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Review article

Polysaccharide-decorated nanoparticles

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Abstract

Surface modified colloidal carriers such as nanoparticles are able to modulate the biodistribution of the loaded drug when given intravenously, but also to control the absorption of drugs administered by other routes. This review presents the different strategies to coat the surface of polymeric as well as inorganic nanoparticles with polysaccharides. Various physicochemical and biological methods have been described to demonstrate such surface modification. The medical applications, mainly in imaging cancer, of polysaccharide-coated nanoparticles are presented, including their abilities to increase the blood circulation time and to target specific tumoral tissues. It has been shown that these coatings allow also to improve drug absorption via nasal or ocular pathways, due the mucoadhesive and/or permeability enhancer properties of the polysaccharides. Finally, the ability of polysaccharide-coated nanoparticles to deliver DNA or oligonucleotides will be discussed.

Keywords: Nanoparticles; Polysaccharide; Surface modification; Surface characterization; Cancer therapy; Tumour imaging; Mucoadhesion; Targeting

1. Introduction

Over the past few decades, there has been considerable interest in developing biodegradable nanoparticles as effective drug delivery systems. These colloidal carriers have shown the following advantages: (i) drug protection against in vivo degradation; (ii) stability; and (iii) ability to control the drug release. However, the main drawback of these carriers is their non-specific interaction with cells and proteins leading to drug accumulation in non-target tissues. This is the reason why research has focused on the development of surface modified nanoparticles.

For a number of applications, the surfaces of nanoparticles have to be highly hydrophilic and able to prevent protein adsorption. In this context, poly(ethylene glycol) (PEG) appeared as an ideal candidate, and was widely used to coat the surface of polyester or polyalkylcyanoacrylate (PACA) nanoparticles [1–3]. Pegylated nanoparticles could successfully avoid the mononuclear phagocyte system (MPS) sequestration. In this way, their blood circulation time was dramatically increased compared to uncoated nanoparticles [4]. Nevertheless, the presence of a PEG coating only delayed the phagocytosis of these nanoparticles, whose final destination was always the MPS [2]. However, pegylated nanoparticles have circulation times compatible with the passive targeting of tumors or inflammatory tissues, where advantage is taken of an increased vascular permeability [5]. In order to achieve an active targeting towards these tissues, specific ligands have to be attached to their surface to enable molecular recognition. However, chemical coupling of such ligands is often difficult, because of the absence of reactive groups at the surface of pegylated carriers [6]. This is one of the reasons why polysaccharide coatings have been considered as an alternative to the PEG coatings. Additionally, oligo- and polysaccharides may achieve active targeting per se since they have specific receptors in certain cells or tissues [7,8]. Moreover, they constitute an important class of physiological materials. They display well-documented biocompatibilities and biodegradabilities, which are the basic characteristics for polymers used as biomaterials [9]

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and have several properties (such as antiviral, antibacterial, antitumoral) not found in other natural polymers [9,10].

Besides, polysaccharides are involved in cell surface properties including tissue addressing and transport mechanisms. They are largely expressed in membrane cells. Sialic acid, as a component of the antigenic determinants of the cell surface, was described to play a role as biological mask [11] since the desialylation of red blood cells led to their immediate and massive uptake by the MPS [12].

In addition, several mucosal surfaces such as the nasal, pulmonary, and peroral mucosae are good targets for polysaccharide recognition. In particular, the nasal route is receiving considerable attention due to its high permeability and the easy access of the drug to the absorption site [13]. As exceptional mucoadhesive properties have been observed with polysaccharide chitosan [14], it appeared interesting to overcoat nanoparticles with this type of polysaccharide. In the same way, concerning ophthalmic dosage forms, some polysaccharides such as chitosan or hyaluronic acid have proven to enhance the contact between drug and ocular mucosa due to their high mucoadhesive properties [15–20].

Thus, with regard to the role of these polysaccharides in terms of biological or biomimetic activities, it appeared interesting to design carriers decorated with polysaccharides. In the liposome field, many examples show the benefit of polysaccharide coatings: (i) dextran, pullulan or glycolipid decreased the uptake of liposomes by the MPS [21,22]; (ii) hyaluronic acid conferred bioadhesive properties for local drug depot [23]; (iii) functionalized dextran enabled targeting specifically of human endothelial cells [24] and vascular smooth muscle cells [25] and (iv) pullulan was useful for oral immunization [26].

In the field of nanoparticles, there are many applications with oligosaccharides, too. For example, when liver targeting is desired, poly(γ-benzyl L-glutamate *N*-carboxyanhydride) nanoparticles, appropriately coated with galactose-containing copolymers, could be recognized by hepatocytes [27,28].

Furthermore, cholesterol-bearing pullulan derivatives conjugated with pluronic® were found to form self-aggregating nanoparticles [29] and self-assembling polysaccharides hydrophobized with cholesterol allowed complexation and stabilization of hydrophobic substances such as proteins [30,31] acting also as a molecular chaperone [32]. More recently, in order to improve the cancer-targeting activity, self-assembled nanoparticles of hydrophobically modified polysaccharide bearing biotin were designed [33].

Drug carrier surface modification with polysaccharides was also applied to polymeric micelles. For example, a novel amphiphilic copolymer (dextran-*g*-polyethyleneglycol alkyl ether) was synthesized which resulted in polymeric micelle formation, encapsulating cyclosporine in the hydrophobic core and providing a hydrophilic corona [34].

Among these various drug delivery systems, nanoparticles have, however, several advantages per se, such as high drug encapsulation efficiency, efficient drug protection against chemical or enzymatic degradation, unique ability to create a controlled release, cell internalization as well as ability to reverse the multidrug resistance of tumor cells [35]. They could also provide one solution to deliver new classes of active molecules such as peptides, proteins, genes and oligonucleotides.

Thus, this paper will review in detail how polysaccharide-decorated nanoparticles may improve drug administration and delivery.

2. Methodology of nanoparticle surface modification with the aid of poly- or oligosaccharide

Coating nanoparticles with polysaccharide was achieved by adsorption, by incorporation, by copolymerization or by covalent grafting (Table 1).

2.1. Single adsorption

It has been shown that the coating of liposomes with sialic acid could reduce their phagocytosis by macrophages in vitro [36,37], therefore increasing their circulation time in the blood stream [38]. As already mentioned in the Introduction, this biomimetic approach was based on the observation that sialylated red blood cells escape MPS recognition. Based on the same concept, Olivier et al. [39], proposed to coat preformed polyisobutyl cyanoacrylate (PiBCA) nanoparticles with orosomucoid, which is a sialic acid-rich serum glycoprotein. For this, orosomucoid was adsorbed onto PiBCA nanoparticles prepared by emulsion polymerization according to the method of Couvreur et al. [40]. The adsorption was found to be dramatically dependent on the pH, reaching a maximum at a pH value close to the isoelectric point (pI 2.8). It was suggested that electrostatic and hydrophobic interactions might both govern the interactions between the surface of nanoparticles and orosomucoid. In addition, the high stability of this coating was demonstrated in the presence of plasma proteins.

