Inorganic Nanoparticles

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PEGylated Inorganic Nanoparticles

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Application of inorganic nanoparticles in diagnosis and therapy has become a critical component in the targeted treatment of diseases. The surface modification of inorganic oxides is important for providing diversity in size, shape, solubility, long-term stability, and attachment of selective functional groups. This Minireview describes the role of polyethylene glycol (PEG) in the surface modification of oxides and focuses on their biomedical applications. Such a PEGylation of surfaces provides "stealth" characteristics to nanomaterials otherwise identified as foreign materials by human body. The role of PEG as structure-directing agent in synthesis of oxides is also presented.

1. Introduction

Healthcare and energy are at the forefront of all major technological advancements of the past few decades. Treatment of diseases and medical care shares complex economical, social, and ethical challenges as they are directly connected to human life. The increased life expectancy has lead to extensive research in the treatment, as well as early stage diagnosis and detection of medical problems. Important advancements in science and technology is slowly transforming the medicinal research from an age of medical uncertainty in treatment of diseases to a stage of continuous monitoring, localized treatment, and early prediction of the effectiveness of treatment methods. Researchers no longer believe in exposing the whole body to a drug or radiation for the treatment of localized problem at a specific site or organ. Thus the new age treatment of diseases requires precise control over the local delivery, localized action, and continuous monitoring of the drug. These stringent requirements of medicine have resulted in a multidisciplinary research requiring molecules and materials to be controlled accurately and precisely.

It is evident that controlling the molecules at the atomic scale can transform the field of medicine and healthcare. The cross-over of medicine and nanotechnology has resulted in a new field of nanomedicine where nanotechnology is applied to medicine.[1] Nanotechnology is the science and technology of materials having one of the dimensions less than 100 nm ($1\ nm = 10^{-9}\ m$). The physical, chemical, biological, and optical properties of nanomaterials are significantly different to bulk materials and only as a result of their smaller dimensions. Nanotechnology has shown tremendous potential in improving technology by tuning the properties of the materials at the atomic and molecular level. Nanomaterials of various shapes, sizes, and dimensionality (0D–3D) of pure metals, metal oxides and sulfides, alloys, and various compounds have been developed that have lead to great improvements in the fields of energy, environmental protection, and healthcare. Both polymeric and inorganic nanoparticles (NPs) have redefined the way the drugs were being used and delivered to target organs.[1–9] The miniaturized size of drug carrying vehicles have made the therapies more patient specific in addition to being disease specific.[5–8] The ability to precisely position and control the matter at the atomic scale through the attachment of various functional molecules has provided added functionality to the nanomaterials and has resulted in a new field of nanovectors.[1,10–13] The surfaces of NPs can be vectorized in various ways, to give, for example, 1) a passive layer of polymer, 2) a fluorescent tag for detection, 3) a biomolecular entity for recognition by target sites, and 4) special conjugation strategies that can make the surface active or inactive depending upon the pH value or the microenvironment of the target cells.

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Both inorganic and polymeric NPs are currently explored for biomedical applications. Soft polymeric NPs have been used in the medicine for a long time however, inorganic NPs such as metals, metal oxides, and semiconductors, form a separate class of nanomaterials that is finding widespread use in biomedical applications.[1,14–20] The main difference in using inorganic NPs as compared to polymeric nanomaterials lies in the active participation of the nanomaterials in therapeutics and detection/diagnosis. Polymeric NPs are mostly designed as carriers of an active drug or a host material for attaching fluorescent tag and can be designed to actively or passively deliver drugs to the target sites. On the other hand inorganic nanomaterials can often provide the same functions of polymeric NPs with additional advantages of being therapeutic (such as gold, silver, and cerium oxide),[21–26] act as a fluorescent tags (such as quantum dots[27–29]) with high resolution, and as imaging and/or magnetic contrast agents[30–33] (such as gold, silver, iron oxide, and gadolinium). To take the advantage of inorganic NPs for therapeutic and drug delivery applications a few key aspects must be controlled:

1) The surface of NPs must be tailored to retain the high surface area and reactivity but reduce or minimize the unintentional reaction of NPs with the human body
2) Extensive biocompatibility and no systemic toxicity to normal cells/tissue at the level of dose administered must be demonstrated
3) The NPs should stay in the blood for a time long enough for active recognition and uptake by the target organs
4) The NPs should demonstrate nonspecific accumulation in body and should be able to clear out of the body by normal means

e) The characteristics of NPs, such as, size, dispersion, and surface charge should remain unaltered in the hostile cellular environment.

To demonstrate the above mentioned properties inside the hostile cellular environment, the surface of NPs needs to be protected and/or modified. Bare, uncoated NPs can agglomerate and are cleared (out of the body) by the reticulo endothelial system (RES) resulting in poor biomedical properties.[34] Often the surface of NPs is covered and modified with various functional molecules to achieve the desired function. Surface modification of inorganic NPs by biocompatible compounds can tailor the surface properties, such as surface charge, biocompatibility, and solubility. Several functional groups have been tried to modify the surface of NPs including different polymers, macromolecules, and bio-molecules. Compounds such as citrates, amines, nucleic acids, peptides, antibodies, and lipids have been tried as ligands for modifying the surface of NPs.[35] In addition several polymers such as polysaccharides, polyacrylamide, poly(vinyl alcohol), poly(*N*-vinyl-2-pyrrolidone), poly(ethylene glycol) (PEG), and PEG-containing copolymers, have been used to coat the surface of NPs for additional stability, water solubility, and modification of surface charges.[34,36,37] Among all the polymers tested for improving the solubility and biocompatibility of NPs, PEG and PEG-copolymers[38,39] are currently most popular and found to be most effective in shielding the surface charge of NPs. The term PEGylation is used specifically for the attaching or coating of the NP surface with PEG molecules through surface adsorption, covalent linkages (by anchoring groups), and entrapment.[39,40] It has been shown extensively that PEGylated NPs have improved stealth properties unparalleled by any other surface coating. In this sense PEG has become a very important technological material that can improve the biomedical properties of NPs and tailor their use in the biological environment.[38,40–43] Several Review articles have documented the PEGylation of polymeric compounds[38–40,42,44] however, PEGylation of inorganic NPs, even though very important, has been largely unnoticed. Herein we outline some of the current PEGylation strategies for inorganic materials coupled with their applications. We also review the importance of PEG as a solvent for the synthesis of inorganic particles with various shapes and sizes. We will also cover some of the interesting properties of polyethylene glycol that makes it an important material for coating surfaces for biomedical applications and a valued solvent in synthesis of nanomaterials.

2. Physical and Chemical Properties of Poly(ethylene glycol)

Polyethylene glycol (PEG) is a polymer of ethylene glycol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$). It is available in a variety of molecular

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weights ranging from hundreds to several thousands of Daltons. The lower molecular weight PEGs are highly soluble in water though the solubility decreases with increasing molecular weight of PEG. The lower molecular weight (less than 800 Daltons) PEGs are liquids at room temperature and are completely soluble in water.[45] PEG has excellent solvating characteristics and has been found to complex with several lanthanides and transition-metal cations.[45–48] This feature is extremely useful in designing NPs using PEG as a solvent for synthesizing PEGylated NPs. Another useful feature which adds to the popularity of PEG is its unique stability against oxidation, reduction, and decomposition by acids, bases, moderately high temperatures, hydrogen peroxide, and sodium borohydride.[45] The terminal OH group of PEG may be selectively oxidized to functionalize PEG with various terminal end groups or to attach large ligands, such as biomolecules. The presence of both hydrophilic and hydrophobic groups in PEG has made it a popular solvent for green synthesis. It is often used in aqueous biphasic system (ABS) because of its ability to display phase separation under controlled conditions which can be exploited in bioseparation.[49,50]

