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Advances in multifunctional glycosylated nanomaterials: preparation and applications in glycoscience

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ABSTRACT

Applications of glycosylated nanomaterials have gained considerable attention in recent years due to their unique structural properties and compatibility in biological systems. In this review, glyco-nanoparticles (glyco-NPs) are defined as compounds that contain a nano-sized metallic core, are composed of noble metals, magnetic elements, or binary inorganic nanoparticles, and that exhibit carbohydrate ligands on the surface in three dimensional polyvalent displays similar to the glycocalyx structures on cell membranes. Nanomaterials decorated with suitable biological recognition ligands have yielded novel hybrid nanobiomaterials with synergistic functions, especially in biomedical applications. This review focuses on strategies for building various types of glyco-NPs and highlights their potential in targeted drug delivery and molecular imaging as well as their uses in bioassays and biosensors. The most recent examples of glyco-NPs as vaccine candidates and probes for assaying enzymes with bond-forming activities are also discussed.

1. Introduction

Carbohydrates are ubiquitous in all organisms and are commonly found on cell surfaces in the form of glycoproteins, glycolipids, and the glycocalyx. Because (oligo)saccharides of the glycocalyx are easily accessible, they are involved in carbohydrate–protein interactions that play essential roles in a range of pathological and physiological cell functions.[1–3] Such interactions are also considered as a prerequisite step in the cycle of host infection by several microbes, including viruses, bacterial pathogens, and their toxins.[4] The interactions between an individual carbohydrate ligand and a carbohydrate-binding protein (lectin) are relatively weak and nonspecific. Consequently, these interactions often (but not always) exhibit a low association constant (K_a) in the range of $\sim 10^3\ M^{-1}$ and are difficult to quantify.[5] To overcome this limitation, simultaneous multi-point contacts between clusters of carbohydrates and a corresponding multimeric protein, or a protein with multiple binding sites, exploit multivalent binding to achieve high avidity as a result of the cluster glycosidic effect.[6] Such interactions are ubiquitous in biology, and the binding affinities are orders of magnitude higher than that of a monovalent binding event.[7] As a result, both the clustering of carbohydrate binding sites in the protein partner and the presentation of the carbohydrate at the cell surface in a polyvalent, oriented fashion have been utilized to overcome the typical low K_a of carbohydrate–protein interactions in living systems.[8] Therefore, understanding the central role that glycans (free carbohydrates or carbohydrate fragments of glycoproteins, glycolipids, and proteoglycans) play in a wide variety of biological recognition events is crucial for developing efficacious drugs and diagnostic tools.

In light of the above factors, numerous well-defined synthetic multivalent glycomimetics with variable valency, topology, and modes of ligand presentation have been introduced. These glycomaterials include peptides,[9] dendrimers,[10] polymers,[11] liposomes,[12] cyclodextrins,[13] fullerenes,[14] calixarenes,[15] and nanoparticles (NPs).[16] Despite many studies on multivalent glycosylated nanoscale systems, the precise requirement for the best scaffold relies heavily on a specific type of glycan binding event. When conjugated to affinity ligands such as glycans, nanomaterials provide excellent opportunities for studying carbohydrate-mediated biological interactions at the molecular level (Fig. 1). Because of their inherent high surface area-to-volume ratio compared to other traditional micrometer-sized counterparts, NPs provide a greater contact surface area capable of producing higher capacity receptor binding. In addition, multiple and different forms of carbohydrate ligands can be incorporated simultaneously on the surface of an NP, thus mimicking the polyvalent glycolipid structure on the cell surface. Such features facilitated the self-assembly of carbohydrate monolayers on the surface of colloidal gold nanoparticles (AuNPs), termed glyco-AuNPs, and were first employed as metal-based multivalent scaffolds to

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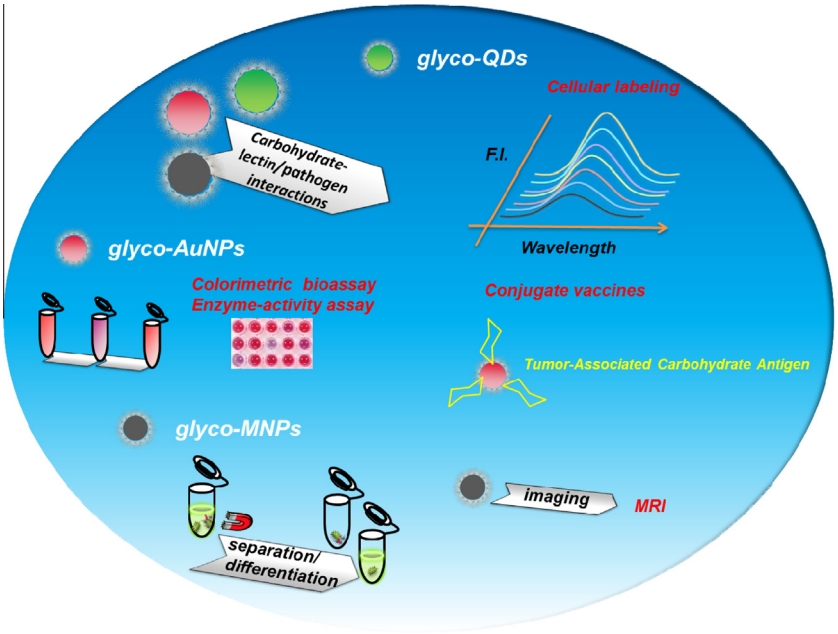

