is even more challenging in 3D cell culturing. Mooney and co-workers demonstrated that IPNs of alginate and reconstituted basement-membrane (rBM) matrix can meet this prerequisite (Figure 23a).[261] Such IPN hydrogels with an alginate−$Ca^{2+}$ network for stiffness modulation can serve as a materials platform to study the influence of matrix stiffness on the induction of malignant phenotypes in mammary epithelium. The hydrogel storage modulus (at 1 Hz) can be adjusted from 30 to 310 Pa through restricting deformation of a rBM matrix with different $Ca^{2+}$ concentrations. They

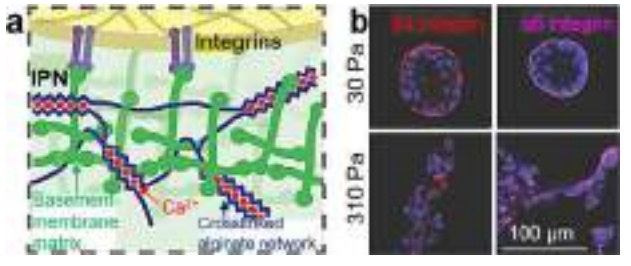


**Figure 23.** Regulation of cell behavior by hydrogel stiffness. (a) Schematic illustration of the IPN of alginate (blue) and basement-membrane matrix (green) and the binding of cellular integrin receptors to ligands. (b) Staining of the different hemidesmosome components in MCF10A clusters at the indicated stiffness. DAPI costain is shown in blue. Reproduced with permission from ref 261. Copyright 2014 Springer Nature.

discovered that for normal mammary epithelial cells, increasing hydrogel stiffness alone induces malignant phenotypes. Moreover, the IPN hydrogel platform allows the investigation of the joint effect of stiffness and composition. The results revealed that an increase in basement-membrane ligands in IPN hydrogels completely abrogated the stiffness effect on phenotype induction. A close examination demonstrated that the combination of stiffness and composition is sensed through $\beta$4 integrin, Rac1, and the PI3K pathway, thus providing a correlation between the cell behavior and the static mechanical properties (stiffness) of alginate-containing IPN hydrogels (Figure 23b). It is noteworthy that although the stiffness of such IPN hydrogels was modulated independently from polymer composition in this study, which studied cell-adhesion ligand density, and the matrix architecture, the possible change of matrix plasticity along with the stiffness induced by different $Ca^{2+}$ concentrations was revealed in a later study.[262] It is likely that the effect of stiffness on the induction of malignant phenotypes could be interfered by the matrix plasticity, which is to be further investigated.

*4.2.1.3. Cell Regulation by the Hydrogel Dynamic Mechanical Properties.* A recent advance in dynamic polymer networks has led to novel designs of adaptable scaffolds, providing opportunities to better mimic the dynamic nature of the ECM.[253,263−265] For conventional static hydrogel scaffolds cross-linked by covalent bonds, degradation is typically required for proper functions of encapsulated cells. However, the requirement of degradation could be contradicted to that of mechanical strength and long-term stability. On the contrary, within adaptable hydrogels cross-linked by reversible interactions, the polymer network can be locally and temporally broken down and repaired through dissociation and reassociation of the reversible linkages, thus making it possible to permit cellular functions while maintain long-term hydrogel integrity (Figure 24). Moreover, the reversible nature

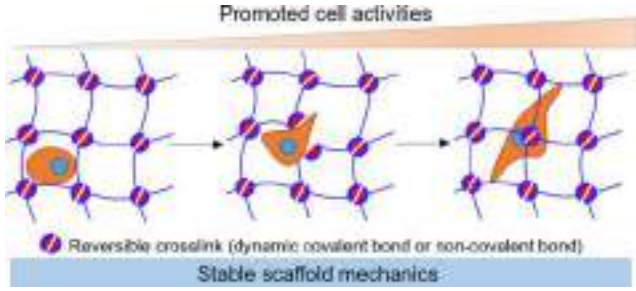


**Figure 24.** Adaptable hydrogel scaffold cross-linked by reversible interactions could promote cell activities while maintaining stable scaffold mechanics during cell culturing.

of the cross-links can give rise to other practically useful features, such as stimuli responsiveness, self-healing, and injectable properties.[266−268] Although studies on the regulation of cell behavior by dynamic mechanical properties were initiated with synthetic polymers, including polyacrylamide[269] and PEG,[270−272] naturally occurring polysaccharides, including chitosan, HA, and alginate, have also been used in constructing dynamic hydrogel scaffolds for regulating cell behavior. For this purpose, polysaccharides can be cross-linked by either dynamic covalent or noncovalent bonds, which should guarantee proper gelation kinetics and cytocompatible gelation conditions.[263]


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In early examples, Wei and co-workers developed a hydrogel with dynamic covalent cross-linking, i.e., Schiff base cross-linking, between the amine groups of glycol chitosan and the benzaldehyde groups of a biocompatible telechelic difunctional PEG.[218,222,273] Such glycol chitosan-based hydrogels were used for encapsulation of HeLa cells. High cell viability after 3D hydrogel encapsulation and subsequent injection demonstrated that such adaptable hydrogels can be potentially used for injectable cell therapies. It is noteworthy that the importance of stress−relaxation of the viscoelastic hydrogels cross-linked by dynamic covalent interactions in regulating cell spreading and differentiation was first revealed using synthetic polymers.[272] The influence of the dynamic mechanical properties on the cell behavior was examined later in biopolymeric hydrogels that better mimic the natural ECM. Chaudhuri and co-workers prepared an IPN hydrogel with HA and collagen I, where HA was cross-linked with dynamic covalent hydrazone bonds. Stress−relaxation of such IPN hydrogels can be adjusted by HA cross-linker affinity, molecular weight, or concentration. It was found that faster relaxation can promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cultures (Figure 25).[228]

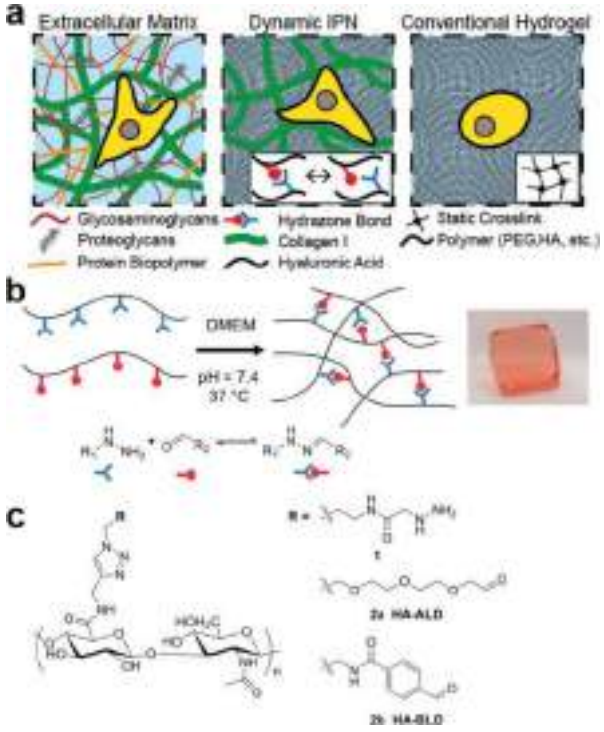


**Figure 25.** Adaptable hydrogels with dynamic IPN structures. (a) Comparison between natural ECM, dynamic IPN matrix, and conventional hydrogels, illustrating that the dynamic IPN hydrogel is a better mimic of the natural ECM. (b) Dynamic covalent cross-linking with hydrazone bonds. (c) Chemical structures of the functionalized HA polysaccharides. Reproduced with permission from ref 228. Copyright 2018 Elsevier.

In addition to dynamic covalent bonds, noncovalent interactions have also played an important role in the development of adaptable polysaccharide scaffolds. For example, Mooney and co-workers intensively studied the stress−relaxation of alginate−$Ca^{2+}$ hydrogels.[227,229] By changing the polymer molecular weight or binding affinity of the calcium chelating domains, the stress−relaxation of alginate hydrogels was facilely tuned. The studies revealed that stress−relaxation can promote cell spreading, nuclear localization of the mechanosensitive transcriptional regulator (e.g., yes-associated protein, YAP), and even osteogenic differentiation of mesenchymal stem cells (MSCs).[227,229] Heilshorn and co-workers demonstrated that alginate−$Ca^{2+}$ hydrogels could be an ideal material platform facilitating matrix remodeling, cell spreading, and stemness maintenance of neural progenitor cells (NPCs).[274] Variation in the ratio of $CaSO_4$ to alginate offers a reliable way of changing the stiffness. It was found that the reversible nature of the adaptable hydrogels supports the stemness maintenance of NPCs regardless of the stiffness variation. In contrast, their covalently cross-linked counterparts blocked matrix remodeling and cell spreading, thus leading to a loss of stemness (Figure 26). Very recently, Sacco and co-

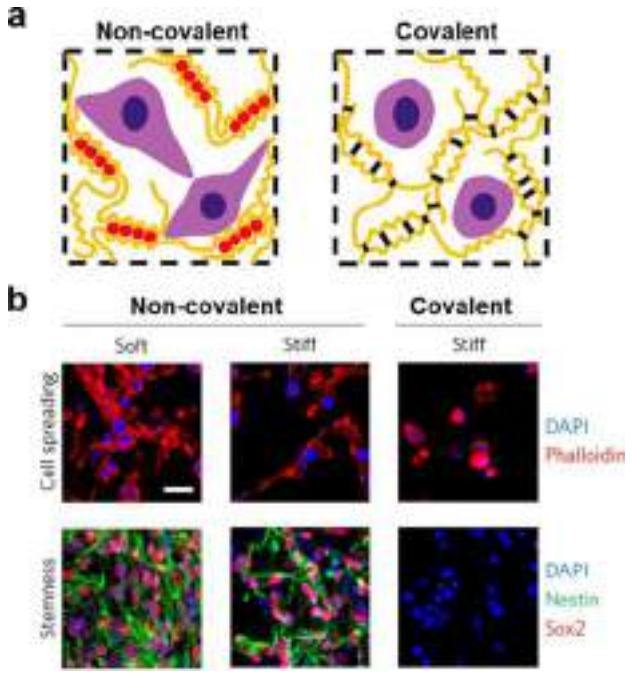


**Figure 26.** Neural progenitor cells (NPCs) culturing in alginate hydrogels with covalent or noncovalent cross-links. (a) Schematic illustration of the network structures of alginate hydrogels cross-linked by noncovalent cross-links ($Ca^{2+}$) or covalent cross-links. (b) Cell spreading visualized by DAPI/Phalloidin staining and stemness evaluation by staining DAPI/Nestin/Sox2 (scale bar, 25 $\mu$m). Reproduced with permission from ref 274. Copyright 2017 Springer Nature.

workers developed another ionically cross-linked chitosan platform in which the energy dissipation property could be decoupled from stress−relaxation and overall stiffness.[275] The study revealed an inverse relationship between substrate dissipation energy and cell response. It was found that cell adhesion and spreading was favored on substrates with a lower dissipation energy.

Macrocyclic host−guest interactions with good biocompatibility have also been used for adaptable 3D hydrogel scaffolds. In the pioneering example, Kim and co-workers developed HA hydrogels cross-linked by cucurbit[6]uril-based host−guest interactions.[220] They demonstrated that such supramolecular hydrogels could be used as a modular platform for 3D cell culturing but did not investigate the influence of the mechanical properties on cell behavior. Later, Burdick and


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co-workers developed injectable and shear-thinning HA supramolecular hydrogels with cyclodextrin-based host−guest interactions showing tunable mechanical properties by adjusting the host−guest contents in the hydrogels.[246] However, due to the relatively weak cyclodextrin-based host−guest interactions compared to their cucurbit[6]uril-based counterparts, the resultant hydrogels need further reinforcement for long-term cell culturing applications. Reinforcement can be achieved by inducing second covalent cross-linking, which enables the application of such host−guest HA hydrogels in 3D bioprinting and 3D cell culturing.[276,277] With this method, Burdick and co-workers recently studied mesenchymal stromal cell mechanosensing in 3D HA hydrogels cross-linked by host−guest interactions with additional covalent cross-links. With an increased ratio of host−guest cross-links relative to covalent cross-links, cell spreading was enhanced, showing greater aspect ratios than those in degradable hydrogels without host−guest cross-links.[278] Another reinforcing strategy for such host−guest HA supramolecular hydrogels is to employ the multivalent effect. Bian and co-workers reported a "Host−Guest−Macromer" (HGM) strategy for in situ formation of multivalent host−guest interactions. The resultant HA hydrogel exhibited fast stress−relaxation and excellent long-term stability, thus enabling in vitro 3D culturing of stem cells. Moreover, promoted cartilage regeneration was validated in a rat model with osteochondral defects (Figure 27).[225] Such a HGM strategy has also enabled the fabrication of other biopolymeric hydrogels with promising applications in tissue engineering.[279−283]


**Figure 27.** HGM strategy for reinforcing host−guest HA supramolecular hydrogels. (a) Schematic illustration of the HGM strategy used to fabricate HA hydrogels cross-linked by multivalent host−guest interactions. (b) Such hydrogels were used as stem cell delivering matrix for cartilage regeneration in a rat model. Reproduced with permission from ref 225. Copyright 2017 American Chemical Society.

Regardless of the above-mentioned efforts in regulating cell behavior with static or dynamic mechanical properties, the coupling effect of both aspects on cell responses has been less understood. Mooney and co-workers developed a 3D cell culture system from alginate, which enabled the independent control of stiffness, stress−relaxation, and adhesion ligand density of the hydrogels, thus allowing for systematic investigation on cell responses to different combinations of these matrix properties. Using the RNA-seq technique, the study revealed dramatic transcriptional coupling between these biomaterial properties as well as the relative contribution of each property to gene expression changes in mouse mesenchymal stem cells and human cortical neuron progenitors.[284]

**4.2.2. Cell Regulation by Biochemical Properties.** The scaffolding polysaccharides can affect cell behavior through diverse biochemical cues, which can induce either direct or indirect cell−scaffold interactions and lead to effective regulation of cell behavior. While the direct interactions are mediated by the direct binding of carbohydrate structures with their cell surface receptors, the indirect interactions could be mediated by the functional peptides conjugated to carbohydrate structures or by the growth factors sequestered within the carbohydrate-based macromolecular scaffolds (Figure 28).

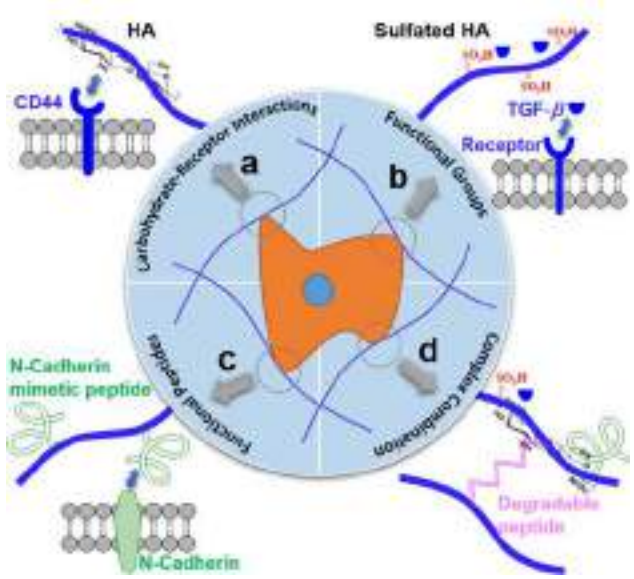


**Figure 28.** Schematic illustration of different strategies in modulating the hydrogel biochemical properties. (a) Biochemical activity of the polysaccharide scaffold can be presented by the carbohydrate structure and mediate cell−scaffold interactions, e.g., HA−CD44 binding. (b) Biochemical properties of nonsulfated polysaccharides (e.g., HA) can be modulated by converting the surface groups to sulfates for growth factor retention. (c) Polysaccharides can be modified by functional peptides, e.g., N-cadherin mimetic peptides containing a His-Ala-Val (HAV) sequence. (d) Biochemical properties of polysaccharide scaffolds can be optimized with a complex combination of the functionalities.

*4.2.2.1. Modulation of Hydrogel Biochemical Properties.* The intrinsic biochemical properties of naturally occurring polysaccharides, such as binding of HA to cell surface CD44 and CD168 receptors, sequestering of growth factors and cytokines by chondroitin sulfate, and binding of heparan sulfate to various protein ligands, have attracted enormous attention in tissue engineering.[285−287] Polysaccharide hydrogel scaffolds could inherit such biochemical properties and show great potential in regulating cell behavior and promoting tissue regeneration (Figure 28). In addition, modulation of the hydrogel biochemical properties can also be achieved by altering the chemical functional groups (e.g., from −OH and


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−COOH to $-SO_3H$) or by conjugating functional peptides to polysaccharides (Figure 28).[288]

Altering polysaccharide functional groups has been proved to be an effective strategy for modulating their biochemical properties. A typical example is to incorporate sulfated groups into naturally nonsulfated polysaccharides to mimic the structure and function of heparan sulfate/heparin. For example, Bian and co-workers revealed that sulfated HA hydrogels can retard HA degradation while enhancing growth factor retention for promoted human mesenchymal stem cell (hMSC) chondrogenesis.[289]

Conjugation of functional peptides has been demonstrated as a versatile and effective strategy in constructing polysaccharide hydrogel scaffolds with various bioactivities due to the flexible design and commercial source of peptides.[290] As the most widely investigated functional peptides, those containing the RGD (Arg-Gly-Asp) sequence have been intensively used to enable cell−scaffold interactions mediated by RGD-binding integrins. Considering that most naturally occurring polysaccharides are not cell adhesive enough to support proper cell attachment and spreading, which is very often a prerequisite for many downstream cell functions and activities, such RGD functionalization plays a crucial role in many polysaccharide hydrogel scaffolds. In addition, for specific tissue-engineering applications, growth factors (GF) or biomimetic peptides have also been conjugated to polysaccharide hydrogels. For example, Crescenzo and co-workers demonstrated that the combination of RGD peptide and the vascular endothelial growth factor (VEGF) could promote the adhesion and selective proliferation of endothelial cells.[291,292]

While RGD represents the predominantly used cell adhesive peptide to mimic cell−ECM interactions, peptides for mimicking cell−cell interactions have also been used in polysaccharide hydrogel scaffolds. Burdick and co-workers conjugated a N-cadherin mimetic peptide on HA hydrogels. The results revealed the importance of artificial cell−cell interactions in promoting stem cell differentiation. More recently, other functional peptides have been employed to modulate cell−scaffold interactions. For example, Bian and co-workers showed that Foxy5 peptides conjugated on the HA hydrogel scaffolds can promote the mechanosensing and osteogenesis of human mesenchymal stem cells by activating noncanonical Wnt signaling.[293]

*4.2.2.2. Cell Regulation by Direct Cell−Carbohydrate Interactions.* The typical direct cell−carbohydrate interaction used in polysaccharide hydrogel scaffolds is represented by the interaction between HA with cell surface receptors such as CD44 and CD168. Such interactions are essential in natural ECM−cell interactions, influencing a variety of intracellular signaling pathways and many cellular processes, such as receptor-mediated HA internalization and degradation, cell adhesion, proliferation, aggregation, and migration.[287] Burdick and co-workers translated such interactions to HA hydrogel scaffolds for 3D stem cell culturing.[294] Compared to inert PEG hydrogels lacking such cell−scaffold interactions, HA hydrogel can promote stem cell chondrogenesis. Such chondrogenic effect can be abrogated by blocking CD44 with antibodies, further confirming the important role of HA−CD44 binding in stem cell differentiation.[295] Very recently, they pointed out that the chemical modification of HA can significantly compromise its binding with CD44, hence leading to the decreased effect of such direct cell−carbohydrate interactions on stem cell chondrogenesis (Figure 29).[296] For instance, hydrogels prepared from norbornene-modified HA (NorHA1) can

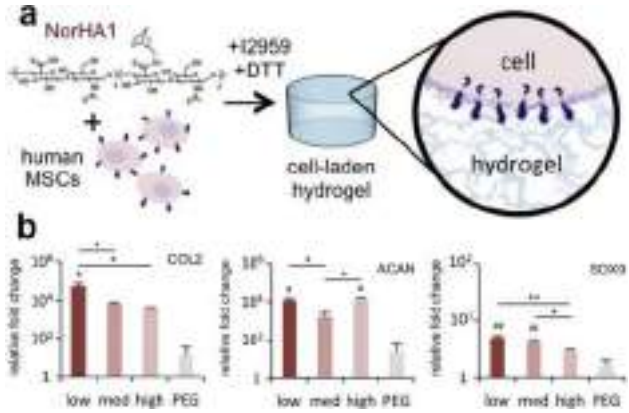


**Figure 29.** Extent of HA modification influences early chondrogenesis in 3D hydrogels. (a) Schematic illustration of the norbornene-functionalized HA (NorHA1) and the preparation of cell-laden hydrogels. (b) Comparison of stem cell chondrogenesis in different hydrogel scaffolds. Reproduced with permission from ref 296. Copyright 2019 Elsevier.

maintain cell−hydrogel interaction via HA−CD44 binding (Figure 29a), hence showing higher expression of chondrogenic markers (COL2, ACAN, and SOX9) than cells in PEG hydrogels. When such cell−hydrogel interaction was interfered by higher modification degrees, its chondrogenic inductive effect was compromised (Figure 29b). It is noteworthy that compared to HA-based scaffolds, much less on direct cell−scaffold interactions has been explored with the other naturally occurring polysaccharides listed in section 2.1. Most of their biochemical activities toward the encapsulated cells within the scaffolds are exerted via indirect interactions mediated by functional biochemical cues, such as functional peptides and sequestered growth factors.

*4.2.2.3. Cell Regulation by Indirect Cell−Carbohydrate Interactions.* Functional peptides have found many applications in biomedicine and tissue engineering.[297] Peptide conjugation offers a powerful method for bioactivating polysaccharide scaffolds, giving rise to indirect cell−carbohydrate interactions mediated by the peptides as linkages.[290] On one hand, thanks to their abundant reactive groups, including hydroxyl, amine, and carboxyl groups, polysaccharides can be facilely functionalized by peptides. On the other hand, a recent advance in cell biology and peptide synthesis has facilitated the design and commercial production of functional peptides that can carry out specific functions. As mentioned, RGD has been the predominantly used functional peptide for activating the cell-adhesive properties of tissue-engineering scaffolds since it was identified in 1984.[298−300] Since then peptide design and production has become a highly specialized and commercialized field, providing a substantial support for biomaterials and tissue-engineering researchers. Since it is out of the scope of this review, readers are referred to recent reviews covering functional peptides for tissue engineering[297,301] and polysaccharide−peptide conjugates for tissue engineering.[244,290]

Nevertheless, we would like to point out the emerging trend of conjugating multiple functional peptides on polysaccharide backbones. Together with the direct cell−carbohydrate interactions mediated by polysaccharides (e.g., HA), the hybrid indirect cell−carbohydrate interactions mediated by


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multiple peptides can enable the development of polysaccharide scaffolds with a closer complexity to natural ECM, where the cells are usually regulated simultaneously by various biochemical cues. In a representative example, Burdick and co-workers demonstrated both direct and indirect cell−carbohydrate interactions and their influence on stem cell differentiation.[295] To hybridize the above-mentioned direct cell−HA interactions with peptide-based indirect interactions mediated by N-cadherin mimetic peptides, the photo-cross-linkable macromers (MeHA) were modified with N-cadherin mimetic peptide (Ac-HAVDIGGGC) (Figure 30a). Consequently, the resultant HA hydrogels can present simultaneously three different bioactivities to the encapsulated hMSCs, namely, HA−CD44, HA−CD168, and N-cadherin−peptide interactions. It was revealed that the N-cadherin-mediated cell−scaffold interaction can supplement the aforementioned HA−CD44/168 effect, thus enhancing expression of chondrogenic markers and promoting long-term cartilage matrix production. In a more recent example, Bian and co-workers showed that Wnt5a mimetic ligand (Foxy5 peptide) immobilized on HA hydrogels can activate noncanonical Wnt signaling, thus leading to enhanced intracellular calcium level, F-actin stability, actomyosin contractility, and development of cell adhesion structures. Such enhanced mechanotransduction of stem cells promoted their in vitro osteogenic differentiation, demonstrating the great potential of such scaffolds in repairing rat calvarial defects. Moreover, this study has also emphasized the synergy effect from copresenting RGD with such secondary functional peptides, which is yet to be explored for establishing the ECM-like complexity of polysaccharide hydrogel scaffolds.[293]

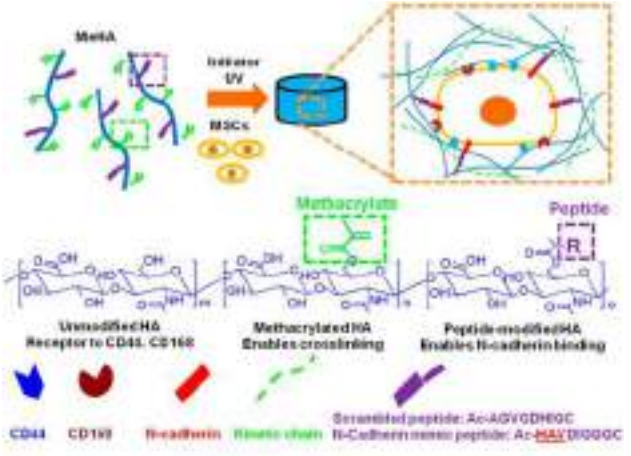


**Figure 30.** Schematic illustration of a photo-cross-linkable HA hydrogel for hMSCs culturing. Hydrogel scaffold can interact with hMSCs via HA−/CD44/168 binding as well as N-cadherin-HAV peptide binding. Reproduced with permission from ref 295. Copyright 2013 National Academy of Sciences.

In another example, an even more complex polysaccharide scaffold was achieved by Carmichael and co-workers.[302] HA−heparin hybrid hydrogels were systematically optimized by employing multiple peptides and growth factors, including a peptide cross-linker sensitive to matrix metalloproteinase (MMP), adhesion peptides, and heparin-bound growth factors (Figure 31). It was demonstrated that the scaffold can be optimized with an iterative approach to achieve the complex combination of mechanical, biochemical, and biological properties, thus promoting the survival and differentiation of encapsulated human neural progenitor cells after transplantation in the stroke brain.

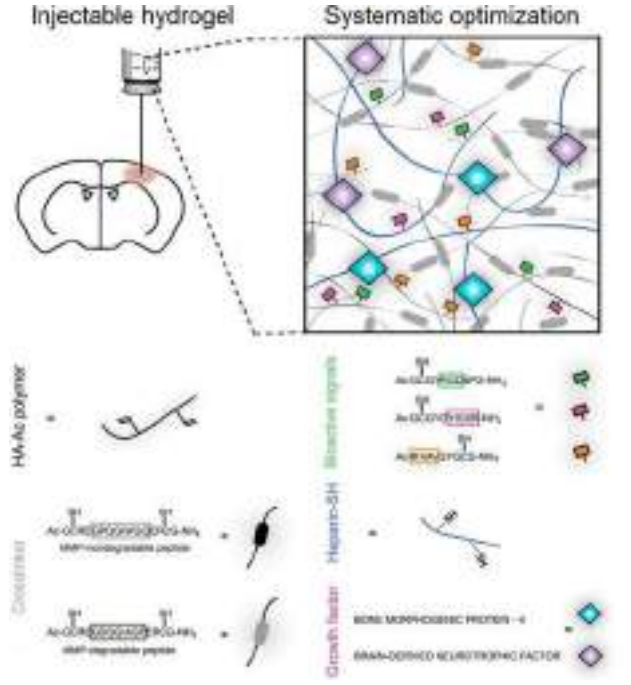


**Figure 31.** Schematic illustration of the injectable hydrogel composed of acrylated HA, MMP degradable or nondegradable motifs, adhesion peptides, and heparin-bound growth factors. Reproduced with permission from ref 302. Copyright 2016 Elsevier.

### 4.3. Glycopolymers in Tissue Engineering

Thanks to the advance of modern polymerization techniques, glycopolymers can be readily synthesized with controlled size, architectures, and functionalities, thus enabling systematic study of their structural features and bioactivities.[63,303] Glycopolymers have great potential in tissue engineering due to their capability to mimic cell−matrix interactions via multivalency. However, although glycopolymers have been used a lot for triggering carbohydrate-mediated cellular signal transduction, their applications in tissue engineering are still limited. Exceedingly rare studies have been found using such synthetic glycopolymer scaffolds for tissue engineering. Therefore, reports on the mechanical properties of glycopolymer scaffolds and their influence on cell behavior are lacking. Glycopolymers have been solely investigated with their biochemical functions based on direct interactions with cell surface receptors as well as the resultant cell behavior.[63] More specifically, a Gal-carrying synthetic glycopolymer was used, which can be recognized by the ASGPR expressed on the surface of hepatocytes.[231,304,305] An initial attempt has been made to use glycopolymers as coatings of tissue culture polystyrene plates in order to improve cell attachment.[306] For example, Akaike and co-workers developed a Gal-derivatized polystyrene, PVLA, to mimic the carbohydrate−ASGPR interaction for regulating hepatocyte cell adhesion signaling.[231,305] The study showed that as an artificial matrix, such synthetic glycopolymer matrix can regulate integrin-mediated signaling, thus supporting hepatocytes cell growth.[231] Cho and co-workers compared such Gal-carrying synthetic glycopolymer with xyloglucan (XG), a polysaccharide derived from tamarind seeds. XG is composed of Glc units in the main chain


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and Xyl and Gal units in the side chains.[307] Since cell aggregation and the resultant spheroid formation is important for maintaining the viability and functions of hepatocyte, after 42 h of incubation of hepatocytes on polystyrene surfaces coated by PVLA or XG, the spheroid formation was evaluated.[308] Compared to hepatocytes seeded on the PVLA-coated polystyrene surface, those on the XG-coated surface aggregated more rapidly to form spheroids, suggesting that the synthetic glycopolymer is not as good as XG for culturing hepatocytes.

In addition to the intrinsic carbohydrate structures, the biochemical properties of glycopolymers can also be modulated by the lengths of the pendant alkyl chain between the polymer backbone and the carbohydrate functionalities. For example, Dhayal and co-workers prepared three types of glycopolymers with varied spacer length between the pendant Glc moiety and the polymer backbone in order to investigate the response of different cell functions, such as adhesion, viability, and proliferation of mouse osteoblast (MC3T3) cells, to such structural variations.[309] It was demonstrated that the glycopolymer with a linker of C6 (GP3) exhibited better osteoblast cell adhesion and proliferation than the counterparts with shorter linkers (Figure 32a and 32b). Moreover, the

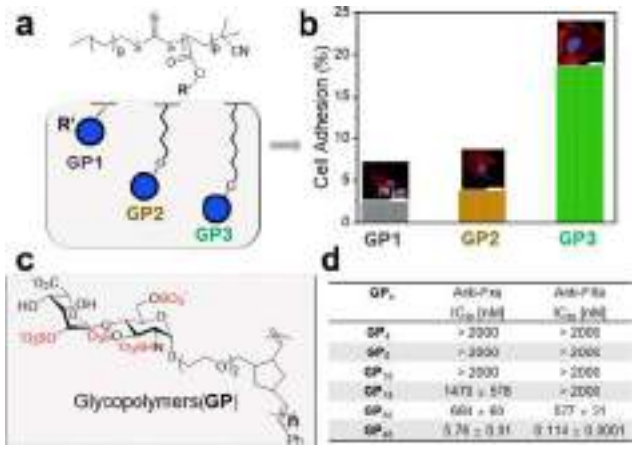


**Figure 32.** (a) Glycopolymers with distinct spacer linkers. (b) Cell attachment results corresponding to the glycopolymer structures. Reproduced with permission from ref 309. Copyright 2014 Royal Society of Chemistry. (c) Chemical structures of tailored sulfated glycopolymers, and (d) corresponding biological activity. Reproduced with permission from ref 310. Copyright 2013 Wiley-VCH.

structural similarity between glycopolymers and their natural blueprints allows for some important functions exerted by natural polysaccharides to be inherited by glycopolymers. As shown in Figure 32c and 32d, homogeneous sulfated glycopolymers composed of tetrasulfated disaccharides were synthesized to serve as alternatives to natural heparins.[310] Consequently, these glycopolymers recapitulated some key functions of natural heparins, as confirmed by their potent anticoagulant activity through inhibiting the serine proteases FXa and FIIa with potentiation of ATIII. With low polymerization degrees ($n$ = 4, 6 and 10), the glycopolymers ($GP_4$, $GP_6$ and $GP_{10}$) showed no significant anti-Fxa and anti-FIIa activities. With increasing degree of polymerization ($n$ = 15, 30 and 45), glycopopolymers ($GP_{15}$, $GP_{30}$ and $GP_{45}$) showed increasing anti-Fxa and anti-FIIa activities (Figure 32d).