A colloidal suspension of NPs is generally charged and provides electrostatic stability to the suspension.[51] However a change in the dispersing medium of NPs to serum or cellular environment leads to loss of surface charge over time and results in aggregation of the NPs. The surface charge on NPs is often recognized by the body's self-defense mechanism which identifies the NPs as foreign objects. Thus NPs face stiff challenges to reach the intended target organs inside the human body and need to be carefully designed to achieve necessary stealth properties. In biomedical applications of NPs, PEGylation of the surfaces has become the most common strategy for providing stealth characteristics to NPs.[38,39,44] The stealth characteristic is known as the "enhanced permeation and retention" (EPR) effect of PEGylated surfaces whereby PEGylated surfaces are able to avoid the non-specific interactions with opsonin proteins and uptake by the reticulo endothelial system (RES).[39] As a result of the EPR effect, PEGylated surfaces can penetrate through the leaky vasculature of the cancer or tumor cells, whereas the normal tissue has a tight vasculature. A longer circulation time in the blood increases the chance of the NPs to reach the cancerous or tumorous tissue/cells.[38] The circulation time is increased by PEGylation which shields the charge of NPs, increases hydrophilicity, and provides the required flexibility (through the flexible PEG molecules attached to the hard NP surface) to the NPs.[39] Non-immunogenicity and the availability of full toxicity profiles of several PEG molecules have also increased the popularity of PEG as passive barrier coatings for NPs. Several theories have been proposed for the improved stealth properties of nanomaterials upon PEGylation and have been covered extensively in the literature,[39] they include:

1) Shielding of surface charge and increase in hydrophilicity leads to reduced interaction and identification by opsonin proteins
2) Decreasing the interfacial free tension of the NPs in fluid media minimizes the interaction with proteins
3) Generation of repulsive forces through the compression of flexible PEG chains on the surface of NPs when encroached by proteins
4) High mobility of flexible PEG chains results in minimizing the interaction time with the proteins to prevent any specific binding
5) The PEGylated surface of NPs increase the attachment of dysopsonin proteins that suppress the phagocytic uptake
6) The high surface density of PEG chains does not offer a specific surface to opsonin proteins for binding and thus uptake by RES is avoided.

The ability of PEG to repel opsonin proteins can be obtained by achieving a minimum surface density of PEG. The protein repulsion tendency of PEG does not depend upon the nature of interaction between the NPs and PEG as both covalently bonded and electrostatically bonded PEG can show good protein rejection tendency.[52] However, covalent bonding can ensure that the PEG functionality is not lost upon long term storage in highly ionic medium and during the blood circulation. There are mixed reports with respect to achieving high density of PEG on the surface of NPs coupled with the effect of branching of PEG (star PEG) and the molecular weight of PEG. It is expected that the higher molecular weight PEG may provide better flexibility through the long-chain molecules but suffers because it is not possible to have a high density of such large polymers on the surface.[52]

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Similarly, branched PEGs may provide steric repulsion to the attachment of neighboring PEG molecules leading to empty spaces on the surface, just enough to attract the non-specific binding of opsonin proteins. Thus a high density of short-chain PEG molecules is preferred for achieving optimum stealth properties though chains that are too short may be too rigid to provide enough flexibility. It was shown that the protein rejection ability of PEG generally increases with increasing in molecular weight, however, efficient protein rejection can also be obtained from a very high surface density of very short PEG chains.[39,40]

To achieve the necessary stealth properties the most suitable molecular weight of PEG has been reported between 1500–5000 Da.[39] The scope and interaction of PEG molecules may vary with the development of PEG block copolymers and attachment of large ligand molecules as well as target specific biomolecules. However, the above mentioned points can only be used as general guidelines while designing a PEGylated system.

PEGylation of NPs give rise to important characteristics, such as biocompatibility, water solubility, decreased enzymatic degradation, and non-immunogenicity. In addition, PEGylation helps in shielding the surface charge of nanoparticles, which is considered an important parameter in imparting PEGylated molecules a prolonged circulatory time in blood stream and enhanced cellular uptake.

Citrates, amines, acrylates, and other carboxy-terminated ligands give rise to positive or negative surface charge on nanoparticles depending upon the pH value of the medium. While the surface charge helps in increasing the solubility or suspension characteristics of NPs it reduces the circulation time in blood and reduces the preferential uptake of nanoparticles. Long-term stability and aggregation in highly ionic and aggressive cellular environment can also be a problem. It can be debated that the mutual attraction of positively charged proteins on cell surface and negatively charged nanoparticles may result in enhanced uptake of nanoparticles however, the chances of charge mediated uptake are relatively low in an in-vivo environment as charged NPs are cleared rapidly by the RES. The pH-value-dependent reversal of surface charge can be used to deliver drug loads and displace ligands from the surface. Interestingly oligonucleotide- and DNA-based approaches have resulted in increased uptake of NPs despite being negatively charged.[35] The mechanism of such an uptake is still elusive and has been ascribed to adsorption of specific proteins and subsequent identification and internalization by cells.

3. PEG as Solvent for Synthesis of Inorganic Nanoparticles: Size and Shape Selectivity

PEG has been used extensively for synthesis of inorganic NPs in aqueous medium to provide an easier and greener method of preparing NPs with varying size and shape selectivity. Self-assembly of NPs to achieve higher ordered structures is emerging as a new technique for the development of compounds that can be used in applications ranging from sensing to catalysis. Higher ordered structures of NPs, such as nanorods, nanowires, nanocubes, nanobelts, and nanoprisms each show different electronic, magnetic, and physico-chemical properties depending upon the final shape of the NPs.[53] These anisotropic nanostructures show improved biomedical properties in detection and therapy compared to spherical NPs and thus have important technological implications.[53]

PEG has shown tremendous potential in the self-assembly of several oxides as well as of metallic NPs into nanostars, nanowires, nanobelts. The ability of PEG to complex with several transition metals, rare-earth elements, and other alkali and alkaline-earth metals can be harnessed to utilize PEG as a soft template for engineering ordered nanostructures.[46,47] The specific surface adsorption of PEG molecules on selective crystallographic planes can also help in generating NPs with anisotropic properties through oriented attachment of NPs in a colloidal medium. Such specific adsorption can stop the growth of NPs along adsorbed crystallographic planes by sterically hindering the interaction and facilitating oriented attachment of NPs. The growth of NPs through the exposed crystallographic planes can then lead to one dimensional nanostructures and can be tailored by the size and the molecular weight of PEG. Similarly, metal nano building blocks can be assembled into spherical shapes through the organization of metal-ion–PEG globules and can result in the formation of mesoporous microspheres composed of several crystalline NPs.[54] Table 1 lists the size and shape selectivity of important inorganic NPs synthesized using PEG as a structure-directing template.

Cerium oxide NPs are used in a variety of applications ranging from catalysis to solid-oxide fuel cell (SOFC) electrolyte. Our group has shown that the self-assembly of cerium oxide NPs into super octahedral structures through fractal assembly is accelerated by PEG.[55] The octahedral morphology was obtained for $M_w = 600$ Daltons PEG in aqueous solutions ranging from 5 to 40 vol% of PEG. A further increase in the concentration of PEG leads to an increase in the viscosity of the solvent. This viscosity impedes the self-assembly of particles by reducing the free orientation and motion of NPs through increased steric hindrance. Similarly, 2 vol% PEG (600 mol wt) was used to synthesize very high surface area ceria–zirconia NPs assisted by a sonochemical process[56] and ceria nanorods were prepared using ultrasonication in 1 wt% PEG (600 mol wt) as a structure-directing surfactant template.[57] Ultrasonication at room temperature for 1 h leads to the synthesis of nanorods 100–150 nm in length and less than 10 nm in diameter. The molecular weight and concentration of PEG played an important role in the formation of nanorods that were found to form only in 0.5–5 wt% PEG solution (M_w PEG < 2000).

Synthesis and self-assembly of zinc oxide nanostructures using PEG as a solvent has been studied extensively. Intriguing three dimensional morphologies, such as stars, prisms, flowers composed of nanorods, and nanotubes can be synthesized by employing PEG as a solvent in hydrothermal synthesis.[58] Straight chain PEGs of molecular weight 300, 600, and 10000 Daltons yield spherical, star, and flower-like arrangements of zinc oxide nanorods, respectively, while zinc oxide nanotubes were observed only with PEG $M_w =$

Table 1: Various synthesis strategies to obtain size and shape selectivity of various metal and oxide NPs using PEG as a solvent as a function of molecular weight.