Figure 1. Examples and applications of different multivalent and multifunctional glyco-NPs.

present carbohydrate ligands.[17,18] In this review, glyco-nanoparticles (glyco-NPs) are defined as compounds containing a nano-sized metallic core that is composed of noble metals, magnetic elements, or binary inorganic NPs and that exhibit biologically relevant carbohydrate ligands on the surface in three dimensional polyvalent displays. Earlier contributions have surveyed the methodologies involving the synthesis and characterization of gold, magnetic iron oxide, and binary inorganic (e.g., semiconductor quantum dots (QDs)) NPs and their applications in specific biomolecular recognition events.[16,19] Metal-based NPs functionalized with natural polysaccharides, such as chitosan, heparin, and dextran, have been reviewed elsewhere[20] and are therefore not covered in this review.

Here, we highlight multivalent glycosylated nanomaterial systems based on gold, iron oxide, and QDs that have demonstrated enhanced binding affinity for glycans and proteins. Specifically, we focus on glyco-NP-based binding assays and biosensors for protein detection and present their potential in targeted drug delivery and molecular imaging. Finally, we discuss the advances in vaccine development and briefly outline the prospective applications of glyco-AuNPs in enzyme activity assays (Fig. 1).

2. A brief overview of the preparation of glyco-NPs

Glyco-NPs consist of an inorganic core of nanoscale dimensions (less than 100 nm in diameter (d)) and an outer surface covered with a flexible organic layer (linker or polymer) connected either covalently or non-covalently to glycans. The chemical stability of gold colloids is advantageous in synthesis for controlling the size and shape of the NPs. The traditional method of fabricating AuNP uses the reduction of metal salts such as chloroauric acid ($HAuCl_4$) in aqueous media to produce neutral Au atoms by modification of the Turkevich or Brust method.[21,22] While the gold-colloid method uses the mild reducing agent sodium citrate in hot aqueous solution to produce colloids of a relatively broad size range (~12–100 nm in d), the Brust method utilizes a strong reducing agent, such as $NaBH_4$, to yield AuNPs with a d of ~2–5 nm. The covalent binding between AuNPs and biomolecules is easily achieved with a ligand-exchange process using thiolated molecules, that is, the generation of a self-assembled monolayer (SAM) on the colloidal gold surface.[23] The ligand density on the metal surface can be controlled by dilution with non-functionalized thiols. Thus, by changing the reaction conditions, the size, shape, and chemical composition of the surface of the AuNPs can be selectively adjusted. In addition, AuNPs have a unique surface plasmon band in the visible region (400–700 nm), making them a suitable tool for studying or monitoring biological recognition processes. Penadés and co-workers were the first to use carbohydrate-functionalized AuNPs, which contained lactose (Lac) and Lewis^X (Le^X), for studying carbohydrate–carbohydrate interactions.[17]

Magnetic cores such as iron oxide (Fe_3O_4/Fe_2O_3) can also be used to functionalize desired glycans. The common methods for synthesizing magnetic NPs (MNPs) are co-precipitation, thermal decomposition, and microemulsion. However, the conjugation chemistries used in the surface modification are critical for ligand assembly and have been reviewed extensively.[24] To attach glycans onto MNPs, carboxymethyldextran-coated MNPs or amine-functionalized dextran-coated MNPs were employed, thus achieving glycofunctionalization. For example, amine-functionalized dextran-coated MNPs have been used as a platform for the incorporation of multiple copies of sialyl Le^X (sLe^X) onto the MNP surface.[25] Methods for the covalent functionalization of pre-fabricated MNPs through peptidic coupling[26,27] or Cu(I)-catalyzed alkyne–azide [2+3] cycloaddition[26,28] have also been reported with a high loading yield of the glycan. With the latter method, azido-functionalized NPs provide improved conjugation efficiency with alkynated carbohydrates compared to alkynated NPs with azide-bearing molecules.[28] The typical size of glyco-MNPs (d 10–100 nm) is an average of two orders of magnitude smaller than that of a bacterium and allows multiple attachments of NPs onto the surface of a cell. In addition, MNPs exhibit high magnetization due to their superparamagnetic properties. These features make them useful in bioseparation and as a contrast agent for Magnetic Resonance Imaging (MRI).

QDs are nano-sized fluorescent semiconductors and are characterized by a narrow emission bandwidth, high quantum yield, and long-term photostability compared to common organic dyes and fluorescent proteins.[29] The most common procedure used to fabricate stable and water soluble QDs is the attachment of a hydro-