### 4.4. Supramolecular Glycopolymers in Tissue Engineering

While the above-mentioned glycopolymers can provide a synthetic platform to mimic direct cell−matrix interactions, supramolecular glycopolymers are able to regulate cell behavior by additional properties. Owing to its structural features as discussed in section 2, it can nicely resemble both the dynamic and the morphological properties of the natural ECM.

Unlike naturally occurring polysaccharides that are covalently or noncovalently cross-linked into 3D scaffolds, supramolecular glycopolymers form hydrogel scaffolds via molecular self-assembly. The hydrogel formation and mechanical properties can be modulated by the self-assembly process determined by carbohydrate amphiphile structures. For example, the effects of the inherent carbohydrate structure on the self-assembly and gelation behavior of supramolecular glycopolymers have been explored through a series of diphenylalanine conjugates tethering with various monosaccharides or disaccharides (Figure 33a).[238] Disaccharide-

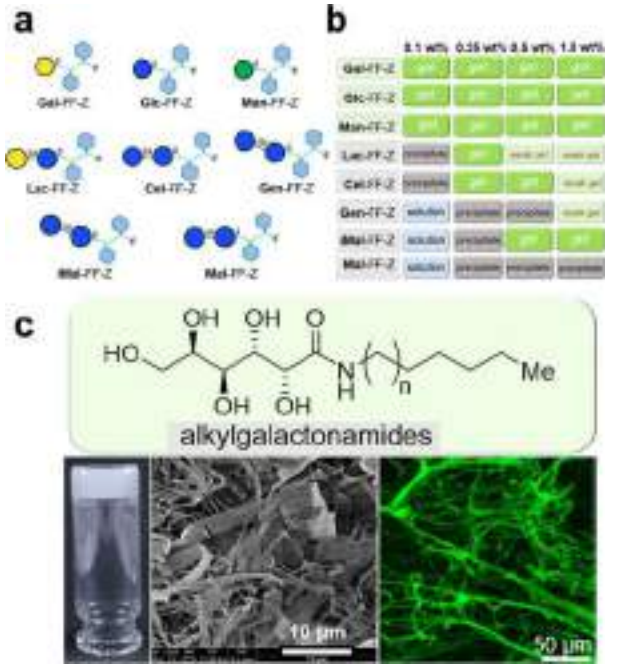


**Figure 33.** Effect of inherent carbohydrate structure on resultant hydrogel properties. (a) Diverse diphenylalanines conjugated with mono- and disaccharides. (b) Summary of gelation tests. Reproduced with permission from ref 238. Copyright 2017 Royal Society of Chemistry. (c) Chemical structures of alkylgalactonamides (top column), photograph of resultant hydrogel (bottom left), SEM image of resultant hydrogel (bottom center), and human neural stem cell differentiation on formed hydrogel (bottom right). Reproduced with permission from ref 239. Copyright 2018 American Chemical Society.

substituted diphenylalanines were more prone to afford a solution or precipitation instead of hydrogel in comparison with the monosaccharide counterparts owing to the imbalanced hydrophobic/hydrophilic ratios (Figure 33b). Moreover, the configuration of substituted disaccharides plays a significant role in the gelation ability. Diphenylalanine conjugates consisting of disaccharides with a "linear" linkage ($\beta$-1,4-linkage), such as Lac (Lac-FF-Z) and Cel (Cel-FF-Z), showed lower solubility and gelation at 0.25 wt %, while the counterparts consisting of a "bent" linkage, such as Mal (Mal-FF-Z, $\alpha$-1,4-linkage), isomaltose (iMal-FF-Z, $\alpha$-1,6-linkage),


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and gentiobiose (Gen-FF-Z, $\beta$-1,6-linkage), displayed higher solubility but resulted in precipitation at an identical concentration (Figure 33b). Another carbohydrate amphiphile example showed that self-assembly of alkylgalactonamides afforded a soft hydrogel (elastic modulus of 8 kPa) (Figure 33c).[239] Benefiting from its large interspace and highly hydrated surface (up to 99.5%), this hydrogel enabled the human neural stem cells to differentiate initially into glia or neuronal cells and finally into a dense neurofilament network.

Such hydrogels consisting of self-assembled supramolecular glycopolymers might be useful as tissue-engineering scaffolds, which can provide functional biochemical cues from carbohydrates for regulating cell behavior. However, these aforementioned examples have barely investigated the mechanical or biochemical regulation of cell behavior. In another example, Pashkuleva and co-workers coassembled carbohydrate amphiphiles and dipeptide amphiphiles into surface glycosylated nanofibers. The heparin-mimicking sulfated structure not only prolonged the stability period of growth factors up to 7 days but also preserved the viability of cultured cells, thus demonstrating the biochemical influence of supramolecular glycopolymers on cell activity (Figure 34).[241]

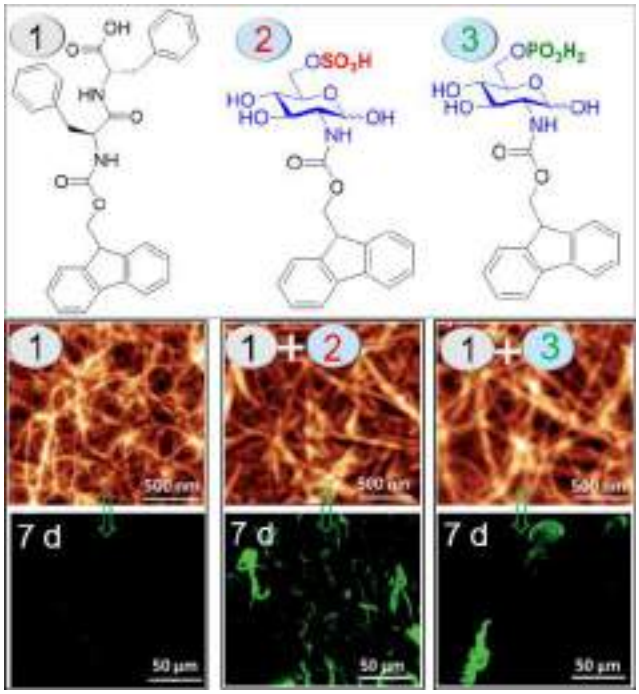


**Figure 34.** Chemical structures of dipeptide amphiphiles and carbohydrate amphiphiles (top column), corresponding AFM height images (middle column), and bioactive FGF-2 (green) distribution and stability within the gels demonstrated 7 days by CLSM (bottom column). Reproduced with permission from ref 241. Copyright 2019 Royal Society of Chemistry.

Other than such supramolecular glycopolymers self-assembled from carbohydrate amphiphiles, peptide-based supramolecular glycopolymers, also known as glycopeptide supramolecular nanofibers, have demonstrated great potential in tissue engineering. For example, the self-assembly of a synthetic glycopeptide into a nanofiber can be induced by the enzymatic dephosphorylation under physiological conditions, leading to nanofiber entanglement and supramolecular hydrogel formation (Figure 35).[240] The resultant nanofiber displayed

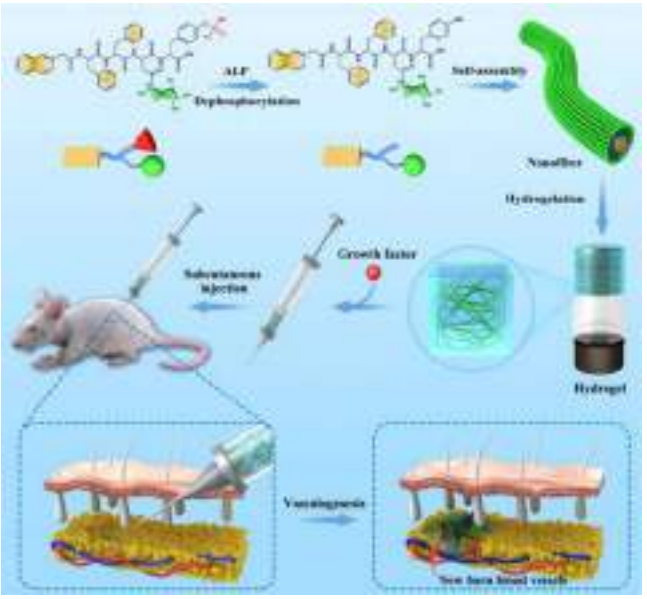

**Figure 35.** Illustration of the molecular structure of a glycopeptide molecule and its self-assembly process for the generation of a supramolecular hydrogel with Glc decoration, which could be exploited for inducing angiogenesis in vivo. Reproduced with permission from ref 240. Copyright 2018 American Chemical Society.

a high density of Glc moieties on the surface for endothelial cell adhesion and proliferation. In vitro study showed a sustainable release of the encapsulated drug deferoxamine (DFO), which further induced endothelial cell capillary morphogenesis. More interestingly, the DFO-loaded glycopeptide hydrogel was injected subcutaneously to trigger the in vivo generation of new blood capillaries in mice.

As a unique feature of self-assembled supramolecular biomaterials, the structural and biochemical properties can be facilely and modularly adjusted by hybridizing different building blocks with specific functions. In particular, the modulation of their biochemical properties can be achieved by presenting different carbohydrate structures on nanofiber surfaces[240] or by adjusting the organization of different bioactive groups on nanofiber surfaces,[236] thereafter influencing the cell−scaffold interactions and subsequent cell behavior. Such a hybridizing process is also known as supramolecular copolymerization, which is effective in adjusting the organization of bioactive groups on the nanofiber surfaces. For example, supramolecular copolymerization of different glycopeptide amphiphile molecules (Figure 36a) can allow the copresentation of Glc and carboxylic groups within the glycopeptide scaffold.[311] The resultant hydrogel scaffolds presenting such bioactivities were found to be good mimics of natural HA molecules for interacting with CD44 receptors. They can act as a synthetic counterpart of HA and be used for stem cell-based cartilage regeneration (Figure 36b). Extensive expression of the cartilage-specific proteins, such as aggrecan, collagen II, and SOX 9, from MSCs cultured in such glycopeptide hydrogel scaffolds was observed, indicating the chondrogenic inductive properties of such hydrogels. Moreover, in vivo cartilage regeneration was promoted by such hydrogels, as demonstrated by the healing of osteochondral defects. In another example, Guler and co-workers reported that the spatial organization of bioactive groups, i.e., Glc, carboxylate, and amine, on glycopeptide supramolecular


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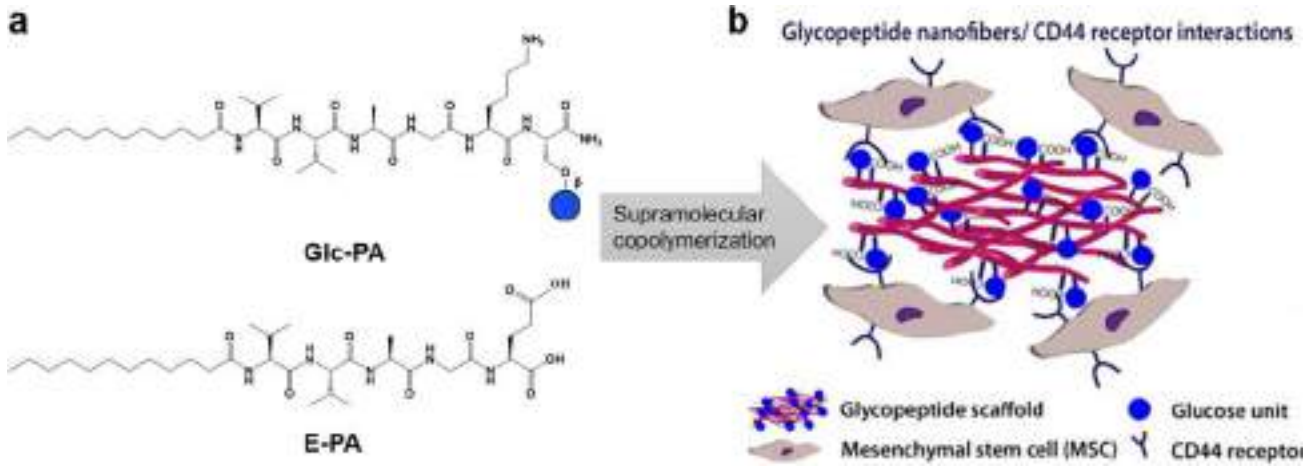


**Figure 36.** (a) Chemical structures of amphiphile molecules of Glc-PA and E-PA. (b) Scheme of the supramolecular glycopeptide scaffold and its interaction with MSCs. Reproduced with permission from ref 311. Copyright 2016 American Chemical Society.

nanofibers was a key factor for regulating stem cell differentiation. The glycopeptide nanofibers with the carboxylate adjacent to Glc could facilitate the differentiation of MSCs into brown adipocytes even in the absence of any differentiation medium, whereas the glycopeptide nanofibers with amine adjacent to Glc could not.[236]

Other than directly interacting with cell surface receptors, supramolecular glycopolymers can also mimic sulfated polysaccharides in retaining growth factors. Stupp and co-workers developed nanofilaments containing sulfated carbohydrates to bind with Bone Morphogenetic Protein 2 (BMP-2) growth factors.[104] Such nanofilaments can amplify BMP-2 activity during bone regeneration.

### 4.5. Carbohydrate-Based Macromolecular Biomaterials in Other Tissue-Engineering Applications

**4.5.1. Organoid Development.** Organoids have emerged as a promising strategy for tissue engineering as well as fabrication of disease models due to their high potential in resembling small units of their organ of origin. To date, Matrigel, a basement-membrane matrix extracted from Engelbreth−Holm−Swarm mouse sarcomas, has been exploited as a scaffold for a myriad of cell-culture applications.[312] However, its complex, ill-defined, and variable composition has limited the applications in organoid development, especially for clinical translation.

Polysaccharide-based scaffolds could afford chemically defined and reproducible alternatives. Compared to Matrigel, the cellulose nanofibril hydrogels showed high performance in terms of differentiating liver organoids into functional hepatocyte-like cells due to its unique mechanical properties such as the rapid self-healing and shear-thinning behavior.[313] In addition, a HA hydrogel was used as a platform to enable the liver-based cell organoids inoculated with colon carcinoma cells to serve as an effective model for monitoring the metastasis growth and response of tumor cells upon drugs.[314]

In addition to scaffolds, synthetic glycomaterials were developed by Godula and co-workers for glycocalyx engineering of stem cells. The synthetic glycomaterials as proteoglycan mimetics can function like natural heparan sulfate proteoglycans in mediating growth factor signaling of stem cells, thus promoting specific cell fate commitment.[315,316] Such a glycocalyx engineering strategy has been demonstrated in the development of stem cell spheroids and their controlled differentiation by tailoring the display of glycomimetics on cell surfaces.[317,318]

**4.5.2. Cancer Spheroids.** Cancer spheroids have emerged as a promising platform for cancer research. By recapitulating the in vivo microenvironment of tumors, carbohydrate-based hydrogels have been developed for culturing cancer spheroids. The resultant cancer spheroids were used for investigating the invasion behavior of tumors as well as for screening anticancer drugs.[319−326]

For example, Kinsella and co-workers fabricated a composite hydrogel of alginate and gelatin. It was revealed that the ratio of alginate to gelatin seemed to be a key determinant that affects the growth trend of cancer spheroids. More gelatin tended to afford larger multicellular tumor spheroids, whereas more alginate yielded smaller and less viable multicellular tumor spheroids (Figure 37).[325]

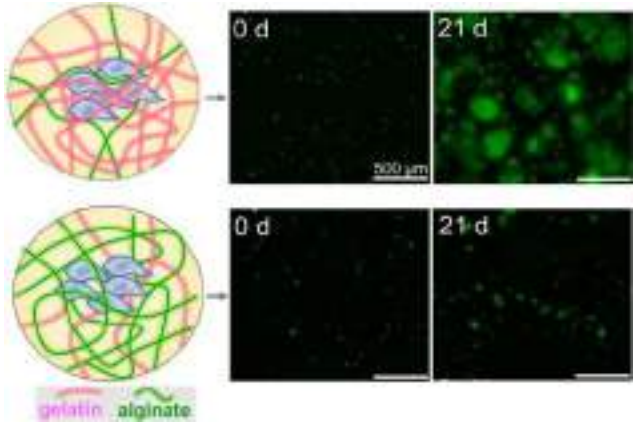


**Figure 37.** Cancer spheroids cultured in hydrogels with different ratios of gelatin to alginate. Reproduced with permission from ref 325. Copyright 2020 IOP Publishing Ltd.

Some versatile hydrogels were also fabricated to promote the development of cancer spheroids. For example, a thermoresponsive hydrogel composed of cellulose nanocrystals surface functionalized with temperature-responsive poly(*N*-isopropylacrylamide) (PNIPAM) was demonstrated not only to promote the growth of individual breast cancer cells into


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cancer spheroids but also to trigger the release of cancer spheroids by cooling-induced hydrogels liquefaction.[326]

## 5. IMMUNOLOGY

The informative role of carbohydrates in macromolecular materials will be emphasized in this section. The natural carbohydrates play crucial roles in immune response, pathogen infection, and cancer metastasis based on their specific recognition with other biomolecules.[327−330] For instance, innate and adaptive immune responses can be modulated by oligosaccharides via specific recognition between carbohydrates and lectins.[330,331] Unique glycans displayed on tumor cells and pathogens have been exploited as ideal targets for vaccination. In addition, blocking the interactions between pathogens and hosts with glycomimetics protects their host from infection.

Most of the current research in the field of carbohydrate vaccines somehow started from carbohydrate synthesis. Indeed, the carbohydrate antigens are complicated and have intrigued many researchers to develop elegant chemical methodologies for their synthesis. Meanwhile, the vaccination function requires multivalent interactions between carbohydrates and proteins, thus pushing such synthesis from the small-molecule to the macromolecular level. Moreover, since the carrier is one of the three basic elements of vaccines besides antigen and adjuvant, some vaccines are developed in the form of molecular assemblies, such as vesicles and fibers. In this part, the macromolecular form of carbohydrate vaccines will be emphasized, where carbohydrates function as either antigens or adjuvants. As such, this part will bridge the research fields of polymeric biomaterials and carbohydrate vaccines. Therefore, we summarize the recent progress of carbohydrate-based macromolecules with immune functions in the following four sections. As CPIs are the initial step of infection, the materials for infection prophylaxis will be first introduced. Then the glycomaterials participating in immunoregulation via CPIs will be presented. Some of the glycomaterials have been solely utilized as adjuvants, of which the performance will be discussed following immunoregulation. Finally, carbohydrate-based macromolecular vaccines and immunotherapies will be presented. It is challenging to systematically summarize this part in terms of macromolecular biomaterials; thus, typical examples and recent progress will be given with emphasis on macromolecular features.

### 5.1. Infection Prophylaxis

CPIs mediate the first step in the infection process of many pathogens, including bacterial infection and viral entry.[332,333] By virtue of specific recognition, extraneous pathogens adhere to the host cellular surface and influence their physiological functions. Thus, it is a rational strategy to construct synthetic glycoconjugates possessing various carbohydrates and multivalency to interfere with interactions between pathogens and host cells. Herein, we tend to give an overview of recent advances in glycoconjugates comprised of synthetic scaffolds and glycosides that assist host cells to prevent or inhibit pathogen infection during the initial stage. There are several pathogens and carbohydrates referred to in this section (Table 1).

**5.1.1. Bacterial Infection.** Since the spatial proximity of the carbohydrate ligands in space enables the "glycoside cluster effect", multivalent glycoconjugates can generate high affinity with lectin receptors expressed by bacteria.[334,335] Hence, glycosylated scaffolds capable of disrupting the interactions between the host cell and bacteria have attracted much attention as inhibitors for toxin entry,[336] bacterial adhesion,[335] and biofilm formation.[337]

**Table 1. Typical Carbohydrate-Protein Interactions during Pathogen Adherence**

| pathogens | proteins | carbohydrates |
|---|---|---|
| *Vibrio cholerae* | cholera toxin | Gal |
| *Escherichia coli* | FimH | Man |
| *Pseudomonas aeruginosa* | LecA, LecB | Gal |
| Ebola virus | DC-SIGN | Man |
| HIV | DC-SIGN | Man |
| influenza virus | hemagglutinin | Neu5Ac |

*5.1.1.1. Bacterial Toxin.* Pathogenic bacteria secrete protein toxins, causing primary pathogenicity around the world. In most cases, the emergence of diarrheal diseases is attributed to toxins produced by bacteria, including shiga-like toxins and cholera toxins.[338] These toxins consist of a single toxic A subunit attached with nontoxic lectin-like B pentamers belonging to the family of $AB_5$ toxins. The B pentamer is a carbohydrate binding domain which facilitates the attachment of such toxins to specific host cell−surface glycans.[336,339] After initial adhesion, the toxins enter the host cells, traffic to the endoplasmic reticulum, and release toxic A subunit into cytosol. To deal with the infection, glycosylated scaffolds with selectivity toward toxins can be used to block the interactions, which has attracted much interest in the past decades. The relevant research has been highlighted in several excellent reviews.[336,340−342]

In general, it is challenging to amplify the interactions via the "glycoside cluster effect" with low molecular weight scaffolds due to the low binding affinity. The common approach to present multivalent glycosylated ligands is to string carbohydrates out along a polymer chain. The polymeric architecture connects multiple carbohydrates together and presents ligands in close proximity simultaneously. Gibson and co-workers reported a series of glycopolymers with precisely controlled carbohydrate density, linker length, and chain length by postpolymerization modification.[88] Furthermore, they studied the interactions between cholera toxins and glycopolymers. The longer linkers could increase the inhibitory activity, which was possibly attributed to the depth of the binding pocket. To mimic host cellular glycocalyx, Gibson and co-workers recently prepared a series of heterogeneous glycopolymers with varying ratios of Gal and Man (Figure 38).[91] A nonuniform distribution of carbohydrates on the polymeric backbone provided suitable space for binding with specific receptors. It was found that heterogeneous polymers exhibited a higher inhibitory activity than their homogeneous counterparts. Glycan with a 3:1 ratio of Gal to Man displayed the best inhibitory potency against cholera toxin B subunit. Apart from the optimum polymeric structure and the specificity of the carbohydrates, different distributions of carbohydrates should be considered while modulating the inhibitory activity.

Although the glycosylated scaffolds have potential application for inhibiting toxin, there have been very few associated references in recent years. Nevertheless, carbohydrate-based materials are promising to provide a straightforward method to prevent infection.

*5.1.1.2. Bacterial Adhesion.* The majority of infectious diseases resulted from pathogenic bacteria are governed by


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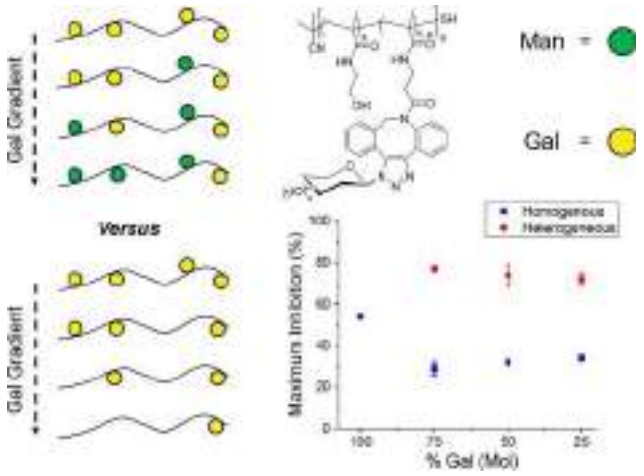


**Figure 38.** Illustration of glycopolymers and comparison of total maximum inhibition achieved. Reproduced with permission from ref 91. Copyright 2019 Wiley-VCH.

interactions between bacterial organelles called fimbriae, and specific glycoconjugates on the cellular periphery. For instance, Type 1 fimbriae expressed by pathogenic strains binds to the corresponding carbohydrate ligands in the urinary tract.[272,273] In particular, much interest has focused on FimH, a Man-modified glycoprotein-specific lectin at the terminal of fimbriae. Different inhibitors have been constructed to compete with the interaction, providing effective approaches to combat bacterial adhesion and infection. An intelligent design of multivalent glycoconjugates requires not only ligands with high affinity but also other factors, such as scaffolds, clusters, steric shielding, etc.[343] On the basis of the above considerations, various glycosylated scaffolds ranging from dendrimers, linear polymers, and nanoparticles to nanofibers (1D) and sheet-like platforms (2D) have been developed rationally in recent years.

In contrast to the low-affinity binding between monovalent molecules to proteins, glycodendrimers exposing Man residues could significantly facilitate the binding with *Escherichia coli* (*E. coli*).[335] Analogously, linear polymeric scaffolds are flexible and therefore able to access lectins at bacterial fimbriae. A library of precisely defined linear and star-shaped glycopolymers bearing multiple pendent copies of *n*-heptyl α-D-Man (HM) with different chain lengths was designed.[344] HM-based glycopolymers could disrupt the binding of invasive *E. coli* to T84 intestinal epithelial cells potently, displaying a more than 100-fold antiadhesive effect to the monovalent HM.

Furthermore, globular nanoparticles grafted with Man residues can also shield sectional areas of the bacteria. In 2010, fullerene bearing 12 peripheral Man moieties was reported as an antagonist of FimH for the first time.[345,346] In addition, polymersomes are an appealing alternative by virtue of their controllable structure as well as their mechanical and biological properties. Perrier and co-workers reported giant glycosylated polymersomes (GGPs) comprised of novel amphiphilic diblock glycopolymers presenting tunable bacterial affinity.[347] In this study, biologically relevant glycopolymers formed nanoscale and microscale morphologies in solution via different self-assembly processes. Specifically, the aggregation of Glc-modified GGPs was observed by confocal microscopy upon addition of FimH-positive *E. coli*. Gal-modified GGPs, however, displayed a different binding behavior that possibly immobilized the bacteria. The research provided an approach to tune the bacterial aggregation through selective ligand–receptor interactions.

Glycosylated nanofibers formed by supramolecular self-assembly are attractive due to multivalent interactions with specific lectins. Lee and co-workers controlled the length of nanofibers by regulating the crystallinity of the aromatic moieties. They observed a more effective *E. coli* agglutination ability from the longer Man-modified nanofibers.[348] The phenomenon demonstrated that the length of the nanofibers was a critical factor in regulating the proliferation and agglutination of bacteria. For antiadhesive therapy, nanomaterials with biologically and environmentally benign components have attracted great interest. Cellulose nanofibrils functionalized with Man were reported to capture fimbriated *E. coli* and prevent adhesion.[349] In order to mimic the dynamic process of specific recognition in biology, Liu and co-workers constructed DNA–dendron supramolecular scaffolds which could be utilized to agglutinate *E. coli* reversibly (Figure 39).[350]

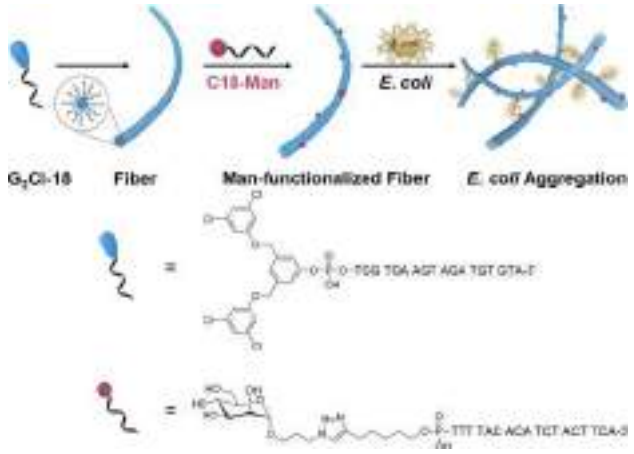


**Figure 39.** Illustration of functionalized DNA–dendron supramolecular fibers and regulation of *E. coli* association. Reproduced with permission from ref 350. Copyright 2015 American Chemical Society.

The carbohydrate–oligonucleotide conjugates (C18–Man) were guided onto DNA–dendron supramolecular fibers via DNA hybridization, thereby forming Man-functionalized fibers. Due to the high-affinity binding between the Man ligands and the FimH receptors on the *E. coli* strains ORN178, Man-functionalized fibers recognized, entangled, and agglutinated the pathogen specifically. In contrast, monovalent C18–Man or nonfunctionalized fibers did not show similar performance. In addition, the association process of *E. coli* was reversible by replacing multivalent Man ligands with competitive unmodified DNA sequences. By virtue of the designable sequence and hybridization properties, glycosylated DNA scaffolds can offer controllable frames to regulate *E. coli* association.

Generally, large sheet-like 2D scaffolds provide more contact area at the interfaces than nanofibers, which could better inhibit pathogen adhesion. Haag and co-workers constructed a multivalent Man-functionalized 2D scaffold by placing cyclodextrin-based sugar ligands on adamantyl-functionalized thermally reduced graphene oxide (TRGO) sheets.[351] These Man-functionalized TRGO sheets could effectively capture and agglutinate *E. coli* with much higher agglutination ability than


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that of the parent macrocyclic host or TRGO itself. The unique infrared (IR) absorption property of graphene was utilized to kill the captured bacteria under IR irradiation. Due to the unique optical and mechanical properties, graphene was utilized to wrap pathogens, taking advantage of the unprecedentedly large surface and flexibility.[343] Multivalent Man residues recognized the FimH receptors on *E. coli* and made 2D scaffolds distinctive in the applications of pathogen inhibition. Besides FimH, there are other types of fimbriae existing on the surface of *E. coli*, such as FimP, FimS, and FimG, which allow bacteria binding with different carbohydrate units.[352] Glycosylated architectures mimicking host glycan are docking points for bacterial entry, which can be employed to expand other functions, including imaging,[353,354] phototherapy,[355] and high-throughput screening.[356] Nevertheless, since bacterial adhesion is always troublesome and poses a threat to human public health, it is essential to explore innovative glycosylated antagonists against adherent-invasive bacteria. Multivalent glycoconjugates provide fundamental guidance for inhibiting bacterial adhesion from the mechanism of infection.

*5.1.1.3. Bacterial Biofilm.* Adhering bacteria are able to embed themselves in a self-generated matrix of extracellular polymeric substances (EPS) consisting of proteins, polysaccharides, humic acids, and eDNA. The formation of EPS, also known as biofilm formation, provides a physical barrier to antibiotics, host immune system, and environmental challenges. To deal with the tolerance of the biofilm, many synthetic materials have been designed.[357]

Disrupting biofilm formation is essential to restore antibiotic efficiency and overcome antibiotic resistance. Since Gal-specific lectin (LecA) and Fuc-specific lectin (LecB) are known to mediate biofilm formation,[337] a promising strategy is to develop multivalent glycoconjugates with Gal and Fuc to inhibit the corresponding lectins. For instance, peptide dendrimers have been demonstrated as multivalent glycoconjugates for this purpose. They can be synthesized by attaching glycosidic groups at the end of the dendrimer branches, thus resulting in glycopeptide scaffolds mimicking glycoproteins. In 2007, Reymond and co-workers reported the first study of *P. aeruginosa* biofilm inhibition by LecB-targeting glycopeptide scaffolds.[358] Later, by attaching 4-carboxyphenyl $\beta$-galactoside (Gal-A) and carboxypropyl $\beta$-thiogalactoside (Gal-B) to peptide dendrimers, they investigated the biofilm inhibition of a glycopeptide family with well-designed building blocks (Figure 40).[359] These glycopeptide dendrimers could bind to specific LecA and inhibit biofilm formation, showing the crucial importance of the multivalency effect for biofilm inhibition. Specifically, it was revealed that both Gal−AG2 and Gal−BG2 inhibited the biofilm formation completely, but the acetylated dendrimer lacking Gal groups displayed unsatisfactory performance.