Material	Synthesis	MW(PEG) [g mol^{-1}]	Morphology	Ref.
ZnO	Hydrothermal treatment in alkaline medium	2000	Star shaped (sixfold symmetry containing parallel nanotubes)	[58]
		10000	Flower-like (containing smooth nanorods as petals)	[58]
	Hydrothermal treatment on a glass substrate to grow nanotubes on glass	2000	Ordered array of nanotubes (dia 40–200 nm, length 2 μm)	[59]
	Hydrothermal treatment in absolute ethanol	400	Nanowires (dia 30–50 nm, length 2 μm)	[60]
	Wet chemical synthesis using carboxy-terminated PEG in absolute ethanol	–	Water soluble 10 nm NPs	[61]
	Hydrothermal in absolute ethanol	20000	Microspheres composed of individual nanorods	[54]
CeO_2	Ultrasonication at room temperature of cerium nitrate hydrolyzed with sodium hydroxide in 0.5–5% PEG	600	Nanorods (dia 5–10 nm, length 50–150 nm)	[57]
	Pulsed ultrasonication to produce CeO_2-ZrO_2 through double alkaline treatment with ammonium hydroxide followed by sodium hydroxide	600	3.7–13.5 nm particles with high specific surface area (226 m^2 gm^{-1})	[56]
	Wet chemical synthesis in aqueous medium using hydrogen peroxide as oxidizer and aging NPs for 7–14 days	600	Super-octahedral morphology of 3–5 nm individual crystallites	[55]
TiO_2	Hydrothermal treatment in absolute ethanol, PEG, and urea (acidic medium)	–	Core shell microspheres	[62]
Fe_3O_4	Hydrothermal treatment in alkaline medium	4000	Nanowires (dia 20–35 nm, length 1 μm)	[63]
Sb	Hydrothermal treatment in presence of a reducing agent and PEG	10000	Nanobelts	[64]
Ag	High temperature reduction on PEG	200	Nanoparticles (4 nm) and nanoprisms	[65]

2000 Daltons as solvent (Figure 1). These results suggest that PEG chains that are too short or too long will not produce a tubular morphology possibly because they do not curl enough to template a tubular structure.[60] Short-chain PEG polymers ($M_w = 400$) were utilized to synthesize long nanowires.[60] The PEG molecules serve as a structure-directing template allowing nanowires as long as 2 μm to be produced during the growth of zinc oxide nanocrystals from alkaline solutions. The wires form because the preferential adsorption of short chains of PEG changes the kinetics of growth of the colloidal NPs in specific crystallographic directions causing the anisotropic growth of the crystals. Solvent molecules can also affect the morphology by solvating PEG and reducing the interaction of PEG with the oxide NPs. Thus changing the solvent from ethanol to water resulted in the formation of more spherical particles than nanorods or nanowires. It was found through a series of control experiments and high-resolution transmission electron microscopy that the soft templating of high molecular weight (20000) PEG resulted in the formation of tubular coils that turned into large globules within few hours.[54] The concentration of zinc ions was much higher in the globules, the ions being bound to PEG, than in the bulk solution. Further hydrothermal processing of these globules resulted in the formation of microspheres containing individual nanotubes. Nanotubes arranged in microspheres of several compounds, such as ZnO, $La(OH)_3$, MnO_2, and Bi_2S_3 were obtained through this templating effect of PEG_{20000}.[54]

Bifunctional PEG was also used to synthesize water-soluble ZnO less than 10 nm in diameter and ZnO ellipsoids were synthesized at 60 °C using PEG_{10000}.[61] Mirkin's group demonstrated a unique behavior of PEG by using it as a sacrificial material for generating positive and negative nanostructures in dip–pen nanolithography (DPN).[66] Short-chain PEG_{2000} was used as a sacrificial photoresist that lead to the formation of positive solid-state features after chemical etching thereby overcoming some of the limitations of the alkanethiols.

PEG has also been employed as a soft template to produce nanostructures of titanium dioxide[62] and also metallic NPs, such as gold, silver,[65] and antimony. Large-scale antimony nanobelts were produced using hydrothermal reduction of antimony in a PEG medium.[64] With ZnO, TiO_2, and Cu_2O it was observed that the low-molecular-weight PEG has a smaller steric effect than long-chain PEG.

Thus it can be inferred that the complexing ability of PEG with metals and its preferential adsorption over selected crystallographic planes can be used for self-assembly of NPs in various shapes and sizes. This primarily stunts the growth of NPs along the PEG adsorbed crystallographic plane to a degree depending upon the length/molecular weight of the polymer and allows the NPs to grow anisotropically along planes on which PEG is not adsorbed. Synthesis of NPs in a PEG-containing medium may have the additional advantage of producing PEGylated NPs directly in a one-step treatment thereby reducing additional steps in the surface conditioning of NPs.

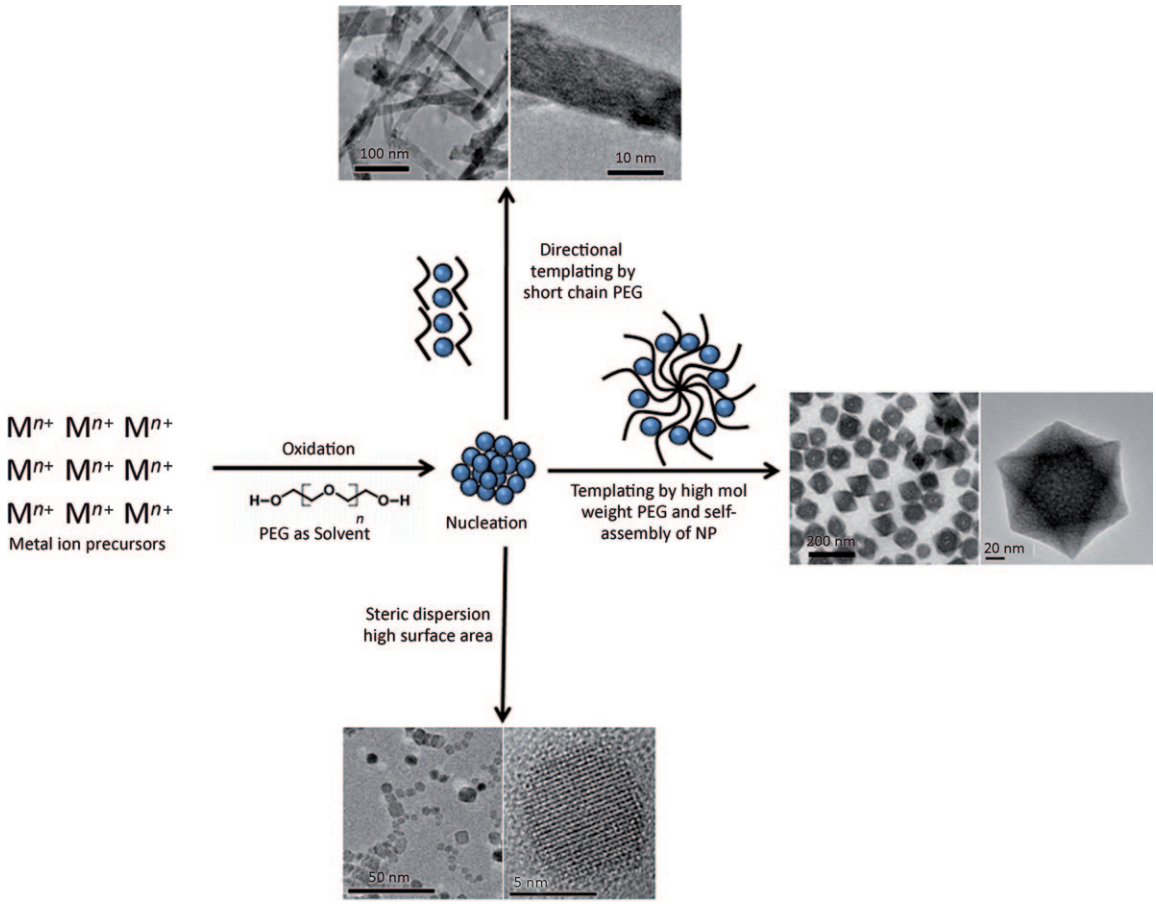

Figure 1. The structure-directing templating effect of PEG, used as solvent, during the synthesis of nanoparticles CNPs). The specific adsorption of PEG molecules along preferred crystallographic planes of NPs leads to self-assembly of NPs as nanorods and nanotubes as a function of molecular weight of PEG. Complexation and association of PEG with metal ions can be used for the self-assembly of nanoparticles into symmetrical shapes, such as superoctahedra (stars). PEG can also assist in oriented attachment of NPs or prevent them from agglomeration to obtain extremely small high specific surface area NPs.

