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# Natural Polysaccharide based Nanoparticles for Drug/Gene Delivery

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**ABSTRACT**

The dawn of the state-of-the-art methods of drug/gene delivery, nano-based delivery systems for delivery of pharmaceutically active agents into the target sites in the body have been developed. Among these, a concerted effort has been directed towards the development of biodegradable/biocompatible nanomaterials with the high potential benefits of passive/active targeting and reduced undesirable side effects. Since polysaccharides offer a large number of available reactive groups, specific targeting could be obtained by the surface coating of nanoparticles with targeting ligands. On the other hand, mucoadhesive properties of polysaccharides can be used for prolonging the residence time of delivery systems at the site of absorption. This study reviews a number of important polysaccharides with a perspective on the challenges, advantages, and disadvantages of their applications as drug/gene delivery systems.

## Introduction

An important part of studies in the medicine has been focused on the production of novel nanoscale particles for developing new drug/gene delivery systems.[1] Macromolecules like proteins and nucleic acids show low stability in the biological media. Their efficient delivery to the target sites has been broadly limited *in vivo*, because these molecules can hardly pass through the various biological barriers.[2] Nanoparticles engineered with particular physicochemical characteristics have been found to be able to overcome cell-associated barriers for delivering the pharmaceutical molecules into cells.

The size of nanoparticles plays a key role in the use of these structures as drug/gene carrier systems as well as trans-membrane transporters.[3] Nanoparticles are ultrafine colloidal particles in the size range between 1-100 nm and exhibit different properties as compared to their source material.[4] Drugs or bioactive molecules can be encapsulated into the interior matrix of nanoparticles or incorporated to the exterior surface by the adsorption or conjugation.[5] Not only the synthetic polymers like Poly(lactide-co-glycolide),[6] polyacrylates,[7] polycaprolactones,[8] and polyethylenimine,[9] but also natural polymers such as albumin,[10] alginate,[11] and chitosan[12] can be used for the preparation of nanoparticles. Among these, biodegradable polysaccharide nanoparticles show excellent properties for a prolonged drug release.[13] Amphiphilic nature of polysaccharides facilitates their self-assembly in an aqueous environment and helps to form specific structures.[14,15] Polysaccharide materials display a high affinity to the mucosal surfaces covering the nasal, pulmonary, and gastrointestinal tracts.[16] The affinity of a drug delivery system to the mucus layer covering the epithelial surfaces assists in guiding the carrier into a particular site and prolonging the residence time of therapeutic agent. Therefore, these systems can improve the permeability and bioavailability of macromolecules such as proteins and peptides in the absorption site.[17]

Polysaccharide materials can be classified into two groups including polyelectrolytes and non-polyelectrolytes. Polyelectrolytes can be further divided into cationic (chitosan), anionic (alginate, heparin, pectin, hyaluronic acid), and neutral (pullulan, dextran) subgroups, based on their intrinsic charge.[18] The surface coating of nanoparticles with polysaccharide and oligosaccharide has also been proposed recently as an alternative strategy for the PEGylation of nanoparticles surface. The coated materials can interact with a particular group of receptors at the

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cell membrane and tissue surface; thereby leading to an active targeting.[16] Notably, polyelectrolyte complexation is known as a simple method for the preparation of polysaccharide-based nanoparticles and is based on the electrostatic interaction of polysaccharides with various oppositely charged polymers.[18]

Hence, due to the increasing applications of biodegradable nanoparticles, here we review the properties of nanocarriers prepared with polysaccharide based materials and derivatives used for the drug/gene delivery.

### *Alginate*

Alginate, an anionic unbranched biopolymer, is comprised of guluronic and mannuronic acid residues.[19-21] Alginate nanoparticles owing to biocompatibility, biodegradability, non-antigenicity, gelation ability, and mucoadhesive advantages have been extensively applied as drug/gene delivery systems.[22-24] The most common techniques used for the preparation of alginate nanoparticles are the reverse microemulsion,[25] desolvation,[26] cross-linking,[27] and evaporation.[28] During the addition of divalent ions (calcium, strontium, and barium), the ionic cross-linkage between residues is formed and water-soluble alginate salts are transformed into the water-insoluble salts.[20] The electrostatic interaction between the carboxyl groups and divalent ions mediates the formation of cross-linked gel. This phenomenon plays an important role in controlling the drug release.[29] Alginate can interact with the cationic components due to its anionic nature. Therefore, it can be used for the preparation of delivery systems due to incorporate positively charged drugs and molecules. Calcium alginate is the most commonly used alginate matrix in the engineering of drug/gene delivery systems.[24] Alginate gel beads coated with chitosan can prolong the residence time through the storage and *in vivo* circulation. Besides, alginate-chitosan complex was shown as a promising system for the controlled release of incorporated agents compared to either alginate or chitosan alone.[30] Alginate nanoparticles have also been developed to promote the bioavailability of antitubercular drugs (isoniazid, pyrazinamide, ethambutol, and rifampicin).[31,32]