In order to coat poly(lactid acid) (PLA) nanoparticles with dextran, Rouzes et al. [41] synthesized amphiphilic dextran, modified by covalent grafting of phenoxy groups. Due to its amphiphilic properties, phenoxy dextran was successfully adsorbed onto the surface of PLA nanoparticles prepared by emulsion-solvent evaporation, according to a Langmuir type isotherm. The mechanism involved in this physical adsorption was based on hydrophobic interactions between the phenoxy groups of the modified dextran and the hydrophobic surface of PLA nanoparticles.

On the contrary, as the hydrophobic interactions were not strong enough to perform a direct adsorption in the case of

Table 1
Methodology and physicochemical characteristics of polysaccharide-coated nanoparticles

Polysaccharide coating	Nanoparticle core	Methodology for coating	Nanoparticle size (nm)	Zeta potential (mV)	Amount of polysaccharide at the surface	Reference
Orosomucoid	PACA	Adsorption	220	ND	4.5 mg/m^2	[39,74]
Dextran	Iron oxide	Coprecipitation	28	−25	ND	[56]
Dextran	PLA	Adsorption on NP (hydrophobically modified dextran)	160		6.6 mg/m^2	[41]
Dextran	PLA	Adsorption during the NP preparation (hydrophobically modified dextran)	170		5.6 mg/m^2	[41]
Dextran	PCL	Direct formation using amphiphilic grafted copolymers	220–280	−15	0.7 mg DEX/mg copolymer	[72,73]
Dextran	PMMA	Radical polymerization	72	−6	ND	[67]
Dextran	PACA	Radical polymerization (pH 1)	200–290	−15	ND	[69]
Degraded dextran	Iron oxide	Coprecipitation	32	−30	ND	[56]
DEAE-dextran	Iron oxide	Coprecipitation	104	+20	ND	[56]
DEAE-dextran	PACA	Emulsion polymerization (pH <2.5)	165	+37	ND	[98]
Dextran sulfate	PACA	Radical polymerization (pH 1)	190–270	−40	ND	[69]
Heparin	PMMA	Radical polymerization	78	−44	ND	[67]
Heparin	PACA	Radical polymerization (pH 1)	93	−45	ND	[69]
Heparin + dextran	PACA	Radical polymerization (pH 1)	190	−24	ND	[69]
Hyaluronic acid	PCL	Adsorption on positively charged nanoparticles	200–500	−45	41.6 ± 18.0 μg HA/mg PCL	[42]
Chitosan, hyaluronic acid, pectin	PACA	Radical polymerization (pH 1)	30–60 μm	ND	ND	[69]
Chitosan	PACA	Emulsion polymerization (pH 2)	60	+39	ND	[63]
Chitosan	PACA	Emulsion polymerization (pH >3)	60	+21	ND	[64]
Chitosan	PLA	Adsorption	500	+21	ND	[44]

ND, not determined.

hyaluronic acid onto poly(epsilon caprolactone) PCL nanoparticles, this coating was performed in a two-step procedure [42]. First, positively charged PCL nanoparticles were prepared by nanoprecipitation using a cationic surfactant, benzalkonium chloride. Adsorption of hyaluronic acid could then be preformed by the single addition of a hyaluronic acid solution to the positive PCL nanoparticles in suspension. Nanoparticles with a size ranging between 250 and 500 nm were produced, as a function of the process and the cationic surfactant chosen. At the highest hyaluronic acid concentrations studied, it was shown that bridging could occur between the nanoparticles, leading to a dramatic size increase. In optimal conditions, free benzalkonium not adsorbed on PCL surface and which could interact with hyaluronic acid was removed by dialysis prior to hyaluronic acid adsorption. In this case, the fabrication yield of the nanoparticles was about 35%.

2.2. Incorporation of polysaccharide in the course of the preparation of the nanoparticles

Since chitosan has a high affinity for cell membranes due to its mucoadhesive properties [17], it was utilized as a coating agent for colloidal carriers. In general, chitosan is dissolved in the aqueous phase where nanoparticles are prepared by solvent displacement or nanoprecipitation method [43]. Using this approach, chitosan-coated poly (lactid-*co*-glycolic acid) (PLGA) nanoparticles may be produced, in which chitosan molecules anchor to the surface due to entanglements with PLGA chains. Moreover, Vila et al. [44] showed that the presence of negatively charged lecithin, used as surfactant, enhanced the interaction of hyaluronic acid with the nanoparticles.

In contrast, it has been shown that nanoprecipitation of PCL in an aqueous phase containing hyaluronic acid failed to prepare coated nanoparticles [42]. These nanoparticles presented a mean diameter around 200 nm but a very high polydispersity index and an unstable coating because both PCL and hyaluronic acid are negatively charged molecules.

Besides nanoprecipitation, another common method to prepare nanoparticles is emulsion-solvent evaporation. It consists of dissolving preformed polymers in an organic solvent non-miscible in water and then in forming an o/w emulsion before evaporation occurs. Surfactants are often required to stabilize these emulsions, which may induce some toxicity after intravenous administration. This is the reason why Coombes et al. [45] proposed using dextran grafted with PEG side chains (PEG–DEX) as stabilizer of PLGA microparticles. Interestingly, dextran or PEG alone failed to stabilize the o/w emulsions, whereas PEG–DEX conjugates efficiently stabilized them. PEG anchored into the microparticle core, whereas dextran extended from the surface to contribute to the steric stabilization.

More recently [41], in an attempt to replace poly(vinyl-alcohol), one of the most commonly used nanoparticle

stabilizers, by polysaccharide derivatives with tensioactive properties, hydrophobized dextrans were synthesized by grafting phenoxy [46] or alkyl chains [47]. These copolymers were able to stabilize o/w emulsions, contrary to the native dextran. The hydrophobic moieties were embedded inside the outer part of the PLA core of the nanoparticles, whereas dextran decorated the external surface [41]. Moreover, this dextran coating provided steric protection against non-specific interactions with proteins and insured particle stability in the presence of salts and after freeze drying [41].

Maruyama et al. [48] proposed a poly(L-lysine)-*g*-polysaccharide copolymer to modify the surface of PLA nanoparticles by using a diafiltration method. The multilayered nanoparticles prepared in this way were composed of a polysaccharide-rich surface, a poly(L-lysine)-rich intermediate surface layer and a PLA core. Furthermore, DNA was successfully adsorbed onto the surface of these nanoparticles.