4. PEGylation of Inorganic NPs: Synthesis and Applications

PEGylation of NPs is desired for improving the stealth properties and biocompatibility of NPs in biomedical applications. The ability to tailor inorganic NPs by precise control at the nanoscale level makes them promising candidates for biomedical applications. In combination with the inherent tunable properties of inorganic NPs, special ligands (such as peptides, proteins, sugars, oligonucleotides, amines, polysaccharides and antibodies) for biorecognition, fluorescence and/or sensing can be attached directly or indirectly to the surface of inorganic NPs increasing their technological importance. Metal NPs, such as gold nanorods and nanoshells are currently being investigated for therapeutic applications in treatment of cancers and tumors. Metal oxides, such as iron oxide and gadolinium oxide, have been investigated as magnetic contrast agents while silica NPs are primarily used to passivate the surface of metals and metal oxides, provide a dielectric core for anchoring gold nanoshells and also serve as functional shells for the immobilization of various ligands for the vectorization of NPs. In addition, semiconductor NPs, such as quantum dots, are currently being developed for diagnostic applications in deep tissue imaging. PEGylation of NPs not only provides the required biocompatibility and solubility in aqueous medium but also increases the circulation time of NPs in blood for improved targeted delivery without any systemic toxicity. As illustrated in Figure 2 PEGylation of NPs can be achieved by 1) direct PEGylation where the PEG molecules are directly adsorbed on the surface through physical bonding. This adsorbsion can be achieved by synthesis of NPs directly in PEG medium or other thermal/hydrothermal means of surface adsorption, 2) monofunctional PEG has also been used to covalently attach NPs surfaces with PEG molecules. This strategy is particularly useful for inorganic materials that show high binding affinity towards selective elements, and 3) bifunctional PEG molecules help in achieving vectorization of NPs with selective ligands for detection, delivery, and therapy in addition to covalent attachment of NPs. These three strategies are common for different types of inorganic NPs, that is metals, metal oxides, and quantum dots, and are explained in the following Sections.

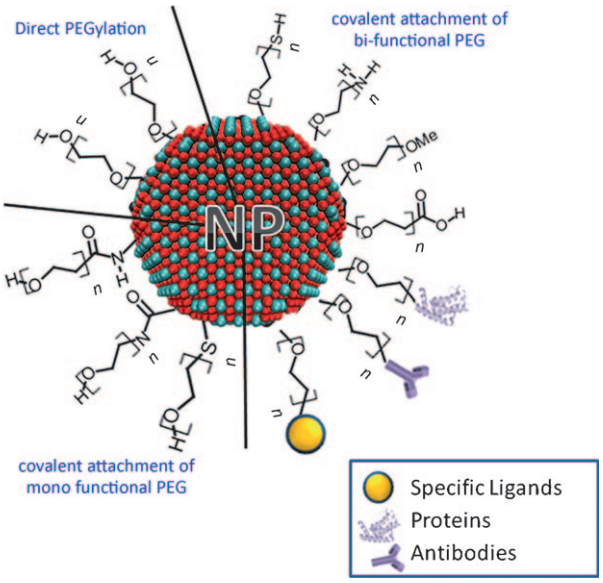

Figure 2. Various strategies for PEGylation of nanoparticle. All the strategies result in nanoparticles that are water soluble and can repel opsonin proteins. Direct PEGylation (by physical or electrostatic adsorption) has the advantage of a simple synthesis. Monofunctional PEGs can be used to achieve covalent bonding between the PEG molecules and the NPs providing long-term stability and high dispersion stability. The vectorization of NPs can be achieved by using bifunctional PEG molecules wherein the free terminal functional groups of PEG can be covalently grafted to other polymers, fluorescent tags, and targeting antibodies or proteins.

4.1. Metal NPs

The use of gold NPs in therapy has largely been the hallmark of the metallic NPs in therapy (see Table 2). Ever since the invention of immunogold in 1971,[67] gold NPs have been used extensively in biomedical applications for labeling of targeting proteins and biomacromolecules.[40,67] The high contrast of gold NPs in electron microscopy provided high imaging quality for visualization of cellular and tissue components. Currently gold NPs are used in a variety of biomedical applications including sensitive diagnostic assays, thermal ablation (photothermal) and augmentation in the radiotherapy of tumors.[68–70] Citrate-, amine-, acrylate-, protein-, antibody-, and DNA-capped gold NPs have been shown to internalize in cells and act as delivery agents.[35] PEGylation strategies for gold NPs have revolved around the use of thiol (SH) terminated PEGs because of the very high specific binding affinity of gold to thiol groups (S–Au bond energy = 47 kcal mol^{-1}). The optimal molecular weight of PEG is a subject of debate and a lower molecular weight PEG (below 5000 Daltons) is often preferred over higher molecular weights. Short-chain lower molecular weight PEG can provide enough surface coverage to completely cover the surface of NPs. However the spacer length of the PEG (that is, the link between the NP surface and the PEG) is also important, especially when fluorescent tags are also attached to the surface of the NPs. Monofunctional PEG-SH can be used to passivate the surface of gold with PEG when no additional surface ligands are required. The thiol group binds strongly to the gold NPs providing a strongly bonded PEG layer on the surface increasing the stability of gold colloids against aggregation in various buffers/mediums and at high ionic concentration. Thioctic acid (TA) containing a cyclic disulfide bond can also be used for covalently linking PEG to gold.[71] Gold NPs PEGylated with TA-modified PEG_{5000} were shown to perform better than thiolated PEG gold NPs. It was also found that the size of the gold NPs also plays an important role in stability through PEGylation. In a comparison of 20, 40, and 80 nm PEGylated gold NPs it was found that the 20 and 40 nm gold NPs were far more stable against aggregation than the 80 nm colloidal gold NPs.[71] The results were supported by the pharmacokinetic study wherein the 20 and 40 nm PEGylated gold NPs showed a delayed clearance from the blood compared to the 80 nm gold NPs. It was found that the 80 nm gold NPs, had the lowest surface PEG density, and underwent nonspecific binding with plasma proteins and were taken up in the liver. The limited oxidative stability of thiolated species in conjunction with exchange reactions with other thiolated compounds inside the body means that the functionality of thiolated PEG-modified gold NPs lasts only for limited time. Polyethylene glycol block poly(2,*N*,*N*-dimethylamino)ethyl methacrylate (PEG-*b*-PAMA) species were shown to improve the long-term stability of PEGylated gold NPs.[72] The tertiary amino group of PAMA in PEG-*b*-PAMA can bind strongly with the surface of gold NPs to give particles that show high dispersion stability even under high ionic concentration ($I = 2.0$). Even though the mechanism of immobilization of PEG-*b*-PAMA over gold surfaces is not clear, it is expected that the branching of terminal nitrogen centers by using secondary and tertiary amino groups can increase the weak N–Au (6 kcal mol^{-1}) bond strength. It was found that when the ratio of the number of tertiary amine groups to gold NPs was higher than 33000, the zeta potential of the NPs was completely shielded and the stability of the dispersion was very high (4 days in 95% human serum). Shorter PAMA chain lengths and three amino segments in the side chain are required to completely cover the NPs with PEG. Acetal PEG-*b*-PAMA can be used to reduce gold NPs directly from a solution containing auric ions. Gold NPs obtained by this procedure have a very narrow range and are covered with PEG layers in a one step process. Various ligands can be attached to the acetal group to give functionalized gold NPs in one step.