Synthetic carbohydrate polymeric structures also possess unique bioactivity for biofilm inhibition compared to individual building blocks. A series of bioinspired PASs was prepared via a controlled anionic polymerization of $\beta$-lactam monomers for this purpose.[58] Grinstaff and co-workers utilized PAS amphiphiles to modulate *P. aeruginosa* biofilm formation without affecting the growth of bacteria. They found that amphiphilic random copolymers could decrease the surface tension of water at interfaces and affect cell−surface and cell−cell interactions, thereby disrupting biofilm formation. Through lectin blocking, biomimetic glycopolymers could

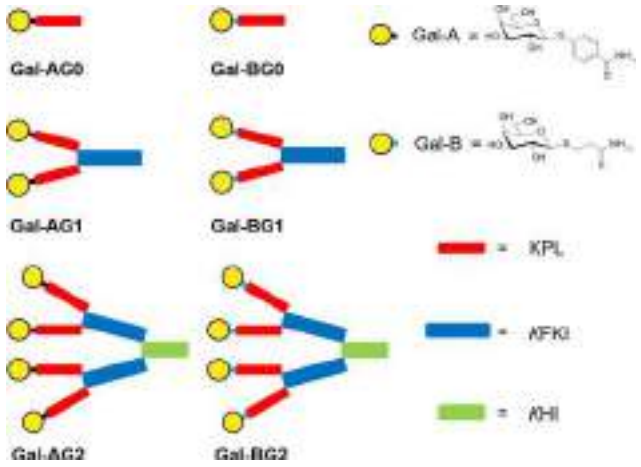


**Figure 40.** Gal-modified peptide dendrimers as inhibitors of *P. aeruginosa* biofilms. *K* = lysine as branching unit, K = lysine, P = proline, L = leucine, F = phenylalanine. Reproduced with permission from ref 359. Copyright 2011 Wiley-VCH.

selectively eliminate the biofilm and eradicate drug-resistant infections. Natural polysaccharides, possessing nontoxicity, good biodegradability, and broad-spectrum antibacterial activity, are widely used in the biomedical field. In recent years, sulfonate chitosan, quaternary chitin−silver nanocomposite, and carboxymethyl chitosan have been reported to inhibit the establishment of bacteria biofilm.[360−362] These results demonstrate safe and effective approaches in biomedical devices and implants. Since biofilms can enhance the resistance to available antibiotics, glycopolymers were combined with a photosensitizer for photodynamic therapy[363] and photothermal therapy.[364]

Moreover, supramolecular scaffolds can provide highly ordered platforms for inhibiting bacterial biofilms. Chen and co-workers constructed glyco-metallacycles combining saccharide functionalization with the positive charges from metallacycles (Figure 41a).[365] Amphiphilic glyco-metallacycles displayed different morphologies via self-assembly, such as nanoparticles, vesicles, and microsized vesicles. After investigating the capacity in *Staphylococcus aureus* biofilms inhibition of glyco-metallacycle assemblies, much better performance of [3 + 3]-Gal assemblies was observed than that of Donor 1 itself and [3 + 3]-$EG_5$ assemblies at the same concentration (Figure 41b). The different biofilm inhibition ability was attributed to the Gal moieties, since [3 + 3]-Gal and [3 + 3]-$EG_5$ shared the same backbone with the only difference being between Gal and $EG_5$. Furthermore, they employed a quartz crystal microbalance to quantify the interaction between assemblies and *S. aureus* (Figure 41c). The results demonstrated that the interaction in the [3 + 3]-Gal group was stronger than that in the [3 + 3]-$EG_5$ group, as a more obvious decrease in frequency (from 0 to −15 Hz) was observed. By virtue of the interaction between Gal and *S. aureus*, glyco-metallacycle assemblies showed more effective inhibition toward the formation of biofilms in comparison with [3 + 3]-$EG_5$. According to these results, glycosylated metallacycle holds great potential as a biofilm inhibitor for pathogen infection. Besides supramolecular metallacycles, glycolipids with moderate hydrophobicity were observed to potentially eradicate the Gram-positive bacteria biofilms, whereas more hydrophobic counterparts could disrupt the established Gram-negative biofilm.[366] These results demonstrated that supramolecular


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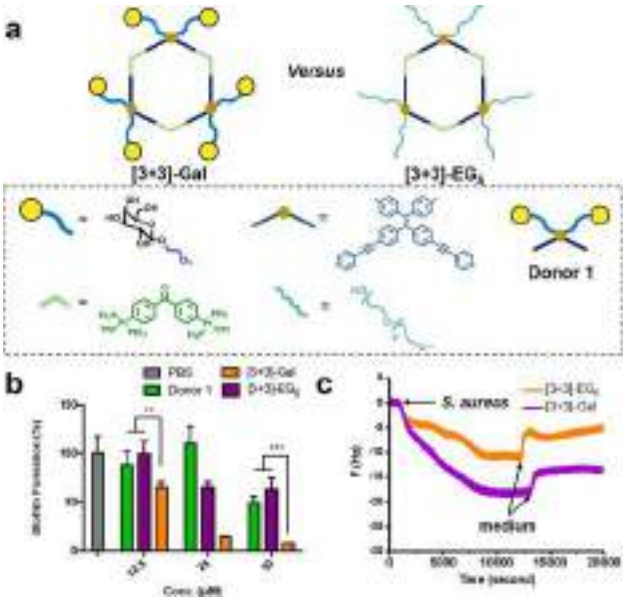


**Figure 41.** (a) Schematic illustration of the formation of amphiphilic metallacycles. (b) Inhibition of *S. aureus* biofilm formation by different [3 + 3] metallacycle assemblies. (c) Frequency response curves of the [3 + 3]-Gal and [3 + 3]-$EG_5$ surface with *S. aureus* medium solution. Reproduced with permission from ref 365. Copyright 2020 American Chemical Society.

scaffolds could effectively disassemble pathogenic biofilms due to CPIs and insertion of appropriate hydrophobic chains in biofilms.

**5.1.2. Virus Infection.** Viruses have always been pathogenic agents and brought about severe infections, such as Ebola, HIV, and influenza virus, etc. Through multivalent CPIs, viruses adhere to the host cellular surface, transport genetic materials, and make viral replication.[331,367] The design of antiviral inhibitors is critical to prevent viruses from escaping the immune defense. However, this issue has not been potently addressed so far probably because monovalent drugs display low binding affinity toward lectin receptors. A valuable strategy has been proposed to prepare new antiviral agents by employing multivalent glycosylated scaffolds in the past decades.

*5.1.2.1. Ebola.* Ebola viruses (EBOV) invade the host through primarily targeting macrophages, DCs, and monocytes.[368] These cells, highly expressing lectin receptors, recognize envelope viral glycoproteins and mediate viral entry. DC-SIGN is a significant C-type lectin which shows potent affinity toward glycoproteins of EBOV.[369] Many reports have demonstrated that Man-modified scaffolds were employed to inhibit EBOV entry.[341,370,371] In early research, the inhibition parameter $IC_{50}$ decreased from the micromolar to the nanomolar range. In addition, fucocluster pseudopeptide-based calixarenes with four pendant Fuc ligands at each molecular surface could also block DC-SIGN active sites at nanomolar $IC_{50}$.[372−374]

Multivalent nanostructures are dominant in competing with viral glycoproteins to target host immune cells. In order to generally mimic the multivalent presentation of carbohydrates at the surface of pathogens, virus-like particles (VLPs) were functionalized with glycodendrimers, providing 1620 pendant Man ligands. These glycodendriproteins strongly prevented EBOV from entering into T cells and DCs via DC-SIGN.[375] Besides, it was evidenced that the replication of some viral infection depended on a low pH in the endosome. Branched polyethylenimine possessing nonprotonated amine groups can neutralize endosomal pH. Man-based dendrimers blocked viral entry and replication, presenting broad-spectrum antiviral activity.[376]

In the past 10 years, fullerenes $C_{60}$ have been widely investigated as biocompatible scaffolds for presenting multivalent carbohydrate ligands since they can mimic natural virus systems with adequate size. Due to the symmetrical and globular structure, innovative Man-modified fullerenes became alternatives to VLP-based nanoparticles. A series of glycosylated 3D fullerenes has been proposed to inhibit EBOV infection; relevant advances were highlighted by Martin and colleagues.[377−380] Furthermore, they utilized other nanocarbon platforms to present carbohydrates multivalently, including single-wall carbon nanotubes (SWCNTs), multiwall carbon nanotubes (MWCNTs), and single-wall carbon nanohorns (SWCNHs).[381] Apart from the unique physical properties and versatile chemistry, these carbon-based nanostructures resemble the form of viruses and could become innovative scaffolds (Figure 42). After connecting asymmetric azide-substituted

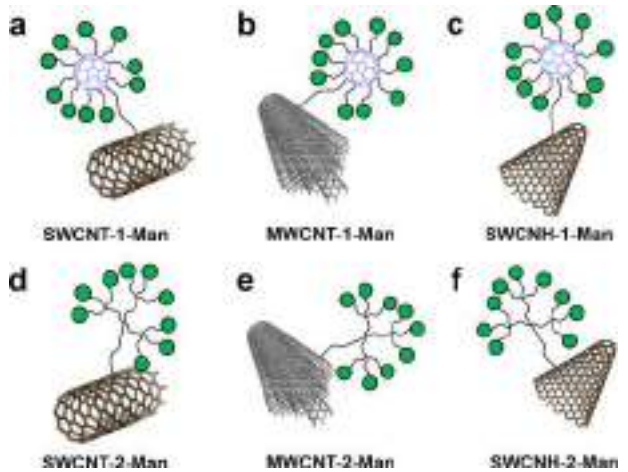


**Figure 42.** Schematic illustration of Man-modified nanocarbon scaffolds. Reproduced with permission from ref 381. Copyright 2018 American Chemical Society.

hexakis adducts of [60]fullerene 1-(Man) or glycodendron 2-(Man), they obtained 6 functionalized glycoconjugates without appreciable cytotoxicity. Most importantly, from the results of the antiviral activity, MWCNT-1-Man was observed to inhibit EBOV infection at concentrations as low as 0.37 $\mu$g/mL. The high antiviral activity was attributed to the presence of a large amount of Man and the appropriate size and shape of the carbon nanoforms compared to the others. In this regard, MWCNTs are appealing materials which serve as efficient antiviral agents. Together, these findings demonstrate that the size, availability, and valency should be considered toward the construction of potent inhibitors against EBOV.

*5.1.2.2. HIV.* HIV is another devastating pathogen and represents a challenge to current fundamental research. The high-mannose glycans of coat protein gp120 play a crucial role in HIV infection via binding to DC-SIGN expressed on DCs. As a critical kind of antigen-presenting cells, DCs migrate to lymph nodes and transmit HIV to eliminate $CD4^+$ T cells, resulting in disruption of the host immune system.[382,383] To


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fight against HIV infection, extraordinary efforts have been made to relieve or prevent viral entry, like microbicides and vaccines. On the basis of the mechanism of infection, carbohydrate-based multivalent scaffolds provide potential opportunities to address this problem by interfering with the binding between gp120 and DC-SIGN. Therefore, designing anti-HIV inhibitors has received much attention in recent years.[331,341,367]

Synthetic glycopolymers become attractive inhibitors and provide multivalent platforms for anti-HIV entry. The proportion of Man in polymeric constitution importantly interferes in the binding of gp120 with DC-SIGN.[86,384] In addition, inorganic nanoparticles and nucleic acids can also serve as multivalent scaffolds.[385] To improve the biocompatibility and resemble natural proteins, glycoconjugated poly(amino acids) are promising alternatives for blocking the interactions between gp120 and DC-SIGN.[82] Chen and coworkers utilized amphiphilic glycopolypeptides (Figure 6) to obtain various morphologies, including nanowires, nanoribbons, and compound micelles. To evaluate the protein binding capacity of mannan-mimicking structures, they chose HIV antibody 2G12 as a model. These alternating amphiphilic glycopolypeptide brush assemblies could effectively inhibit 2G12-gp120 binding at a Man residue concentration of 2 mM.[386]

Glycodendritic compounds are anti-HIV inhibitors because these compounds can display multivalency and spatial presentation of carbohydrate ligands simultaneously. Man-modified glycolipids, pseudomannoside-based dendrimers, and rod-like spacers were designed to potently inhibit HIV transinfection with a concentration from micromolar to nanomolar $IC_{50}$.[387−389] Cyclodextrin-based glycoclusters have attracted considerable interest because the branched structures possessed high affinity with proteins and served as carriers for delivering hydrophobic drug. Becer and colleagues prepared a series of $\beta$-cyclodextrin-based glycoclusters and $\beta$-cyclodextrin-based star glycopolymers (Figure 43).[87] These

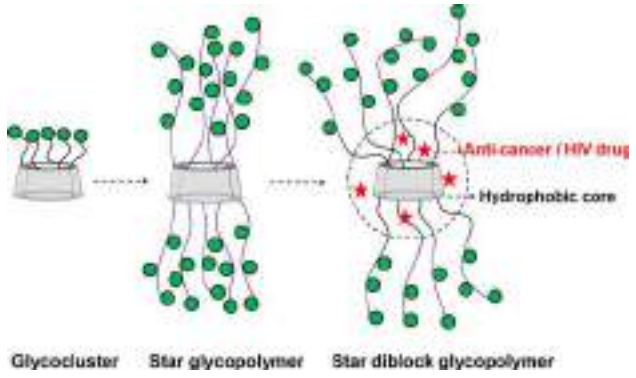


**Figure 43.** Illustration of $\beta$-cyclodextrin-based glycoclusters and $\beta$-cyclodextrin-based star glycopolymers. Reproduced with permission from ref 87. Copyright 2014 American Chemical Society.

Man-based dendritic compounds could effectively prevent the binding of HIV coat protein gp120 to DC-SIGN at nanomolar concentrations. Most importantly, $\beta$-cyclodextrin-based star glycopolymers were able to load hydrophobic anti-HIV drugs into hydrophobic core, indicating that this strategy is promising for inhibiting and eradicating HIV entry.

Despite the fact that many multivalent scaffolds show great potential in anti-HIV entry, this strategy has not been employed in a clinical setting. Some anti-HIV vaccines have been investigated, and associated advances are discussed in the following section of this review.

*5.1.2.3. Influenza Virus.* As one kind of highly contagious pathogen, influenza virus (IV) annually lead to periodic epidemics worldwide. Unfortunately, influenza has caused substantial mortality and economic cost in the past decades. In order to combat this pandemic outbreak, it is essential to investigate the mechanism of viral infection and develop anti-IV inhibitors. However, the development of antiviral reagents is limited by emerging viral resistance.[390] Currently, vaccines provide an unsatisfactory solution because of the long research/development time with usually controversial effects.[391] The viral envelope protein, hemagglutinin, is abundantly distributed on the virion surface for binding with Neu5Ac-containing glycans presented on the epithelial cell surface.[392−394] Hence, Neu5Ac-based multivalent scaffolds are able to target and shield IV, preventing viruses from agglutinating on host cells.

Several Neu5Ac-functionalized nanoparticles have been constructed rationally to inhibit IV entry, including gold nanoparticles and polymeric nanoparticles.[395−399] Due to the high loading capacity, optical properties, and controllable size, these scaffolds not only present multivalency of Neu5Ac to bind with IV but also possess functions of detection. It was also reported that multimeric sialosides may increase the binding efficiency,[400] but optimum spacing among the ligands should be considered to afford strong inhibitors.[401]

In particular, glycopolymers exhibit the "glycoside cluster effect"[402−404] and represent a contribution to the development of nanomedicines for pathogens.[405] Topological design, number of saccharides, and molecular mobility of glycopolymers have been investigated in detail for inhibiting IV entry in recent years.[406−408] For example, highly sulfated synthetic glycomimetics have been utilized to act as natural polysaccharide analogues to inhibit viral binding/infection.[409] To precisely mimic the multivalent biological properties of natural glycoproteins, a route based on the combination of global amino acid substitution and a peptide glycosylation platform was presented. Engineered with a high density of homopropargylglycine residues, an elastin-like-peptide backbone could couple protected and deprotected mono-, di-, and trisaccharides via CuAAC reaction. Neu5Ac-functionalized glycoprotein mimetic materials show the ability to protect cells against influenza A virus entry, demonstrating a comparable effectiveness to natural mucins.[410] This work provides a robust strategy to synthesize mucin mimics and replicate critical biophysical properties, which differs from conventional glycopeptides.

For the design of optimal antiviral inhibitors, the spatial activity of Neu5Ac-containing scaffolds should be considered. Hydrophobic chains in molecular structures can facilitate penetration toward lipid bilayer membranes, which improve the spatial activity of inhibitors.[411] From the perspective of a protein, hemagglutinin is a trimeric protein, providing three binding sites for Neu5Ac. The spatial distance among the binding sites on a single hemagglutinin is about 4−5 nm. In addition, the diameter of the typical IV is 120 nm, and the distance between the centers of the adjacent heads of the hemagglutinin trimer is 10−12 nm. The spatial distance between adjacent carbohydrates is critical to the activity of the scaffolds. Inspired by this, a series of multivalent 6′-sialyllactose-polyamidoamine conjugates was designed.


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Among these scaffolds, S3−G4 conjugates (Figure 44) showed the strongest interaction with hemagglutinin and the lowest

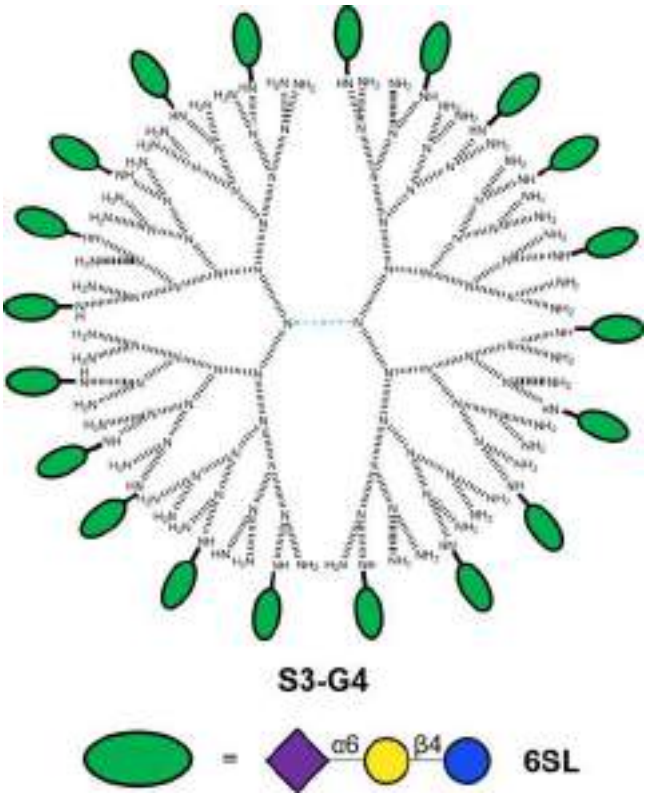


**Figure 44.** Structure of dendrimer conjugates S3−G4. S represents the number of 6SL ligands, and G represents generation in the S−G conjugates. Reproduced with permission from ref 401. Copyright 2017 Springer Nature.

$IC_{50}$ against H1N1 viral infection as S3−G4 conjugates display a near-optimal ligand spacing with an interligand spacing of about 3.1 nm. The glycan architectures of antiviral inhibitors should be emphasized when considering potent efficiency.[401] Furthermore, bacteriophage capsids carrying carbohydrate ligands were investigated to inhibit viral infection. Icosahedral bacteriophages Q$\beta$ are suitable for a presentation of Neu5Ac residues. The triangular distance of amino acid residue K16 in capsid structure is 5−6 nm, which matches the distance between the individual binding sites of trimeric hemagglutinin (4.7 nm).[412] The rational geometric arrangement of capsid could be utilized to anchor Neu5Ac ligands for binding hemagglutinin on the surface of IV effectively (Figure 45). The spatially defined arrangement of the carbohydrate ligands in scaffolds facilitates virus targeting. This work is superior to traditional scaffolds since the distance of the carbohydrate ligands could be designed precisely on a nanoscale, which may become novel antivirals for the treatment of IV.

## 5.2. Carbohydrate-Based Immunoregulation by Targeting Lectins

In the previous section, inhibition of CPIs to prevent various infections has been presented, which requires different multivalent scaffolds with enhanced binding ability. In this section, the immunoregulation of glycomaterials targeting lectins will be introduced. Although multivalency is still dominating in the presented glycomaterials, including glycopolymers, glycodendrimers, glycoliposomes, glyconanoparticles

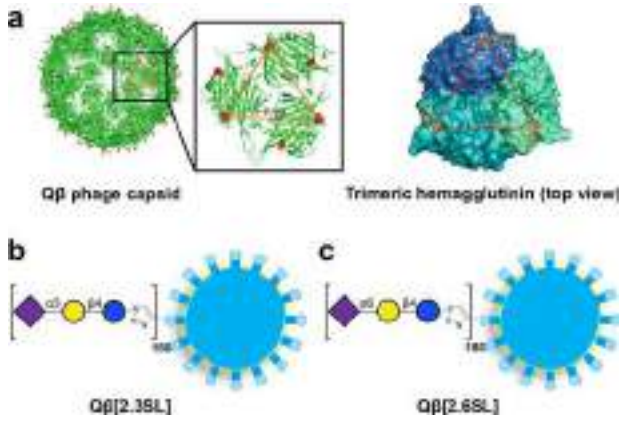


**Figure 45.** (a) Structural match between distances of Neu5Ac attachment points (position K16 of wild-type Q$\beta$ coat protein, red dot) on the capsid surface and distances of hemagglutinin-Neu5Ac binding pockets. (b and c) Recombinantly expressed Q$\beta$ was conjugated with Neu5Ac. Reproduced with permission from ref 412. Copyright 2020 Springer Nature.

(GNPs), etc., the response from immunological cells will be emphasized. For clarity, the immunoregulation effect of glycomaterials in this section will be categorized by receptors.

**5.2.1. Immunoregulation by Targeting Siglecs.** Siglecs, i.e., Neu5Ac-binding immunoglobulin (Ig)-type lectins, are transmembrane proteins of the Ig superfamily. They are mainly expressed on immune cells and can recognize Neu5Ac-containing glycans (sialoglycan) via the *N*-terminal Ig domain. Siglecs can be divided into two groups: those conserved across mammals, including Siglec-1 (CD169), -2 (CD22), -4, and -15, as well as variable CD33-related Siglecs, including Siglec-3 (CD33), -5, -6, -7, -8, -9, -10, -11, -14, and -16, for humans.[413,414] Among this family, extra attention has been paid to Siglec-2 expressed on B cells and Siglec-7 expressed on natural killer cell (NK), DC, and macrophage, etc.

Recent investigations have shown that sialoglycan−Siglec interaction can serve as immune checkpoints to help immune cells to distinguish between self and nonself.[415] Due to the widespread presence of Siglecs on immune cells and Neu5Ac on all mammalian cells, sialoglycan−Siglec interactions are involved in diverse cellular immune responses and thus related with various kinds of diseases, such as neurodegeneration, asthma, allergy, and cancer, etc. Thus, the interactions between sialoglycan and Siglec have become an attractive target for therapeutic interventions. For example, tumor cells can utilize sialoglycan−Siglec interactions to modulate immune cell function by promoting the creation of the immunosuppressive tumor microenvironment. In this regard, multivalent scaffolds, including liposomes, polymers/polysaccharides, VLPs, and other nanoparticles, have been exploited to target Siglecs with high affinity, improving or alleviating immune response via sialoglycan−Siglec interaction.[416−418] A series of studies has been reported by the Paulson and other groups, with detailed information (e.g., chemical structures of ligands for every Siglec) provided in the previous reviews.[414,415] Some representative polymeric glycomaterials for modulation of the immune response are highlighted below.

High expression of Neu5Ac on the cell surface is a character shared by many tumor cells.[419] To better understand the correlation between hypersialylation and an immune-suppressive environment, Bertozzi and co-workers engineered cells'


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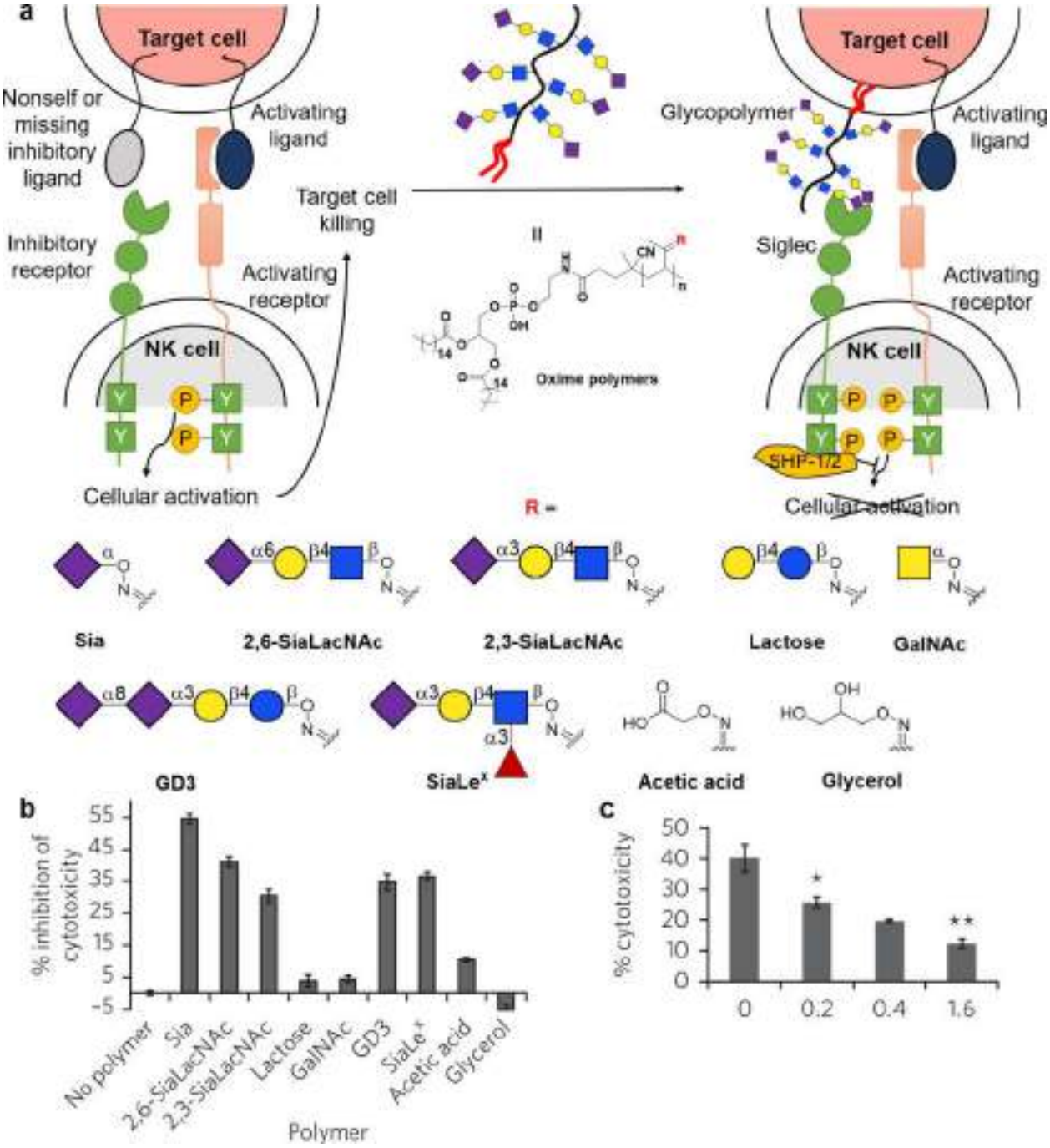


**Figure 46.** (a) Study of sialoside-dependent NK inhibition by a glycocalyx-engineering approach. (b) Glycopolymers protect target cells from NK-mediated cytotoxicity. (c) Sia density dependence for protecting target cells from NK cell cytotoxicity. Reproduced with permission from ref 420. Copyright 2014 Springer Nature.

glycocalyx with sialoglycan-containing glycopolymers, wherein phospholipids at the end of the glycopolymers can be passively inserted into cell membranes (Figure 46).[420] The resulting cells were used to investigate the roles of sialoglycan in mediating Sigelec-based immunosuppression. The engineered cells were first constructed by incubating several cancer cell lines (Chinese hamster ovary (CHO), Jurkat, MCF-7, and so on) with fluorescently labeled glycopolymers. The incorporation efficiencies were determined by flow cytometry and fluorescence microscopy. The results showed that only sialoside-functionalized glycopolymers can protect engineered Jurkat cells from NK cell killing, whereas the NK cell cytotoxicity could not be inhibited when polymers without Neu5Ac were evaluated (Figure 46b). In addition, in the presence of Siglec-7-blocking antibody, comparable cytotoxicity was observed under similar conditions, confirming that Neu5Ac-based NK cell inhibition was mediated by Siglec-7. Notably, it was also demonstrated that the NK cell cytotoxicity to target cells was highly dependent on the density of polymer containing sialic acid (Sia) (Figure 46c). With increasing concentration of this polymer, NK cell-mediated cytotoxicity was largely reduced. The above investigation was eventually exploited to protect engineered allogeneic and xenogeneic


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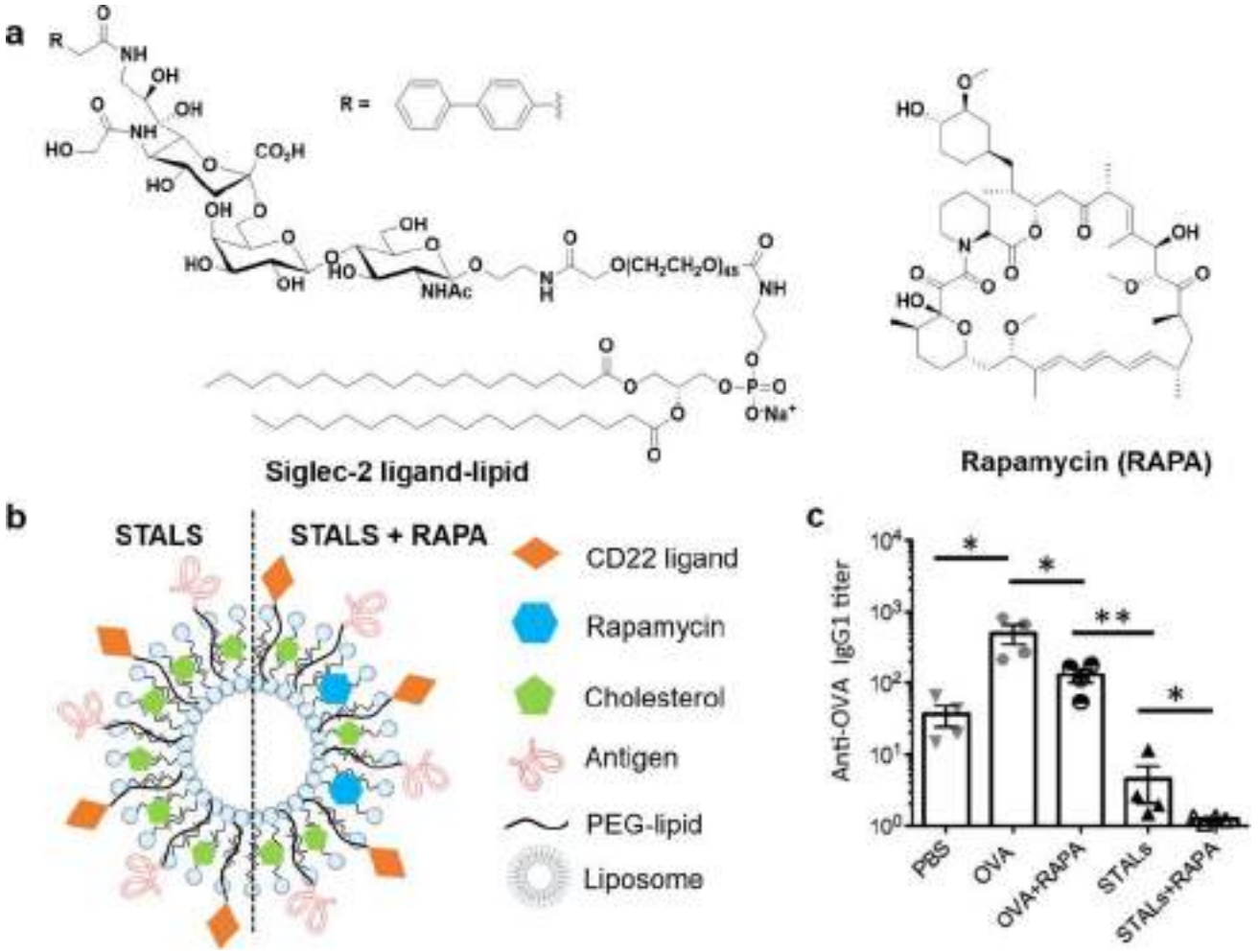


**Figure 47.** Enhancement of immune tolerance by Siglec-engaging tolerogenic liposomes. (a) Chemical structure of compound Siglec-2 ligand−lipid and RAPA. (b) Illustration of STALs. (c) Anti-OVA titers (IgG1) on the 28th day (0.1% OVA was added in all groups). Reproduced with permission from ref 422. Copyright 2017 Wiley-VCH.

primary cells from NK-mediated killing, indicating that Siglecs might be used as targets in cell transplant therapy.