The most commonly used synthesis route to obtain polysaccharide-coated iron oxide particles involved the alkaline precipitation of magnetite-like compounds from Fe^{2+} and Fe^{3+} salt solutions in water in the presence of a colloid stabilizing agent such as carboxymethyldextran [49], dextran or its derivatives [50–53]. This superparamagnetic iron oxide (SPIO) colloids were made by coprecipitation of monocrystalline particles, i.e. Endorem®, or polycrystalline aggregates, i.e. Sinerem®, with polysaccharides. The nanoparticles were easily separated from unbound polysaccharide by gel filtration chromatography [50]. The synthesis conditions such as pH and temperature could influence the structure of the dextran coating, and the nanoparticle persistence time in the blood [54]. The polysaccharide coating provided a better stability of the SPIO particles in biological media [54], because the stable dextran coating isolated the central iron oxide core from macromolecular solutes and minimized protein adsorption onto the particle surface [54]. According to the preparation, using monocrystalline particles or polycrystalline aggregates, the dextran coating was more or less dense [55]. Moreover, according to the type of dextran coating, the diameter of iron particles increased significantly [56], i.e. the use of DEAE-dextran increased the size 3–4 fold compared to the native dextran. In addition, compared to the iron oxide particles just stabilized by polysaccharide adsorption, colloids grown in the presence of the polymer had a higher stability [54,57].

2.3. Polysaccharide-based nanoparticles obtained by copolymerization

2.3.1. Emulsion polymerization

Emulsion polymerization is a very popular approach to produce nanoparticles. The polymerization of alkyl cyanoacrylate is initiated by hydroxyl ions in water, and elongation of the polymer chains occurs according to an anionic polymerization mechanism (Fig. 1). Nevertheless, the anionic polymerization of PACA always requires the presence of a stabilizer such as dextran [40]. The addition of cyclodextrins to the polymerization medium could promote the association of poorly water-soluble drugs with PACA nanospheres [58,59] and also played a role of stabilizer [60]. Zeta potential measurements suggested that part of cyclodextrin molecules were located at the nanoparticle's surface and that they could play a role concerning the interactions with biological media [61].

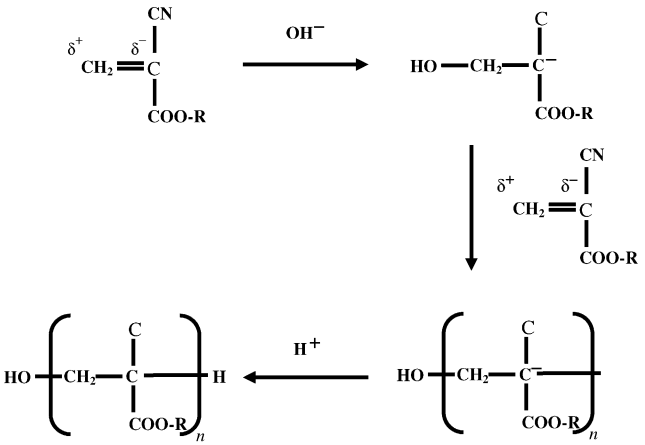

Fig. 1. Anionic polymerization mechanism of alkyl cyanoacrylate.

According to the studies of Illum and coworkers [62], the presence of hydroxy groups in the stabilizer (dextran, cyclodextrin) might also initiate the polymerization of alkyl cyanoacrylates and the stabilizer might thus covalently bind to the PACA polymer (Fig. 2). The type and concentration of the stabilizer are very important factors for controlling particle size, surface properties as well as drug release [63]. PACA nanoparticles are characterized by a negative surface charge, which might be attributed to adsorption of anions from the aqueous phase [63]. Nevertheless, as discussed before, positively charged nanoparticles are gaining increasing importance for drug delivery following intravenous, oral or ocular administration [63]. Such particles may be prepared by emulsion polymerization of ACA in the presence of the cationic polysaccharide, chitosan [63]. The effect of different physicochemical factors such as pH, amount of the chitosan, chitosan molecular weight and temperature on the mean particle size and turbidity of chitosan-PACA nanoparticles was investigated. Regarding these factors, the highest percentage of chitosan grafted onto PACA occurred at pH 2. Furthermore, the stabilizing efficiency of chitosan-PACA increased with increasing molecular weight and concentration of chitosan. In this study, Yang et al. [63] concluded that chitosan, containing amino and hydroxyl groups, could act as a nucleophilic agent to initiate the polymerization of ACA, leading to an irreversible attachment between chitosan and the nanoparticles through different multipoint linkages.

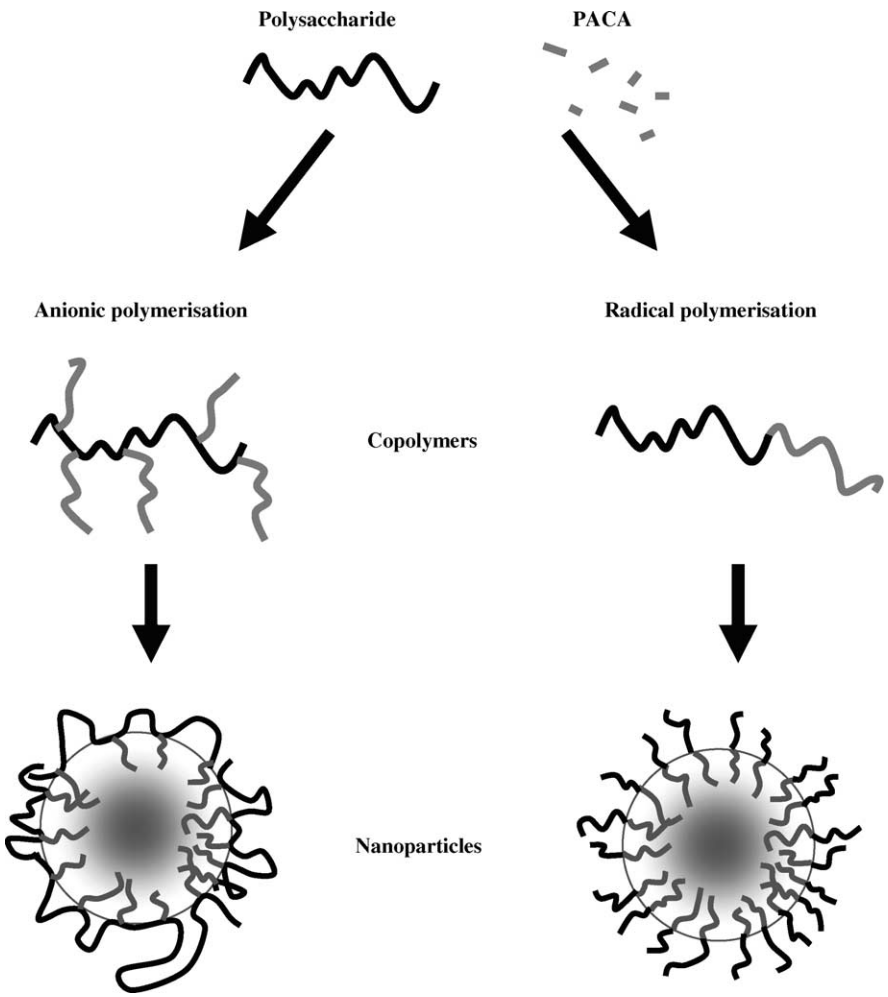

Fig. 2. Hypothetical conformation of the polysaccharide chains at the surface of nanoparticles depending on the mechanism of polymerization of alkyl cyanoacrylate (anionic or radical) applied for the synthesis of nanoparticles (adapted from Ref. [77]).