The cornea drainage and tear flow limit the penetration and residence time of drugs at the eye for the treatment of ocular diseases. Cornea and conjunctiva have a negative charge like any other biologic membrane in human body, therefore the positively charged mocuadhesive polymers such as chitosan can interact with these negative surfaces. Hence, polyelectrolyte complexes consisting alginate and chitosan have been prepared for the effective ophthalmic drug delivery.[21]

### *Chitosan*

Chitosan, composed of D-glucosamine repeating units, is known as a non-toxic, biodegradable, bioadhesive polysaccharide and is structurally similar to the cellulose except that amine groups give chitosan its net positive charge. The main advantages of chitosan nanoparticles used for developing gene/drug delivery systems are summarized in Table 1.

The intrinsic degradation and imperfect absorption of protein drugs through the mucosal barriers of gastrointestinal tract restrict medical applications of this group of drugs. The tight junction between the epithelial cells is another limiting cause for the transition of hydrophilic drugs across the intestinal membrane.[33] Chitosan exhibits superior mucoadhesive properties and is able to open tight junctions between the epithelial cells due to its disruptive effect on intercellular lipid packing in epithelium.[34,35] The mucoadhesive properties of chitosan is related to the interaction between its positively charged groups and negatively charged mucin.[36] As a result, it can be used as an absorption enhancer in mediating the residence and penetration of nanocarriers through the epithelial barriers.[14] In addition, its functional groups can be modified chemically due to the design of carrier systems with high potential for the nasal, oral, ocular, and transdermal administrations.[34] Notably, factors involved in the preparation and physicochemical conformation of chitosan nanoparticles (e.g. molecular weight, deacetylation degree, concentration of chitosan and protein) affect the ability of nanoparticles to enhance mucoadhesive property as well as facilitating drug/gene delivery.[37] Owing to the convenience and compliance of patients, the oral administration of drugs has recently attracted many attentions.[38]

**Table 1.** The advantage of chitosan nanoparticles used in gene/drug delivery.

| Advantages | References |
|---|---|
| Biocompatibility, biometabolizability, and non-toxicity make it a safe carrier system | 96 |
| Excellent capability in controlled release of encapsulated agents | 97 |
| High potential for complexation with negatively charged DNA and macromolecules | 98 |
| Prohibition of toxic organic solvents during preparation process | 98 |
| Prolonged residence time at the site of absorption because of its mucoadhesive nature | 40 |

However, a proper strategy to solve the problems associated with oral delivery like pre-systemic metabolism and mucosal barriers presence is the development of novel carrier systems.[39] As mentioned above, the ability of chitosan to adhere to the mucosal surface is a potential approach in improving the efficiency of drug delivery by oral administration.[38,40] Nanoparticles potentially improved the intestinal absorption of insulin compared to aqueous solution of chitosan after oral administration.[41] In addition, a polyelectrolyte complex consisting of chitosan/alginate bearing opposite charges and different ratios has been reported for the efficient delivery of insulin.[42] On the other hand, ocular delivery of drugs is limited by the barriers present in the precorneal site. Therefore, the improvement of intraocular penetration and residual time of drug at the site of action is critical. Hence, chitosan based systems are attractive candidates for use in ocular delivery.[43] Besides, chitosan can be modified by specific targeting ligands for the rapid and efficient interaction of carrier with cell membranes.[40]