Recently, Huang and Li's groups reported the enhancement of anticancer immunotherapy utilizing glycoengineered NK cells (Figure 47).[421] NK cells with CD22 ligands were constructed by metabolic glycoengineering or insertion of glycopolymers on the NK cell membrane. Although the CD22 ligand level on NK cells decreased rapidly in the latter method, the presentation remained detectable for 3 days. The glycopolymer-modified NK cells also showed enhanced cytotoxicity toward CHO cells, which were genetically engineered to express human CD22 on the cell surface.

To induce antigen-specific immune tolerance and alleviate undesired immune responses, including organ transplant rejection, allergies, autoimmune disease, etc., copresentation of antigen and ligands of Siglec was used as the strategy to induce immune tolerance by Paulson's group (Figure 47).[422] Siglec-engaging tolerance-inducing antigenic liposomes (STALs, Figure 47b) were prepared with lipid-conjugated Siglec-2 ligand (i.e., Siglec-2 ligand−lipid, Figure 47a) and ovalbumin (OVA). In addition, an immunomodulator rapamycin (RAPA, Figure 47a), which is clinically used to prevent acute renal allograft rejection, was also formulated into liposomes to induce strong antigen-specific immune tolerance (Figure 47b). Mice were immunized on day 0 and then challenged with immunogenic OVA liposomes 14 days later. Anti-OVA titers (IgG1) on day 28 showed that the stronger tolerance was induced by STALs than the OVA + RAPA group. The best tolerance was achieved by STALs + RAPA group (Figure 47c). This result indicated that the combination of antigen and ligands of Siglec could be employed for treating allergy and autoimmune diseases.

In addition, ligands of Siglecs were also used for targeted delivery of antigens to antigen-presenting cells (APCs). For example, liposomes were formulated with both mycobacterial lipid antigen C80 Glc-6-monomycolate (**C80 GMM**) for activating Group 1 CD1-restricted T cells and a modified ligand of Siglecs-7 (Figure 48a).[423] It was demonstrated that the liposomes were presented to human monocyte-derived DCs (MO−DCs) and delivered to lysosome through Siglec-7-mediated endocytosis (Figure 48b). The CD1b-restricted T cells line LDN5 were efficiently activated to produce significantly more IFN-$\gamma$ than the free **C80 GMM**.

Recently, high molecular weight HA, a kind of nonsialic acid containing glycan, was also reported to be recognized by CD33-related Siglecs. Nizet and co-workers discovered that Group A *Streptococcus* could recognize human Siglec-9 via its high molecular weight HA capsule to block neutrophil extracellular trap formation and oxidative burst, thereby promoting bacterial survival. Thus, inhibition of the interaction


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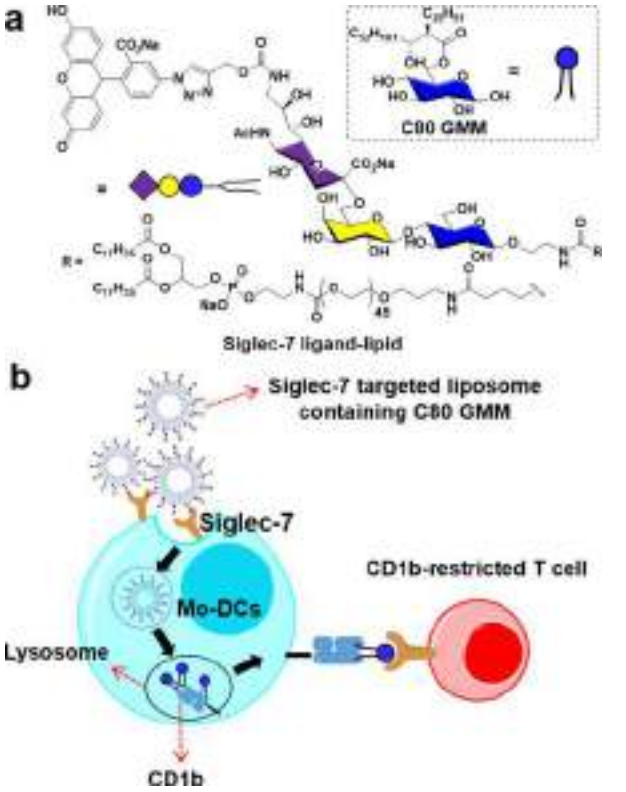


**Figure 48.** (a) Structure of ligand of Siglecs-7, i.e., Siglec-7 ligand−lipid. (b) Activation of CD1b-restricted T cells through Siglec-7-mediated endocytosis.

between high molecular weight HA and human Siglec-9 may be used to manipulate the neutrophil function in infectious and inflammatory diseases.[424]

**5.2.2. Immunoregulation by Targeting Galectins.** Galectins are one of the most widely expressed lectins, which typically bind $\beta$-D-Gal-containing glycoconjugates and share highly homologous CRD. Galectins have the capabilities to oligomerize glycoproteins and glycolipids on the cell surface into ordered aggregates and play essential roles in many biological processes, including differentiation, cell binding, cellular trafficking, migration, cell signaling, apoptosis, and so on. There are 15 mammalian galectins inside and outside the cell. Galectins are subdivided into 3 types: (1) Proto-type galectins, including galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15, are noncovalently dimerized homodimers containing two identical CRDs; (2) Tandem-repeat galectins, including galectin -4, -6, -8, -9, and -12, are covalently linked containing two different CRDs; (3) Chimera type (galectin-3) assembles into a pentamer through a N-terminal domain. Among these galectins, galectins-1 and -3 have been extensively explored owing to their capabilities to induce T-cell apoptosis. Overexpression of these galectins on many tumors are correlated with tumor immune privilege and various steps of the metastatic cascade.[425]

Putnam and co-workers prepared a series of glycopolymers with different carbohydrate densities by grafting amino-functionalized saccharide onto poly(acrylic acid).[426] Binding between glycopolymers and galectins was measured by surface plasmon resonance (SPR). It was shown that only Lac-containing glycopolymers displayed binding affinities to galectin-1 and -3. Under a similar grafting degree of Lac, glycopolymers with a higher molecular weight exhibited higher binding affinities due to the increased cluster valency. In addition, owing to pentameric structures, the glycopolymers presented higher binding affinities toward galectin-3 than galectin-1 and a significantly lower equilibrium dissociation constant $K_D$ ($10^{-11}$ M) than monovalent CPI ($10^{-3}$ −$10^{-4}$ M). Recently, glycopolymers were also used for selectively binding to galectin-1.[427] Not only the density but also the structures of the carbohydrate (mono-, di-, and trivalent) were tuned for the selectivity. It was revealed that dense presentation of individually distributed LacNAc on the copolymer with *N*-(2-hydroxypropyl)methacrylamide (HPMA) was strongly preferred by galectin-1 with up to 300-fold discrimination.

In 2015, self-assembled nanofibers were employed as a modulator of galectin-1 bioactivity by Hudalla's group (Figure 49).[428] The monosaccharide GlcNAc was modified at the N-

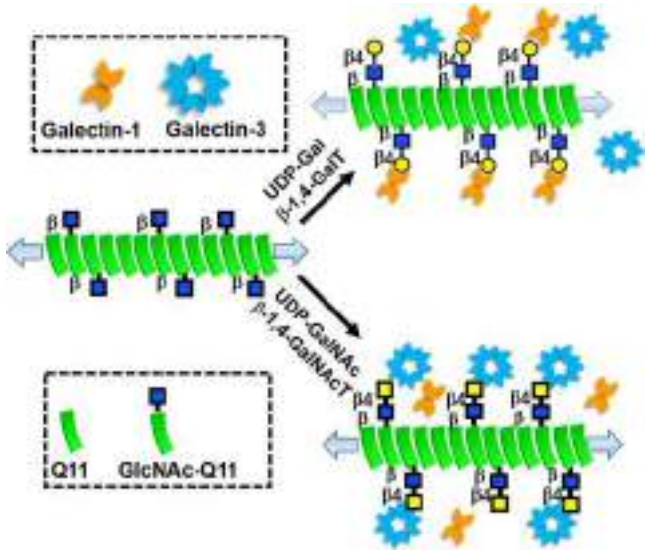


**Figure 49.** Modulator of galectin-1 and galectin-3 bioactivity by self-assembled glycopeptide.

terminus of the QQKFQFQFEQQ (Q11) peptide which could self-assemble into $\beta$-sheet nanofibers. The morphology was retained after introduction of the carbohydrate, and the carbohydrate concentration could be easily tuned by coassembly of GlcNAc−Q11 and Q11 at different molar ratios in the preassembled state. Treatment of the resulting nanofibers with $\beta$-1,4-galactosyltransferase ($\beta$-1,4-GalT) provided the LacNAc−Q11 nanofibers in situ. As expected, nanofibers with the highest molar ratio of LacNAc−Q11 to Q11 exhibited the highest binding affinity to galectin-1 and the best inhibition to T-cell apoptosis. However, only a low binding affinity and no inhibition to T-cell apoptosis were observed in the case of galectin-3. To develop effective galectin-3 inhibitors, self-assembled peptide nanofibers modified with disaccharide *N*,*N*′-diacetyllactosamine (LacDiNAc), which preferentially binds to galectin-3, were constructed through modular coassembly.[429] Unexpectedly, the serum glycoproteins could block the interaction between LacDiNAc and galectin-3 in the binding assay. When competitive serum glycoproteins are minimized, the newly developed nanofibers can selectively bind to galectin-3 in the presence of galectin-1. The efficacy of LacDiNc−Q11 nanofibers to inhibit galectin-3 pro-apoptotic activity was also demonstrated. Moreover, Hudalla's group also investigated the effect of the carbohydrate


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density of the glycosylated $\beta$-sheet peptide nanofibers on the binding of the protein. Similarly, the nanofibers were prepared by coassembly of glycosylated Q11 (N(GlcNAc)-SGSG-QQKFQFQFEQQ, GQ11) and Q11 at different molar ratio. It was shown that nanofibers with low to moderate carbohydrate densities exhibited increased binding ability to lectin and improved efficacy for inhibiting wheat germ agglutinin (WGA)-induced T-cell death than that with high carbohydrate densities.[430]

Recently, Chen and co-workers explored a new strategy to prepare protein assemblies by dual supramolecular interactions, including lectin-carbohydrate interaction and $\pi-\pi$ interaction of RhB.[431] With this strategy, a small molecule **R4L** (Figure 50a) comprised of Lac and RhB has been proved to induce the self-assembly of galectin-1 into microribbons (Figure 50b).[432] When **R4L** was added to the galectin-1 agglutinated T cells, agglutination was dissociated due to the formation of such microribbons. In contrast, addition of Lac with a higher molar ratio than **R4L** could not dissociate the agglutinated T cells. This result indicated that coassembly of **R4L** and galectin-1 could compete with the binding between galectin-1 and glycan on the T-cell surface. Finally, galectin-1-associated T-cell apoptosis was also successfully inhibited through the formation of such ribbons.

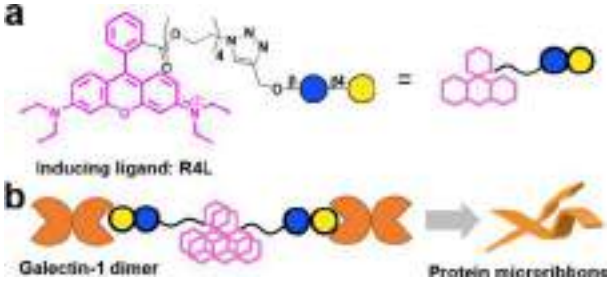


**Figure 50.** (a) Chemical structure and schematic illustration of the inducing ligand **R4L**. (b) Formation of protein microribbons through specific recognition between galectin-1 and **R4L** and RhB dimerization.

In addition, glycoproteins,[433] glycodendrimers,[434−436] glycoliposomes,[437] glyconanoparticles,[438,439] and calixarenes/cyclodextrin-based ligands[440] have also been developed to investigate the binding affinities with galectins. The interaction between galectins and the multivalent ligands was used to target tumor cells.

**5.2.3. Immunoregulation by Targeting C-Type Lectin Receptors.** C-Type lectin receptors (CLRs) are expressed by several immunologically relevant cell types, such as DCs and macrophages. They are comprised of a subfamily of pattern-recognition receptors (PRRs) that bind to carbohydrates.[441,442] There are several CLRs which recognize the pathogen-associated molecular patterns (PAMPs) composed of carbohydrate residues on the surface of bacteria, virus, and parasites (Table 2).

Selectins are special calcium-dependent transmembrane glycoproteins, mediating cell−cell adhesion by binding to fucosylated and sialylated ligands. Selectins are classified into three types: E-, L-, P-selectins. E-selectin is exposed on endothelial cells after being activated by cytokines. L-selectin is expressed on leukocytes. And P-selectin is highly expressed on platelets and endothelial cells. They are key players in chronic and acute inflammatory processes.[443−446]

**Table 2. C-Type Lectin Receptors: Distribution, Character, and Functions**

| CLRs | cellular distribution | no. of CRD | PAMPs |
|---|---|---|---|
| selectin (P-, E-, L-selectin) | leukocytes, platelets, endothelial cells | | fucosylated and sialylated ligands |
| MR | macrophages, DCs | 8 | Man, Fuc, GlcNAc |
| MGL | macrophages, DCs | 1 | Gal, GalNAc |
| DC-SIGN | DCs | 1 | Man, Lewis-type antigen, etc. |
| Dectin-1 | DCs, macrophages, monocytes, etc. | 1 | $\beta$-1,3-glucan |
| Dectin-2 | DCs, macrophages, monocytes, etc. | 1 | high-mannose oligosaccharide |

The mannose receptor (MR, CD206) is expressed on macrophages and immature DCs with 8 CRDs. CD206 specifically binds Man, Fuc, and GlcNAc on the surface of bacteria, virus, and parasites. Macrophage galactose-type binding lectin (MGL, CD301) is mainly expressed on macrophages and DCs. It has one CRD that binds Gal and GalNAc to stimulate T-cell signaling.[447] DC-SIGN is expressed on the surface of DCs with only one CRD to bind with Man and other molecules. Dectin-1 and Dectin-2 are also expressed on DCs and macrophages. They bind $\beta$-glucan and a high-mannose oligosaccharide, respectively, with only one CRD.[448] All of these CLRs play important roles in mediating endocytosis and influencing the intracellular signaling pathways. Since many CLRs are expressed on APCs, they are promising targets for the investigation of vaccine adjuvants and antigen delivery.[449]

*5.2.3.1. Immunoregulation by Targeting Selectins.* Selectins mediate the tethering and rolling of leukocytes and lymphocytes along the blood vessel wall and are associated with the initial stage of the leukocyte adhesion cascade. Thus, inhibition of selectin ligand binding is useful for the therapy of inflammation-related diseases, such as stroke, cancer, sclerosis, rheumatoid arthritis, and so on.[446,450]

P-selectin ligand-1 (PSGL-1, P-selectin $K_D$ = 0.3 mM) and E-selectin ligand-1 (ESL-1, E-selectin $K_D$ = 62 mM) are natural ligands of selectins within the inflammatory cascade. The sialyl Lewis$^X$ (SLe$^X$) tetrasaccharide is the common structure required for binding with P- and E-selectins.[446,451] Owing to the susceptibility to glycosidases and peptidases in vivo, natural ligands failed to become anti-inflammatory drugs. In this regard, significant effort has been directed toward exploring SLe$^X$ mimetics[452] in the form of small molecules, glycopeptides,[453,454] glycopolymers,[455−461] glycoliposomes,[462−464] etc., with some of these works emphasized below.

In 2017, polymeric selectin ligands for inhibiting cell adhesion were developed by Zentel's group (Figure 51).[455] In this work, PHPMA was used as the backbone. For comparison, SLe$^X$ or the three individual monosaccharides (Fuc, Gal, and Neu5Ac) as well as sulfated tyramine as the mimetic of *O*-sulfated tyrosines in the natural ligands were conjugated onto the polymer backbone. The in vitro binding affinities of four glycopolymers **P1**−**P4** (Figure 51a) toward P- and L-selectins are a factor of 10−100 higher than those to E-selectin, and the sulfated glycopolymers **P2** and **P4** showed an obviously higher binding affinity than their nonsulfated counterparts **P1** and **P3**. Compared with glycopolymers bearing SLe$^X$, the SLe$^X$ mimetics **P3** and **P4** showed lower binding affinity (Figure 51c). However, the sulfated mimetic


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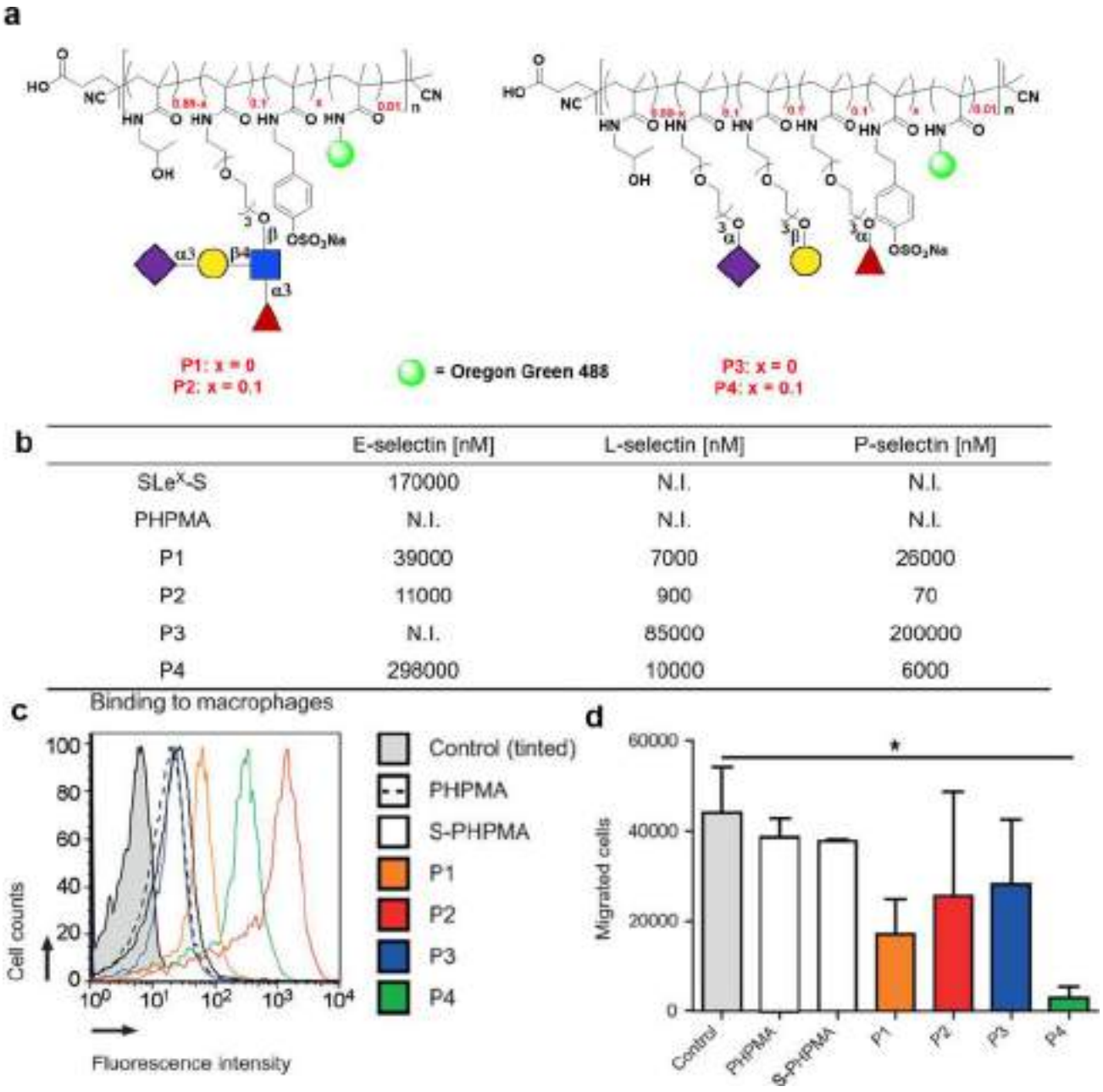


**Figure 51.** Polymeric selectin ligands and their binding to macrophages: effects on macrophage migration and cell adhesion. (a) Chemical structure of four selectin ligands. (b) $IC_{50}$ values of glycopolymers and carbohydrate conjugates toward binding of E-, L-, and P-selectins. N.I., no inhibition up to 1 mM. (c) Flow cytometry-based quantification of binding of the four ligands to macrophages. (d) Quantification of macrophage migration after 16 h. Reproduced with permission from ref 455. Copyright 2017 Wiley-VCH.

**P4** displayed a similar binding affinity as $SLe^X$ to E-, L-, and P-selectins. In addition, the binding activities of the glycomimetics, **P2** and **P4**, were also demonstrated to activate endothelial cells and macrophages. Flow cytometry-based quantification (Figure 51b) of ligand−macrophages binding affinity showed the highest cell-associated fluorescence with **P2**, which is 1 order of magnitude higher than that of **P4.** Interestingly, the mimetic **P4** significantly inhibited the migration of macrophages (Figure 51d), while **P2** and the other two mimetics only moderately reduced the macrophage migration. This investigation showed that even simple glycomimetics may exert some useful biological function as natural glycans, showing their potential for treating inflammatory diseases.

After this work, the glycopolymer mimetics **P1**−**P4** were also used in the therapy of inflammatory liver disease.[465] The in vivo biodistribution and treatment efficacy were analyzed. It was demonstrated that four polymers can accumulate in the liver without causing hepatotoxicity. In particular, the non-sulfated random glycopolymer **P3** displayed the best effect to protect mice from acute toxic liver injury, whereas the sulfated counterpart aggravated immune-mediated liver injury. These experiments implied that selectin-binding glycopolymers could be used to treat inflammatory disease or improve the efficacy of immunotherapies for cancers.

High selectin binding can also be achieved using polysaccharides as the polymeric backbone instead of synthetic polymers. The $SLe^X$−chitosan conjugate was prepared by


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incorporating GlcNAc onto the amino groups of chitosan, followed by three steps of enzymatic reactions.[457] The resulting glycomaterials exhibited high affinity to E-selectin with a $K_D$ of 920 nM.

In addition to polyvalent $SLe^x$ glycomimetics, sulfated glycopolymers and polysaccharides were also used as a selectin-targeting agent due to the existence of sulfated esters, which could promote selectin binding when they are appropriately positioned on carbohydrates.[466−473] As early as 1998, sulfated glycopolymers have been reported by the Kiessling group, wherein the synthetic glycomimetics (Figure 52a and 52b) could induce the release of the extracellular

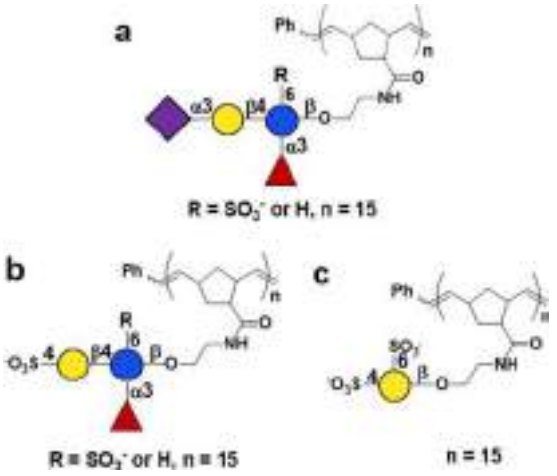


**Figure 52.** Chemical structure of synthetic glycomimetics.

portion of L-selectin in the presence of some endogenous protease.[473] Furthermore, a simplified structure (Figure 52c) was developed, which could also inhibit L-selectin-mediated cell rolling and promote L-selectin shedding.[472]

The heparinoid mimics, dendrimer-like PEO glycopolymers, have also been explored as selectin-binding antagonists.[471] Among these glycopolymers, which were decorated with sulfated Lac at the terminal of PEO, the 12-arm glycomimetics displayed inhibition of neutrophil and macrophage recruitment, whereas the 3- and 4-arm glycopolymers were ineffective. In addition, polyglycerol dendrimers modified with other sulfated saccharides, including Gal, Fuc, Lac, Man, and GlcNAc, were also developed with $IC_{50}$ on the nanomolar scale for inhibition of L- and P-selectin.[469,470]

Recently, sulfated HA nanoparticles were also employed to effectively target both P-selectin and CD44, which are highly expressed on tumor cells and responsible for tumor cell metastasis.[466] High binding affinities to P-selectin were achieved with an increase of the sulfate content, indicating the necessity of sulfate groups and the high sulfate ratio for P-selectin binding.

*5.2.3.2. Immunoregulation by Targeting Antigen Presenting Cells via CLRs.* Dectin-1 and Dectin-2 play important roles in antifungal and antibacterial immunity. In 2011, Underhill's group demonstrated that only particular $\beta$-glucans can activate Dectin-1 signaling.[474] To clarify the relationship between the glycan structure and their immune response, the first chemically defined ligands for Dectin-1 and Dectin-2 were reported by Bertozzi's group. Glycopolypeptides were successfully prepared by *N*-carboxyanhydride polymerization (Figure 53).[475] The $\beta$-1,3-glucan fragments (**Glc2**) were used

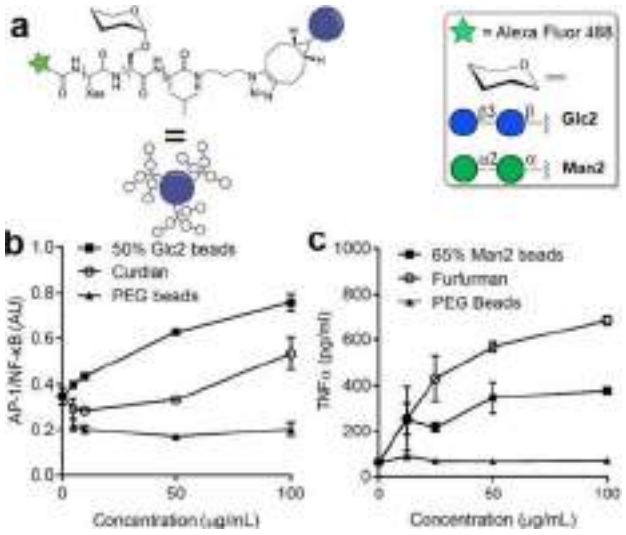


**Figure 53.** (a) Glycopolypeptide−bead conjugates induce cellular response through interaction with CLRs. (b) Dose-dependent AP-1/NF-kB activation in RAW-Blue cells after incubating with **Glc2** glycopolypeptide-decorated beads for 16 h. (c) TNF-$\alpha$ secretion in JAWSII cells after incubating with **Man2** glycopolypeptide-decorated beads for 16 h. Reproduced with permission from ref 475. Copyright 2018 Wiley-VCH.

to target Dectin-1, whereas the $\alpha$-1,2-dimannoside (**Man2**) was used to target Dectin-2. It was shown that polystyrene bead-immobilized glycopolypeptides, which contain **Glc2** fragments, elicited a comparable immune response to bacterially derived Dectin-1 agonist Curdlan (Figure 53b). Similarly, **Man2** glycopolypeptide-decorated beads also induced dose-dependent TNF-$\alpha$ secretion (Figure 53c).

DC-SIGN is significantly involved in human disease through an interaction with the oligosaccharides on viral (e.g., HIV, mycobacterial lipoarabinomannans), microbial, parasitic pathogens. Adaptation of pathogens to target DC-SIGN may help them to escape from the immune system. In addition, DC-SIGN can recognize the host glycoproteins and regulate DC migration and DC−T-cell interactions, functioning as cell-adhesion receptor. Thus, DC-SIGN is a promising target for modulating immune response.[382,476]

To study the internalization pattern and influence on the innate immune response of the DC-SIGN ligand, a hexavalent dendrimer **Polyman26** composed of modified $\alpha$-1, 2-Man and a rod-like spacer were reported by Berzi and co-workers (Figure 54).[477] Experiments showed that **Polyman26** can selectively bind to DC-SIGN via CRD. After incubation with human immature monocyte-derived dendritic cells (iMDDCs), the dendrimer was internalized by receptor-mediated endocytosis and then transported to endolysosomal compartments. Notably, **Polyman26** can upregulate multiple effectors, including $\beta$-chemokines and pro-inflammatory cytokines (IL-1$\beta$, IL-6, and TNF$\alpha$), which are important in antiviral responses. In addition, the ability to polarize adaptive immune responses toward the Th1 phenotype was also demonstrated by significant upregulation of IL-12 and IFN-$\gamma$. The cytokines and receptors involved in DC maturation, homeostasis, and activation were also modulated.

Multivalent DC-SIGN ligands were also used to reduce inflammation. Puzo and co-workers reported the prevention of acute lung inflammation by mannodendrimers.[478] Proinflammatory cytokines produced by lipopolysaccharide (LPS)-


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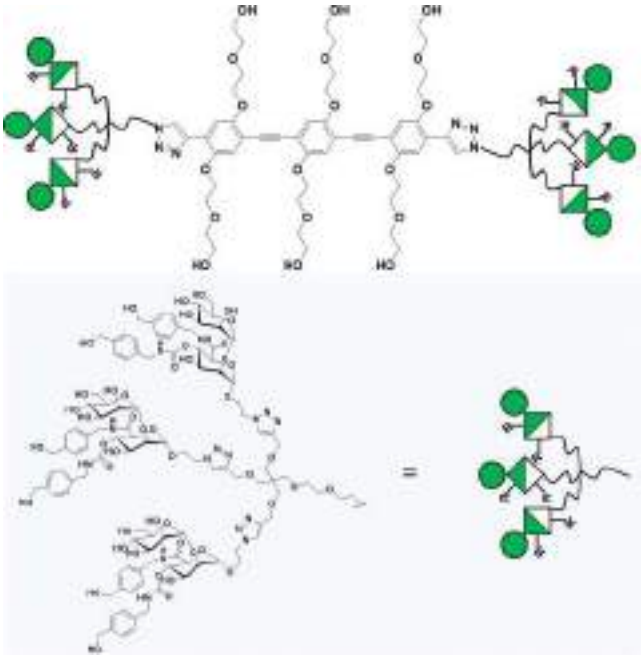

**Figure 54.** Structure of **Polyman26**.

stimulated DCs were inhibited by a third-generation dendrimer bearing trimannosides and a fourth-generation dendrimer bearing dimannosides. Furthermore, the anti-inflammatory activity was tested in a mouse model with acute lung inflammation caused by exposure of mice to aerosolized LPS. Neutrophil recruitment was significantly reduced after per os administration of the third-generation dendrimer bearing trimannosides, and acute lung inflammation was thus prevented. Becer's group reported manipulation of cytokine secretion in DCs using 5- and 8-arm star-shaped as well as linear glycopolymers.[479] The star-shaped glycopolymers displayed higher binding affinities to DCs than the linear ones due to stronger multivalent interactions with the oligomeric DC-SIGN. More importantly, they invoked the increase of IL-10 and decrease of IL-12p70, which is associated with anti-inflammation and active wound healing.