More recently, positively charged PACA nanoparticles were developed [64] by emulsion polymerization of alkylcyanoacrylate with low molecular weight chitosan from 5000 to 30,000 g/mol. Chitosan-coated nanoparticles were successfully prepared with a mean diameter of 60 nm and a unimodal distribution. This method presented several advantages such as the pH of reaction for the polymerization (higher than pH 3) which is more compatible with drugs stability, the ability of this cationic surface to adsorb more efficiently nucleic acids, and the better biocompatibility of low molecular weight chitosan (MW $<$ 30,000 g/mol) [65].

In order to purify proteins such as lectins, Chern et al. [66] have also proposed the use of dextran modified nanoparticles, which offered an extremely large particle surface area able to interact with proteins. Stable poly (methyl methacrylate) (PMMA) nanoparticles were prepared by emulsion polymerization using dextran as stabilizer. The reaction, leading to the formation of surface modified nanoparticles, was started by sodium persulfate as initiator. The influence of the dextran proportion in the reaction mixture on the nanoparticle stability and their lectin binding properties was investigated.

2.3.2. Radical polymerization

In a biomimetic approach to overcome the fast capture of the nanoparticles by the MPS, it has been proposed to coat them with dextran or heparin [67]. For this, a novel amphiphilic copolymer was synthesized by radical polymerization of methyl methacrylate initiated by cerium IV ions in the presence of dextran or heparin. The acidic conditions of the reaction lead to the cleavage of the polysaccharide by cerium ions. This enabled the radical polymerization of methyl methacrylate, leading to the formation of diblock copolymers. Because of their amphiphilic properties, the copolymers spontaneously formed small and monodisperse nanoparticles in aqueous media. These nanoparticles possessed polysaccharide moieties on their surface in a brush-like structure and a more hydrophobic PMMA core. One advantage of these nanoparticles is their preparation in the absence of toxic solvents and surfactants, which could interfere with biological media. Furthermore, the polysaccharidic surface conferred a high stability to the nanoparticles over several months. However, PMMA is a non-biodegradable polymer, which is not acceptable for the development of a drug delivery system for systemic

administration to humans. Therefore, Chauvierre et al. [68] prepared polysaccharide-decorated biodegradable nanoparticles using PACA. As in the case of PMMA, redox radical polymerization in acidic conditions (pH 1) leads to the formation of a copolymer between PACA and the polysaccharide. As the reaction was performed in aqueous medium, the copolymers were able to self-organize in the form of nanoparticles with a PACA core and a polysaccharide coating (Fig. 2). A large variety of polysaccharide coatings could be obtained with this methodology: dextran, heparin, dextran sulfate, chitosan, hyaluronic acid, pectin, as well as mixtures of polysaccharides [69]. The particle properties depended on the nature of the polysaccharide used. For example, dextran, dextran sulfate and heparin lead to the formation of nanoparticles with a size range between 93 and 800 nm, whereas hyaluronic acid, chitosan or pectin formed microparticles from 30 to 59 μm under the same conditions of preparation. Nevertheless, the polymerization rate was the same whatever the polysaccharide used. The high molecular weight of hyaluronic acid, pectin and chitosan were considered to be responsible for the increase of particle size [69]. Indeed, long polysaccharidic chains would be more deployed in water, thus contributing to an increase of the size of the particles.

2.4. Polysaccharide-based nanoparticles obtained from preformed copolymer

Gref et al. [70] described the synthesis of new comb like copolymers composed of polysaccharidic backbones on which preformed polyester chains were grafted by means of ester bridges. In the case of dextran–PCL (DEX–PCL) copolymers it was shown that this synthesis route led to the formation of well defined materials with controlled structures, i.e. the number of PCL linked to the dextran backbone could be controlled. A family of amphiphilic DEX–PCL copolymers with various hydrophilic–lipophilic balances was successfully obtained. These copolymers could even efficiently stabilize O/W emulsions [71]. Despite the insolubility of these copolymers in aqueous or organic phases, they were able to form nanoparticles with a core–shell structure [72]. The dextran backbone of the copolymer was indeed soluble only in water, whereas PCL was only soluble in organic solvents such as ethyl acetate or methylene chloride. Therefore, the copolymer migrated to the O/W interface to form a stabilizing layer around the solvent droplets. Taking into account these solubility properties, dextran preferentially migrated to the water interface, whereas PCL remained inside the droplet. Upon solvent evaporation, the copolymer precipitated in the form of nanoparticles made of PCL surrounded by a dextran shell. This new methodology allowed the entrapment of proteins such as albumin or lectins [73].

3. Characterization of the polysaccharidic surface-modified nanoparticles

3.1. Determination of the amount of polysaccharide-associated nanoparticles

The quantification of the amount of polysaccharide at the particle surface represents the more direct method of demonstrating the existence of a polysaccharide coating.

Barbault-Foucher et al. [42] set up a dosage method of hyaluronic acid based on a radioimmunology technique generally used to dose hyaluronic acid in serum. Using a specific hyaluronic acid binding protein, this very sensitive method allowed quantification of intact hyaluronic acid adsorbed on the surface of the nanoparticles. The yield of fixation of hyaluronic acid (hyaluronic acid attached/total hyaluronic acid) depended on the nanoparticle preparation method and was found equal to 41.6 ± 18.0 μg hyaluronic acid/mg PCL in optimal conditions. Elemental analysis enabled also to determine the amount of hyaluronic acid in the freeze-dried nanoparticles, but this method could only be applied in the case of nanoparticles prepared without any surfactant.

Olivier et al. [74] used two analytical methods for the determination of orosomucoid adsorbed on PACA nanoparticles. High performance capillary electrophoresis was used to determine the amount of orosomucoid adsorbed onto the surface and HPLC allowed quantification of free orosomucoid in the supernatant. A good agreement was found between these two techniques: 3.51 versus 4.15 mg/m^2 of orosomucoid adsorbed per nanoparticle surface.

Rouzes et al. [41] determined the amount of dextran grafted with phenoxy groups associated with the PLA nanoparticles by spectroscopy, since phenoxy groups absorbed at 269 nm. This amount reached 5.6–6.4 mg/m^2 according to the method of coating (adsorption or emulsification).

The conformation of the polysaccharide depended on the coating methodology [41]. For example, when dextran was used as stabilizer in emulsion-solvent evaporation process, a great number of phenoxy groups of phenoxy-dextran were anchored in the PLA core, and dextran adopted a deployed (distance of 10 nm between the shear plane and the surface) conformation in solution with a density of 0.5 g/cm^3 [41]. On the contrary, when phenoxy-dextran was adsorbed onto preformed PLA nanoparticles, it adopted a more compact conformation (density of 1 g/cm^3, distance of 6 nm between the shear plane and the surface) [41].