4.1.1. Gold Nanoshells

PEGylated gold nanoshells are used to treat tumor cells by exploiting the NIR absorption properties of gold nanoshells.[23] Gold nanoshells are described as compounds with tunable optical properties composed of a dielectric silica core covered with a very thin layer of gold. By adjusting the ratio of core to shell thickness these NPs can absorb light in NIR region with a very high cross section of absorption. Optical penetration of light through the tissue is optimal in NIR and can be used for the treatment of cancers embedded deep in tissues. The nanoshell surfaces coated with monofucntional thiolated PEG were used to treat tumor cells in mice and

Table 2: PEGylated gold NPs as a function of molecular weight and terminal functional groups of PEG for applications in biomedical sciences.

Functionality	MW(PEG) [g mol^{-1}]	Anchoring group	Application/Properties	Ref.
Monofunctional (MeO-PEG-SH)	5682	SH	High stability against aggregation in higher ionic strength/salt concentration	[73]
Monofunctional (PEG-TA)	2000	Bisulfide linkage S-S	TA modified PEG shows higher stability than SH modified PEG on gold colloids. 20 and 40 nm gold NPs show high circulation time in blood by avoiding non-specific uptake by plasma proteins	[71]
Monofunctional (PEG-*b*-PAMA)	4800–13300	Tertiary amino group	Stability depends upon chain length of PAMA—shorter chain high stability. High dispersion stability—4 days in 95% serum	[72]
Monofunctional (PEG-SH)	5000	SH	PEGylated gold nanoshell were used to treat tumor by NIR induced photothermal ablation and showed complete regression of tumor	[22]
Pure PEG	6000	Physisorption	Direct PEGylation was observed during synthesis in synchrotron X-ray irradiation. PEGylated gold NPs demonstrated high colloidal stability and high efficiency in increasing cell mortality under irradiation	[74, 75]
Monofunctional PEG (N6-PEG)	2210	N-termination	One step reduction and PEGylation of gold colloids using atmospheric pressure dielectric barrier discharge (DBD) plasma jets	[76]
Homo bifunctional (HS-PEG-SH)	3878	SH	Self assembly of gold nanorods with gold colloids. Demonstrated receptor ligand system using biotin–streptavidin interaction	[73]
Hetero bifunctional (HO-PEG-SH)	1427	SH	Coumarin derivatized PEG-SH was attached to gold NPs and showed low cytotoxicty, non-specific endocytotic internalization and could be tracked with high resolution through dye fluorescence	[77]
Hetero bifunctional (acetal-PEG-*b*-PAMA)	5100 + 3800	Tertiary amino group	PEG-*b*-PAMA can directly reduce auric ions to form PEGylated gold NPs. Ligands, such as biotin, can be attached to PEG end to provide functionalized PEGylated gold NPs	[78]
Hetero bifunctional (HOOC-PEG-2SH)	1347	Dithiol	Monoclonal antibody attached to PEGylated gold NPs was used to develop highly stable optical contrast agents for pancreatic cancer tissue labeling	[79]

resulted in complete regression of tumors within 10 days after the treatment.[22] The EPR of PEGylated nanoshells was demonstrated by thermal measurements at the tumor and non-tumorous locations several millimeters away which showed a similar temperature profile to the non-treated mice.

4.1.2. Vectorization of Gold NPs with PEG

Gold NPs modified with homo and hetero bifunctional PEG can be used when additional functionality or vectorization of NPs is required. Self-assembly of gold colloids linked to gold nanorods was demonstrated using homo bifunctional PEG (SH-PEG-SH).[73] PEGylated gold nanorods were anchored to the colloidal gold NPs through the free thiol group. Increasing the density of bifunctional PEG molecules on the nanorods leads to an increase in the number of colloids attached to the gold nanorods. Similarly, biotin was attached to thiol-terminated PEGylated gold NPs using a thiol reactive biotin derivative which in turn was shown to attach to streptavidin as a proof of concept of formation of a receptor ligand system through bifunctional PEG.[78] (A thiol reactive biotin derivative is one that reacts with thiol groups to be anchored to gold NPs.)

Using a hetero bifunctional PEG (such as HS-PEG-TA, HS-PEG-NH_2, HS-PEG-COOH,) coumarin dye molecules were attached to the surface of gold NPs and the uptake, cytotoxicity, and fluorescence confocal analysis of the resulting particles was performed.[77] It was shown that the PEGylated gold NPs were taken up through non-specific endocytotic pathway and the fluorescent probe allowed the particles to be tracked with nanometer accuracy. Using heterobifunctional PEG it has also been possible to immobilize various antibodies, peptides, and proteins on the surface of gold NPs.[77,79–81] By covalently linking PEGylated gold NPs with monoclonal antibodies, a label for human cancer tissue was developed.[79] The strong scattering properties of PEGylated gold NPs were used to image the tumor and its stromal tissue with actual spatial distribution.

4.1.3. Non-Thiol PEGylation of Gold NPs

Apart from thiol based approaches several other methods have been explored for the synthesis of PEGylated gold NPs. Direct PEGylation of gold NPs driven by synchrotron X-ray irradiation has been reported.[74,75] By this method well dispersed water-soluble PEGylated gold NPs were obtained from auric solution in PEG (6000 Daltons) within 5 min. PEGylation resulted in substantial internalization of NPs in cells without exocytosis for longer times and with no detectable toxicity. In addition, the efficacy of radiotherapy was increased by enhancement of the X-ray mediated damage in cancer cells. Furusho et al[76] demonstrated atmospheric-pressure plasma-jet-based single-step synthesis of PEGylated gold NPs. Highly stable PEGylated NPs with a narrow size distribution were prepared by the interaction of dielectric-barrier discharge plasma jets with auric chloride.

PEGylated dendrons have also been used to encapsulate gold NPs. The gold nanoclusters can grow in the intermolecular space of PEGylated PAM dendrons.[82,83] Complete core–shell type morphology can be achieved by increasing the number of dendron generations. For a narrow size distribution of gold NPs hemispherical shaped dendrons gave the best results. The size distribution of gold NPs increased as the shape of dendrons changed from hemisphere to corn shaped.

Double phase transfer of gold nanorods was also shown as a promising alternative to develop PEGylated NPs. Gold NPs synthesized in CTAB (cetyl trimethyl ammonium bromide) were PEGylated in a one-step phase transfer by ligand exchange and subsequent entrapment in PLGA-*b*-PEG-COOH.[84] The lipophillic core of poly(lactic co-glycolic acid) (PLGA) was used as a guiding molecule to trap the ethyl 11-mercaptoundecanoate-coated gold NPs.

4.1.4. PEGylated versus Non-PEGylated Gold NPs

Mirkin and co-workers[35] have recently reviewed a range of simple and designer ligand molecules for applications in medicine and biology. Diverse surface chemistry, gives rise to different surface charge on, and size of NPs resulting in selective uptake of the nanoparticles. While the citrate- and amine-functionlized gold NPs can penetrate the cells the shortcomings of such charged nanoparticles were clearly identified. In this sense PEGylation provides a better alternative as PEG molecules completely shield the surface charge of nanoparticles. The internalization of charged molecules was suggested to take place by the interaction between the positively charged NPs (amines) and the negative cell surface, or for negatively charged nanoparticles (citrate-, polyacrylic acid (PAA)-coated NPs) through their interaction with specific proteins.