### *Heparin*

Heparin is an anionic and extremely sulfated polysaccharide that exhibits strong anticoagulant properties.[44] A number of protein components like different growth factors have heparin-binding domains which can simply interact with heparin.[45] Heparin-based nanoparticles can serve as novel systems to protect against the proteolytic and chemical degradations as well as controlling the release of heparin binding growth factors by enhancing the interaction between the growth factors and their receptors.[46] Moreover, similar to chitosan, heparin has demonstrated anticancer effect.[47] The antitumor property of heparin is related to its ability to suppress the tumor angiogenesis and metastasis via disrupting the activity of VEGF and bFGF.[44] However, small heparins with low molecular weight show higher efficacy in suppressing the tumor growth.[48]

### *Hyaluronic acid*

Hyaluronic acid (HA) is a non-sulfated linear and negatively-charged polysaccharide that has been widely used for the fabrication of nanoparticulate carriers.[49] HA shows good solubility and stability in the aqueous environments and is a suitable component for the development of biodegradable and biocompatible systems, because of its non-toxic and non-immunogenic nature.[50] HA can form various structures depending on its concentration.[15] When HA dissolves in water, it forms a gel similar to lubricant. Hence, HA has been regarded as an ideal lubricant in the joints and other tissues due to its ability to decrease postoperative adhesion formation following abdominal and orthopedic surgeries. In addition, water absorbing potential of HA leads to its hygroscopic and homeostatic properties, which is believed to be important for modulating tissue hydration as well as osmotic balance. These outstanding properties expand its application in pharmaceutical and medical sciences.[51] In the field of drug delivery, a small number of systems are able to accumulate at the tumor site through the carrier/receptor interaction. HA is one of the biopolymers having the good potential for passive tumor targeting.[52] HA is also introduced as one of the main constituent components of extracellular matrix and is found throughout connective, epithelial, and neural tissues.[53] It shows high affinity to hyaluronan receptors, CD44 and RHAMM, over-expressed in a large number of tumor cells. Therefore, specific targeting of these receptors can be considered as an effective strategy in tumor therapy for increasing the accumulation of nanoparticles in the tumor site.[54-56] In aqueous environments, the high molecular weight HA can form a sponge-like structure with radius about 100 nm. This property makes HA an ideal candidate for passive targeting.[52] HA has been proposed for the preparation of ophthalmic dosage forms, since it can improve the interaction between the drug and ocular mucosal surface.[16] The chemical modification of' HA makes it a favorable material for potential therapeutic/diagnostic applications.[50] In addition, the enzymatic degradation of HA-based carriers by hyaluronidase mediates the sustained release of encapsulated drugs.[49] To improve the treatment efficiency of cancer after oral administration, nanoparticles must be able to overcome the limitations of this route. Polyelectrolyte nanocomplexes composed of chitosan and HA have been investigated to improve the oral delivery and the intracellular accumulation of anticancer drugs using both mucoadhesive and passive targeting characteristics of chitosan and HA, respectively. Ovalbumin loaded polyelectrolyte nanocomplexes were proposed to improve antigen delivery after nasal and intradermal administrations.[57] Similarly, HA nanoparticles have been investigated as carriers to increase the permeability and cellular uptake, and minimize the enzymatic degradation of insulin through the oral route in animal models.[58]

### *Dextran*

Dextran is another unbranched polysaccharide that shows high water solubility and is extensively applied in the area of food and medicine.[59] The presence of hydroxyl groups significantly modulates the incorporation of proteins, aptamers, or pharmaceutical agents into the dextran skeleton. Disintegration of dextran occurs mainly in the

liver, spleen, kidney, and bottom section of the gastrointestinal tract by dextranase enzyme.[60] Dextran can be used in designing stealth nanoparticles to escape from the reticuloendothelial system and enhance the blood circulation time. Gelation of bovin serum albumin (BSA) and dextran conjugate after heating at BSA isoelectric pH yields a structure with BSA core and dextran shell. One recent study demonstrated that the conjugation of dextran to BSA facilitates the dispersion of doxorubicin-loaded nanoparticles and increases the stabilization of encapsulated cargo in solution.[61] In addition, it has been shown that BSA-dextran conjugates can encapsulate hydrophobic drugs such as ibuprofen.[62] Undivided attention has been payed especially to the decoration of dextran nanocarriers for the therapeutic applications.[63] Biocompatibility, biodegradability, non-antigenicity, non-immunogenicity, and other properties of dextran make it an attractive candidate for the fabrication of nanoparticles to be used for the delivery of the therapeutic targets.[60] For example, dextran nanoparticles have been designed for the delivery of doxorubicin into the nuclei of cancer cells as intelligent drug delivery systems in chemotherapy.[64]

