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SURFACE MODIFIED NANOPARTICLES: A MPS OPPOSING SYSTEM

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ABSTRACT

Text mining was used to extract the recent advances in the surface modification of nanoparticles to oppose uptake by Mononuclear Phagocyte System (MPS). First, we described the process of opsonization in which, non-stealth nanoparticles allowing macrophages of the MPS to easily recognize and remove them from the circulation before they attain their designated therapeutic function. Then to address these limitations, various methods have been elaborated to camouflage nanoparticles from the MPS. Of these methods particular focus has been done on the literature concerning adsorption of different polymers (like PEG, Poloxamers, Poloxamines etc.) to the surface of nanoparticles. This review highlights the advances and applications of surface modified nanoparticles along with their characterization.

Keywords: Stealth Nanoparticles, Long Circulating Nanoparticles, MPS-avoidance system.

1. INTRODUCTION

In the updated study of this introductory paper the main focus has been emphasized on the methods and advances in the surface modifications of nanoparticles. Nanoparticles have received much attention in the last decade as it presents revolutionary opportunities to fight against many diseases.[1] Nanoparticles given by IV route are easily removed by MPS macrophages due to rich blood supply and specialized architecture. This problem necessitated modification of the surface of nanoparticles in order to evade them from being phagocytosed and make them long circulating particles. The design of long circulating particulate systems is therefore reliant upon a proper understanding of mechanism(s) by which particulates are

cleared by the macrophages of the reticuloendothelial system (RES), a process which is still poorly understood.

2. OVERVIEW

2.1 Mononuclear Phagocytic System (MPS) or Reticuloendothelial System(RES)

The mononuclear Phagocytic system is one of the body's innate defenses. MPS is the system in which the phagocytosis process occurs. Phagocytosis is the engulfing and eventual destruction or removal of foreign particles from the blood stream. Macrophages are the important constituent of the MPS which have the ability to filter and eliminate any injected particulate matter including nanoparticles from the blood stream within seconds of intravenous administration, rendering them ineffective as site specific drug delivery device.[2] Removal of nanoparticles by the MPS is a major obstacle to the active targeting. Surface non-modified nanoparticles are rapidly opsonized and massively cleared by the macrophages of MPS rich organs.[3] IGg compliment C3 components are generally proteins used for recognition of foreign substances, especially foreign macromolecules.

2.2 Phagocytosis

Phagocytosis is the clearance process which is regulated by the balance between two groups of blood components i) Opsonins: which promotes the phagocytosis ii) Dysopsonin: which suppress the process.[4-7]: Immunoglobulins and components of the compliment system such as C3, C4 and C5 are known to be common classical opsonins molecules while fibronectin, C reactive protein and tuftsin have also shown to enhance the process of phagocytosis. Moghini and Patel recently proposed organ specific opsonins which enhance the uptake of particulates by Kupffer cell and spleen macrophages.[5] iii) The process of phagocytosis occur by different methods:

a. **Opsonization:** Opsonins are the proteins present in blood stream and binding of these opsonins on to the surface of nanoparticles is known as opsonization. It is the initial and critical step to the process of Phagocytic recognition. As a general rule opsonization of hydrophobic particles as compared to hydrophilic particles shown to occur more quickly due to enhanced adsorbility of blood serum proteins on the surfaces.[8-10] Some of the nanoparticles along with their interaction with protein are discussed in Table 1.

Table 1: Studies of the Opsonization of different polymeric nanoparticles.

Formulation	Report	References
Poly (D, L-lactide) Nanoparticles	On incubation of PLA NPs in human plasma and serum, the major protein which were adsorbed on to the surface of NPs were found to be IgG along with albumin, apolipoprotein–E.	[11]
Polystyrene Nanoparticles	Studied the kinetics of protein adsorption on to Polystyrene NPs and concluded that albumin and fibrinogen were adsorbed in highly diluted plasma.	[12]
PLGA and PLA Nanoparticles	Shown that the interaction of proteins with NPs depend upon the method of NP preparation. As the spray-dried PLGA nanoparticles and PLA nanoparticles produced by w/o/w emulsion technique, the amount of apolipoproteins in plasma proteins adsorption pattern were higher in former as compared to later.	[13]
PEG coated Sterically stabilized Nanospheres	Made an attempt to correlate the adsorption results with the *in-vivo* circulation of NPs and concluded that there was decrease in protein adsorption on to PEG-coated NPs and making them Long-circulating particles.	[14]

b. Attachment of phagocyte to NP via surface bound opsonin

The process of attachment of the phagocytes to the nanoparticles via opsonins may occur by different methods:

- **Conformational Changes:** When the bound opsonin proteins undergo conformational changes from an inactive protein present in the blood stream to an activated protein structure that can be recognized by phagocytes. Phagocytic cells surface contain specialized receptors that interact with the modified conformation of opsonins thus alerting them to the presence of a foreign material. [15]
- **Non Specific Adherence of Phagocytes:** This attachment to surface adsorbed blood serum proteins can result in the stimulation of phagocytosis as well. This process is typically due to the association of opsonin proteins with a more hydrophobic particle surface. [16]
- **Compliment activation:** It is also one of the methods of phagocyte attachment and occurs due to the presence of compliment activating group or nanoparticles e.g. hydroxyl group which activate the compliment C3 components present in the blood. These components are part of immune system used for the recognition of foreign particles. [17-18]

2.3 Surface characteristics of Nanoparticles

Apart from size of nanoparticles, the factor that determines adsorbility of proteins to solid surface basically depends upon surface properties such as surface chemistry, charge and

hydrophilicity which effects opsonization process. The hydrophilicity/hydrophobicity of the particles influence the opsonization process as higher protein adsorbility is seen with hydrophobic particles as compared to hydrophilic. Hydrophobic particles are rapidly removed in-vivo by phagocytes. [19-23] DLVO theory in the form of a potential energy diagram rationalized some basic aspects of the electrostatic interaction between particles and blood components. [24-25] The surface charge has been recognized as a determinant of particulate clearance from the circulation. It is a general view that negative surface charge increases the clearance of nanoparticles from the circulation relative to neutral or positively charged one.[26]

3. SURFACE MODIFICATION TECHNIQUES

The earliest strategies to overcome rapid uptake by liver was through suppression of the MPS phagocytic function by saturating it with a dummy dose of colloidal particles (nanoparticles or liposomes). The other techniques used was phagocytosis depressants like Dextrane Sulphate, Methyl Palmitate etc. [27] But such approaches were not clinically acceptable due to their impaired MPS function and progression of diseases. Apart from these the other methods of camouflaging masking nanoparticles is by surface modification. The surface modifications of the nanoparticles minimize the opsonization and prolong the circulation of nanoparticles *in-vivo*. It can be achieved by following methods:

i. Adsorption and self assembly strategies for surface modification: Adsorption basically implemented for good stability and hydrophilicity whereas self assembly deals with making one, two and three dimensional structures of nanomaterials. [28-31]
ii. Organic reaction strategy for surface modification: Direct chemical reaction or covalent attachment is done under this technique after the formation of primers which activate the surface of nanoparticles. (3-amino- propyl) triethoxysilane (APS) can be used as primers for this purpose. [32-35]
iii. Inorganic layer based surface modification strategy: These layers are generally incorporated to introduce new electronic, magnetic, mechanical and surface chemical properties of particles. Silica and Titania are the common inorganic layers used. [36-39]
iv. SOL-GEL method for surface modification: SOL-GEL are the small colloidal nanoparticles in solution which form gel like network on further polycondensation .in these different precursors are used for coating of different substances like silica for silica network , zirconia for zirconia coating.
v. Surface coating with hydrophilic polymers/surfactants.

Most of the practical work has been carried out using nanoparticles. The surface coating material studied up to now include following materials discussed below:

3.1 PEG coated Nanoparticles

The coating of a particle surface by the covalently grafting, entrapping or adsorbing of PEG chain is known as PEGylation. These chains create a barrier layer to block the adhesion of opsonins so that particles remain masked or invisible to Phagocytic cells. [15] Peracchia *et al* [40] experimentally demonstrated the protein rejecting capabilities of PEGylated surface using freeze-fraction transmission electron microscopy. A representative listing of nanoparticles which have been coated with PEG have been shown in Table 2.

Table 2: Studies of Polymeric nanoparticles with surface adsorbed PEG.

Nanoparticles	Surface Coating	Molecular Weight	Outcome	References
Poly(Ɛ-caprolactone)	PEG	6,000 and 20,000	Typical plasma proteins, heat labile serum proteins (e.g. complement components) and IgG are involved in the opsonization.	[41]
Poly Lactic Acid (PLA)	PEG	6,000 and 20,000	Nanoparticles PEO coatings were produced by the salting-out process and purified by the cross-flow filtration technique with combinations of PLA and diblock or triblock copolymers of PLA and PEO. The influence of the PEO molecular weight and surface density on the particle uptake was especially marked for the diblock and triblock copolymer formulations, with a decrease in uptake of up to 65% with one of the diblock copolymer formulations.	[42]
	PEG-b-PLA	2,000 & 5,000	Nanoparticles were prepared from methoxy poly(ethylene glycol)poly(d,l-lactic acid) block copolymers (Me.PEG-PLA) or blends of Me.PEG-PLA and PLA by the precipitation-solvent diffusion method. *In vivo*, the half-life in plasma of the Me.PEG-PLA nanoparticles that were intravenously administered to rats is increased by a factor 180 compared with the F68-coated PLA nanoparticles.	[43]
	PEG-b-PLA	10,000, 15,000 & 20,000	A series of corona/core nanoparticles of sizes 160-270 nm were prepared from diblock PEG-PLA. 2-D PAGE studies showed that plasma protein adsorption on	[44]