In the case of DEX-coated nanoparticles prepared using PCL–DEX amphiphilic copolymers by the interfacial migration technique, the most probable structure was a core–shell exhibiting dextran on the surface [72,75]. An anthrone assay confirmed that all DEX in the copolymer was recovered in the form of nanoparticles. Moreover, in order to determine the amount of dextran specifically located on the surface, a strategy based on a selective

degradation of dextran using endodextranase was set up. This enzyme allowed selective degradation of only accessible dextran chains, located in the corona. The resulting dextran fragments, as well as dextran entrapped in the core of nanoparticles, were further assessed by the anthrone method. It was shown that about 70% of the total amount of dextran was located at the nanoparticle surface and formed a shell of about 20–30 nm thick.

3.2. Physicochemical techniques

3.2.1. Zeta potential

Generally, zeta potential measurement is the most common technique used to establish the presence of a polysaccharidic coating. However, as hyaluronic acid is a negatively charged polysaccharide, zeta potential measurement did not allow determination of the surface modification of PCL nanoparticles, already negatively charged [42]. On the other hand, neutral dextran provided slightly negatively charged particles (−25 mV), when it was used as stabilizer of iron oxide particles [56]. However, when polyester or PACA nanoparticles were coated with dextran, the zeta potential tended towards neutrality, probably because of masking the negative charge of the core [41,67,69,72,73] as shown in Table 1. The molecular weight of dextran (15,000 or 71,000 g/mol) seemed not to influence the value of the zeta potential (−15 mV) [69]. As heparin or dextran sulfate were negatively charged, their use led to the formation of highly negative nanoparticles exhibiting zeta potential values as low as −45 mV [67,69].

The presence of a chitosan coating could also be highlighted using zeta potential measurements. Indeed, chitosan-coated nanoparticles had positive zeta potential values, ranging between +20 and +39 mV [44,63,64], whereas the cores had negative zeta potential values, around −30 mV.

In order to determine the effects of the surface charge onto the biodistribution of paramagnetic particles, Chouly et al. [56] have used different types of dextran. Negatively charged dextran produced by oxidation or enzymatic degradation of native dextran led to the formation of particles with a zeta potential of −30 mV, whereas positively charged dextran such as diethylaminoethyl-dextran led to particles with a zeta potential of +20 mV.

3.2.2. Hydrophobic interaction chromatography

The hydrophilicity of nanoparticles, due to the polysaccharidic coating could be estimated using hydrophobic interaction chromatography [39]. For example, in a column filled with propylamine-agarose, orosomucoid-coated PiBCA nanoparticles passed through the column, whereas hydrophobic PiBCA nanoparticles were retained [39]. However, this method did not allow separation of nanoparticles with different hydrophilic protein coatings.

3.2.3. Molecular architecture and mobility

Since electronic paramagnetic resonance (EPR) can provide useful information at the molecular level on the structure and organization of polymers at interfaces, Fournier et al. [76] used this method to study the interfacial properties of hydrophobic derivatives of dextran adsorbed onto polystyrene surface and the influence of this dextran conformation preventing protein adsorption. Chauvierre et al. [77] proposed to apply this technique to study the conformation of the dextran coating at the surface of PACA nanoparticles. For this purpose, dextran chains were labeled by covalent linkage with TEMPO, a label containing a nitroxide free radical, and their mobility was investigated using EPR. Anionic and radical polymerization of ACA led to the formation of core–shell nanoparticles presenting different conformations of dextran. Analysis of EPR spectra confirmed that dextran was located at the outside of the nanoparticles. Dextran formed a repelling outermost brush structure on the PACA core when obtained by radical polymerization, which conferred a high stability to the colloidal dispersion, even after freeze-drying. In contrast, when anionic polymerization was carried out, dextran was anchored by several points resulting in the formation of loops and trains very close to the core of the nanoparticle, corresponding to more dextran chains becoming collapsed onto the nanoparticle surface.

3.2.4. Analysis of the chemical composition of the surface

X-ray photoelectron spectroscopy (XPS) or electronic surface chemical analysis (ESCA) provides information about chemical composition of the top layer of nanoparticles. This quantitative technique gives the average composition over a 5–7 nm depth inside the nanoparticle [78]. Fitting the four carbon functionalities, Rouzes [41] determined the composition of coated PLA nanoparticles and confirmed the presence of phenoxy-dextran on their surface. In the same way, the analysis of freeze-dried nanoparticles using ESCA showed a dramatic difference of the oxygen to carbon atomic ratio between PiBCA nanoparticles and dextran- or heparin-coated PiBCA nanoparticles [69].

3.3. Interaction with proteins

The adsorption of model proteins was used to indirectly demonstrate the presence of a polysaccharidic coating, by giving evidence of the steric protection conferred by the polysaccharide layer [41,72]. The presence of a hydrophilic layer of dextran onto the surface of nanoparticles resulted indeed in protein rejecting abilities. For example, adsorbed BSA was not detectable at the surface of phenoxy-dextran-coated PLA nanoparticles after their incubation with this protein [41]. Only 0.2 mg/m^2 BSA was adsorbed in the case of PCL–DEX nanoparticles [75], and this amount was comparable with the amount detected on the corresponding pegylated nanoparticles taken as positive control [75,78].

Passirani et al. [79] and Chauvierre et al. [69] have chosen to measure complement activation to characterize the nanoparticle surface. Since heparin is known to increase the activity of protein H, which controls the complement system, its presence on the surface of PMMA or PACA nanoparticles dramatically inhibited complement activation [69,79]. On the other hand, the dextran coating induced only a weak activation of the complement system [69,79].

4. Application of surface-modified polysaccharide nanoparticles in medicine

4.1. Pharmacokinetics and biodistribution

As it has been previously pointed out, conventional colloidal drug delivery systems are rapidly removed from the blood stream after their intravenous administration. Thus, the development of surface-modified nanoparticles appears crucial to increase the circulation time of the colloidal carriers after their intravenous administration. As complement activation and opsonization by plasma proteins are key factors involved in the uptake of particles by MPS, modified nanoparticles should present surfaces which inhibit complement activation and opsonin adsorption. Surface modification with heparin or with dextran appeared particularly appealing, as heparin was shown to increase the activity of protein H, the control protein of the complement system [79], and, depending on its configuration, soluble or crosslinked, dextran was shown to be a weak or a strong activator, of the complement system [79]. Thus, in a biomimetic approach, nanoparticles made of copolymers of dextran or heparin covalently bound to PMMA were synthesized [79].

The in vivo half-life of these surface modified nanoparticles was dramatically increased from only a few minutes in the case of uncoated PMMA nanoparticles, up to 5 h in the case of heparin-coated PMMA nanoparticles [80], in accordance with the least take up by macrophage-like cell lines in vitro compared to uncoated PMMA nanoparticles [81]. It was suggested that the steric hindrance effect caused by dense brush-like arrangement of end-attached polysaccharide chains could contribute to the long-circulating properties of the heparin-coated PMMA nanoparticles [80].