### *Pullulan*

Pullulan is a linear, highly water soluble, nontoxic, and neutral polysaccharide that is produced by the fermentative activity of the strains of fungus Aureobasidium pullulans.[65-67] Pullulan is known as a suitable material with many therapeutic applications such as drug/gene delivery, tissue engineering, diagnosis, imaging, and also the generation of compression moldings, fibers, and edible films.[68,69] The surface modification with hydrophobic molecules such as cholesterol can facilitate the self-aggregation and nanoparticle formation of pullulan.[70] The process of acetylation can be used for producing the hydrophobic pullulan. The self-assembled pH-sensitive nanoparticles of pullulan acetate/sulfonamide conjugates were proposed as efficient delivery systems.[66] Pullulan-based nanoparticles have been successfully used for the enhanced delivery of doxorubicin into tumor cells.[70] Similarly, pullulan nanoparticles have been designed as mocuadhesive ocular drug delivery systems.[71] Pullulan has also been reported for the oral delivery, because of its biodegradable nature.[69] Besides, pullulan has shown anticancer activity.[70] Cholesteryl group-bearing pullulan (CHP) is a universal protein-based antigen delivery vehicle for adjuvant free nasal vaccination. CHP can self-assemble into nanoparticles in aqueous environment and encapsulate a large amount of therapeutic payloads in the interior space through hydrophobic interactions. In addition, the cationic type of CHP nanoparticles (cCHP) can be prepared by adding amine groups to the CHP nanoparticles. It has been reported that CHP nanoparticles are efficiently transferred to antigen-presenting cells like dendritic cells and/or macrophages, and this provides a stronger immune response.[4] Figure 1. represents the uptake of cCHP nanoparticle-vaccine antigen complex by nasal dendritic cells that induce antigen specific immune responses.

### *Pectin*

Pectin is a structural polysaccharide extracted from the plant cell walls. A significant factor in determining solubility, gelling, and film forming capability of pectin is the degree of esterification of its galacturonic acid residues.[72] Pectin can pass intact through the stomach and small intestine, while it is degraded by pectinases secreted from bacteria present in the large intestine. Upon the mentioned properties, pectin based delivery systems would be suitable for protecting the protein and polypeptide based drugs to be passed through the gastrointestinal tract until they reach the large intestine.[73,74] However, the high hydrophilic nature of pectin may limit the ability of pectin based delivery systems in the protection of loaded drug along the gastrointestinal tract.

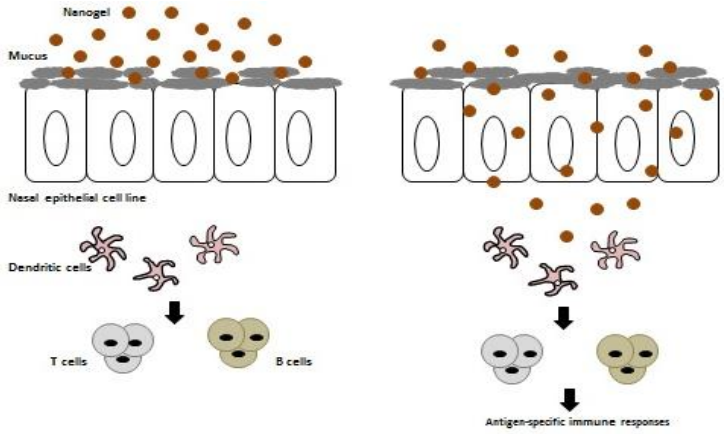


**Figure 1.** The uptake of cCHP nanoparticle-vaccine antigen complex by nasal dendritic cells for inducing antigen specific immune response.