			PEG-coated PLA nanospheres strongly depends on the PEG molecular weight (Mw) (i.e. PEG chain length at the particle surface) as well as on the PEG content in the particles (i.e. PEG chain density at the surface of the particles).	
Poly(lactic-co-glycolic) acid (PLGA)	PEG-b-PLA	2,000 & 5,000	Coating polymers with PLA:PEG ratio of 2:5 and 3:4 (PEG chains of 5000 and 2000 Da. respectively) were studied. The results reveal the formation of a PLA:PEG coating layer on the particle surface resulting in an increase in the surface hydrophilicity and decrease in the surface charge of the nanospheres. The PLA:PEG coating also prevented albumin adsorption onto the colloid surface.	[45]
	PEG-b-PLGA	12,000 & 20,000	Biodegradable nanospheres were developed from amphiphilic copolymers composed of two biocompatible blocks. The nanospheres exhibited dramatically increased blood circulation times and reduced liver accumulation in mice. Furthermore, they entrapped up to 45 percent by weight of the drug in the dense core in a one-step procedure and could be freeze-dried and easily redispersed without additives in aqueous solutions.	[2]
PolyStyrene (PS)	PEG-b-BSA	5,000	A two-step approach is described to chemically camouflage the inert surface of model polystyrene nanospheres of 60 nm in diameter against recognition by the body's defenses. The average poly(ethyleneglycol) (PEG) content for a 60-nm nanospheres was found to be 13.7±0.4 μmol PEG/Amol BSA and 3.6±0.3 Amol PEG/μmol IgG. Only nanospheres with the most hydrophilic phenotype (approximately 70% of the total population) exhibited stealth properties after intravenous injection to rats.	[46]
Gelatin Type B	PEG	5,000	Poly(ethylene glycol) (PEG)-modified gelatin was synthesized by reacting Type-B gelatin with PEG-epoxide. The nanoparticles, prepared by pH and temperature controlled ethanol-water solvent displacement technique. Cytotoxicity assays indicated that both	[47]

			gelatin and PEGylated gelatin were completely non-toxic to the cells. A large fraction of the administered control gelatin and PEGylated gelatin nanoparticles were found to be concentrated in the perinuclear region of the BT-20 cells after 12 hours indicating possible vesicular transport through initial uptake by endocytosis and endosomal processing.	
Polyalkylcyanoacrylate (PACA)	PEG-b-Polyhexadecylcyanoacrylate	2,000	It was observed that [14C]-radiolabeled PEGylated nanoparticles remained for a longer time in the blood circulation after intravenous administration to mice, compared to the non-PEGylated poly(hexadecylcyanoacrylate) (PHDCA) nanoparticles. The PEGylation degree of the polymer seemed not to affect the in vivo behavior of the nanoparticles.	[48]
Poly(isobutyl 2-cyanoacrylate) (PIBCA)	PEG-b-PIBCA	4,500	Nanoparticles were formed by chemical coupling of PEG during emulsion/polymerization of isobutylcyanoacrylate (IBCA). A polyethylene glycol (PEG)-coating onto injectable particles showed to reduce either protein adsorption and complement consumption, as a function of the PEG density.	[49]

3.2 Poloxamer and Poloxamine coated Nanoparticles

Poloxamers and Poloxamine are non-ionic surfactants also known as Pluronic® and Tetronic® macromolecules respectively. Both have diverse applications in various biomedical fields ranging from drug delivery and medical imaging to management of vascular diseases and disorders. [50] Poloxamers basically consists of a central polyoxypropylene (POP) molecule having two hydrophilic chains of poloxyethylene (POE) on both sides. Similarly poloxamines have a slightly different structures consist of tetra functional block copolymer with four POE-POP blocks joined together by a central ethylenediamine bridge. The hydrophobic section of the polymer which contain PO unit can be used to adsorb the surfactant molecules to the nanoparticles surface, while the hydrophilic EO containing polymer can extend in to solution and shield the surface of the particle. [51] The recent applications of these polymers in nanoparticulate engineering are given in Table 3.