Iron oxide nanoparticles have been developed for diagnostic purposes [82]. According to their surface properties, charge and size, their pharmacokinetics and biodistribution could be modulated (Table 2). As explained before, SPIO are particles of about 150 nm surrounded by a dextran layer [82]. The first application of SPIO as MRI contrast agents was for the MPS organs [56]. Once injected by the intravenous route, SPIO were recognized as foreign particles and were taken up massively by macrophages. The half-life of these nanoparticles was less than 30 min [82]. Endorem®, the commercial SPIO, increased the contrast between the healthy and the diseased tissues, like tumors or metastases, devoid of Kupffer cells [82], and demonstrated its efficacy in the detection of local lesions in the liver in preclinical and clinical studies [82], as well as lesions in the spleen [82].

In order to avoid the uptake of these contrast agents by the MPS, monocrystalline iron oxides (MION) or ultra small paramagnetic iron oxides (USPIO) were designed [82], since it is well recognized that the elimination of the nanoparticles from the bloodstream strongly depends on their size. The larger particles had the shortest blood half-life. However, Chouly et al. [56] showed that under the threshold of 50 nm, the nanoparticle diameter no longer influenced the blood persistence time. For example, MION consisted of a single crystal stabilized by dextran, exhibiting a diameter of 20 nm [55,83,84]. The dextran layer minimized the interactions between the iron core and plasma proteins and the blood half-life was about 4.5 h [54]. Moreover, the thick dextran coating, as well as the unimodal particles size, may be responsible for the slow clearance

Table 2
Medical applications of polysaccharide-coated nanoparticles

Polysaccharide coating	Nanoparticle core	Nanoparticle size (nm)	Application	In vitro/in vivo	Reference(s)
Dextran	PMMA	72–78	Long circulating	In vivo	[67]
Dextran	Iron oxide	28	Long circulating	In vivo	[56]
Dextran	Iron oxide	50–150	MRI liver, spleen	In vivo	[56]
Dextran	Iron oxide	20–30	MRI lymph nodes	In vivo	[54,82,84,87,88]
Dextran	Iron oxide	20–30	MRI brain	In vivo	[85,86,89–91]
Dextran	Iron oxide	20–30	Hyperthermia	In vitro	[95]
Dextran	PCL	200	Ligand adsorption (lectin)	In vitro	[73]
DEAE-dextran	PACA	165	ODNs adsorption	In vitro/in vivo	[97,98]
PLL-dextran	PLA	60–300	DNA adsorption	In vitro	[48]
Chitosan	Gadolinium	430	Irradiation	In vivo	[96]
Chitosan	PACA	60	Nucleic acids adsorption	In vitro/in vivo	[64]
Chitosan	PLA	500	Mucoadhesion (nasal route)	In vivo	[44]
Chitosan	PCL	250	Ocular delivery	In vivo	[103]
Heparin	PMMA	72–78	Long circulating	In vivo	[67]
PVLA	PLA	300	Liver targeting	In vitro	[28]

from the intravascular system of MION or USPIO [55]. Concerning their biodistribution, MION were not only taken up by macrophages but also by tumor cells [85] or lymph nodes [84]. The accumulation of MION in tumor cells was explained by the enhanced extravasation of the particles into the interstitium of solid tumors [86].

In magnetic resonance lymphography, dextran-coated SPIO need some requirements to be 'lymphotropic' such as small size, long circulation time and hydrophilic surface properties [87]. The combination of these physicochemical characteristics allowed the nanoparticles to avoid uptake by MPS, to circulate during several hours and to have a better opportunity to reach the interstitial space by passive diffusion. Despite their short blood half-life (13 min) and their bigger size (60–90 nm), starch-coated SPIO particles were at least as efficient in lymph node accumulation as long circulating dextran-coated SPIO particles exhibiting only a 25 nm diameter [87]. The major pathway of SPIO lymph node accumulation was recognized as the endothelial transcytosis, with the subsequent phagocytosis by macrophages in the lymph nodes [87]. Sinerem®, the commercial USPIO has been used for lymphography of hyperplasic or metastatic lymph nodes in rats after intravenous administration [82]. The histological analysis of lymph nodes showed that USPIO particles accumulated mainly in lymphatic sinuses, in which phagocytes were abundant [54,88]. However, the differentiation of malignant and normal lymph nodes could sometimes be hindered by the slight accumulation of Sinerem® in tumor tissues due to the diffuse extravasation across the hyperpermeable vasculature [88], and the lack of contrast between the follicles of normal lymph nodes and the nodes with micrometastases [54].

Long-circulating iron oxide nanoparticles (LCDIO) accumulated also in other tissues, such as intra cerebral implanted tumors (9L gliosarcoma) after intravenous administration [89]. The mechanism involved was an endocytosis of the LCDIO by tumor cells. Moreover, particles were found also in tumor-associated macrophages as well as in endothelial cells in the area of active angiogenesis [89]. Sinerem® was also used for the detection of human brain tumors [90]. These USPIO could extravasate and accumulate thus in the brain tumoral interstitium. They remained localized in the tumor over a period of several days, due to their low diffusion coefficient and partly to their local endocytosis by tumor cells [89,90]. They constituted a major improvement on the performance of gadolinium chelate, another contrast agent, permitting the delineation of human brain tumors to be prolonged for MR imaging. These results have favorable implications for the targeting of diagnostic biopsies and the planning of surgical resections [90]. More recently, a clinical comparative study showed that UPSIO enhanced the contrast in imaging intracranial tumor, mostly malignant tumors, comparable to the common clinically used gadolinium chelate, but with a longer persistence of the signal [91]. The long plasma half-life (25–30 h) may create enough time for these iron oxide particles to 'search for' the most leaky areas of the damaged blood brain barrier and to cross it resulting in good detectability with MR imaging [91].

The surface charge of iron oxide particles altered the circulation time of these colloids too [54,56]. Thus, introduction of positive charges induced a decrease of the blood half-life, reaching 1–2 min [54]. Adsorption of negatively charged compounds on MION nanoparticles increased their uptake by liver and spleen, compared to neutral MION [54]. On the other hand, unmodified and negatively charged MION were both mainly accumulated in lymph nodes [54]. Moreover, the nature of surface coating as well the geometric arrangement of the polysaccharide on the iron oxide core determine not only the overall size of the colloid, but also play a significant role in uptake kinetics and blood persistence [92]. For example, the particles with the highest density of the dextran coating had the longer blood half-lives [85]. Dextran-rich MION circulated 3–4 times longer than dextran-poor nanoparticles [54]. Protein adsorption studies revealed that five major proteins (transferrin, vitronectin, fibrinogen, immunoglobulin G and complement C3) implicated in uptake by MPS adsorbed on dextran-poor MION [54]. Considering clinical safety of SPIO such as Endorem®, precautions for intravenous administration were recommended, in order to avoid anaphylactic-like or hypersensitivity reactions which might be related to the presence of dextran [93].

Furthermore, it is noteworthy that MION with galactose terminals were found to distribute to hepatocytes via the asialoglycoprotein receptor whereas MION without galactose were taken up by macrophages [92].