As previously demonstrated, DC-SIGN and other CLRs not only mediate the recognition, uptake, and processing of antigens but also are implicated in pathogen infection. To understand the influence of the antigen physical properties on the antigen fate upon DC-SIGN-mediated internalization, glycopolymers differing in length (**Gp1**−**Gp4**) were prepared by ROMP (Figure 55).[480] All of the soluble glycopolymers were routed to endosomal compartments, and the longer glycopolymers were more efficiently internalized than the shorter ones. However, when glycopolymers **Gp2** and **Gp3** were prepared into nanoparticles, the resulting particulate antigens were trafficked to invaginated pockets (cell−surface-accessible compartment) just as with HIV-1. This investigation will be useful for rational design of synthetic vaccines.

In addition, DC-SIGN can be used to target antigen to DCs and thus modulate the immune response. For example, multivalent glycopeptide dendrimers comprised of DC-SIGN ligand Lewis$^b$ and peptide antigen were reported by García-Vallejo and co-workers.[481] The glycopeptide dendrimers could be efficiently recognized and internalized by DC-SIGN and routed to lysosomes for antigen processing and presentation. The antigen-specific $CD4^+$ and $CD8^+$ T-cell responses were

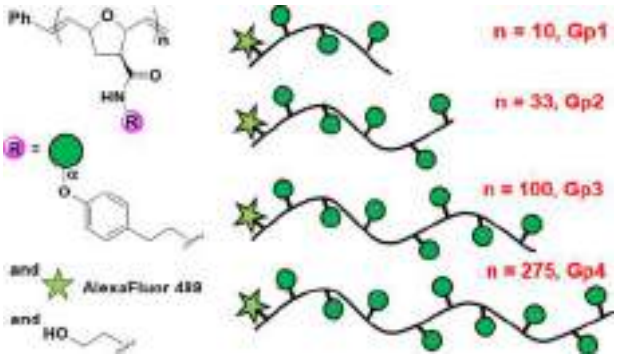


**Figure 55.** Glycopolymers with defined length bearing aryl Man ligand for DC-SIGN and Alexa Fluor 488 for visualization.

robustly inducted by the DC-SIGN-targeting glycopeptide dendrimers.

In addition, due to wide expression of MR on M2-type macrophages and DCs, Man-modified nanoparticles have been used to target tumor-associated macrophages (TAMs) or enhance antigen presentation by DCs. For example, Man-modified and PEG-shedded poly(D,L,-lactide-*co*-glycolide acid) (PLGA) nanoparticles were described by Cui and co-workers. After the acid-sensitive PEG shedding was removed in the acidic tumor microenvironment, the nanoparticles could be selectively accumulated in tumor tissue via an interaction between Man and MR.[482] In 2011, a pH-sensitive and Man-modified dextran nanoparticle was reported by Fréchet and co-workers.[483] Compared with nontargeted particulate formulation, enhanced presentation of model antigen OVA by MHC class I molecules was observed. In 2019, Mayorga and co-workers reported glycodendropeptides that were comprised of a food allergen peptide (i.e., Pru p 3) and multivalent Mans.[484] The introduction of Man led to maturation of monocyte-derived DCs (Mo-DCs). After coculturing of the preincubated Mo-DCs with autologous lymphocytes, proliferation of T cells and B cells was significantly induced in allergic patients.

Recently, systematic studies on the immunoregulation effect of self-assembled polymeric glycomaterials have been performed by Chen and co-workers. Various effects in the design of GNPs, including the size, shape, and architecture on immunoregulation have been explored. As shown in Figure 56a, GNPs with different shapes and dimensions including spherical micelles (**Sp**) as well as short and long cylinder micelles (**SC** and **LC**) were prepared by functionalization of poly(DL-lactide)-*b*-poly-(acrylic acid) (PDLA-*b*-PAA) or poly-(L-lactide)-*b*-poly-(acrylic acid) (PLLA-*b*-PAA) micelles with Man (Figure 56).[97] Cellular uptake experiments showed that fluorescently labeled **Sp** were more efficiently internalized than cylindrical micelles due to different endocytosis pathways (Figure 56b). Interestingly, the longer cylindrical GNPs with weaker internalization induced a more significant inflammatory response than shorter cylindrical and spherical GNPs (Figure 56c). Moreover, the macrophage-interacting ability of glycomaterials with different shapes was investigated. Using the same Man-containing and PLLA-based diblock glycopolymer, diamond-shaped and cylindrical glycoparticles were prepared by changing the solvent ratios (Figure 56d).[99] After removal of the organic solvent by dialysis, the resulting nano-objects, including a cylinder (**Cy**) as well as small, medium, and large platelets (**SP**, **MP**, **LP**), were incubated with RAW264.7 macrophages. The endocytosis experiment showed


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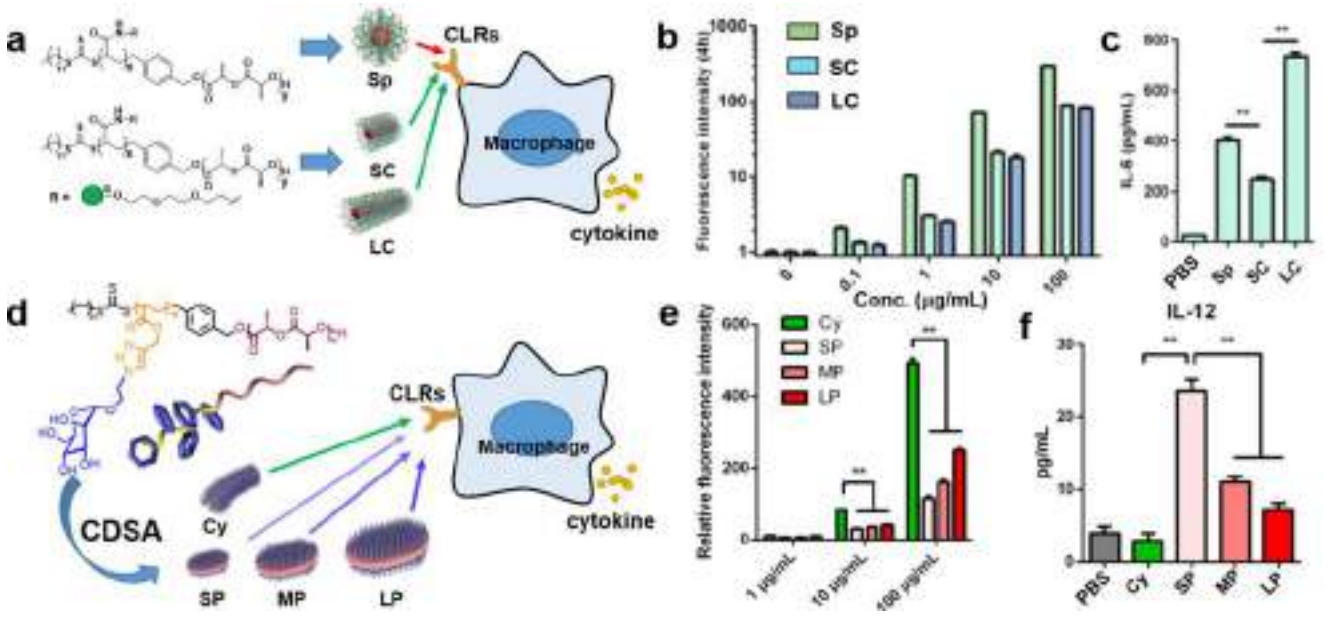


**Figure 56.** (a) Illustration of glycomaterials with different shape and size to induce immune response. (b) Dose dependence of the binding of GNPs with RAW264.7 macrophages. (c) GNPs (10 $\mu$g/mL) promoted the IL-6 secretion of macrophages after 24 h incubation measured by enzyme-linked immunosorbent assay (ELISA). (d) Immune response induced by GNPs with different shape by interaction with macrophages. (e) Endocytosis of different fluorescent $PLLA_{33}$-*b*-$PMan_{12}$ GNPs by RAW264.7 macrophages. **Cy**, cylinder; **SP**, small platelet; **MP**, medium platelet; **LP**, large platelet. (f) GNPs with different size/shape (10 $\mu$g/mL) promoted IL-12 secretions of macrophages after 24 h incubation measured by ELISA. Reproduced with permission from refs 97 and 99. Copyright 2016 and 2019 American Chemical Society.

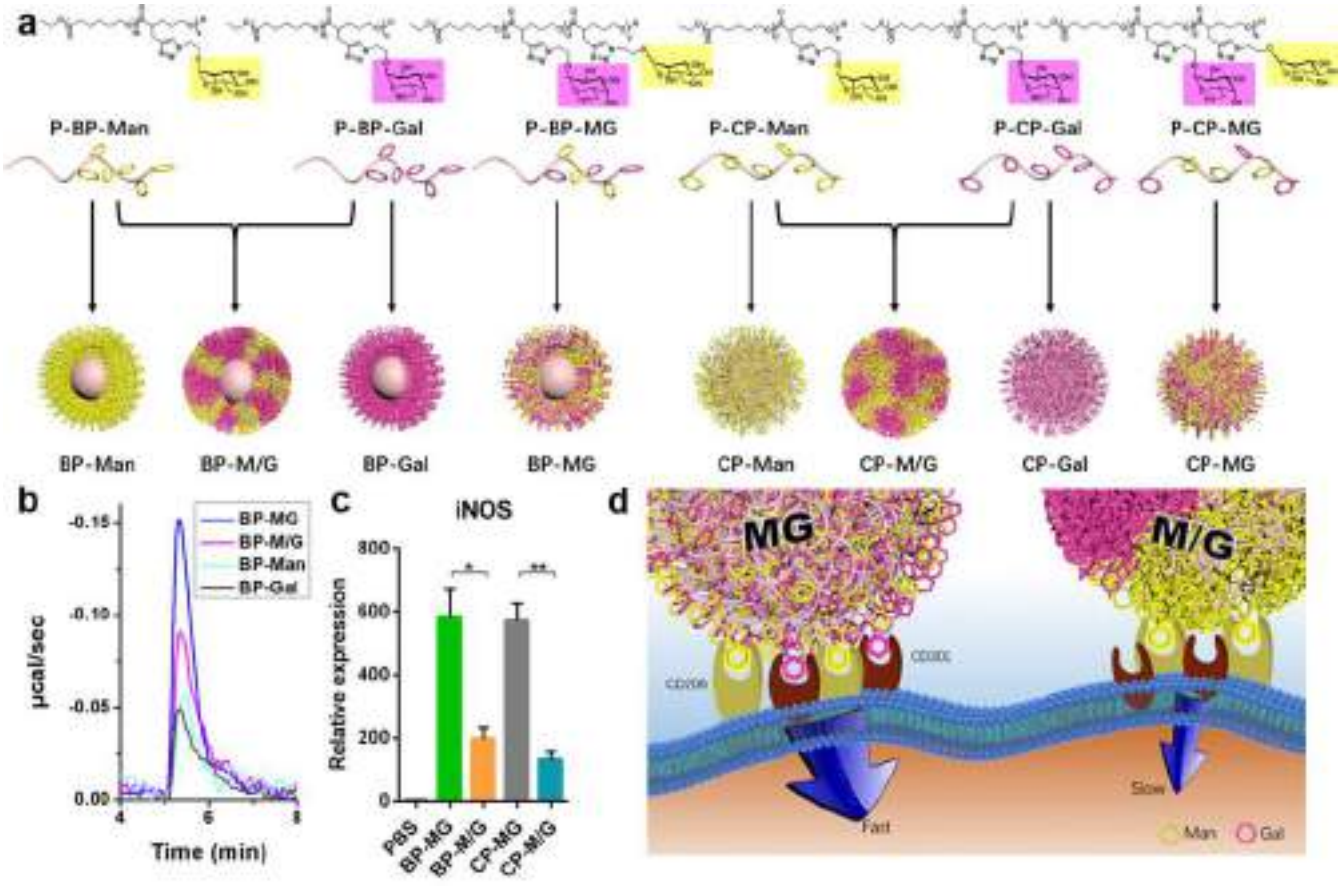


**Figure 57.** (a) GNPs with different shell architectures made by self-assembled glycopolymers. (b) Heat release of block copolymer (BP) series GNPs binding with lectins (ConA:PNA = 1:1). (c) iNOS release under equal endocytosis amounts. (d) Interaction between two different saccharides and their receptors. Reproduced with permission from ref 98. Copyright 2018 American Chemical Society.


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that **Cys** were more efficiently uptaken by macrophages (Figure 56e). However, glyco-platelets, especially **SP**, displayed better immunostimulatory activities than the other groups (Figure 56f).

Besides the effects of the shape and size of glycomaterials for immunoregulation, interestingly, GNPs with various architectures were also investigated in cellular uptake and lectin binding abilities. As shown in Figure 57a, the blend-mixed GNPs (i.e., **BP−M/G**, **CP−M/G**) were prepared by mixing two homoglycopolymers (i.e., **P-BP/CP-Man** and **P-BP/CP-Gal**), whereas the homogeneously mixed GNPs (**BP−MG**, **CP−MG**) were prepared by glycopolymers containing both Man and Gal, i.e., **BP−MG** and **CP−MG**.[98] The endocytosis of these GNPs by RAW264.7 macrophages showed that a homogeneous mixture, **BP−MG** and **CP−MG**, exhibited higher efficiency in cellular uptake and binding abilities toward MGL and MR as well as other plant lectins (Figure 57b) than **BP−M/G** and **CP−M/G**. Meanwhile, macrophages were also more efficiently activated by the homogeneous mixtures, **BP−MG** and **CP−MG**, than GNPs with blend-mixed coronas, **BP−M/G** and **CP−M/G**, which could be characterized by enhanced release of arginase 1 and iNOS (inducible nitric oxide synthase) (Figure 57c). It was speculated that GNPs with a homogeneous corona allowed for simultaneous interactions between two different saccharides and their corresponding receptors (Figure 57d).

Macrophages are important APCs in innate and cell immunity, which have been traditionally divided into two groups according to distinct activation pathways: the classical M1 type (activated by IFN-$\gamma$ and TLR ligand) and the alternative M2 type (stimulated by IL-13/IL-4). The M1-like macrophages are mainly responsible for removing invaded pathogens and tumor cells, secreting proinflammatory cytokines. However, M2-like macrophages are related to wound healing and tumor progression, secreting anti-inflammatory cytokines, and expressing high CD206. TAMs generally exhibitan M2-like phenotype, facilitating angiogenesis, tumor cells invasion, migration, and suppression of antitumor immune response.[485,486]

Considering the plasticity of TAMs upon the confronted microenvironments, Chen and co-workers explored the effect of glycocalyx-mimicking nanoparticles on the polarization of the thioglycolate broth-treated mouse primary peritoneal macrophages (normally regarded as M2-like macrophage).[487] As shown in Figure 58a, three GNPs, **M−Man**, **M−Gal**, and **M−Fuc** together with a PEGylated NP as control were prepared through self-assembly of block copolymer PR-*b*-PS (R represents Gal, Man, Fuc, or PEG), showing a similar hydrodynamic diameter. All three GNPs displayed specific binding toward M2-like macrophages at 4 °C and receptor-dependent internalization at 37 °C due to the presence of C-type lectins (MGL and MR) on M2-like macrophage. Most importantly, the expression of CD86 was significantly upregulated, while CD206 and CD23 were downregulated after GNPs incubation, indicating the polarization from M2 to M1 (Figure 58b). Furthermore, the polarization was also confirmed by the increase of cytokine IL-12 secretion in vivo (Figure 58c).

In combination with the above inspiring results, enhancement of anti-PD-L1 cancer immunotherapy through reversion of TAMs was also demonstrated (Figure 59).[488] The signaling pathways involved in TAMs reversion by GNPs were carefully investigated; it was shown that the reversion of TAMs was

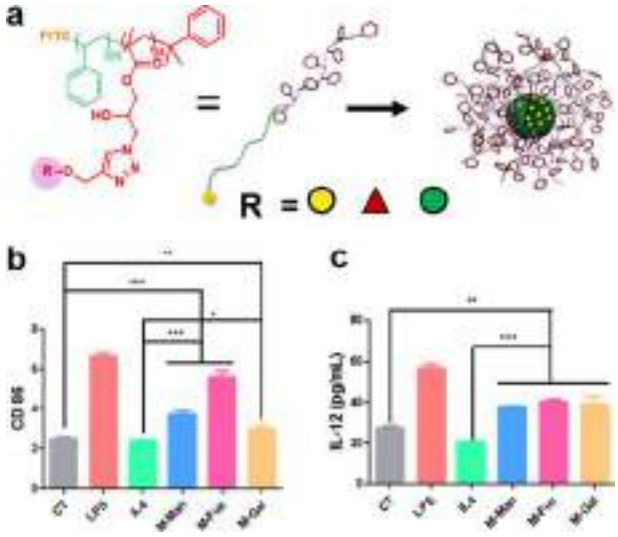



**Figure 58.** (a) Self-assembly of glycopolymers into GNPs. (b) GNPs increased expression of CD86 analyzed by FACS. (c) GNPs altered the cytokine IL-12 secretion of primary peritoneal macrophages measured by ELISA. Reproduced with permission from ref 487. Copyright 2015 Wiley-VCH.

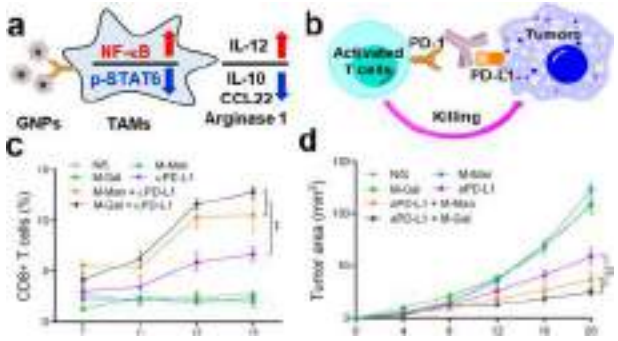



**Figure 59.** Enhancement of anti-PD-L1 cancer immunotherapy through reversion of TAMs by glycocalyx-mimicking nanoparticles. (a) TAMs reversion by GNPs. (b) Anti-PD-L1 cancer immunotherapy. (c) Infiltration of cytotoxic T cells ($CD8^+$) in tumor. (d) Tumor progression was monitored by measuring tumor volume ($n$ = 5). Reproduced with permission from ref 488. Copyright 2018 American Chemical Society.

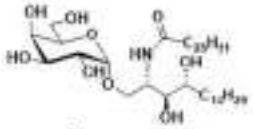
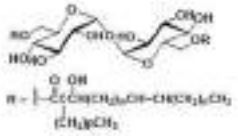
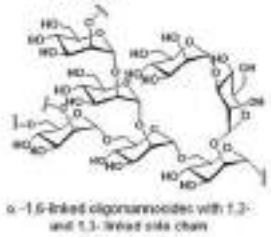
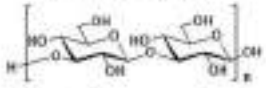
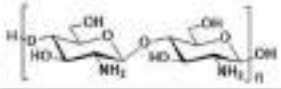
mainly attributed to upregulation of NF-$\kappa$B p65 phosphorylation and downregulation of STAT-6 (Figure 59a). In addition, cell experiments also indicated that TAMs reversion induced by these GNPs was helpful for T-cells activation and proliferation (Figure 59c). Moreover, continued injections with $\alpha$PD-L1 and GNPs led to a significant reduction of tumor burdens, implying that GNPs-induced TAMs reversion was beneficial to $\alpha$PD-L1 therapy (Figure 59d).

### 5.3. Carbohydrate-Based Immunologic Adjuvant

Adjuvants are mostly required in the preparation of vaccines to improve the immunogenicity of antigens. They can induce significant immune response and thus reduce the dosage and production cost of vaccines. Although alum-based adjuvants have been approved for use in United States, other candidates including emulsions, cytokines, nucleic acids, saponins, polysaccharides, and microorganism-derived components, etc., have been developed for possible applications in vaccines due to the weak adjuvancy of alum to stimulate Th1 immune

AQ

**Table 3. Carbohydrate-Based Adjuvants**

| Name | Structure | Sources | Receptors | Immune response |
|---|---|---|---|---|
| MPLA |  | Gram-negative bacteria | TLR-4 | Stimulate antigen presentation, activate Th cells |
| KRN7000 (αGalCer) |  | Marine sponges | iNKT receptor | Activate iNKT cells and APCs |
| TDM |  | Mycobacterium tuberculosis | C-type lectin mincle | Activate APCs and Th1 / Th17 response |
| α-MOS |  | Yeast | CLRs, TLRs | Activate NF-κB, DC maturation, Proinflammatory |
| β-Glucan |  | Euglena gracilis, Saccharomyces cerevisiae | Dectin-1 | Activate NF-κB, complement and inflammasome, proinflammatory |
| Chitosan |  | Crustaceans | Dectin-1, TLR-2, macrophage MR | Activate macrophage, proinflammatory |

response. Thus, adjuvants with high adjuvancy but low toxicity have been pursued in this field.[489−491]

As most carbohydrates are safe, low toxic, biodegradable, and widely found in plants, bacteria, and yeast, the carbohydrate-based adjuvants are promising candidates for development of human vaccines. Carbohydrates act as adjuvants through binding to specific innate immune receptors (e.g., Toll-like receptors (TLRs), NOD2, C-type lectins, etc.) or by activation of complement, NF-κB, inflammasome, and so on.[490] Carbohydrate-based adjuvants could be mainly divided into 3 types (i.e., glycolipid, polysaccharide, and saponin) according to their structural features. Some of them are summarized in Table 3.

The well-known glycolipid-based adjuvants include MPLA, KRN7000, TDM (trehalose-6,6′-dimycolate), and their derivatives.[490,492] MPLA is the 1-*O*-dephosphorylated form of lipid A, which is the most conserved and immunologically active portion of LPS, located on the outer membrane of Gram-negative bacteria. Removal of the specific phosphate groups could significantly reduce the toxicity yet retain the strong immunostimulatory ability. In this regard, MPLA has been widely investigated as an adjuvant in vaccines. The immunostimulatory ability of MPLA is achieved by interacting with TLR-4, which stimulates antigen presentation and activates T-helper (Th) cells, thereby helping to elicit further T-cell-mediated immunity.[493] α-Galactosylceramide (αGalCer), a glycolipid known as KRN7000, was isolated from marine sponges.[494] It can activate invariant natural killer T (*i*NKT) cells through presentation by CD1d molecules which are expressed on APCs, initiating "T-cell-dependent type II response".[495] Due to the amphiphilic structures, adjuvants in glycolipid form, including MPLA, KRN7000, and TDM, together with carbohydrate antigens as well as their covalent conjugates are always prepared into liposomes. The immunological functions of such liposomes based on multivalent structures will be introduced in the vaccine section.

Polysaccharides can activate macrophages, DCs, B lymphocytes, T lymphocytes, and NK cells, promoting the production of immune-related molecules, such as cytokines, antibodies, and complement molecules.[489] The mostly investigated polysaccharides for adjuvants are chitosan, α-MOS (α-mannanoligosaccharide), glucan, Chinese medical herb polysaccharide, and so on. Their structures, functions, advantages, and disadvantages as adjuvants have been summarized in relevant reviews.[496−498]

Recently, polysaccharides were employed as components of nanomaterials for modulation of the immune system.[499] For example, α-MOS can induce immune response by binding to CLRs (such as CD206)[500] and TLRs.[501] Haddadi and co-workers modified mannan (α-MOS) with PLGA; the resulting nanoparticles could induce enhanced phenotypic and functional maturation of DCs compared with soluble mannan.[502]

To enhance the antigen uptake and increase the vaccine immunity, polysaccharides were also used as carriers for vaccines. Wu and co-workers attached α-MOS to the capsid protein of porcine circovirus type 2 (PCV2) through an acid-sensitive Schiff base reaction considering that mannan could target the formulated vaccine to APCs via interaction with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MRs. The Man-modified PCV2 protein elicited a significantly stronger humoral immune response than any other control groups in mice.[503] In addition, α-MOS was also coated on nanoliposomes via an aminooxy coupling reaction. The α-MOS-liposomes can promote DC maturation with a comparable immunostimulatory ability to LPS.[504] Lu and co-workers also reported the immunostimulatory properties of chemically modified phytoglycogen nanoparticles (a dendrimer-like α-D-glucan), which were derived from a genetic variant sweet corn.[505] The positively charged dendrimer-like nanoparticles could adsorb negatively charged protein antigens. In vitro studies showed that the phytoglycogen nanoparticles could not only efficiently deliver antigens to DCs but also induce activation of DCs, leading to the strong immune response.

Well-defined fragments of polysaccharides were also conjugated to some scaffolds for immunological studies. The resulting polymeric materials and their assemblies were used as mimetics of natural polysaccharides, facilitating investigation of the structure−activity relationship. Dong and co-workers prepared glycopeptide-based nanoparticles, displaying different monosaccharides in multivalent form (Figure 60).[506] Activation of the macrophages by these nanoparticles was then evaluated. It was shown that CD40 and CD86 were significantly expressed on macrophages (Figure 60b), and secretion of pro-inflammation cytokines, IL-6 and IL-12, was induced by Man-modified nanoparticles. However, nanoparticles modified with other monosaccharides only displayed low activation effects. Notably, with OVA as a model antigen, the immunostimulatory capabilities of Man-modified nanoparticles were also demonstrated in vivo. Compared with other control groups, the combination of OVA antigen and Man-modified nanoparticles elicited the highest IgG antibodies titer (Figure 60c). It was also demonstrated that the macrophage MR is the major target for Man-modified nanoparticles.

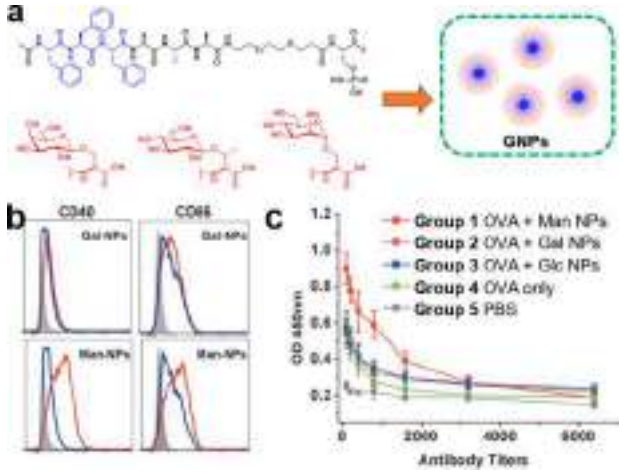


**Figure 60.** Self-assembled glycopeptide as adjuvant for mimicking complex polysaccharides. (a) Chemical structure of synthetic glycopeptides. (b) CD40 and CD86 expression levels after RAW264.7 macrophages are stimulated by different glycopeptides for 48 h. Red line, GNPs; blue line, nonglycosylated shell nanoparticles. (c) IgG antibody titers of immunized mice. Reproduced with permission from ref 506. Copyright 2020 Wiley-VCH.

## 5.4. Carbohydrate-Based Vaccine and Immunotherapy

Carbohydrate-based vaccines have been developed utilizing discrimination between self and nonself in biological immune system. The early vaccines were prepared by isolation of capsular polysaccharides from pathogens. However, since most pure carbohydrate antigens are T-cell independent, such vaccines usually have weak immunogenicity by directly interacting with B cells, producing low titers of low-affinity IgM antibodies. To overcome this problem, carbohydrate antigens are traditionally conjugated to carrier proteins possessing T-cell epitope peptides, such as tetanus toxoid (TT), bovine serum albumin (BSA), and OVA, to enhance the presentation of carbohydrate antigens to the immune system and improve the immunogenicity of vaccines.[507,508] Recently, chemical, enzymatic, and chemoenzymatic synthesis as well as engineered biosynthesis of oligosaccharides have been explored to boost the development of carbohydrate-based vaccines.[328,509−513]

Over the past several years, carrier protein-based carbohydrate vaccines have achieved great success.[514,515] However, the carrier protein-based vaccines have some drawbacks. The most obvious problem is the significantly suppressed immune response against carbohydrate antigen moieties induced by the strong immunities against the carrier proteins, leading to poor vaccine efficacy.[493] In addition, conjugation of the carbohydrate antigens to carrier proteins is difficult to control, while ill-defined and heterogeneous conjugates were sometimes afforded, which lead to largely batch-dependent physical and biochemical properties. For these reasons, a variety of new platforms, including VLPs, polysaccharides, polymers, lipids, and so on, have been explored, especially in developing cancer vaccines.[331,516] In this section, we will focus on the results categorized by different diseases.

**5.4.1. Cancer Vaccine.** TACAs are aberrant or overexpressed saccharides on tumor cells and can be divided into two classes: glycoprotein antigens (e.g., Tn, TF, and STn) and glycolipid antigens. The glycolipid antigens could be further classified into several families: the blood group, gangliosides, and the Globo class (Figure 61). The special status makes TACAs promising targets for the design of cancer vaccines. To induce a high affinity of IgG antibodies and amplify the function of the oligosaccharide, TACAs and T (Th)-cell epitopes, immunostimulatory molecules as well as some targeting molecules are always simultaneously conjugated to a variety of scaffolds to present these components in multivalent forms.[517−521]

*5.4.1.1. Glycoprotein Vaccines.* To improve the immunogenicity of carbohydrate antigens, TACAs are always conjugated to carrier proteins through various linkers (Figure 62). Carrier proteins not only provide T-cell epitopes to transform a T-cell-independent immune response to a T-cell-dependent type but also present carbohydrate antigens in multivalent form to promote aggregation of IgM on the surface of B cells, facilitating class switching of antibodies from IgM to IgG. The key points in the design of TACAs-based vaccines include (a) appropriate TACAs antigens, (b) proper carrier proteins, (c) suitable linkers between TACAs and carriers, and (d) the number and type of TACAs. With the development of carbohydrate chemistry, well-defined and high-purity oligosaccharides with huge potential in the rational design of safe and efficacious vaccines can be afforded by different chemical methods.[513,522] TACAs with either one type, clusters of one type, or multitypes have been chemically synthesized and


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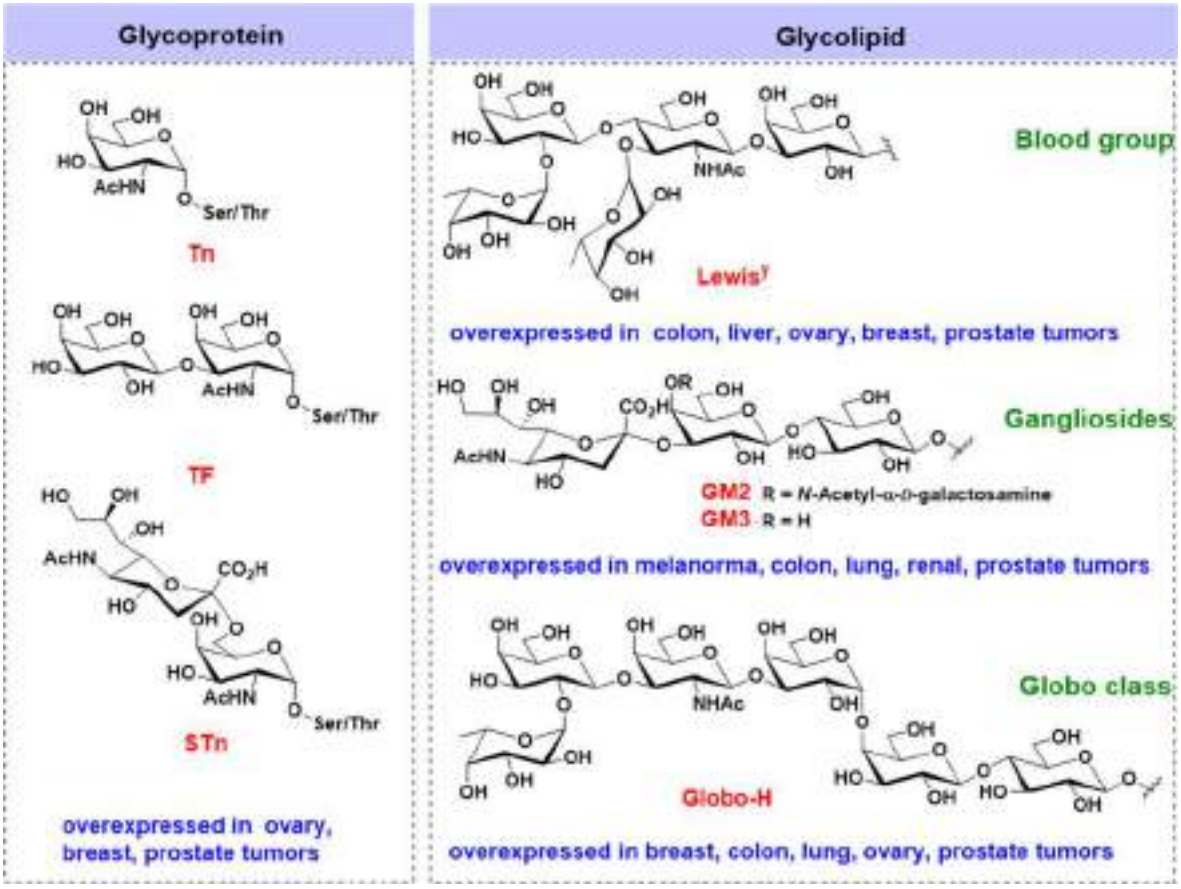


**Figure 61.** Chemical structures of some TACAs.


**Figure 62.** General structure of glycoprotein cancer vaccines.

conjugated to carrier proteins to provide glycoprotein vaccines.[518] TACA derivatives or analogues were also prepared to improve the immunogenicity of carbohydrate antigens.[523−527] The key points are crucial in the design of not only protein vaccines but also other vaccines, which will be referred to in the following discussion. In order to facilitate a better understanding of the existing protein vaccine structures, the representative works are listed in Table 4.