**Table 2.** Classification of polysaccharide-based nanoparticles used as carriers for drug delivery.

| Nanoparticle system | Physicochemical properties | Comments | References |
|---|---|---|---|
| Chitosan | Shows mucoadhesive property and ability for opening the tight junctions between epithelial cells | Its cationic nature mediates delivery of negative molecules such as DNA | 34,79 |
| Alginate | Shows mucoadhesive and gelling properties | Its anionic nature mediates delivery of cationic agents | 20,24 |
| Heparin | An anionic and highly sulfated polysaccharide that shows anticoagulant properties | Ideal system for delivery of growth factor | 14,46 |
| Hyaluronic acid | Affinity to water absorption and gel forming | Facilitates passive tumor targeting through CD44 receptor-mediated endocytosis | 49,51 |
| Dextran | A neutral polysaccharide with lower cytotoxicity | Degradation of nanoparticles occurs by dextranase | 60 |
| Pulluan | A neutral polysaccharide produced by a specific fungus | Relative high cost of pullulan has limited its application | 65 |
| Pectin | An anionic polysaccharide with gelling and film forming ability | Degradation of nanoparticles occurs by pectinase secreted from bacteria present in the colon | 72,73 |

Thus, the discovery of pectin derivatives with low water solubility must be focused in future studies.[73] For example, this problem can be bypassed by increasing the thickness of the coating polymers, inactivation of proteases, application of crosslinking calcium ions, and incorporation of the physical layers that separate drug-pectin.[75] The classification of polysaccharide-based nanoparticles used as carriers for the drug delivery has been summarized in Table 2.

### *Polysaccharide nanoparticles for gene delivery*

Generally, gene therapy is a therapeutic technique to transfer foreign genes into host cells to treat or prevent genetic diseases and dysfunctions.[76] There are different viral vectors such as retroviruses, adenoviruses, adeno-associated viruses (AAV), and herpes simplex viruses that can transfect a wide range of dividing and non-dividing cells. Recently, the attention of researches has been directed to the identification of safe and efficient alternatives for the viral vehicles. Non-viral vectors have many advantages as compared to the viral vectors such as simple preparation process, low immunogenicity, flexibility in loading of genes with different sizes, and less clinical risks of using viral vectors.[77] Nanoparticles constitute a new class of non-viral vectors and have shown a high potential to deliver the genetic material across the cell membrane and ultimately to the cell nucleus.[78] In this regard, polysaccharides and other cationic polymers are cases of the most extensively-used polymers for the gene delivery.

Chitosan is a suitable candidate for developing gene vectors with high efficiency. The cationic nature of chitosan mediates the ionic interaction of amine groups present on its backbone with negatively charged DNA. Coating of chitosan with anionic alginate reduces the interaction between chitosan and DNA, thereby mediating the dissociation of DNA upon entry into cell.[23,79] Therefore, encapsulated p-DNA and siRNA can be preserved from nuclease degradation without being damaged. In addition, chitosan nanoparticles may be more efficient in delivering encapsulated siRNA into target cells as compared with liposomes.[80] Stable cationic chitosan/carboxymethyl cellulose nanoparticles coating the plasmid DNA have been tried as non-viral vectors for gene transfection, after direct injection into the mice skin.[81] However, binding capability of chitosan with nucleic acid may be limited due to its low water solubility. One strategy to improve the solubility and stability of chitosan nanoparticles is grafting PEG.[82]Acidic hydrolysis is another method to improve the water solubility and to provide a better access to the internal binding sites. Chitosan nanoparticles have been previously developed to transform human papilloma virus type 16 into CHO cells. The effective expression of E7 proteins was proved via SDS-PAGE and western blot analysis. Finally, this study suggested the application of chitosan nanoparticles as an efficient and safe non-viral system for the delivery of nucleotides into CHO cells.[83] Interleukin-12, an immunomodulatory cytokine, plays a key role in promoting strong antitumor activities. For this reason, mannosylated chitosan nanoparticles have been designed to transfect interleukin-12 gene into the dendritic cells by targeting the mannose receptor-mediated endocytosis pathway. This strategy may be suitable to induce a long-term and cell-mediated immune response.[84] The $\alpha v\beta 3$ integrin is an overexpressed receptor in a wide range of cancer tissues, therefore

this property can be used in the active delivery of drug into the cancer cells. Conjugation of cyclic Arg-Gly-Asp (RGD) peptide to chitosan nanoparticles was reported as a strategy for the specific delivery of siRNA via target αvβ3 integrin on tumor cells.[85] However, in order to reduce the affinity between chitosan and entrapped gene and to increase the transfection efficiency, the complex of chitosan and a negatively charged polymer such as alginate was previously suggested.[86]Alginate-chitosan complexes were shown to be more efficient in the protection of entrapped agents compared to alginate or chitosan alone. Besides, the effect of different parameters on the preparation of alginate/chitosan nanoparticles as carriers for antisense oligonucleotides have been investigated.[30] After the endocytosis of alginate nanoparticles, rapid erosion and osmotic swelling results in the endosomal escape and intracellular localization of gene.[87] For example, coating of chitosan nanoparticles with alginate increased the gene escape before arriving digestive endolysosomal vesicles and enhanced the transfection rate in NIH 3T3 cells.[88]