4.2. Cancer therapy

4.2.1. Hyperthermia

In experimental or clinical oncology, hyperthermia takes advantages of the higher sensitivity of tumor tissues to heat. The technique consists of heating biocompatible coated superparamagnetic particles with application of external electromagnetic field. The particles dissipate the energy of radiowaves into heat. It could be associated with chemotherapy to further increase the therapeutic effects, because malignant cells are more sensitive to treatments at higher temperature. In this strategy, particles must be targeted specifically in the tumor zone, in order to minimize heating of the rest of the organism. Furthermore, hyperthermia should occur after internalization of the nanoparticles inside the tumor cells. However, the particle uptake could lead to the loss of their dextran coating due the lysosomal enzymatic dextran digestion, which might result in particle aggregation [94]. This aggregation decreases the specific power absorption rate (SAR) and by this way the cellular heating. Despite the fact that dextran-coated particles were classified as not suitable for intracellular magnetic fluid hyperthermia [94], Brusentnov et al. [95] proposed evaluating the potential of novel ferromagnetic fluids

or suspensions in radiofrequency-induced hyperthermia. In these studies, the type of coating appeared to be crucial to increase SAR. Dextran and carboxymethyl dextran revealed to be very stable coatings of ferrite particles, and were not detached even after the sonication of the particles. Due to low toxicity combined with high SAR, these ferrofluids were considered as very promising materials for hyperthermia applications [95]. As endocytosis is the first step in the particle internalization process, Jordan et al. [94] demonstrated that particle uptake was dependent not only on the coating of particles (dextran or silan) but also on the cell types. The mechanism of unspecific endocytosis of cancer cells is not well known. However, the adhesion abilities of the particle to the cell surface was identified as a precondition to unspecific endocytosis [94].

4.2.2. Irradiation

Gadolinium neutron-capture therapy is a cancer therapy, using gadolinium neutron-capture reaction in vivo by thermal neutron irradiation. The major problem with gadolinium is that it remains in insufficient quantity in the tumor tissue after intratumoral administration. This is the reason why gadopentetic-chitosan nanoparticles were conceived to allow a better localization of this compound and to control its release [96]. Gadopentetic-chitosan nanoparticles were injected by the intratumoral route to mice having developed B16F10 melanoma tumors. The therapeutic treatment consisted of a thermal-neutron irradiation, 8 h after administration of gadopentetic-chitosan nanoparticle. Despite the radioresistance of the melanoma model, the gadopentetic-chitosan nanoparticles allowed significant suppression of the tumor growth, with a mean tumor volume variation less than 15% after 14 days. This efficacy was attributed to the ability of the chitosan nanoparticles to retain a high concentration of gadolinium in the tumor tissue, compared to the gadolinium solution which was very rapidly eliminated from the tissue.

4.3. Gene/ODN delivery

The efficacy of genes and oligonucleotides (ODN) is hampered by their (i) rapid degradation by nucleases and (ii) poor intracellular diffusion.

In view of this, novel polysaccharide-bearing multilayered nanoparticles were proposed as a DNA carrier, in which poly(L-lysine)-*g*-dextran copolymer covered PLA nanoparticles [48]. The presence of dextran led to a modified conformation of poly lysine, resulting in an increase of the number of amino groups on the nanoparticle surface. This increased the adsorption of plasmid DNA or ODN onto the nanoparticle surface through ionic interaction. Moreover, it was found that both the adsorption of DNA and the stability of the carrier were improved when the dextran content in the copolymer was increased.

Nanoparticles prepared from PACA and diethylaminoethyl-dextran exhibiting positive charge allowed the ODN loading capacity to be increased with 35 μmol ODNs adsorbed per gram of polymeric material [97,98] and ODN to be protected against degradation by endonuclease [97]. In vivo these carriers targeted ODN to the liver [98].

Nucleic acids, such as antisense ODNs, plasmids, interfering RNA or DNA were also successfully associated with chitosan-coated PACA nanoparticles [64]. The use of a low molecular weight biocompatible chitosan, instead of the usual cationic surfactant, such as cetyl triethyl ammonium bromide (CTAB), contributed to a decreased cell toxicity of the carrier and increased the adsorption of nucleic acids. Indeed, chitosan-coated PACA nanoparticles improved significantly the adsorption of ODN of 80% as compared to only 10% adsorbed via CTAB-coated nanopaticles. The concentration inducing cell toxicity was increased from 1 (CTAB-coated nanoparticles) to 10 μg/ml (chitosan-coated PACA nanoparticles). The final size of nanoparticles with adsorbed nucleic acids was less than 300 nm, which is compatible with an intravenous administration. The adsorbed ODN was stable after 24 h of incubation in culture medium. In vitro, surface-associated plasmids could be successfully transfected in NIH-3T3 cells, as demonstrated by cell growth inhibition. In vivo, GFP Hela cells were implanted in nude mice and the intratumoral delivery of RNAsi by chitosan-coated PACA inhibited the expression of tumor GFP protein as well as the expression of its ARN messenger. Thus, these nanoparticles enable enhancement of nucleic acid cell internalization and protection against fast intracellular degradation.

4.4. Mucoadhesion and targeting

The main problem in ocular therapeutics is to provide and maintain an adequate drug concentration at the site of action, since the poor ocular bioavailability of many drugs implies repetitive instillations. Nanoparticles have been developed in ophthalmology and have demonstrated promising results over the last 10 years [99]. They were able to enhance the corneal uptake and the intraocular half-lives of drugs. Furthermore, biocompatibility and good ocular tolerance was demonstrated with PACA and PCL nanoparticles which have improved the corneal penetration of both hydrophilic and lipophilic drugs, as well as of macromolecules [100]. However, PCL nanoparticles did not succeed in prolonging corneal graft survival [99].

Nanoparticles made of chitosan, a biodegradable and cationic polysaccharide, were proposed as an alternative nanotechnology for ocular delivery [101]. Again, the presence of positive charges of this biopolymer contributed to reaction of the drug in the corneal and conjunctival surface, by electrostatic interactions with the negatively charged mucosa [17,102]. Based on these observations and in order to improve the ocular delivery of indomethacin, Calvo et al. [103] proposed to combine the advantages of the PCL nanoparticles (good drug loadings) with those of the chitosan coatings (good adhesion to the mucosa).

After topical ocular instillation, nanoparticles of chitosan-coated PCL allowed the bioavailability of indomethacin in the cornea as well as in the aqueous humor to be significantly enhanced. This was explained by the combining effects: (i) penetration of the particles in the corneal epithelial cells, (ii) mucoadhesion to the cornea epithelium, and (iii) absorption enhancer effect of chitosan [103].