Ligands, such as lipids, peptides, oligonucleotides/DNA, and antibodies can be coated over gold nanoparticles for specific functions such as delivery, cell targeting therapeutics, and imaging. Directly adsorped antibodies on NPs are relatively unstable and usually require an anchoring molecule. Bifunctional PEG can also be used as an anchoring agent for larger biomolecules and can provide the required targeted delivery properties. The most interesting properties are shown by DNA–Au nanoconjugates.[85] It was shown that at a greater surface loading of DNA the cellular uptake of gold nanoparticles can be as high as one million Au NPs per cell.[35,86] The density of DNA on the NP surface is one of the deciding factors for internalization, analogous to the higher internalization of NPs with a high surface density of PEG molecules. A direct comparison of the cellular uptake ability both in vivo and in vitro between the PEGylated and DNA-modified surface has not been reported to date however negatively charged DNA-coated Au NPs are internalized at a much higher rate than the negatively charged citrate-coated NPs.[87] Unlike the PEG molecules that repel opsonin proteins and thus improve the NP circulation time, a change in zeta potential from negative to positive and the adsorption of specific proteins is currently debated as the mechanism of internalization of DNA-coated gold NPs.[86] While DNA-modified gold NPs have several interesting properties, the surface modification of NPs with such biomolecules requires special precautions, such as buffer control, exposure time to chemicals, use of chemicals compatible with DNA, and tedious purification procedures in comparison to PEG. It has been discussed previously that PEG is chemically stable against mild oxidizing and reducing conditions and is stable at moderately higher temperatures. These physico-chemical properties make PEG a popular choice over highly sensitive biomolecules and proteins.

4.1.5. Toxicity and Biocompatibility of PEGylated Gold NPs

Despite the fact that PEGylation was used to increase the biocompatibility and normal excretion of gold NPs from the body, isolated reports of toxic responses to PEGylated gold NPs have raised some concerns about PEGylation process. Acute toxicity from 13 nm PEGylated gold NPs leading to acute inflammation and apoptosis in a mouse liver was reported.[88] Despite PEGylation, the NPs were found to accumulate in the liver for up to seven days post injection. In contrast, it was reported that by replacing the toxic surfactants, such as CTAB, with PEG-coated gold nanorods provided colloidal stability and biocompatibility. The very high value of LC_{50} is debated in the context of encountering such a high dose of NPs in other organs of the body where the uptake of NPs is non-specific.[89] While several articles have been published that have demonstrated high biocompatibility and longer residence time of gold NPs in blood; the isolated reports on toxicity, demand a full-scale investigation of PEGylated NPs. The bond between PEG and the gold NP surface through thiols or tertiary amines need to be well characterized in terms of stability. The surface functionalization must be demonstrated to be stabile for the length of time the NP is expected to remain inside the body. In addition, the chemistry involved in the functionalization of NPs can lead to the adsorption of other chemicals on the surface of NPs. Thus it is extremely important to purify all materials to avoid toxicity arising from undesired surface-adsorbed chemicals that may be falsely ascribed to the PEGylated NPs.

4.2. Nano Metal Oxides

Metal oxides also form a versatile class of technologically important compounds. The inertness of metal oxides to various chemicals prompted their biomedical use as dental and bone fillers or implant materials. However, with technological advances, the role of oxide materials transformed from passive inert fillers to active delivery compounds, magnetic contrast agents, and therapeutic materials. Shrinking the size of inert oxide materials to the nano regime converted the passive oxide particles into active nanomaterials and increased the range of applications of metal oxides. Oxides such as alumina, silica, iron oxide, gadolinium oxide, and cerium oxide, have received special attention. Since the discovery of mesoporous nanosilica in 1992, it has been used in several applications including cosmetics and drug delivery.[90] Hollow silica NPs have found widespread use in a variety of drug-delivery applications.[18,91] Iron oxide NPs have also shown great potential for various nano-biomedical applications including the hyperthermia treatment of tumor cells, magnetic drug targeting, and as magnetic contrast agents.[3,33] Recently there has also been an upsurge in studies on the antioxidant-like behavior of cerium oxide NPs.[21] Such an increase in the biomedical applications of oxide NPs has naturally resulted in the development of their PEGylated

counterparts. This Section will focus on PEGylation of iron oxide NPs and briefly discuss other oxide systems.

Surface PEGylation of oxide NPs can be achieved through surface adsorption, electrostatic interaction, and by using covalent linkages. The presence of hydroxy groups in the surface of oxide NPs (synthesized using aqueous routes) can be used for direct PEGylation. When the NPs are synthesized in organic medium, additional surface treatments to activate the surface is necessary. Direct synthesis of oxide nanoparticles in PEG can also lead to direct PEGylation through the formation of M-O-C bonds as has been reported for cerium oxide, iron oxide, as well as silica NPs.[21,43,92,93] The use of functional PEG terminating in OH and COOH groups or grafted with copolymers, such as PEI (polyethylene imine),[94] PMMA (polymethyl methacrylate),[93] PAMAM (polyamido amine),[95] has also been reported.

4.2.1. PEGylation of Iron Oxide Using Silane Chemistry

Magnetic iron oxide NPs (MIPs) are frequently coated with a combination of PEG–silane. The very strong bonding ability of the silane group with the surface of oxide NPs is exploited in this technique. PEGylated MIPs have been synthesized either by coating the NPs first with a silane group using APTMS (amino propyl trimethoxy silane) or APTES (amino propyl triethoxy silane) and then functionalizing the amine terminal group with a carboxy terminated PEG. Similarly, PEG–silane can be synthesized first and then treated with MIPs to obtain PEGylated MIPs in one step with silane as the primary shell and PEG as the external shell.[96] Using DLVO (Derjaguin and Landau, Verwey and Overbeek) theory it was shown that the PEG–silane stabilizes MIPs by steric repulsion.[97] The external PEG coating can also protect fluorescent dyes attached to a silica coating of the MIPs from photobleaching.[98] The motion of particles under the influence of a magnetic field can then be easily tracked by virtue of the fluorescent dye. Similarly, PEGylation of magnetic gadolinium oxide coated in a fluorescent silane shell has also been reported.[99] A comparison of four different PEGs PEG_{250}-COOH, PEG_{2000}-COOH, PEG_{2000}-OCH_3, and PEG_{2000}-NH_2 showed that the gadolinium oxide coated with methoxy terminated PEG_{2000} demonstrated the longest blood circulation time and greatest accumulation in tumor. Changing the methoxy group to amine or carboxy functionality resulted in an increased accumulation of NPs in the liver and spleen as a result of the higher surface charge in these compounds. Despite the small size, highly dense PEG_{250}-COOH showed free circulation in blood and least adsorption in liver and spleen. Using a similar silane strategy bifunctional PEG could be attached to gadolinium oxide NPs.[100] The terminal carboxy group could be used to attach rhodamine dye as a fluorescent tag. It was shown that the PEGylated gadolinium oxide NPs showed decreased relaxivity compared to the bare NPs however, the relaxivity increased as a function of dialysis time possibly through removal of loosely attached PEG molecules.