*5.4.1.2. Virus-Like Particles-Based Vaccines.* VLPs are highly organized structures which are self-assembled from subunit proteins. They harness high immunogenicity of viruses but are safe to humans and animals owing to the lack of viral genome. The size of VLPs ranges from 20 to 200 nm, which are beneficial for being uptaken by APCs and trafficking to lymph nodes. In addition, the repetitive structures facilitate efficient cross-linking of B-cell receptors and recruiting members of the innate humoral immune system for the sake of enhancing innate and adaptive immune responses. For these reasons, VLPs have been widely used as carriers in vaccines.[543−545]

Recently, VLPs have been developed as carriers for carbohydrate-based cancer vaccines. In 2007, cowpea mosaic virus (CPMV) was first used as the platform for carbohydrate antigens to elicit anticarbohydrate antibodies.[546] Huang and co-workers reported a polyvalent display of Tn antigen on the CPMV scaffold in 2008.[547] High titers of IgG were afforded, and the antibodies were able to recognize MCF-7 and NCIADR RES breast cancer cells presenting native Tn antigens. Afterward, different VLPs, including tobacco mosaic virus[548] and bacteriophage Q$\beta$ capsids,[549] were used as carriers for the development of Tn-based cancer vaccines. Among the three VLPs, the Q$\beta$ elicited higher levels of IgG antibodies than the other two types. Later, it was demonstrated that immunogen structures have a large effect on the diversity of antibodies.[550] The triazole linker formed by CuAAC reaction, which is prevalent in conjugate vaccines, could dramatically reduce the diversity of antibodies in humoral responses. This result was verified not only in small antigen Tn-based conjugates but also in the more complicated antigen GM2.[551] It was speculated that the antitriazole antibodies may hinder the binding of Tn-specific B cells to the vaccine construct.

Furthermore, to activate both specific antibodies and cytotoxic T cells for cancer immunotherapy, MUC1 with a tandem repeat region containing CTLs (cytotoxic T lymphocyte cells) epitopes was conjugated to bacteriophage Q$\beta$. The antitumor humoral and T-cell responses of the resulting vaccine were subsequently investigated (Figure 63). Over 1 million IgG titers and MUC1-specific cytotoxic T cells were elicited. Importantly, the high IgG antibody levels


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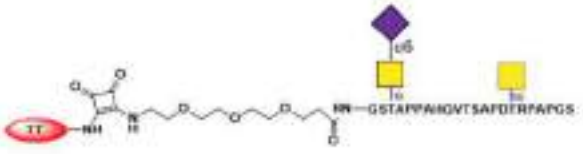
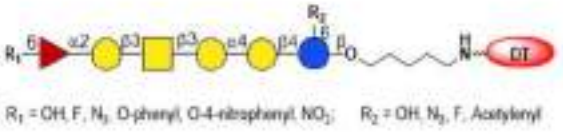
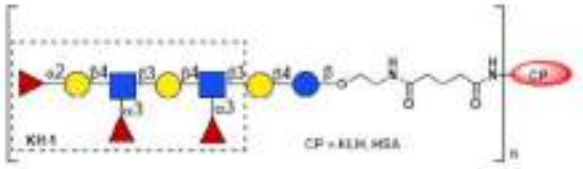
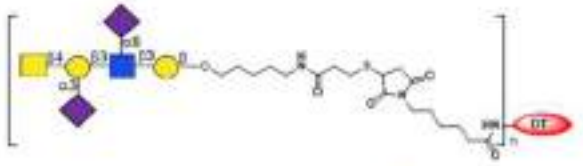
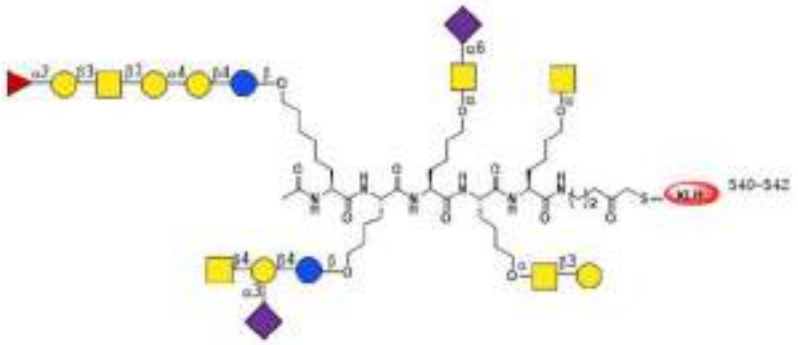
**Table 4. Recent Representative Works on Developing Glycoprotein Cancer Vaccines**[528]

| TACA | Carrier protein | Vaccine | Ref |
|---|---|---|---|
| MUC1[125,528−553] Tn[530,534] TF[533] STn[527,535−537] | TT |  | 529 |
| Globo-H | DT |  | 526 |
| Lewis[x] Lewis[y] KH-1 | KLH HSA |  | 538 |
| GM3 | KLH HSA BSA |  | 524,525 |
| RM2 | DT |  | 539 |
| Globo-H GM2 STn TF Tn | KLH |  | 540−542 |

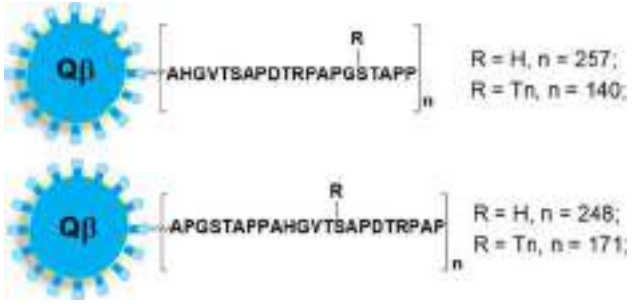



**Figure 63.** Illustration of Q$\beta$−MUC1 conjugation.

persisted for over 6 months, and the CTLs selectively killed tumor cells presenting MUC1.[552]

To mimic the tolerant condition toward MUC1 in humans, human MUC1 transgenic mice (MUC1.Tg) were bred and immunization studies of the above-mentioned Q$\beta$−MUC1 were performed. It was demonstrated that although high IgG titers were elicited by Q$\beta$−MUC1 in MUC1.Tg mice, the antibodies only exhibited weak recognition of MUC1-expressing tumor cells, which implied that the Q$\beta$−MUC1 conjugates were ineffective in providing protection against tumor development (Figure 64). After profiling the binding selectivity of antibodies, the immunodominant but non-protective epitopes were removed. Considering that triazole was detrimental to antibody generation, amide-linked Q$\beta$−MUC1 based on the protective epitope was designed. It was shown that high antibody titers and tumor cell binding were induced by the new Q$\beta$−MUC1 conjugates (i.e., **Q$\beta$−MUC1-**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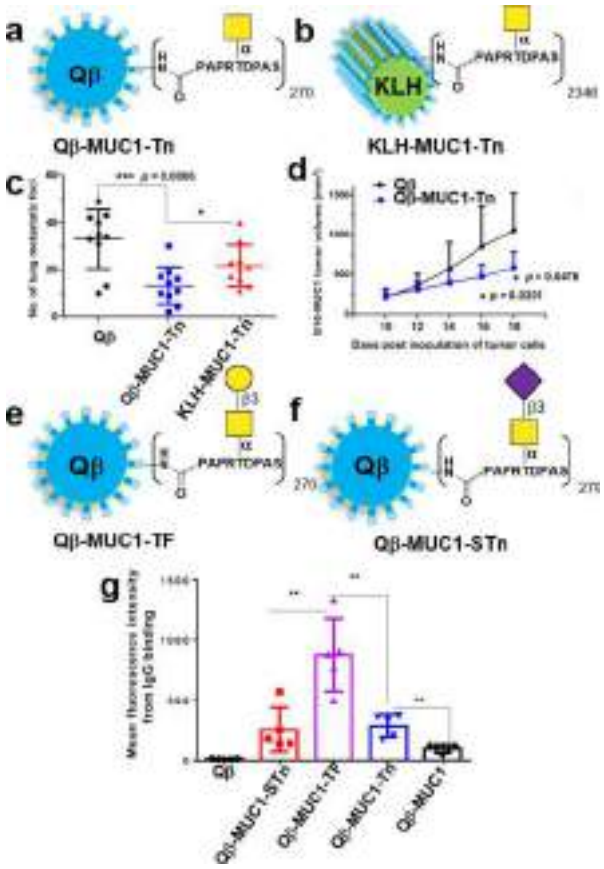


**Figure 64.** Design of MUC1-based vaccine for effective tumor protection in immunotolerant mice. Illustration of newly designed Q$\beta$−MUC1 conjugates (a) **Q$\beta$−MUC1-Tn** and (b) **KLH-MUC1-Tn**. (c) Immunization of **Q$\beta$−MUC1-Tn** significantly protected MUC1.Tg mice from formation of metastatic-like lung tumors. (d) **Q$\beta$−MUC1-Tn** immunization regressed solid tumor growth compared to the control group receiving Q$\beta$. Reproduced with permission from ref 553. Copyright 2018 American Chemical Society. Structural illustrations of (e) **Q$\beta$−MUC1-TF** and (f) **Q$\beta$−MUC1-STn**. (g) Flow cytometry analysis of anti-MUC1 IgG antibodies. Reproduced with permission from ref 554. Copyright 2019 American Chemical Society.

**Tn** in Figure 64a) in MUC1.Tg mice; the values were 2−3 times higher than those of the **KLH-MUC1-Tn** case (Figure 64b).[553] Moreover, the newly designed Q$\beta$−MUC1 conjugate provided significant tumor protection in both solid tumor and metastatic models (Figure 64c and 64d). On the basis of these results, Q$\beta$−MUC1 conjugates with MUC1 bearing disaccharide TF (Figure 64e) and STn (Figure 64f) were also investigated.[554] Over 2 million IgG was elicited, and the antibodies can recognize a variety of MUC1 glycopeptides. **Q$\beta$−MUC1-TF** can largely reduce the number of tumor foci in a lung metastasis model (Figure 64g), implying the translational potential of this structure as cancer vaccines.[522]

*5.4.1.3. Polysaccharides-Based Vaccines.* Zwitterionic polysaccharides (ZPSs) are polysaccharides comprised of both negative and positive charges.[555,556] They are isolated from the capsule of commensal anaerobic bacteria and have the ability to elicit MHCII-mediated, T-cell-dependent immune responses and invoke production of IgG and IgM.[557,558] Their effects are comparable to carrier proteins. There are several kinds of ZPSs (**PS A1**, **PS A2**, **PS B**, **Sp1**, **CP5**, **CP8**) that have been isolated from different bacteria, which have been exploited as vaccine carriers (Figure 65).

In 2009, Andreana and co-workers first reported an entirely carbohydrate vaccine candidate (Figure 66). The Tn antigen was introduced by oxime formation with oxidized ZPS **PS A1**. High titers of immunoglobulins were generated in the absence of adjuvant.[559] Specific IgG3 antibodies were also elicited, implying a T-cell-dependent immune response. Furthermore, STn has also been conjugated to **PS A1** by the same protocol (Figure 66). A strong immune response was induced, and the specific antibodies were able to efficiently recognize STn-expressing cancer cells.[560,561] Recently, a bivalent conjugate comprised of both Tn and TF antigens was studied.[562] The immunological results in C57BL/6 mice indicated that the bivalent vaccine candidate **Tn-TF-PS A1** displayed a better immune response than the monovalent **TF-PS A1**. High titers of IgG antibodies were elicited by the bivalent conjugate, whereas the monovalent **TF-PS A1** only generated an exclusive IgM response. In addition, TACAs on the basis of other ZPSs (such as **PS B**[561]) and even different connection[563] types between ZPSs and TACAs were also developed. Most of the immunological investigations of newly designed conjugates are still in progress.

Apart from ZPSs, nonionic polysaccharides have also been used in vaccine investigations. For example, a Tn-antigen mimetic was conjugated to single-chain polymeric nanoparticles (DXT-SCPNs), which were prepared by intrachain cross-linking of a dextran-methacrylate derivative (DXT-MA) (Figure 67). In vitro stimulation of human peripheral blood mononuclear cells indicated that the biocompatible and water-dispersible nanoparticles elicited TLR-2-mediated IL-6 and IL-10 secretion.[564]

Recently, $\beta$-1,3-glucan polysaccharide, which serves as an immune activator, was used as a carrier to construct synthetic MUC1 vaccine (Figure 68). It was shown that uniform nanoparticles formed by the conjugation induced high titers of anti-MUC1 antibodies as well as high levels of IFN-$\gamma$ and IL-6.[565]

*5.4.1.4. Polymer-Based Vaccines.* The multivalent effects of pendant carbohydrates on the glycopolymer backbone can not only promote the binding of these carbohydrates to receptors but also facilitate the applications of glycopolymers in design of cancer vaccines, although the latter research is still in its infancy.

In 2013, Cameron and Davis's groups reported the synthesis of gold nanoparticles decorated with Tn antigen-appended glycopolymers; the afforded nanomaterials showed a strong and long-lasting immune response in vivo in the absence of typical vaccine components, i.e., T (Th)-cell epitope and adjuvants.[566] Almost at the same time, nanosized polymer-linked vaccines were prepared by coupling MUC1 glycopeptides and T-cell epitope peptides to water-soluble methacrylamide polymers (Figure 69). The attachment of the T-cell epitope **P2** onto the hydrophilic polymer vaccines led to self-assembly of the resulting glycopolyers into nanoparticles. The immunological evaluation showed that the significant IgG titers were induced by the two kinds of nanoparticles in the presence of complete or incomplete Freund's adjuvant. In particular, antibodies induced by the block copolymer (Figure 69b) containing additional nanostructure-promoting domains exhibited high affinity to the tumor cells.[567]

In addition, hyperbranched polyglycerol (HPG) was also used to realize multiple display of MUC1.[568,569] In contrast to


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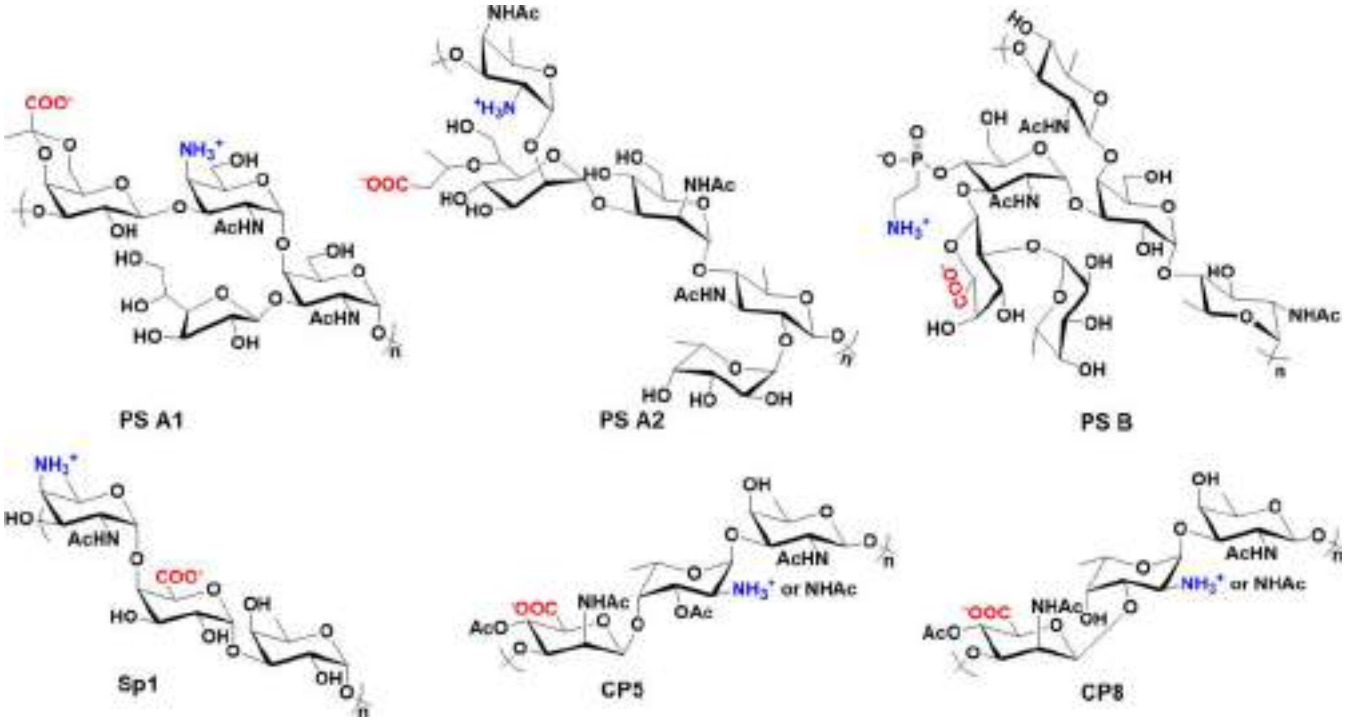


**Figure 65.** Structure of zwitterionic polysaccharides.

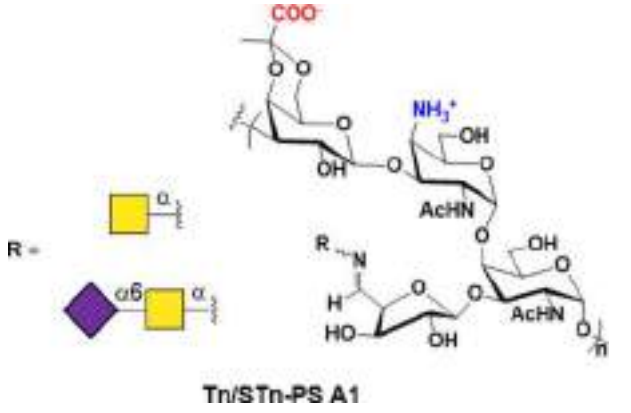


**Figure 66.** Entirely carbohydrate antigen **Tn/TF/STn-PS A1** conjugates.

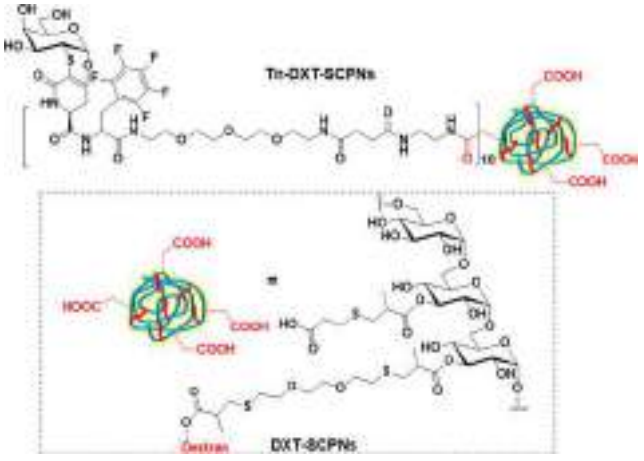


**Figure 67.** Preparation of Tn-DXT-SCPNs.

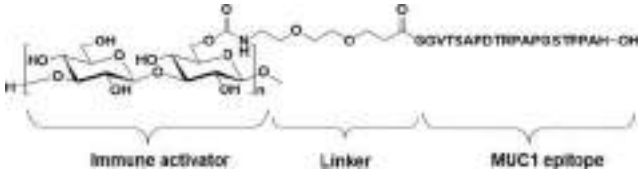


**Figure 68.** Polysaccharide glucan-based vaccine.

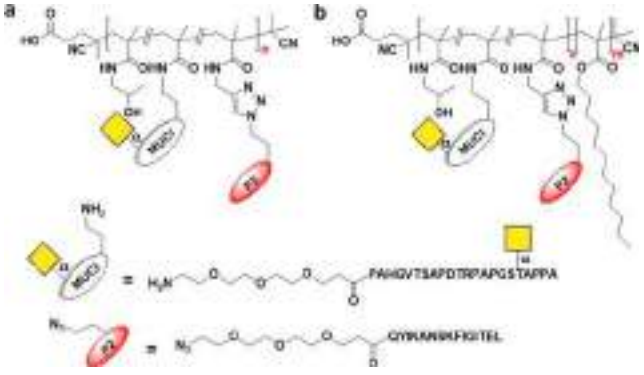

**Figure 69.** Structure of water-soluble polymers coupled with glycopeptide antigens and T-cell epitopes as potential antitumor vaccines, including polymers without (a) or with (b) additional hydrophobic block.

linear polymeric carriers PHPMA, the hyperbranched structures can provide enough space to expose antigen on their surface (Figure 70a).[568] Therefore, when MUC1 and T-cell epitope peptides with covalent linkage were clicked onto the HPG, which carried an average of five alkyne groups, the fully synthetic glycopeptides induced strong immune responses in mice and the IgG antibodies recognized human breast cancer cells.[569] Furthermore, in order to improve multiantigen presentation, HPG-based cancer vaccine with an average of


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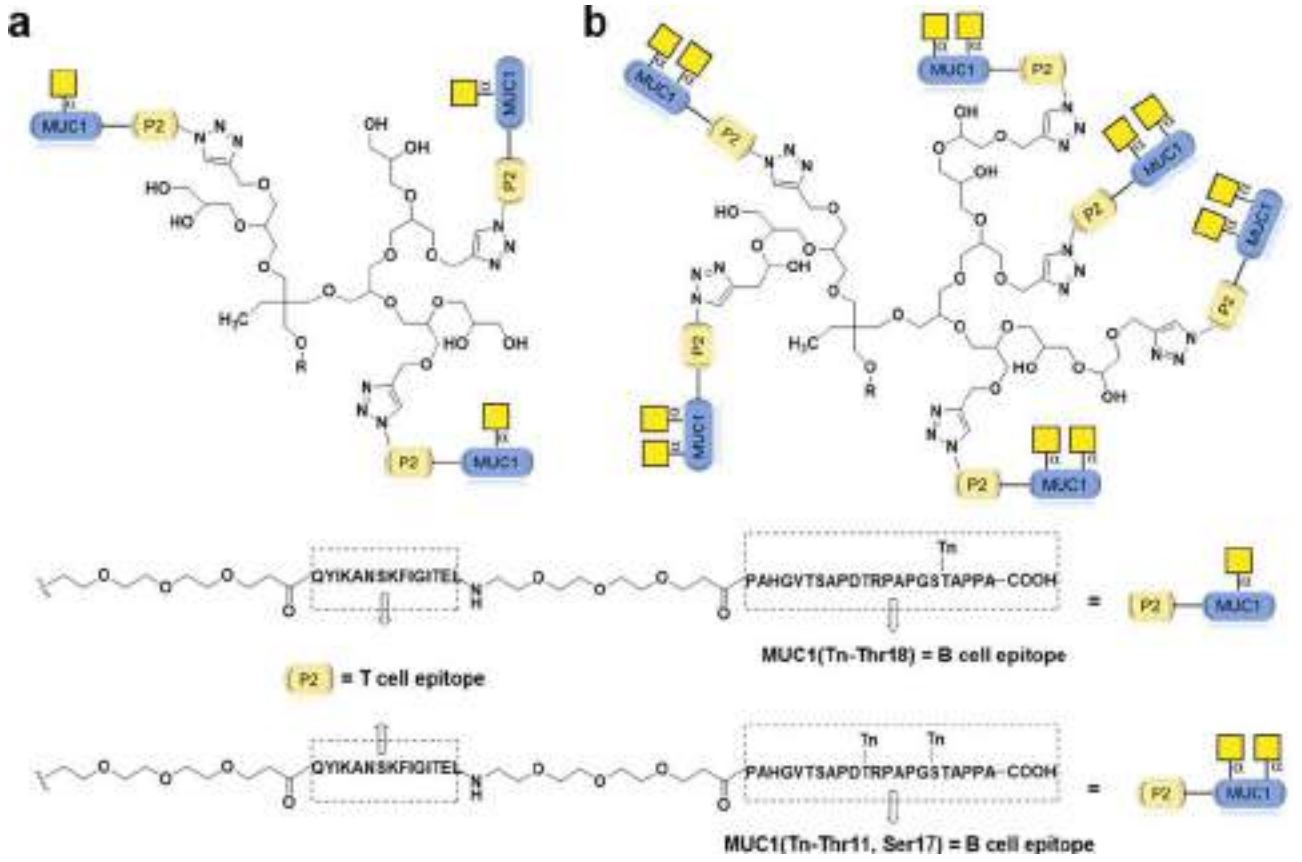


**Figure 70.** MUC1 glycopeptide cancer vaccines based on hyperbranched polymers. (a) HPG-based cancer vaccine containing on average five MUC1 glycopeptides. (b) HPG-based cancer vaccine containing on average eight MUC1 glycopeptides. R can contain additional glycerol units with two MUC1 glycopeptides on average.

eight MUC1 glycopeptides (Figure 70b) was prepared and the stronger immune response was induced by the optimized conjugate than the vaccine which has only about five binding sites (Figure 70a).[568]

Huang and co-workers also reported a glycopolymer vaccine incorporating bothTACA and Th-cell epitope (Figure 71).[570]

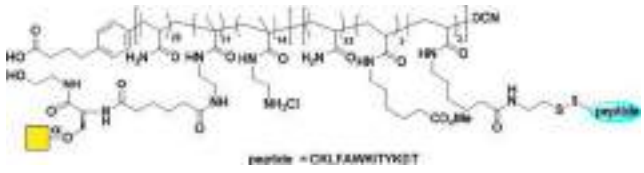

**Figure 71.** Water-soluble copolymers as potential cancer vaccines.

Humoral immunity of mice showed that specific and high IgG antibodies were induced by Tn-based glycopolymers in the presence of Freund's adjuvant. Meanwhile, the antibodies exhibited significant binding with Tn-expressing Jurkat cells. The polymer backbone displayed very low immunogenicity, indicating B cells activation was not significantly suppressed. Furthermore, due to the flexible and adjustable structure of polymers, the effects of valency and density of Tn on B-cell activation were also investigated.[571] It was shown that 40 Tn antigens in a 450 kDa polymer chain gave the strongest stimulation to B cells in vitro. On the contrary, only decreased antibody or even non-responsiveness of Tn specific B cells was induced when the valency and density deviated from the optimal one. The results are helpful for guiding the design of effective carbohydrate-based vaccine constructs targeting certain diseases.

In addition, Yamazaki and co-workers also investigated immune activation with polypeptide assemblies carrying Lewis$^y$ (Figure 72).[572] Carbohydrate antigen Lewis$^y$ was conjugated

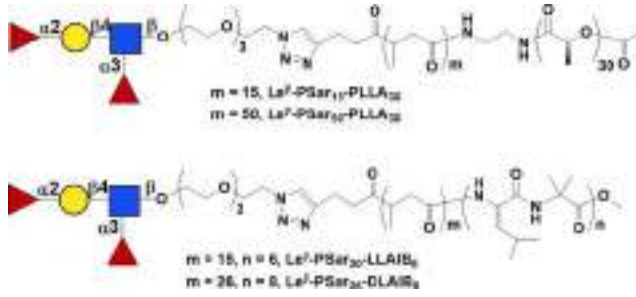

**Figure 72.** Structures of glycopolypeptides as cancer vaccines.

to poly(sarcosine)$_n$-*b*-poly(L-lactic acid)$_{30}$ ($m$ = 15 or 50) and poly(sarcosine)$_m$-*b*-(D/L-Leu-Aib)$_n$ ($m$ = 22 or 30, $n$ = 6 or 8). It was shown that the presentation and the antigenicity of Lewis$^y$ are largely dependent on the morphologies of the molecular assemblies. Among the various morphologies, including nanosheet, curved sheet, nanotube, and vesicle, a nanosheet possessing the highest density of Lewis$^y$ displayed the best effect to produce anti-Lewis$^y$ IgM. Further investigation also demonstrated that nanosheets with a dense display of Lewis$^y$ induced production of the TACA-specific IgM in only one immunization.[573]


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*5.4.1.5. Self-Adjuvanting Glycolipid, Glycopeptide, and Glycolipopeptide.* Self-adjuvanting vaccines are vaccine molecules incorporating both antigens and built-in adjuvants in which regular adjuvants could be avoided. The mostly used built-in adjuvants are lipopeptides, such as the $Pam_3C$ series (including $Pam_3C$, $Pam_3CS$, and $Pam_3CysSK_4$, Figure 73a),

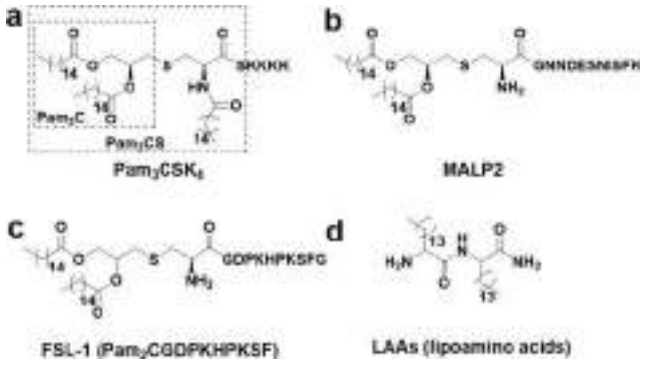


**Figure 73.** Chemical structure of ligands to TLRs-2: (a) $Pam_3C$ series, including $Pam_3C$, $Pam_3CS$, and $Pam_3CysSK_4$, (b) MALP2, (c) FSL-1, and (d) LAAs.

MALP2 (Figure 73b), FSL-1 (Figure 73c), lipoamino acids (Figure 73d), and glycolipid MPLA (Figure 9c), all exerting their activities through interaction with TLRs.[493,574] In addition, KRN7000 as well as other nonlipid molecules, such as CpG and D-peptide, are also used as built-in adjuvants in the multicomponent vaccine investigation due to their immunostimulatory activity. Considering that most carbohydrates have only low immunogenicity, additional exogenous helper T epitopes derived from OVA, tetanus toxoid, etc., were commonly used to induce high titers of IgG. Thus, fully synthetic self-adjuvanting vaccines are generally composed of one or more TACA antigens, built-in adjuvants, and T (Th)-cell epitope peptides (Figure 74a), which do not require

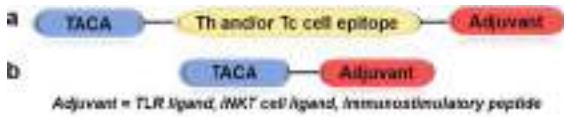


**Figure 74.** (a) Three- and/or four-component vaccines with Th-cell epitopes. (b) Two-component vaccines without Th-cell epitopes.

additional adjuvants. To further induce cellular anticancer immunity, the aberrant mucin MUC1-derived peptides with cytotoxic T (Tc)-cell epitope are also used to construct four-component vaccines.