The interaction with the ocular mucosa of PEG and chitosan coatings has also been studied [104]. After ocular administration, it was shown that PEG-coated PCL nanocapsules accelerated the drug transport across the whole epithelium, whereas chitosan-coated nanocapsules favored the retention of the carrier in the superficial layers of the epithelium. Another bioadhesive polysaccharide, hyaluronic acid, was proposed for coating PCL nanoparticles in order to enhance the bioavailability of drugs in ocular delivery [42]. Various studies demonstrated the mucoadhesive properties of hyaluronic acid in ocular delivery [16,18–20], but, contrary to chitosan, no permeability enhancing properly has yet been described.

Previously, Illum et al. [14] have investigated the permeability enhancer mechanism exerted by chitosan on the nasal mucosa. It was observed that chitosan could increase the permeability of insulin, failing to cause any damage in the nasal membrane [14]. Recently, it has been shown that chitosan-coated PLGA nanoparticles could enhance the transport of tetanus toxoid protein across nasal epithelium versus uncoated particles [44]. The mechanisms involved were the attachment of the particles to the nasal mucosa and the permeability enhancing properties of chitosan. Moreover, chitosan allowed the stability of the nanoparticles to be increased, protecting them from aggregation in the presence of lysosyme. Taking into account the positive charges in chitosan and lysosyme, the adsorption of the enzyme could have also been limited. Nevertheless, PEG coating was more efficient than chitosan concerning the antigen transport, probably because chitosan stick hardly to the mucus layer, whereas PEG did not interact with it [44].

It was suggested that chitosan could have a transient effect on the gating function of tight junctions. Along the same line, Artursson et al. [105] demonstrated that chitosan could also increase the transport of drugs across monolayer intestinal epithelial cells Caco-2 exploiting the following mechanisms: (i) chitosan macromolecules interacted with apical membrane according to a saturable mechanism, due

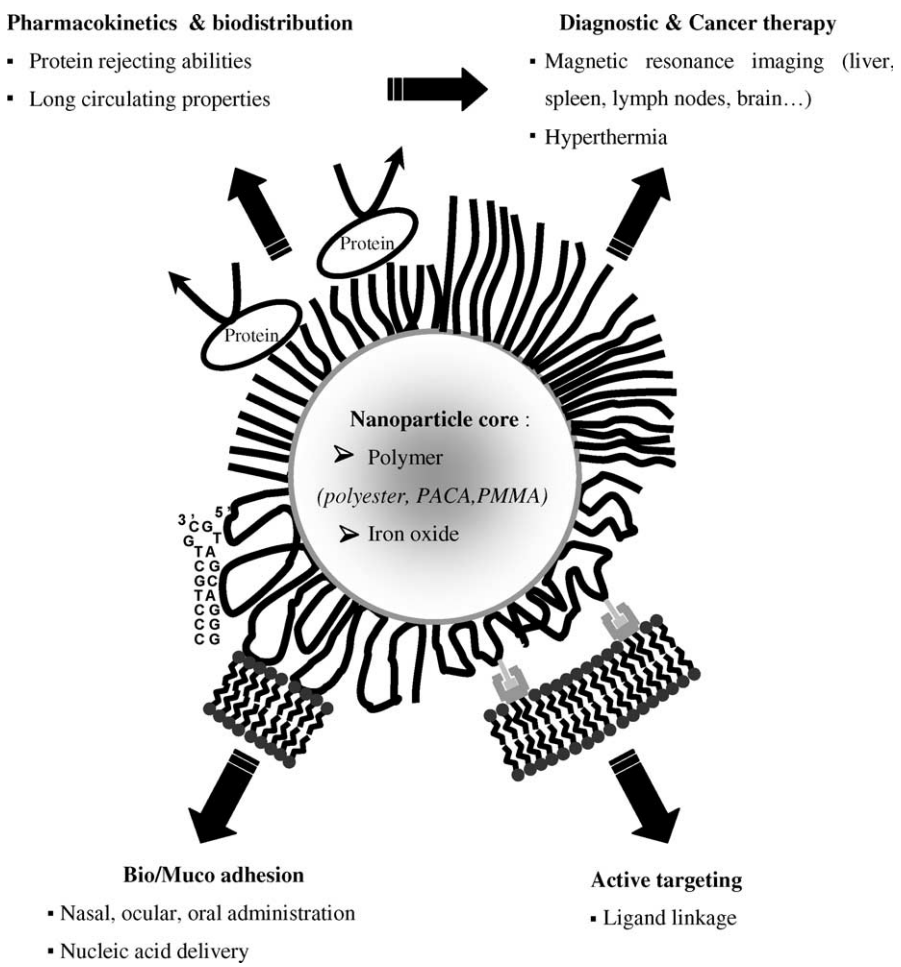

Fig. 3. Schematic representation of polysaccharide-decorated nanoparticle with different polysaccharide conformations (brush or loops) and main applications in medicine.

to interactions with anionic epithelial glycoproteins such as sialic acid, (ii) as the interior of tight junctions channel are negatively charged and hydrated, a moderate opening of tight junctions could occur. This study highlighted too that the intestinal permeability enhancement was dependant on the pH, i.e. the degree of ionization of chitosan.

Rodrigues et al. [73] exploited the dextran coating of PCL nanoparticles in order to adsorb lectins as ligands onto their surface. It was shown that the hemagglutination properties of the adsorbed lectins were maintained. This result suggested the application of this type of carriers for targeted oral administration.

5. Conclusion

As shown in Fig. 3, there are numerous technologies for coating nanoparticles in different configurations (loops or brush). The polysaccharide coating may provide steric protection against protein adsorption and macrophage uptake. Additionally, as polysaccharides offer many available reactive groups, active targeting could be obtained by grafting ligands onto the nanoparticle surface (Fig. 3).

The advantages of polysaccharide-coated nanoparticles for medical applications have been discussed in detail. For example, dextran-coated nanoparticles showed prolonged blood residence time, which depended not only on their size, but also on the properties of their coating layer. Furthermore, this type of dextran-decorated particles allows targeting to specific tissue, such as lymph nodes or brain tumors (Fig. 3). The direct targeting of solid tumors was also made possible by taking advantage of the uptake by tumor cells and tumor-associated macrophages and/or EPR effect. These properties were mainly obtained with carriers loaded with contrast agents like ferrofluids for magnetic resonance imaging in vivo, showing very promising results in tumor delineation.

Moreover, the use of the polysaccharide-coated systems is not only restricted to intravenous administration. Indeed, nanoparticles coated with polysaccharides also showed interesting bioadhesive properties and were able to retain the encapsulated drugs at the site of administration. For example, very promising results were found for the administration of peptides by nasal route and for various other drugs by the ocular route. In particular, chitosan coatings presented a high interest, showing cumulative properties such as mucoadhesion and permeability enhancement, without any irreversible membrane damage. Furthermore, chitosan-coated nanoparticles also interact with nucleic acids (oligonucleotide, DNA,…), improving their nanoparticle loading efficiency, their stability in biological fluids as well as their transfection properties (Fig. 3).

Finally, considering the very large variety of polysaccharides in nature, one could imagine the preparation of surface-engineered nanoparticles, presenting a specific surface, adapted to the targeting purpose.

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