4.2.2. PEGylation by Ligand Exchange

MIPs synthesized in high-boiling, non-aqueous solvents can be PEGylated by ligand exchange methods. MIPs synthesized in non-aqueous medium and stabilized with oleic acid, hexane, or trioctyl phosphine oxide (TOPO) as the surface groups can be directly transferred from non-aqueous medium to aqueous medium using, for example PEG-silanes, PEG-PEI, PEG-PAMAM, PO-PEG (PEG-derivatized phosphine oxide), PEG-fatty acid.[94–97,101,102]

Various PEGylated magnetic NPs (some of which are obtained by ligand exchange) are listed in Table 3 along with their properties/applications. PEGylated magnetic NPs can retain their full relaxometric properties upon PEGylation. The size of the NPs increases after the ligand exchange reaction with PEG as a function of the molecular weight of the PEG coating. The small increase in particle size may decrease the cellular uptake, nevertheless the PEG coating compensates for the small increase in particle size by decreasing the non-specific adsorption by RES. Thus, the overall uptake of NPs without any tumor targeting ligands has been shown to increase in tumor cells through EPR by taking the advantage of leaky vasculature of tumor cells and enhanced circulation time provided by PEG.[96]

4.2.3. One-Step PEGylation of Magnetic NPs

Direct synthesis of MIPs and magnetic NPs in specially designed PEG molecules can also result in the formation PEGylated NPs. In addition to being PEGylated, such one-pot syntheses have the additional advantages of providing small and uniform particle size NPs. PEGylated magnetite NPs as small as 4 and 9.8 nm have been prepared directly from mono- and dicarboxy terminated PEG through the covalent binding to the surface hydroxy groups by thermal decomposition of $[Fe(acac)_3]$ in pyrrolidone and PEG.[104] The solubility of MIPs increases as a function of molecular weight of PEG from 550 to 5000, however the increase in solubility results in an increase in particle size as well as a loss of the magnetic properties. Graft copolymerization of PEGMEA (PEG–methyl ether acrylate) with polymethyl methacrylate using atom transfer radical polymerization have been used to synthesize MIPs in PPEGMEA-PMMA by co-precipitation of Fe^{2+} and Fe^{3+} in the presence of polymer.[93] The surface charge was shielded effectively as a function of increase in PMMA side-chain length. MIPs were also synthesized directly in antibiofouling polymer TMSMA (trimethoxysilyl propyl methacrylate) linked to PEG and silane.[107] The in-situ synthesized NPs showed high dispersibility in PBS buffer and in a pH range from 1–10. They also demonstrated a very low uptake by macrophages and extremely low cytotoxicity making then interesting candidates as magnetic resonance contrast agents.

PEG-gallol (PEG mol wt 5000 and 550) can be coated onto iron oxide NPs in a one-step process. The resulting NPs can be freeze dried as nanopowders that can be easily redispersed in water, a feature that offers great long-term stability of the PEG NPs.[92] One of the terminal ends of PEG can also be coupled to other functional groups, such as biotin

Table 3: Various PEGylated systems for magnetic NPs.

Functionality	MW(PEG) [g mol^{-1}]	Anchoring group	Application/Properties	Ref.
Bifunctional (Mal-PEG-NHS)	5000	(3-mercaptopropyl)trimethoxy-silane (MPTS)	PEGylated Gd_2O3 showed coating dependent magnetic relaxivities	[100]
Mono and bifunctional NH_2-PEG-NH_2, HOOC-PEG-COOH, HOOC-PEG-OCH_3,	250 and 2000	Polysiloxane shell	Methoxy terminated PEGylated Gd_2O_3 showed highest stability and uptake by tumor cells. Uptake is a function of molecular weight of polymer and/or surface density	[99]
Monofunctional PEG-$SiOMe_3$		Silica shell	Fluorescent dye on silica shell was protected from photobleaching. The magnetic drug targeting in vitro could be tracked visually	[98]
Bifunctional trifluoroethyl ester PEG silane (TFEE-PEG-Silane)	600	Silane	TFEE-terminal PEG modified nanoparticles are soluble in both aqueous and non-aqueous solvents. TFEE can be converted into amine and attached to targeting groups such as folic acid.	[103]
Monofunctional MPEG-COOH	1100	Direct attachment	One-pot synthesis to achieve PEGylated magnetite NPs that demonstrate long circulation time	[104]
Monofunctional (MPEG-silane) and bifunctional TFEE-PEG-Silane	–	Silane	PEGylated NPs show enhanced uptake as compared to non-PEGylated cells. Folic acid coated NPs show higher uptake in tumor cells	[105]
PEG-PEI	5000	PEI	Transverse relaxivity higher for micellar system for same size NPs. Relaxivity increases with increase in particle size for polymer-coated NPs.	[94]
PEG-PAMAM	2000	PAMAM	The phase-transfer process of magnetic NPs is sensitive to the composition of PEG-PAMAM copolymer	[95]
Monofunctional PO-PEG (Phosphine oxide-PEG)	550, 1100	PO	Simple process to yield water-soluble iron oxide NPs. PEG_{1100} could not avoid complete adsorption by serum proteins but maintained relaxometric properties	[102,106]
Mono and bifunctional MPEG-gallol and biotin-PEG-gallol	550 and 3400	Gallol	Ability to freeze dry and resuspend magnetic nanoparticles. No corrosion of iron oxide in dopamine system	[92]
PEGMEA-PMMA (polyethylene glycol methyl ether acylate-PEG methyl acrylate)	–	Direct attachment	Direct synthesis of Fe_3O_4 in double hydrophilic polymer composites in one-pot reaction with narrow size distribution	[93]
TMSMA-PEGMA (trimethoxysilyl propyl methacrylate)	475	Silane	Polymer coated NPs could be detected in tumor cells leading to enhanced contrast. NPs have antibiofouling property	[107]

and dopamine, and could still be coated on iron oxide in a one step process. The dopamine functionalized MIPs did not show any iron corrosion owing to the stability offered by PEG-gallol system.

4.2.4. Therapeutic PEGylated Metal Oxide NPs

Cerium oxide nanoparticles (CNPs) have recently shown promising results in the treatment of diseases caused by reactive oxygen species (ROS).[108] The ability of cerium to switch its oxidation state between +III and +IV can catalyze the single electron reduction and oxidation of ROS. It was shown that PEGylation of CNPs enhances its activity towards scavenging both superoxide and peroxide radicals. While the protein repelling tendency of PEGylated CNPs was not tested, the use of PEG as an active coating instead of passive protecting group was shown for the first time.[21] It was shown that CNPs react with hydrogen peroxide in presence of PEG to form a complex which facilitates the quenching of peroxide. These results open up a new dimension of PEGylation where the PEG molecules can also be involved actively in addition to providing a resistance to non-specific protein adsorption.

4.3. Quantum Dots

Quantum Dots (QD) are a class of semiconductor NPs that have the potential for biological applications owing to their outstanding fluorescence properties. Recent advances in water soluble QDs has resulted in the synthesis of target-specific QDs that can be used in imaging (cellular and deep-tissue) and also as efficient fluorescence resonance energy transfer (FRET) donors.[109] QDs are generally made of semiconductor core (e.g., CdSe, CdTe) coated with a shell (e.g., ZnS, CdS) to improve their optical properties.[110] QDs also serve as a tool for investigating the interactions between NPs and cells owing to the availability of QDs in different sizes, shapes, they can be covered with various surface coatings, are easily detected, and give high fluorescence yield.[111] Different strategies have been used to attach PEG to QDs and some of the popular ones are 1) linking NH_2-PEG molecules to amphiphilic-polymer-coated QDs which have free carboxylic acid groups by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)[112,113] or *N*-hydroxysuccinimide (NHS) and EDC coupling,[114] 2) thiol modified PEG molecules attached through a thiol exchange reaction,[115] and 3) phospholipid PEG molecules attached by physical adsorption on trioctyl-phosphineoxide coated QDs.[116]

4.3.1. Organ Uptake and Interaction of PEG–QD with Cells

PEG molecules on the surface of QDs reduce the interaction of the particle with the cell surface and extracellular matrix proteins leading to a decrease in non-specific binding to the cell. The surface density of PEG molecules on QDs may also influence the interaction between QDs- and cell, it was reported that a small number of surface PEG molecules is sufficient to reduce non-specific binding.[114] Interaction of QDs with cells can also vary depending on the surface modification for example a much lower interaction was reported for PEGylated QDs than for QD-COOH and QD-NH_2.[111] It was shown in an in-vitro study that the magnitude of the interaction between the cells and PEGylated QDs was twofold higher in medium with 10% fetal bovine serum as compared to media without supplementation.[117] Surface coating by PEG also influences the organ uptake and blood circulating time of QDs. In-vivo studies in mice showed subcutaneously injected CdSe/ZnS-PEG-coated QDs cleared from the site of injection and accumulate in lymph nodes.[118] Interestingly, QDs were found to deposit in liver, skin, and bone marrow depending on the surface coating.[119] Liver is the primary organ of deposition of PEG-QDs.[120] Reduced organ uptake, longer circulation time in blood, and slow accumulation of QDs in tumors have been reported for PEG-QDs.[113,121–124] The size of the PEG molecule also influences the blood circulating time and biodistribution of the QDs. Lower molecular weight PEG-QDs showed a circulating life time of 12 min or less whereas higher molecular weight PEG-QDs had reduced macrophage recognition, much longer circulating lifetime, and showed reduced uptake by the lymph nodes and liver.[112,119] Similarly higher molecular weight PEG, with a specific ligand or peptide linked at the distal end of the PEG-QDs lead to improvements in tumor and subcellular site targeting.[115] However, in contrast to this report large QDs (like PEG-dihydrolipoic acid-QDs) were trapped in the organs such as the liver, lungs, and spleen but were not found in bladder.[125]

Clearance from the organ system is another requirement for QDs to be a potential candidate for in-vivo imaging. The organ clearance study of PEG-QDs showed the clearance of QDs in the order of liver, spleen, bone marrow and finally from lymph nodes.[119] Nevertheless, PEG-tumor-targeting peptide-CdSe/ZnS QDs reduced nonspecific elimination of QDs through lymphatic system.[117] The extent of cellular interaction and toxicity of QDs functionalized with PEG molecules of different molecular weights and terminal functional group is summarized in Table 4.