Jin-Oh You et al reported the transfection of plasmid-loaded calcium-alginate nanoparticles by NIH 3T3 cells. These nanocomplexes condensed plasmid DNA higher than polyethyleneimine (PEI).[22] Owing to the anti-inflammatory gene therapy, tuftsin-labeled alginate nanoparticles were reported for the transfection of interleukin-10 gene into the macrophages.[89] Considering that HA is abundant in ocular tissues, current efforts have been focused on mediating the gene delivery into the ocular tissues using HA.[90] A novel gene nanocarrier made of two mucoadhesive polysaccharides, HA and chitosan, was intended for the specific delivery of genes into the cornea and conjunctiva via CD44 receptor mediated endocytosis mechanism.[91] HA/chitosan nanoparticles were investigated as efficient vectors for gene transferring into chondrocyte for the treatment of joint diseases.[92] In fact, anionic nature of HA limits the encapsulation of negative molecules such as siRNA. However this problem can be readily handled by the modification of nanoparticles with components such as fatty amines and cationic polyamines that decrease negative charge density in the polymer structure and enhance the loading capacity of siRNA.[70] In addition, HA nanoparticles loaded with siRNA and cisplatin were shown as promising carriers that could target CD44 receptors overexpressed in the cisplatin-resistant tumors.[93] Figure 2. is a schematic illustration of HA target-mediated endocytosis for the gene delivery. Transfection of pBUDLacZ plasmid with hydrogel pullulan nanoparticles leads to high β-gal expression in COS-7 cells. The efficiency of these nanoparticles to induce protein expression was comparable with that of Lipofectamine 2000 formulation.[94] The isotropic gelation of a divalent cation (Ca, Mg, and Mn) with the pectin was used for the preparation of pectin nanoparticles as gene delivery vectors. These particles exhibited a superior ability in increasing the viral transduction, with minimum cytotoxicity.[95]

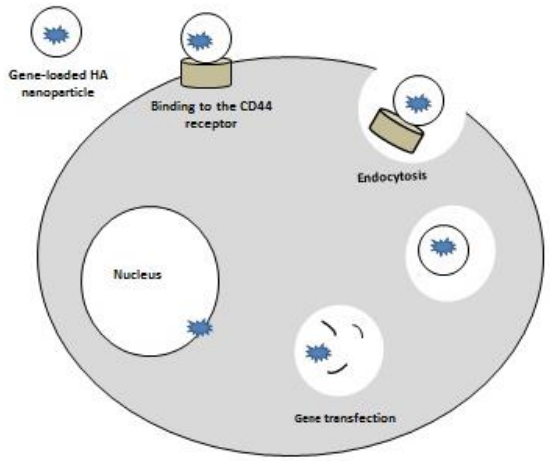


**Figure 2.** A schematic illustration of HA target-mediated endocytosis for gene delivery.

## Conclusion

Mucoadhesive nature of some polysaccharides may overcome the problems of relatively short residence time and weak penetration of hydrophilic drugs and macromolecules at the absorption site. Cationic polysaccharides are ideal molecules for preparing the nanosystems for loading the negatively charged oligonucleotides and accordingly delivering them to the target site. Polyelectrolyte complexation of polysaccharides with opposite charges can be proposed as an appropriate strategy to take the advantages of each substance, simultaneously. Besides, the use of receptor targeting polysaccharides can increase the localization and accumulation of nanocarriers at the target site and settle down many limitations of the drug/gene delivery. However, knowledge about the interaction between polysaccharide and loaded drug/gene at the molecular level, the mechanism of action of interacting molecules, and the fate of polysaccharide nanoparticles *in vivo* can be considered as important challenges for future studies.

## Conflict of interests

The authors claim that there is no conflict of interest.

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