Although T-cell epitopes are conventionally regarded as indispensable components in carbohydrate-based vaccines, more research has demonstrated that carbohydrates can participate in T-cell stimulation as components of T-cell epitopes or they can be directly recognized by T cells.[575] Therefore, fully synthetic carbohydrate-based vaccines without T-cell epitopes (Figure 74b) have also been appreciated by more and more researchers. Thus, carbohydrate-based vaccines with or without T-cell epitopes will be separately discussed in the following sections.

*5.4.1.5.1. Vaccines with T-Cell Epitopes.* Fully synthetic multicomponent carbohydrate vaccines have been systematically studied by Boon's group.[576−578] To improve high titers of IgG and induce cellular anticancer immunity, they explored fully synthetic multicomponent vaccines composed of aberrantly glycosylated MUC1 peptide, T-cell epitope, and TLR agonist $Pam_3CysSK_4$, which induced a robust immune response not only in wild-type mice but also in a humanized mouse model (Figure 75).[579] The IgG antibodies elicited by

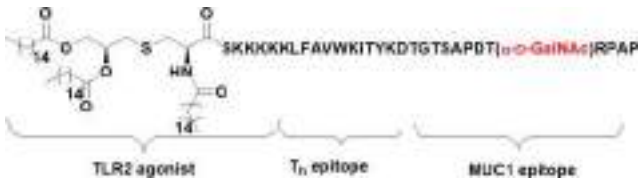


**Figure 75.** Chemical structures of a three-component vaccine.

this vaccine can lyse MUC1-expressing cancer cells. Moreover, the vaccine can disrupt immune tolerance and activate CTLs in transgenic mice. It was also shown that the covalent linkage between the T-cell epitope and B-cell epitope and the type of TLR agonist were critical to the efficacy of the vaccines. Inappropriate choice of TLR agonist may lead to low titers of IgG.[578] On the basis of aforementioned results, STn-based three-component vaccine was also synthesized. The resulting vaccine candidates can disrupt immune tolerance and induce both humoral and cellular immune responses.[580] Furthermore, vaccines containing long MUC1 glycopeptide without any artificial linkers or exogenous Th epitopes but combining functions of activating B cells, helper T cells, and cytotoxic T-lymphocytes have been developed.[581] The robust immune response was elicited by this glycolipopeptide, demonstrating the attractiveness of using endogenous helper T epitope for vaccines.

Meanwhile, a variety of multicomponent vaccines containing other TLR agonists,[582−584] clustered antigens,[585] and multiple or multitype antigens[586] have been developed.

In addition, multicomponent self-assembled vaccines have been reported by Li and co-workers.[587−589] For example, the positively charged T-cell epitope **P1** was explored as a hydrophobic part of the assemblies.[588] Negatively charged immunostimulant $\gamma$-polyglutamic acid ($\gamma$-PGA) and positively charged MUC1 peptide **P2** were successively added to afford self-assembled vaccines driven by the electrostatic interactions (Figure 76a and 76b). It was shown that the assembled vaccine **V1** triggered significant immune responses in mice (Figure 76b). The IgG titers induced by self-assembled vaccine **V1** were even similar to the covalent vaccines **P5**. In addition, the antibodies elicited by **V1** can recognize and neutralize MCF-7 cells (Figure 76c). The novel vaccines largely saved time in purification, providing a new pathway for development of chemically synthetic vaccines.

*5.4.1.5.2. Vaccines without T-Cell Epitopes.* As early as 1994, Toyokuni and co-workers proved that Tn antigens coupled with $Pam_3C$ could induce significant IgG antibodies without additional adjuvants (Figure 73).[590] In 2010, Kunz and co-workers reported fully synthetic vaccines prepared by conjugation of MUC1 glycopeptide with $Pam_3CSK_4$ lipopeptide (Figure 73).[591] The vaccine candidate elicited a specific humoral immune response in all immunized mice.

Afterward, multiple antigen peptide strategies have been explored, and the effect of the conjugates on the killing of tumor cells was examined.[592,593] As shown in Figure 77, the multivalent vaccines were synthesized by coupling tumor-


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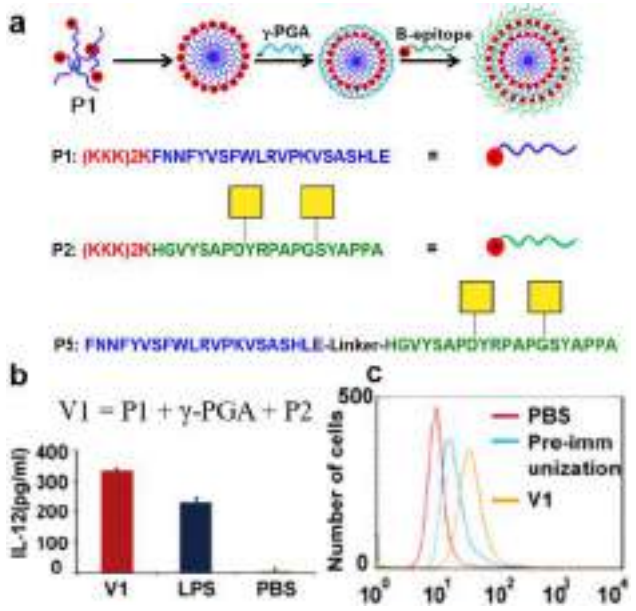


**Figure 76.** Glycopeptide nanoconjugates based on multilayer self-assembly as an antitumor vaccine. (a) Structure of glycopeptide, and illustration of self-assembled GNPs. (b) IL-12 release from RAW264.7 cells cultured with **V1** (54 $\mu$M) or LPS (10 $\mu$g/mL). (c) Binding of antisera to MCF-7 cells. Reproduced with permission from ref 588. Copyright 2015 American Chemical Society.

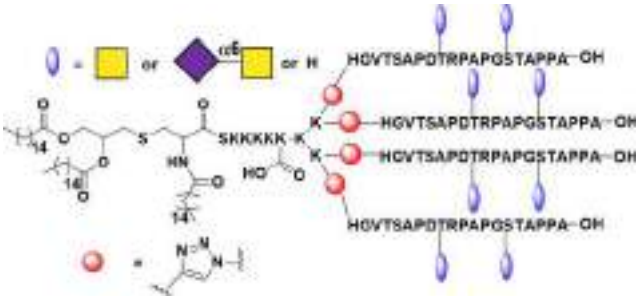


**Figure 77.** Structure of a vaccine containing the TLR2 ligand lipopeptide conjugated to tetravalent MUC1 glycopeptides.

associated MUC1 glycopeptide to a $Pam_3Cys$ lipopeptide through click reactions. Immune responses were elicited in mice without the use of external adjuvant. As expected, the cluster effect of the tetravalent MUC1 glycopeptide–lipopeptide vaccine was well displayed. The antibodies induced by the tetravalent vaccine bound to MCF-7 breast tumor cells and killed these tumor cells by activation of the complement-dependent cytotoxicity complex.

In addition, other self-adjuvanting carbohydrate vaccines without T-cell epitopes have also been explored, including MPLA-based carbohydrate vaccines, $\alpha$GalCer-based carbohydrate vaccines, and immunostimulatory peptide-based carbohydrate vaccines. These vaccines are in macromolecular form and will be discussed in detail.

*5.4.1.5.2.1. MPLA-Based Carbohydrate Vaccines.* As a TLR4 ligand, MPLA can provoke T-cell-dependent immune response.[594] Due to their safe and effective character, MPLA have been recently approved for clinical use.[595]

In 2012, an immunological study of carbohydrate antigen GM3–MPLA conjugates was reported by Guo's group (Figure 78).[596,597] Combined with 1,2-distearoyl-*sn*-glycero-3-phosphocholine and cholesterol (DSPC), the glycolipids were prepared into liposomes (Figure 78a).[596] It was shown that robust IgG antibody responses were induced by the liposomal vaccine in the absence of external adjuvant (Figure 78b and 78c). To disclose the structure–activity relationship of the vaccine candidate, some conjugates with protected hydroxyl groups and/or phosphate were also prepared and used in the immunological evaluation (Figure 78a). The modified glycolipid vaccine **MPLA-GM3-2** led to a 3.8 times increase of the total antibodies mainly contributed by IgG3, and the antibodies were specific to the reformed TACA structure. However, almost no obvious immune response was elicited when all functional groups in the MPLA part of **MPLA-GM3-3** were protected, indicating that the hydroxyl groups and phosphate are critical for interacting with the immune system. In addition, the pure glycoconjugate **MPLA-GM3-2** instead of its liposomal form was also examined, but an inconsistent result was afforded due to the poor water solubility. Moreover, it was demonstrated that addition of external adjuvant (Titermax Gold) was unnecessary, which can lead to the reduced immunological activity of the conjugates (Figure 78d).

To evaluate the impact of the MPLA structure on the immunology, MPLA derivatives containing additional hydroxyl groups and/or lipid chains have been prepared, and these derivatives were further coupled with modified STn.[598] Similarly, the resulting conjugates were formulated into liposomal vaccines. The experiments indicated that all of the conjugates formed by MPLA and STn derivatives successfully induced production of IgG antibodies. However, even minor changes in the MPLA structure have a significant effect on the immune response.

On the basis of the above structure–activity relationship analysis, self-adjuvanting cancer vaccines involving other TACAs, including Globo-H[587] and GM2,[599,600] have been investigated. Compared to the KLH-loaded conjugates, all of the MPLA-based vaccines induced a fast and strong immune response in the immunological evaluation of mice. In particular, the titers of IgG antibodies induced by MPLA-Globo-H conjugates were significantly higher than those of KLH-Globo-H. Moreover, the Globo-H-based vaccine mainly induced production of IgG1 and IgG2 antibodies, which are usually associated with protein conjugates. The phenomenon was different from the case of MPLA conjugates with other TACAs, wherein IgG3 antibodies were mainly observed. This result implied that MPLA conjugates with different TACAs could induce an immune response through different immunological pathways. It was speculated that the structures of carbohydrate antigens may be responsible for switching of immunological pathways.

*5.4.1.5.2.2. αGalCer-Based Carbohydrate Vaccines.* $\alpha$GalCer is a glycolipid which can activate *i*NKT cells through antigen-presenting molecules CD1d (see section 5.3), exerting Th epitope-like function. In view of this, $\alpha$GalCer was covalently conjugated to carbohydrate antigen STn (Figure 79) by Guo and co-workers to serve as a synthetic self-adjuvanting vaccine candidate.[601] The resulting glycolipid was prepared into a liposomal formulation by ultrasonic treatment of the mixture of **STn-α-GalCer**, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, and cholesterol with a molar ratio 1:5:4 in buffer. Without any external adjuvant, the liposomal vaccine was intraperitoneally injected into BALB/c mice three times with a 2 week hiatus. A significant STn-specific antibody response was observed by ELISA in antisera on day 14.


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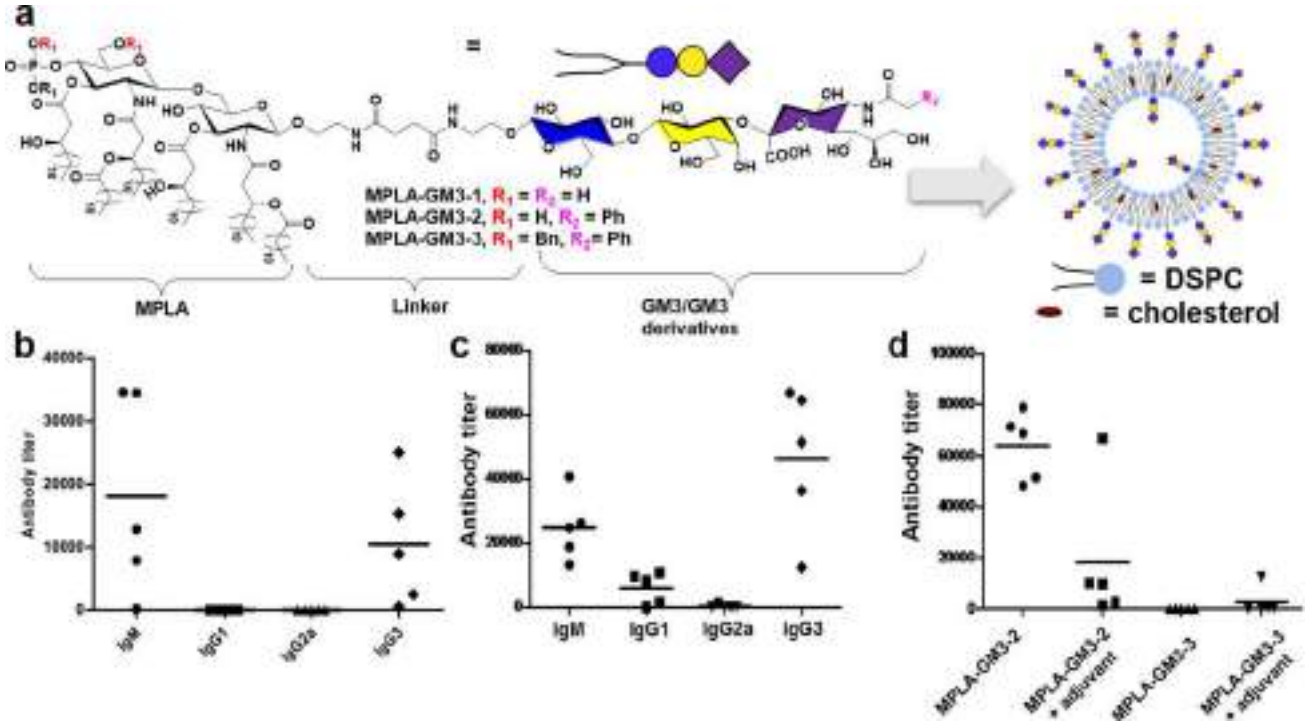


**Figure 78.** MPLA-based carbohydrate vaccines. (a) Liposome formed by synthetic self-adjuvanting GM3-MPLA conjugates. (b) Antibodies elicited by liposomes prepared by compound **MPLA-GM3-1**. (c) Antibodies elicited by liposomes prepared by compound **MPLA-GM3-2**. (d) Antibodies elicited by MPLA conjugates in the presence of adjuvant. Reproduced with permission from ref 596. Copyright 2012 American Chemical Society.

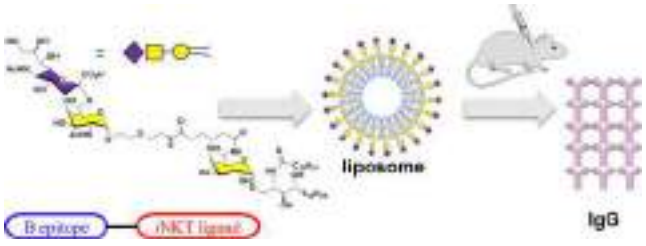


**Figure 79.** Carbohydrate-based cancer vaccine candidate with $\alpha$-galactosylceramide as built-in adjuvant.

Subtype analysis showed that the IgG antibodies were mainly IgG1 and IgG3, indicating a mixed Th1 and Th2 response, which was consistent with the typical behavior of $\alpha$GalCer alone.

For the sake of simplifying the structure−activity relationship studies of vaccines, minimalistic carbohydrate vaccine candidates based on $\alpha$GalCer have been explored by Seeberger's group (Figure 80).[602] As a model TACA, Tn containing a threonine residue was coupled to the linker-equipped $\alpha$GalCer. As a control, Tn equipped with a saturated C18 carbon chain was also prepared. Both of them were formulated into liposomes to display glycan antigen in multivalent form (Figure 80a). In addition, liposomes with average diameters of 120 and 400 nm were prepared to evaluate the size effect on the glycan antibody responses. A robust IgG immune response was generated not only by two-component vaccine **Tn-$\alpha$GalCer** but also by the Tn antigen containing only a C18 carbon chain (i.e., **Tn-$C_{18}$**) (Figure 80b). The size of liposomes can influence the antibody titers, affinity maturation, and Th skewing. The larger liposomes induced the production of Th1-type antibodies with higher

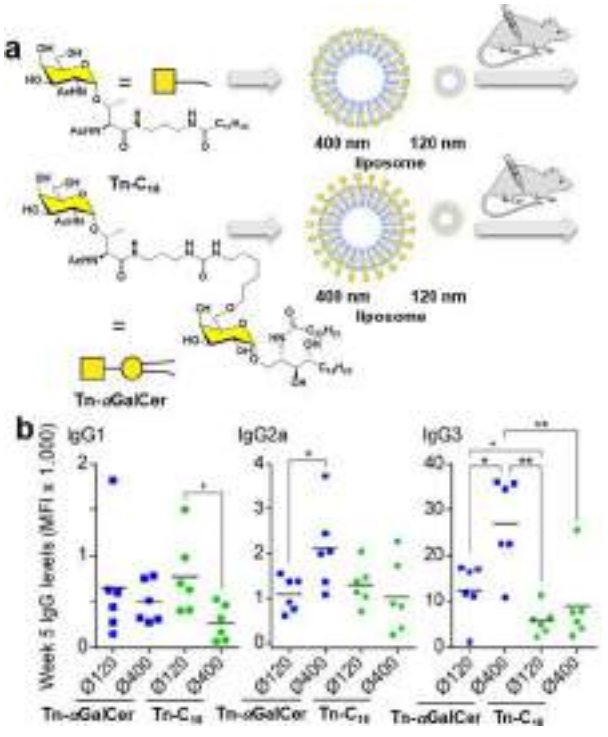


**Figure 80.** (a) Structure and (b) immunological evaluation of a minimalistic carbohydrate−$\alpha$GalCer vaccine candidate. Reproduced with permission from ref 602. Copyright 2018 American Chemical Society.

affinity, whereas the smaller ones were more inclined to elicit Th2-type immunity.

In addition, Li and co-workers reported fully synthetic invariant *i*NKT-cell-dependent carbohydrate vaccines to elicit


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an immune response in mice. It was demonstrated that high levels of tumor-specific IgG antibodies were induced by the αGalCer and MUC1-conjugated glycopeptide. The efficacy of αGalCer as a built-in adjuvant was validated again.[603]

*5.4.1.5.2.3. Immunostimulatory Peptide-Based Carbohydrate Vaccines.* Recently, nanomaterials formed by self-assembly of peptides have been widely explored in vaccine developments.[604,605] Inspired by the self-assembling peptide acting as an immune adjuvant,[606] Li's group applied self-assembled peptide Q11 in the construction of an adjuvant-free MUC1 glycopeptide vaccine (Figure 81).[607] Influenced by the

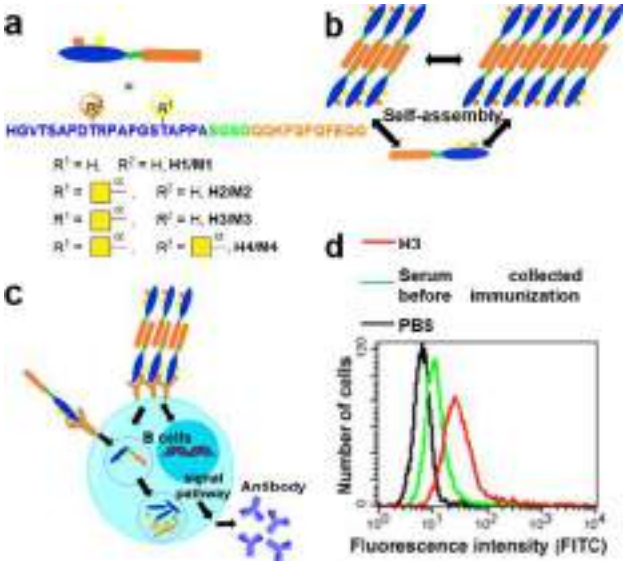


**Figure 81.** (a) Adjuvant-free MUC1 glycopeptide vaccine candidates **H1**, **H2**, **H3**, and **H4**, including the 20-mer B-cell epitopes M1, M2, M3, and M4 from the MUC1 VNTR (peptide sequence in blue). (b) Self-assembly of vaccine candidates into fibers. (c) Activation of B cells by self-assembled fibers. (d) FACS analysis on MCF-7 cell was performed to evaluate the antibody elicited by **H3**. Reproduced with permission from ref 607. Copyright 2012 American Chemical Society.

Q11 domains, the fully synthetic and well-defined conjugate MUC1-glycopeptide−Q11 assembled into fibrils (Figure 81b) and presented multivalent B-cell epitopes (Figure 81c) in a mixture of water and PBS solution. Immunological evaluations indicated that the vaccines elicited a significant immune response in mice, and the antibody can recognize human MUC1-expressing tumor cells (Figure 81d).

Considering that the nanofiber formed by the D-peptides Nap-GFFpY-NMe in the presence of enzyme can serve as an adjuvant and has been employed in anti-HIV DNA vaccines,[609] Zhao and co-workers covalently conjugated Nap-G$^{D}$F$^{D}$F$^{D}$Y$^{D}$K to MUC1 glycopeptide to potentiate the immune response (Figure 82).[608] Transmission electron microscopy (TEM) images showed that the resulting peptides self-assembled into nanoparticles with diameters of ca. 40−200 nm and some nanofibers with a diameter of 40 nm. The immunological evaluation of the assemblies showed both humoral and cellular immune responses. The covalent conjugation **C-MUC1(Tn)-Nap** exhibited better efficacy than the mixture of **MUC1(Tn)** and Nap-G$^{D}$F$^{D}$F$^{D}$Y$^{D}$K (i.e., **M-MUC1(Tn)**) as well as **MUC1(Tn)** in the presence of complete Freund's adjuvant (Figure 82a and 82b). In addition, more cytokines were produced by the **C-MUC1(Tn)-Nap** (Figure 82c), and the

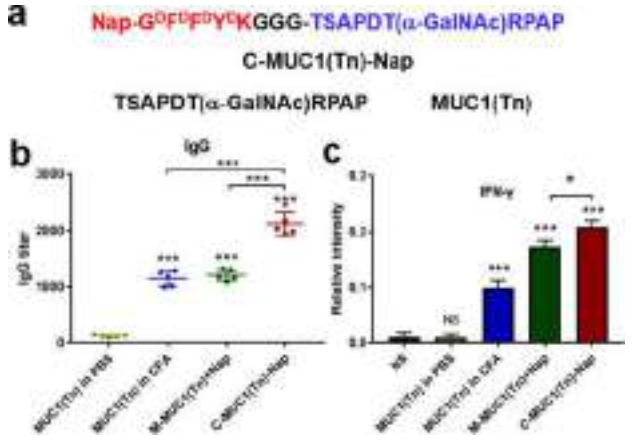


**Figure 82.** (a) Peptide sequences of **C-MUC1(Tn)-Nap** and **MUC1(Tn)**. (b) IgG titers. (c) Expression levels of IFN-γ. NS, normal mouse sera. Reproduced with permission from ref 608. Copyright 2017 Royal Society of Chemistry.

antibodies produced by this conjugation displayed higher binding to MCF-7 breast cancer cells than the others.

*5.4.1.5.2.4. Inorganic Nanomaterials.* In addition to AuNPs bearing TACAs as cancer vaccines,[610,611] iron oxide NPs which are widely utilized in drug delivery studies and molecular imaging have been explored as carriers for glycoconjugate-based cancer vaccines owing to their good biocompatibility and large surface areas.[612] As shown in Figure 83, the representative antigen MUC1 peptide that presented

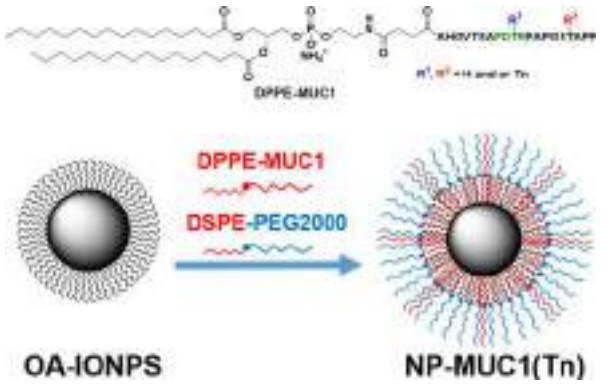


**Figure 83.** Iron oxide NPs as potential glycoconjugate-based synthetic cancer vaccines.

Tn in different positions and numbers was synthesized by SPPS and then conjugated to dipalmitoyl phosphoethanolamine. The amphiphilic MUC1 lipo(glyco)peptides can readily self-assemble onto oleic acid-coated iron oxide NPs (OA-IONPs) for antigen presentation to the immune system. It was shown that the NPs could drain into the local lymph nodes after subcutaneous injection into mice and activate the immune system to produce MUC1-specific antibodies. It was also demonstrated that one Tn in the PDTR region (green part in Figure 83) of MUC1 peptide exhibited the best immunological effect. This strategy can be extended to display multivalent antigens of other glycolipids for the development of TACA-based anticancer vaccines.

**5.4.2. Antimicrobial Vaccines.** Carbohydrates as antimicrobial vaccines can be dated back to the 1970s. Since then, capsular polysaccharides (CPS) isolated from *Haemophilus*


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*influenzae type b* (Hib), *Streptococcus pneumoniae*, and *Neisseria meningitidis* have been used to prevent infections in adults.[613−615] However, the CPS-based vaccines failed to protect children under 2 years old owing to their immature immune systems. To overcome the limitation and improve the immunogenicity of the carbohydrates, glycoconjugate vaccines based on a carrier protein have been explored. A variety of antimicrobial vaccines have been licensed on the market, and more are being developed.[507] Following success of bacterial CPS-based bacterial vaccines, the scope of carbohydrates has been extended to exopolysaccharides, *O*-antigens of LPS from Gram-negative bacteria, teichoic acids from the cell wall of Gram-positive bacteria, and polysaccharides from fungus.[615−617] In addition to glycoproteins, oligosaccharides carried by VLPs, synthetic glycolipids, and GNPs, etc., have also been used in antimicrobial vaccines.

Glycoprotein antimicrobial vaccines are generally prepared by conjugation of carbohydrate antigens, such as CPS, *O*-antigen of LPS, teichoic acid and cell wall polysaccharide, to carrier proteins (e.g., TT, KLH, HSA, CRM197, etc.) (Figure 84). Recently, Berti and co-workers reviewed antimicrobial

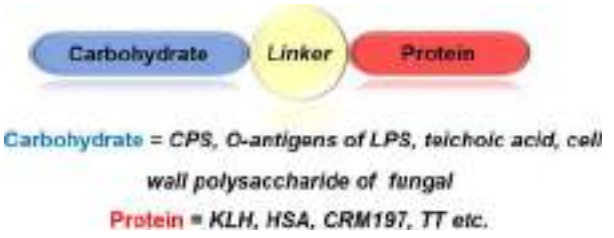


**Figure 84.** General structure of glycoprotein antimicrobial vaccines.

glycoconjugate vaccines in terms of classical and modern approaches for protein modification.[613] The review presented by Khatun and co-workers highlighted different kinds of variables, including the structure of the carbohydrate antigen, the presence or absence of some functional groups, the nature of the carrier protein, the spacer and conjugation pattern, as well as the ratio of carbohydrate to protein, and their influences on immune efficacy.[618] In addition, recent advances and potential targets for exploration of antimicrobial vaccines have been reviewed.[615] Thus, only recent representative works using different carbohydrate antigens for the development of glycoconjugate vaccines are listed in Table 5.

Heterogeneity and batch-to-batch variations are some unavoidable problems in glycoprotein vaccines; thus, well-defined glycoconjugations were also pursued for antimicrobial vaccines.

Recently, Huang's group reported a synthetic carbohydrate-based anti-*Salmonella enteritidis* vaccine (Figure 85).[626] In this vaccine, a repeating tetrasaccharide of *O*-polysaccharide on the cell surface of *S. enteritidis* was chemically synthesized and then conjugated to bacteriophage Q$\beta$. It was shown that high levels of long-lasting and specific antiglycan IgG antibodies were induced by this vaccine candidate, providing 100% protection against a lethal dose of *S. enteritidis* bacteria in the mouse model.

To avoid using peptide or protein for multivalent carbohydrate antigen presentation, Savage's group developed a liposome-based oligosaccharide vaccine with a NKT-cell antigen as adjuvant (Figure 86a).[627] A tetrasaccharide repeating unit from the *Streptococcus pneumoniae* serotype 14 polysaccharide was attached to diacylthioglycerol to give **PBS-150**. The glycosylceramide **PBS-57** was a highly potent stimulator of NKT cells, and the activated NKT cells can provide effective help to the B cell for antibody class switching (from IgM to IgG). It was shown that vaccination with a liposomal formulation of **PBS-150** and **PBS-57** induced higher titers of IgG in C57BL/6 mice than a simple mixture of the two components. Notably, the liposomal vaccine provoked a response superior to the clinically used vaccine (Prevnar), while substantial IgG was produced after a single vaccination (Figure 86b).

Cavallari and co-workers also reported a carbohydrate−lipid vaccine against *S. pneumoniae* in mice.[628] In this vaccine strategy, CPS from *S. pneumoniae* were conjugated to the lipid antigen $\alpha$-galactosylceramide, which stimulated invariant natural killer T (*i*NKT) cells (Figure 87). After immunization of lipid−carbohydrate conjugate **CPS−$\alpha$GC** to mice, IgG antibodies (Figure 87b) specific to pneumococcal polysaccharides with high affinity were produced. The CPS-specific antibodies produced in immunized mice can largely improve the survival rate when mice were challenged with *S. pneumonia* (Figure 87c). Only a negligible weight loss was caused in mice vaccinated with **CPS−$\alpha$GC** (Figure 87d)

In addition, a strategy for MPLA-based carbohydrate cancer vaccines was also expanded into antibacterial vaccines (Figure 88).[629] The repeating units of $\alpha$-2,9-PolyNeu5Ac from capsular polysaccharide of serotype C *Neisseria meningitides* were conjugated to MPLA. To improve the water solubility and presentation, all of the compounds were prepared into liposomes (Figure 88a). The immunological studies of the resulting liposomes displayed robust immune responses in C57BL/6J mice, which were comparable with that induced by the corresponding KLH conjugate vaccines (Figure 88b). Structure−activity relationship analysis also showed that the immunogenicity of these compounds decreased with longer sugar chains (Figure 88c).

Just like carbohydrate-based cancer vaccines, multicomponent vaccine,[630] glycosylated AuNPs,[631] and polysaccharide vaccine[632] have also been explored for development of antimicrobial vaccines. They will not be detailed here.

**5.4.3. Antiviral Vaccines (Anti-HIV Vaccines).** The emergence of diseases caused by viruses such as HIV, SARS, Zika virus, EBOV and the highly contagious COVID-19, etc., have become significant threat to human health. Numerous viral pathogens have utilized host−cell processes to glycosylate their proteins, facilitating immune evasion and persisting infection.[633] As glycans are relatively exposed and structurally conserved, virus-specific oligosaccharides have become attractive targets for the development of novel vaccines. In recent years, several carbohydrate-based antiviral vaccines against HIV, influenza, cholera, and hepatitis C virus have been investigated.[634] Among them, the HIV vaccines have received the greatest attention.[635−642] The recent development of HIV vaccines will be introduced in this section.