Table 4: Properties, bio-compatibility, cellular interaction, and cytotoxicity of QDs functionalized with PEG molecules of different lengths and with different terminal functional groups.

QD	PEG	MW(PEG) [g mol^{-1}]	Properties/Bio-compatibility/Cellular interaction/organ uptake/application	Ref.
Amphiphilic polymer coated CdSe-ZnS	Methoxy terminated PEG	2000 550 350	Molecular weight of the PEG is not important to reduce non-specific binding, lower molecular weight PEG sufficient to eliminate most of the non-specific binding	[114]
Amphiphilic polymer coated CdSe-Cds	NH_2 terminal PEG methyl ester	750 6000	Both cytotoxicity and cellular internalization decrease with higher molecular weight PEG-QDs	[126]
Amphiphilic polymer coated CdSe-ZnS	Methoxy terminated PEG Carboxy terminated PEG	750 5000 3400	Higher molecular weight methoxy terminated PEG-QDs show longer blood circulation time (140 min)	[112]
Amphiphilic polymer coated CdSe-ZnS	Methoxy terminal PEG	5000 700	No accumulation in lymph node in case of QDs functionalized with higher molecular weight PEG	[119]
TOPO coated QDz	PEG	2000	Decease the rate of RES uptake and encourage the excretion compared to bare QDs	[127]
CdSe-ZnS				[128]
Spherical (4.6 nm diameter)	PEG PEG-Amine	–	No cytotoxicity over 48 h Cytotoxicity was observed after 48 h and increase in IL-6 and IL-8 release	
ellipsoid [diameters 6 nm (Minor axis) and 12 nm (Major axis)]	PEG PEG-Amine		Cytotoxicity was observed after 48 h Cytotoxicity was observed after 48 h and increase in IL-8 release	
CdSe-ZnS				[111]
Spherical (4.6 nM diameter)	PEG PEG-Amine	–	Lower hydrodynamic diameter of the PEG-Amine-QDs allowed the penetration through skin and localization in dermal layer whereas PEG-QDs were localized at epidermal layer after 8 h	
ellipsoid [diameters 6 nm (Minor axis) and 12 nm (Major axis)]	PEG PEG-Amine		Higher size restrict the localization of both PEG and PEG-Amine-QDs at epidermal layer after 8 h	
CdSe-CdS QDs	PEG	–	PEG-QDs can only penetrate into the body through damaged skin. They are thus nontoxic to intact skin	[129]
Mercaptoacetic acid- CdSe/ZnS	Methoxy PEG	5000	Possible to target subcellular site using QD-PEG-peptide conjugate	[115]

4.3.2. Cytotoxicity of QD-PEG

For biological applications of QDs it is essential to develop a fundamental understanding of the interaction of QDs with cells and to determine the cytotoxic effects of QDs (if any). Toxicity of QDs is mainly dependent upon the size, charge, concentration, bio-compatibility, and stability (oxidative, photolytic and mechanical) of QDs. The common factors causing toxicity of QDs are core degradation, increase in free-radical generation, and interaction with subcellular components and proteins rendering these components nonfunctional.[118] A cytotoxicity study of PEG-silane-coated QDs revealed a minimal impact and molecular response to the exposed cells.[130] A dose–response study showed cytotoxicity for QD-PEG-amine and COOH-QDs, however no cytotoxicity was observed in case of unfunctionalized PEG-coated QDs. Similarly, cell cytotoxicity was minimum with PEG-coated QDs as compared to the bare CdSe/CdS QDs.[126] PEGylation resulted in decreased internalization into cells.[131] Size of the PEG molecules on the surface of the QDs also plays an important role in determining cellular cytotoxicity. High molecular weight PEG-coated QDs were found to be less cytotoxic than low molecular weight PEG-coated QDs.[126] In addition to the cytotoxicity, the cell immune response of different surface modifications of QDs were also studied using PEG, PEG-amine, and polyacrylic acid (PAA). Carboxylic acid coated QDs (PAA) induced a strong immune response and increased the release of IL-1β, IL-6, and IL-8 by two- to fivefold over 48 h, whereas no such increase in the release of ILs was found for PEG-coated QDs. Interestingly, QDs with PEG-amine coating also showed a slight increase in the release of IL-6 and IL-8.[128]

PEG-coatings on QDs reduce nonspecific binding and uptake by the organs and also reduces the cytotoxicity and immune response of QDs towards living cell. The biocompatibility of hetero and homo bifunctional PEG-QDs terminating in amine or carboxy groups have not been established unanimously and need additional work. Despite the fact that PEGylation was used to increase the stability of the NPs and obtain good dispersion, aggregation of PEG-QDs in high concentration in buffers has been reported[125,132] limiting their scope and applicability. PEG polymer also increases the size of the particles, which may restrict the application of QDs in vivo. Moreover, the effect of PEG density and conformation at the nanoparticle surface on its antibiofouling capacity is largely unexplored.

5. Summary and Outlook

Herein we have outlined some of the current practices and methodologies in the PEGylation of inorganic NPs that have expanded the scope, applicability, and biocompatibility of NPs in various biomedical applications. The huge potential of inorganic NPs in therapy, diagnostive imaging, treatment, and prevention of diseases has been augmented by the stealth properties provided by the PEG coatings. Smarter design and meticulous chemistry have allowed the vectorization of NPs in conjunction with PEGylation. Thus a major thrust in the design of PEGylated NPs is focused on the synthesis of multifunctional PEG molecules that can be grafted with antibiofouling molecules, fluorescent agents, and other functional polymers. Such a derivatization of PEG molecules can turn PEG into an additional probe attached to the inorganic NPs for obtaining a multitude of information from a single platform. In this respect, the major focus in the PEGylation chemistry currently is not limited to the passive use of PEG as a coating for the repulsion of opsonin proteins but to use it as an active attachment that can act as an inherent part of the therapeutic, diagnostic, and imaging platform. In addition, one-step PEGylation strategies for the direct synthesis of PEGylated nanoparticles are highly desirable and will become be more wide spread in the future. Processes such as X-ray irradiation and DBD in the presence of PEG as a solvent are likely to gain more popularity and novel one step PEGylation techniques will be developed. As the research becomes more multidisciplinary, new synthetic methodologies, conjugating agents, and linking molecules will be developed for coating smaller and more diversely shaped NPs leading to efficient encapsulation of the NPs. A clear understanding of the effect of PEG surface density and molecular weight on the cellular uptake of PEGylated NPs for various types of nanoparticles is still under exploration. More research can be expected in determining the specific interaction of various proteins with PEGylated surfaces and the role of such proteins in increasing or decreasing the cellular uptake of nanoparticles. As the intended use of these PEGylated NPs is inside the body, detailed information on the long-term stability, cytotoxicity, and efficacy of the PEGylated NPs is required before they can be realized as a possible alternative on a commercial scale.

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