Table 3: Studies of polymeric nanoparticles with surface adsorbed poloxamers and poloxamines

Polymer	Molecular Weight	EO units	PO units	Nanoparticles	References
Poloxamer-188	8,400	2 X 52	30	Poly Lactic Acid, Poly(lactic-co-gylcolic) acid, Poly(methylmeth acrylate), Polystyrene	[53] [10] [53-54] [55-57]
Poloxamer-401	2,000	2 X 5	67	Polystyrene	[58]
Poloxamer-402	2,500	2 X 11	67	Polystyrene	[58]
Poloxamer-407	12,600	2 X 98	67	Poly(Ɛ-caprolactone), Poly Lactic Acid, Poly(lactic-co-gylcolic) acid, Poly Lactic Acid-Ethylene-co-vinylacetate (50:50), Poly(methylmeth acrylate)	[59] [10] [60-62] [59] [54]
Poloxamine-904	6,700	4 X 15	4 X 17	Poly(lactic-co-gylcolic) acid, Poly(methylmeth acrylate), Polystyrene	[60-61] [63] [64]
Poloxamine-908	25,000	4 X 119	4 X 17	Poly Lactic Acid, Poly(lactic-co-gylcolic) acid, Polystyrene	[10] [47] [9, 65]

3.3 Polysorbate-80 coated Nanoparticles

Polysorbates (Tween-80, T-80) coated nanoparticles represented tools used for delivering drugs to brain. Polysorbates-80 plays a specific role in brain targeting. The poor blood brain barrier (BBB) penetration causes the problem of drug targeting to the brain highly difficult. [66] Alyautdin *et al* [67] explained the number of mechanisms for enhancement of drug transport from the coated nanoparticles through BBB by binding the nanoparticles to the inner endothelial lining of the brain capillaries and brain endothelial uptake by phagocytosis. Cavallaro *et al* [68] studied the effect of surfactant coated nanoparticles on drug permeation across the biological membrane and found that nanoparticles over-coated by polysorbates

especially polysorbate-80 were capable of transporting the loaded drugs across BBB after administration. It seemed that brain targeting of nanoparticles was concerned with the interaction between TAT coating and brain micro-vessel endothelial cells. All the evidences regarding the polysorbate-80 coated nanoparticles indicate that the surface modification of nanoparticles by coating with polysorbate-80 is effective in drug delivery through BBB.

3.4 Cyclodextrin/carbohydrate Nanoparticles

The surface modification of nanoparticles by carbohydrate was found to avoid the MPS uptake. MPS-avoidance characteristics to carbohydrate/cyclodextrin coated nanoparticles were reported by different researchers. [1] Duchene *et al* [69] demonstrated the work to increase the loading of water soluble drugs and bioavailability of the poor water soluble drugs by using ampiphilic cyclodextrin nanoparticles for targeted delivery by oral or parenteral route. Cho *et al* [70] developed the NPs of PLA and Poly(L-lysine) grafted polysaccharide for the delivery of DNA and also found that these nanoparticles were resistant against self aggregation and non specific adsorption of the serum proteins.

4. SURFACE CHARACTERIZATION METHODS

Various techniques have been developed to study the surface modification of nanoparticles. Different methods have been enlisted below which are used to measure the surface modification:

4.1 Zeta potential analysis

It is a technique for determining the surface charge of nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticles surface. The electric potential at the boundary of the double layer is known as the zeta potential of the particles and has values that typically range from +100 mV to -100 mV. Nanoparticles which have zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. The extent of surface hydrophilicity can be predicted from the values of Zeta potential. [71]

4.2 Electron Spectroscopy

It is also known as X-Ray Photoelectron Spectroscopy (XPS) used for chemical analysis. This is based on the emission of electrons from materials in response to irradiation by photons of sufficient energy, to cause ionization of the core-level electron. These electrons are emitted at energies characteristic of the atoms from which they are emitted. Since photons

have low penetration energy only those electron pertaining to atoms at or near the surface (up to 100 A^o) and these can be counted. By this method, surface elemental analysis was performed. [72]

4.3 Hydrophobic interaction chromatography

This method is used to measure the surface hydrophobicity of nanoparticles. It involves the column chromatography, which is able to separate materials based on the interaction with a hydrophobic gel matrix. The interaction between nanoparticles and the gel is a function of surface hydrophobicity of nanoparticles. Propyl agarose gel is used as a stationary phase and elution of nanoparticle can be achieved by using the phosphate buffer. Eluent sample can be collected and optical density is measured spectrophotometrically. [8]

5. CONCLUSION

The literature review provides "stealth" properties of different surface coated polymeric nanoparticles along with their surface characterization. In conclusion, the concept of surface modification method has proven to be valuable for imparting stealth or MPS-avoidance characteristics to nanoparticles. The forgoing showed that the study of stealth nanoparticles and their opsonization by the mononuclear phagocytic system remains a developing area of research.

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