The envelope protein of HIV consists of a glycoprotein trimer, i.e., gp120−gp41. Gp120 is one of the best known glycosylated proteins, mediating HIV attachment to host cells by interaction with CD4 T cells. Chronic HIV-positive patients are able to produce potent and broadly neutralizing antibodies (bnAbs), and many of these bnAbs, such as 2G12, PG9, PG16, and PGTs, bind to *N*-glycans on gp120.[638] A high-mannose patch populated by $Man_9GlcNAc_2$ glycans (Figure 89) is


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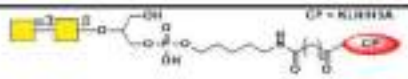
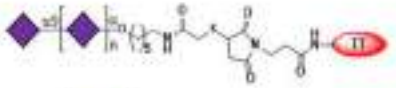
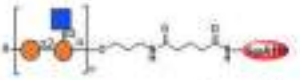
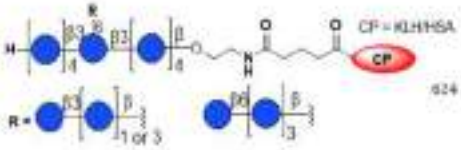
Table 5. Recent Representative Work for Development of Glycoconjugate Antimicrobial Vaccines[619625]

| Carbohydrate | | Carrier protein | Vaccine | Ref |
|---|---|---|---|---|
| Type | Source | | | |
| Teichoic acid | *E. faecium* U0317 | KLH/HSA |  | 619 |
| CPS | *Streptococcus pneumoniae* type 3 | TT |  | 620 |
| CPS | *Neisseria meningitidis* serogroup C | TT |  | 621 |
| Cell wall polysaccharide | Group A *streptococcus* | ScpA193 |  | 622 |
| CPS | *Staphylococcus aureus* | CRM197 |  | 623 |
| Cell wall polysaccharide | Fungal (*Candida albicans*) | KLH/HSA |  | 624 |
| Cell wall polysaccharide | Fungal (*Candida albicans*) | HSA/KLH |  | 625 |

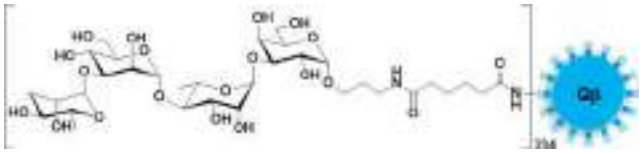


**Figure 85.** Anti-Salmonella vaccines with bacteriophage Q$\beta$ as scaffold.

mostly targeted by bnAbs. Therefore, a multivalent high-mannose glycan cluster has been used to mimic these epitopes.

2G12 isolated from serum of an infected person is the first reported bnAbs which can bind to a cluster of high-mannose $Man_9GlcNAc_2$ on gp120. Although the local structures including the D1 arm $Man_4$ and $Man_9$ exhibit binding affinity with 2G12, the conjugation prepared by coupling of the simplified structure with a carrier protein or Q$\beta$ only induce low or no affinity antibodies.[638,640,643]

Krauss's group created a directed evolution method[644] to construct numerous glycopeptides or glycosylated DNA presenting multivalent $Man_9$ or $Man_4$ clusters that bind 2G12.[645−647] On the basis of the previous work, the immunogenicity of the selected glycopeptides that bind tightly to 2G12 was studied.[648] However, antibodies produced by rabbits vaccinated with glycopeptides containing $Man_9$ primarily bound to the glycan core and linker rather than the whole glycan or $\alpha$-1,2-Man termini of $Man_9$ glycans. It was speculated that the structure of the immunogen can be processed by serum mannosidases. To delay trimming of glycopeptides by serum mannosidase, three different modes of administration were used, and the respective antibody responses were investigated. It was shown that antibodies produced by standard bolus immunization displayed the strongest binding abilities to the HIV envelope protein.

Wang's group reported the minimal epitope of several bnAbs by antibody binding studies.[649] The glycosylation type and site of the third variable (V3) domain of the HIV-1 gp120 envelope glycoprotein were crucial to high-affinity binding to bnAbs. With that, glycopeptide immunogen derived from the V3 domain together with a TLR agonist $Pam_3CSK_4$ and a Th epitope derived from TT, were coupled to form a three-component glycopeptide vaccine (Figure 90a).[650] The self-adjuvant synthetic glycopeptides elicited substantial glycan-dependent antibodies with broad recognition to several gp120s across clades in rabbit immunization. Furthermore, three-component trivalent HIV-1 V3 glycopeptides (Figure 90b) were constructed.[651] The immunogenicity of the V3 glycopeptide was substantially enhanced by the multivalency effect. Glycopeptide-specific antibodies were induced, and the antisera induced by the trivalent glycopeptide displayed stronger binding to gp120 and trimeric gp140 than the monovalent one.

**5.4.4. Antiparasitic Vaccines.** Parasitic infections are one of the most prevalent and life-threatening diseases in humans and other mammals. Every year, millions of people died from diseases like malaria, leishmaniasis, chagas, toxoplasmosis, schistosomiasis, and so on. However, the complexity of parasite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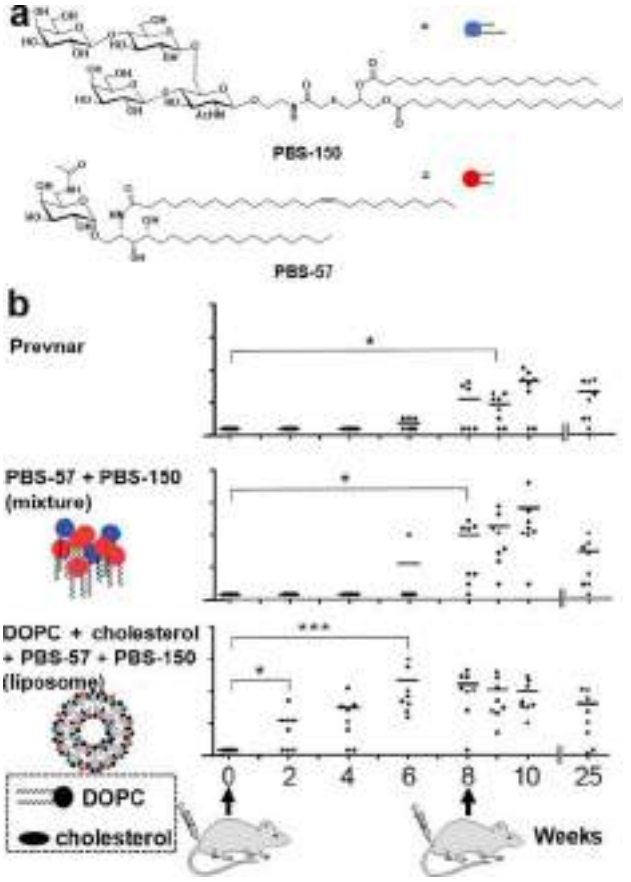


**Figure 86.** (a) Chemical structures of lipidated tetrasaccharide **PBS-150** and NKT-cell agonists **PBS-57**. (b) Antibody IgG1 formation in response to vaccination. Reproduced with permission from ref 627. Copyright 2014 Royal Society of Chemistry.

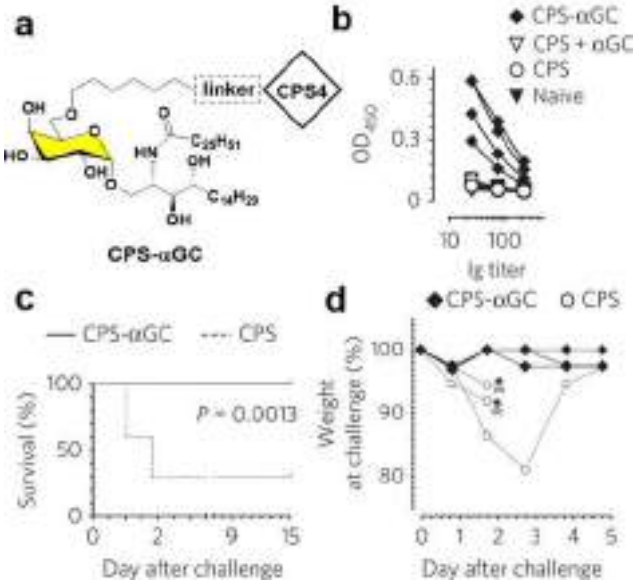


**Figure 87.** (a) Semisynthetic carbohydrate−lipid vaccine **CPS−αGC**. (b) IgG titers. (c) Survival rate of mice in the presence of *S. pneumonia*. (d) Change in body weight of mice in the presence of *S. pneumonia*. Reproduced with permission from ref 628. Copyright 2014 Springer Nature.

biology and poor understanding of the antigens associated to parasitic virulence seriously restrict development of vaccines for protection against parasitic infections.

With the development of glycoscience and glycomics, carbohydrates have been proved to be abundant on the surfaces of parasites and play a vital role in host−parasite interactions. In particular, great advances in the chemical synthesis of oligosaccharides in recent years have made carbohydrate-based vaccines promising objects against parasites.[652,653] The current strategy for parasite vaccine follows the same formulations in antibacterial and anticancer vaccines. The key points in terms of choices of carrier protein, linkers, adjuvants, etc., should be appreciated.

On the one hand, all the surfaces of pathogens have common structures, i.e., D-galactopyranosyl-$\alpha$-1,3-D-galactopyranosyl ($\alpha$-Gal) (Figure 91), which is relevant to their toxicity. Due to the lack of $\alpha$-1,3-galactosyltransferase in humans, we can produce large amounts of antibodies against the $\alpha$-galactosyl epitopes. Therefore, $\alpha$-Gal-containing glycan could be employed as a pan-vaccine against multiple infectious diseases such as malaria, leishmaniasis, ChD, tuberculosis, and so on.[654−656]

On the other hand, the pathology of parasitic diseases is mainly caused by the host's own immune responses. Neutralization of the parasitic toxin has been developed as an alternative way to protect against parasites. Seeberger and co-workers provided a detailed depiction of the present developments in the field of parasite carbohydrate vaccines.[653] The main strategies and new works on parasitic diseases (e.g., malaria and chagas) for the development of carbohydrate-based vaccines will be introduced below.

*5.4.4.1. Carbohydrate-Based Malaria Vaccine.* Malaria is a serious health hazard in humans caused by *Plasmodium* protozoan species. Although quinine and artemisinin derivatives have achieved great success in fighting against *Plasmodium*, the appearance of the drug-resistant parasite forced us to look for new methods to combat this fatal disease. Thus, using a vaccine to prevent malaria has become an important work.

Glycosylphosphatidylinositols (GPIs) are glycolipids which are present on all eukaryotic cells serving as surface protein anchors. Parasite-specific GPIs commonly exist in parasitic protozoa and have been identified as a prominent toxin in malaria. Anti-GPI antibodies are found in the sera of malaria patients, and it was envisioned that the toxic activity of GPIs can be blocked by GPI-mediated signaling and specific antibodies.[657]

For a long time, the heterogeneity of isolated GPI and purity problems led to the inconsistent relationship among the GPI structure, anti-GPI IgG response, and infection.[658−660] To make their relationship clear, chemically synthesized GPI was used to establish the specificity between anti-GPI antibodies and GPI glycans.[661] To reveal the influence of GPI glycan modifications on their immunogenicity, various kinds of GPI fragments evolved from two main *P. falciparum* (Figure 92a) were synthesized to evaluate their structure−immunogenicity relationship.[662] These GPI glycans were then coupled to authorized carrier protein CRM197; the resulting glycoconjugates (Figure 92b) were used to evaluate the production of anti-GPI antibodies, T-cell activation, and protection of mice from experimental cerebral malaria. The experiments showed that the number of Man and the presence of *myo*-inositol and phosphoethanolamine (PEtN) have a moderate influence on


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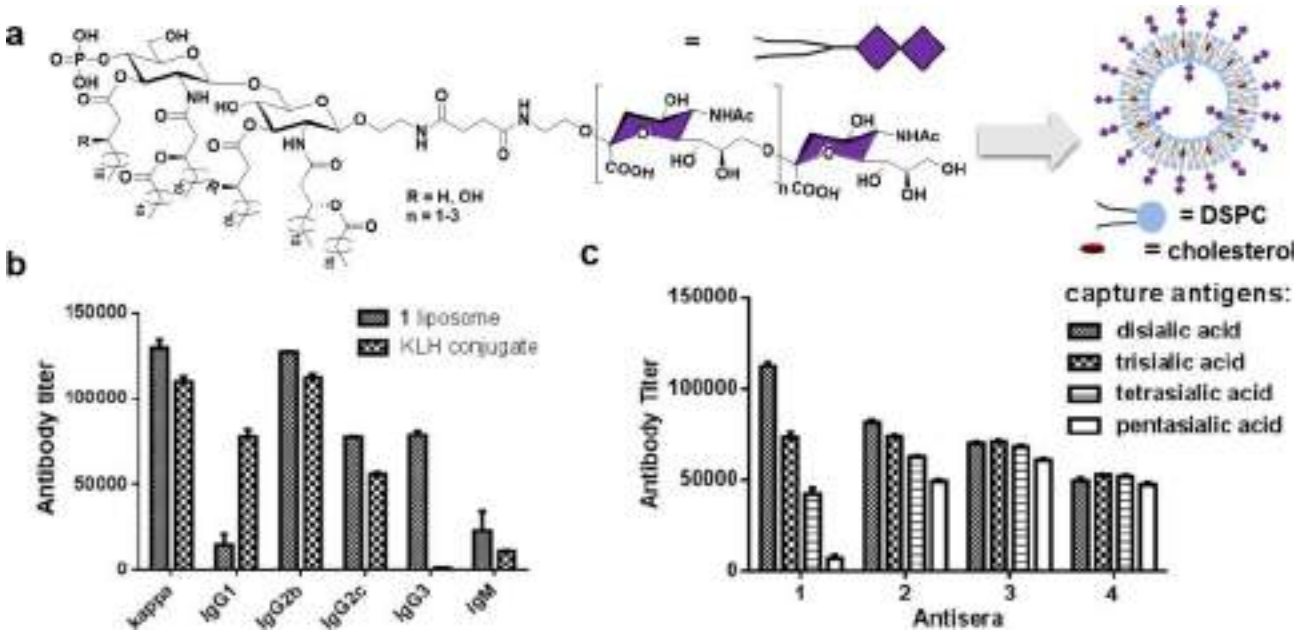


**Figure 88.** (a) Liposome formed by synthetic self-adjuvanting $\alpha$-2,9-oligoNeu5Ac-based conjugates. (b) Antibodies elicited by liposomes or KLH conjugates. (c) Antibodies elicited by MPLA conjugates with a longer sugar chain. Reproduced with permission from ref 629. Copyright 2016 American Chemical Society.

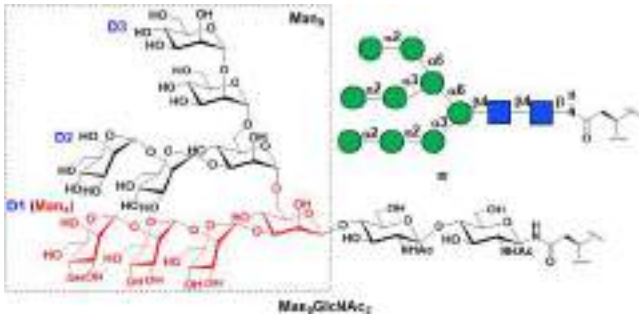

**Figure 89.** Chemical structure of $Man_9GlcNAc_2$.

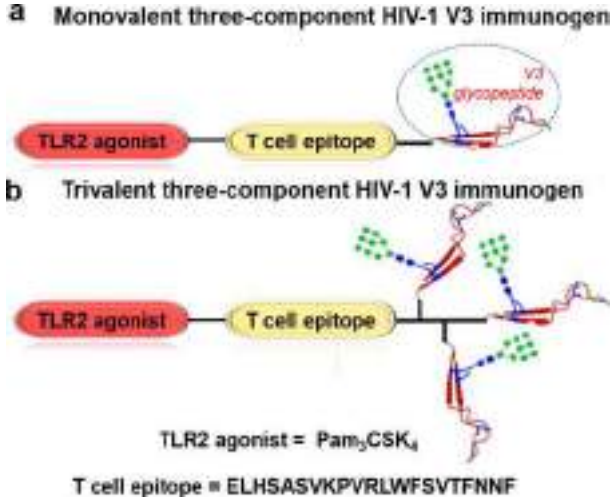


**Figure 90.** Structures of (a) monovalent and (b) trivalent three-component glycopeptide immunogens. Reproduced with permission from ref 651. Copyright 2018 American Chemical Society.

the malaria incidence for mice. The best survival occurred in mice immunized with glycoconjugates **CRM-5** containing the

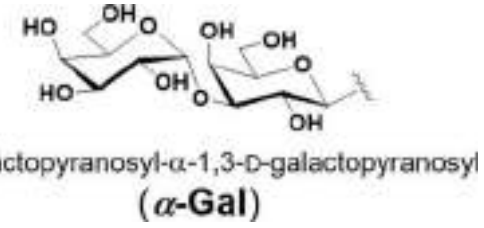


**Figure 91.** Chemical structure of $\alpha$-Gal.

GPI glycan core with the PEtN at a non-natural position (Figure 92b and 92c). Compared with the glycoconjugate with complete GPI structure (**CRM-6**), activation of $CD4^+$ and $CD8^+$ T cells as well as release of pro-inflammatory cytokines IFN-$\gamma$ and TNF-$\alpha$ (Figure 92d) by synthetic glycoconjugate **CRM-5** were reduced, which is consistent with the enhanced survival of mice.

*5.4.4.2. Carbohydrate-Based Chagas Vaccine.* Chagas disease (ChD) is a devastating vector-borne disease caused by the protozoan parasite *Trypanosoma cruzi*. It was estimated that about 7 million people are at risk of infection world-wide; 20−30% of infected individuals will suffer from disability or death owing to the lack of a therapeutic or prophylactic vaccine for ChD.[663,664]

Considering that the membrane surface of *T. cruzi* parasites is heavily coated with GPI-anchored glycoproteins, which exhibit highly immunogenic $\alpha$-Gal-containing glycotopes that are absent in humans. The $\alpha$-Gal-based glycoconjugates have beenexplored as antichagas vaccine.[665]

In 2013, a series of $\alpha$-Gal-containing oligosaccharides derived from *T. cruzi* was chemically synthesized and then covalently linked to BSA (Figure 93). Chemiluminescent enzyme-linked immunosorbent assay showed that the disaccharide Gal$\alpha$-1,3-Gal$\beta$ is the immunodominant glycotope.[666] When the reducing end of the natural carbohydrate epitope $\alpha$-D-Gal-1,3-$\beta$-D-Gal-1,4-$\beta$-D-GlcNAc (Figure 93a) was replaced with Glc, the resulting trisaccharide (Figure 93b) was effective


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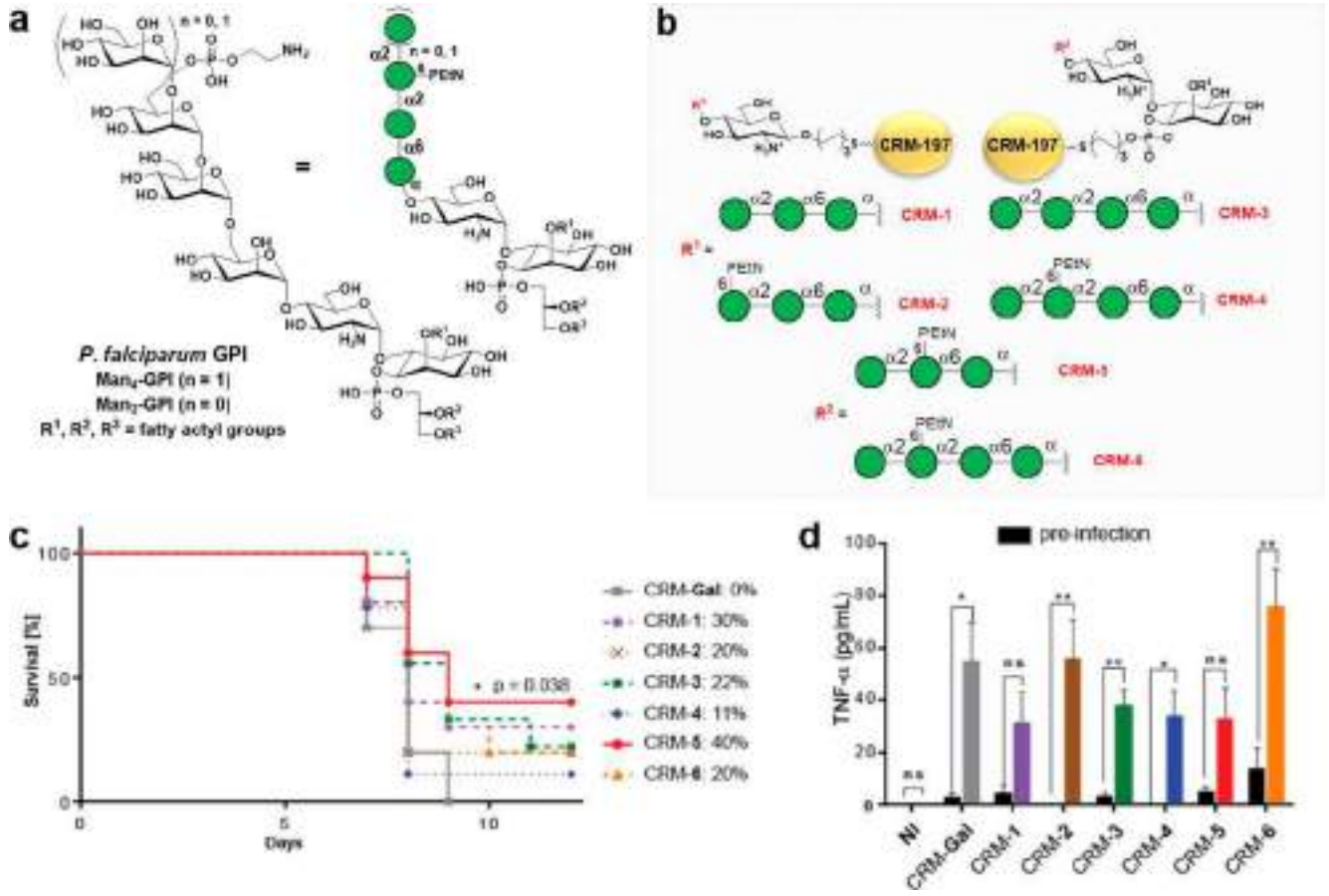


**Figure 92.** (a) Natural GPI structures of *P. falciparum*. (b) Synthetic glycosylphosphatidylinositol glycoconjugates as vaccine candidates against malaria. (c) Mice immunized with GPI conjugates exhibited an increased survival. (d) Serum levels of pro-inflammatory cytokines TNF-$\alpha$. Reproduced with permission from ref 662. Copyright 2020 American Chemical Society.

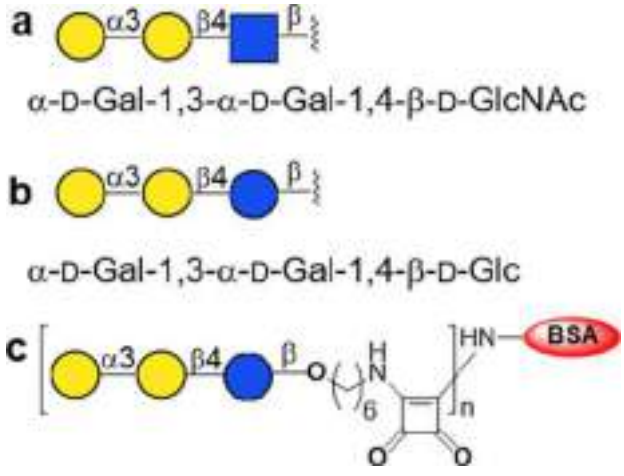


**Figure 93.** Structures of $\alpha$Gal glycans and $\alpha$Gal-BSA.

for recognition of chagasic antibodies. On the basis of this work, Lopez and co-workers conjugated the trisaccharide (Figure 93a) and the disaccharide (Figure 93b) to BSA using the squarate method (Figure 93c). Both of them can be recognized by serum samples of *T. cruzi*-infected individuals.[667]

In 2019, Almeida and co-workers reported a proof-of-concept study to demonstrate the efficacy of $\alpha$-Gal-based glycovaccine for mice infected by Chagas disease.[665] To mimic the human immunoresponse to $\alpha$-Gal glycotopes, the $\alpha$-1,3-galactosyltransferase-knockout ($\alpha$1,3GalT-KO) mice were used in the experiment. Compared with the response to *T. cruzi* infection of wild type ($\alpha$1,3GalT-WT), $\alpha$1,3GalT-KO produced significantly higher levels of anti-$\alpha$-Gal antibodies during the infectious stage, and most of the antibodies were produced against the nonreducing terminal $\alpha$-Gal glycotope. Furthermore, the immunological effect of glycoconjugate Gal$\alpha$3LN-HSA prepared by coupling trisaccharide $\alpha$-D-Gal-1,3-$\beta$-D-Gal-1,4-$\beta$-D-GlcNAc (Gal$\alpha$3LN) to carrier protein HSA was investigated. The experiments showed that a significantly reduced parasitic burden in all analyzed tissues was observed in mice vaccinated with Gal$\alpha$3LN-HSA in the presence or absence of adjuvant.

## 6. CONCLUSIONS AND PERSPECTIVES

In this review, comprehensive coverage of the recent progress on carbohydrate-based biomaterials has been included from the perspective of fundamental investigation, delivery system, tissue engineering, and immunology. As a crucial type of biomaterial, we consider carbohydrates not only as matter or a structural component but also as information or signaling molecules. Although most of the discussed applications are still far from clinical use, carbohydrates deserve to be developed into next-generation macromolecular biomaterials with great potential for the following reasons. (1) The conformation variations and multiple hydroxyl groups render the carbohydrate-based macromolecules tunable and even intelligent


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physical properties, including strength, stiffness, and resilience. These properties are adjustable under physiological conditions with different temperatures, ionic strengths, pressures, etc. (2) The variations in terms of monosaccharides and glycosidic linkages from human beings to animals, plants, and bacteria allow for adjusting the degradability, biocompatibility, and immune response of the biomaterials. The tunable degradation rate and immunogenicity in a wide scope fit the requirements from different biomedical devices and delivery vehicles to vaccines. (3) The biosafety of carbohydrate-based macromolecular biomaterials is the first criteria for any medicine, vaccine, and biomedical device. Meanwhile, the functional groups, including hydroxyl, amino, and carboxylic acid, provide the opportunity for further modification and functionalization. These advantages make carbohydrates the ideal components of next-generation biomaterials, holding great potential for precision medicine.

On the other hand, challenges and scientific questions on carbohydrates are obvious and worth being discussed. There are still many fundamental questions on carbohydrates or glycans in Nature that we do not understand well. "Big" questions include the following. How can we understand the right-handedness and huge structural variation of carbohydrates? How can we fully understand the microheterogeneity of the glycan chains and the molecular weight, monosaccharide building block variations of polysaccharides. Finally, how can we "connect" (not chemically connect) structures of carbohydrates as matter and carbohydrates as information?

To fully tap the potential of carbohydrate biomaterials and answer these questions, multiple tools for different research fields are demanded. "Sweet" science indeed is an interdisciplinary research field with indispensable contributions from synthesis, molecular biology, immunology, structural biology, polymer characterization, and physics. Currently, we still need breakthroughs from chemical and chemoenzymatic synthesis to solve the problems for the preparation of well-defined glycans and polysaccharide chains, breakthroughs to understand the amphiphilicity and hydrogen bond originating from the different carbohydrate building blocks and glycosidic bonds, and breakthroughs to understand the hierarchical structures under macroscopic and microscopic levels. Then it will be time to make carbohydrate biomaterials extensively work for us. We believe that day will come to fruition.

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### Notes

The authors declare no competing financial interest.

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Yingle Feng obtained her Ph.D. degree in Glycochemistry under the supervision of Professor Yonghai Chai in 2016 at Shaanxi Normal University in Xi'an, China. Then she joined Professor Guosong Chen at Fudan University as a postdoctoral fellow, where she focused on carbohydrate-based self-assembly and biological functions. Recently, she went back to Shaanxi Normal University, where she works on carbohydrate-based chemistry, materials, and function.

Kongchang Wei is a scientist at Empa, Swiss Federal Laboratories for Materials Science and Technology. He obtained his B.S. degree in 2008 from the Department of Macromolecular Science, Fudan University, where he also received his Ph.D. degree in Polymer Chemistry and Physics under the supervision of Professor Ming Jiang and Professor Guosong Chen. He moved to the Chinese University of Hong Kong in 2013, where he worked on supramolecular hydrogels for tissue engineering. He moved to Empa in 2018 with a Marie Skłodowska-Curie Fellowship. With his research interests in polymer biomaterials, biofabrication, and tissue engineering, he joined the Swiss collaborative skin research initiative Skintegrity. CH as a Young Investigator in 2021.

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Guosong Chen is a professor in the Department of Macromolecular Science, Fudan University, Shanghai. She studied chemistry at Nankai University, where she obtained her B.Sc. degree in 2001. In 2006, she received her Ph.D. degree from the same university in Supramolecular Chemistry. After postdoctoral studies in carbohydrate chemistry at Iowa State University, she moved to Fudan University in 2008, where she joined the department as a lecturer, working on the interface of macromolecular self-assembly and supramolecular chemistry. Then she was promoted to Associate Professor in 2011 and Professor in 2014. Recently, her research focus has been reoriented toward carbohydrate-based macromolecular self-assembly and its biological functions. She was elected a Fellow of the Royal Chemical Society (FRSC), serves as an associate editor of *ACS Macro Letters*, and is an international board member for several journals.

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## ABBREVIATION

| | |
|---|---|
| APCs | antigen-presenting cells |
| $\alpha$-MOS | $\alpha$-mannan oligosaccharide |
| ASGPR | asialoglycoprotein receptor |
| ATRP | transfer radical polymerization |
| BBB | blood−brain barrier |
| BMP-2 | bone morphogenetic protein 2 |
| BSA | bovine serum albumin |
| BTA | benzene-1,3,5-tricarboxamide |
| CCIs | carbohydrate−carbohydrate interactions |
| CLRs | C-type lectin receptors |
| ConA | concanavalin A |
| CPIs | carbohydrate−protein interactions |
| CPS | capsular polysaccharides |
| CPT | camptothecin |
| CRD | carbohydrate recognition domains |
| CS | cationic chitosan |
| CSCs | cancer stem cells |
| CTLs | cytotoxic T lymphocytes |
| CuAAC | copper-catalyzed azide−alkyne dipolar cycloaddition |
| DCs | dendritic cells |
| DC-SIGNs | dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrins |
| DFO | seferoxamine |
| DnL | dock-and-lock |
| DPn | degree of polymerization |
| DT | diphtheria toxin |
| ECM | extracellular matrix |
| ELISA | enzyme-linked immunosorbent assay |
| EPS | extracellular polymeric substances |
| FACS | fluorescence-activated cell sorting |
| GAGs | glycosaminoglycans |
| GBPs | glycan-binding proteins |
| GGPs | glycosylated polymersomes |
| GLUT | glucose transporter |
| GM | growth factor |
| GNPs | glyconanoparticles |
| HA | hyaluronic acid |
| HepG2 | hepatocellular carcinoma |
| HGM | host−guest−macromer |
| HIV | human immunodeficiency virus |
| hMSC | human mesenchymal stem cell |
| HP | heparin |
| HS | heparan sulfate |
| HSA | human serum albumin |
| Ig | immunoglobulin |
| IL | interleukin |
| *i*NKT | invariant natural killer T |
| IPN | interpenetrating polymer network |
| KLH | keyhole limpet hemocyanin |
| LPS | lipopolysaccharide |
| MGLs | macrophage galactose-binding lectins |
| MMP | matrix metalloproteinase |
| MPLA | monophosphoryl lipid A |
| MR | mannose receptor |
| MWCNT | multiwall carbon nanotubes |
| NK | natural killer cell |
| NPCs | neural progenitor cells |
| NSPCs | neural stem/progenitor cells |
| OVA | ovalbumin |
| PASs | poly-amido-saccharides |
| PGCs | poly(D-glucose carbonate)s |
| PISA | polymerization-induced self-assembly |
| PNA | peanut agglutinin |
| PSGL-1 | P-selectin glycoprotein ligand 1 |
| PVLA | poly(*N*-*p*-vinylbenzyl-4-*O*-L-D-galactopyranosyl-D-gluconamide) |
| RAFT | reversible addition−fragmentation chain transfer polymerization |
| rBM | reconstituted basement membrane |
| RhB | Rhodamine B |
| ROMP | ring-opening metathesis polymerization |
| ROP | ring-opening polymerization |
| SPECT | single-photon emission computed tomography |
| SPPS | solid-phase peptide synthesis |
| TACAs | tumor-associated carbohydrate antigens |
| TAMs | tumor-associated macrophages |
| Tg | glass transition temperature |
| TGF-$\beta$ | transforming growth factor $\beta$ |
| TLRs | toll-like receptors |
| TRGO | thermally reduced graphene oxide |
| TT | tetanus toxoid |
| TTTs | triggerable tough hydrogels |
| VEGF | vascular endothelial growth factor |
| VLPs | virus-like particles |
| XG | xyloglucan |
| YAP | yes-associated protein |
| ZPSs | zwitterionic polysaccharides |

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