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Recent developments in multifunctional hybrid nanoparticles: opportunities and challenges in cancer therapy

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1. ABSTRACT

Multifunctional hybrid nanoparticles combine some of the unique physical and chemical characteristics of two or more classes of materials, such as polymers, liposomes, metals, quantum dots and mesoporous silica among others, to create a versatile and robust new class of nanoparticles. Here we discuss the most recent synthetic strategies to create these hybrid systems and analyze four key design aspects: stability, encapsulation of therapeutic and imaging agents, controlled release of encapsulated agents, and biocompatibility. Through the combination of multiple nanomaterials, hybrid nanoparticles aim to expand the functionality of single-component systems, using the strengths of one material to improve on weaknesses of another. We then examine how hybrid nanoparticle platforms provide unique opportunities in cancer therapy, specifically in the treatment of multidrug resistant cancer. Finally, we discuss some of the challenges hybrid nanoparticles systems might face in their large scale synthesis and commercialization in the biopharmaceutical industry.

2. INTRODUCTION

Nanotechnology has emerged as a potentially novel and promising way of combating cancer in early and late stages - from cancer cell detection and targeting, to drug delivery and cell imaging (1-3). Several approaches have been taken to develop various nanoparticle designs to maximize efficiency in fighting cancer while simultaneously minimizing toxicity. Multifunctional nanoparticles (i.e. nanoparticles that can be used for more than one purpose such as therapy, imaging, or diagnostics) are the most recent novel approaches in cancer treatment (4, 5). Different multifunctional nanocarrier platforms composed of organic materials have recently been developed. Polymeric nanoparticles (6), micelles (7), liposomes (8), dendrimers (9) and carbon nanotubes(10) are common examples. Inorganic synthetic methodologies have also been used in the engineering of nanoparticles through the production of metal nanoparticles (11), silicon nanostructures (12) and nanocrystals (13) (i.e., quantum dots). The bridge between two or more organic or inorganic components in the particle structure has led to the

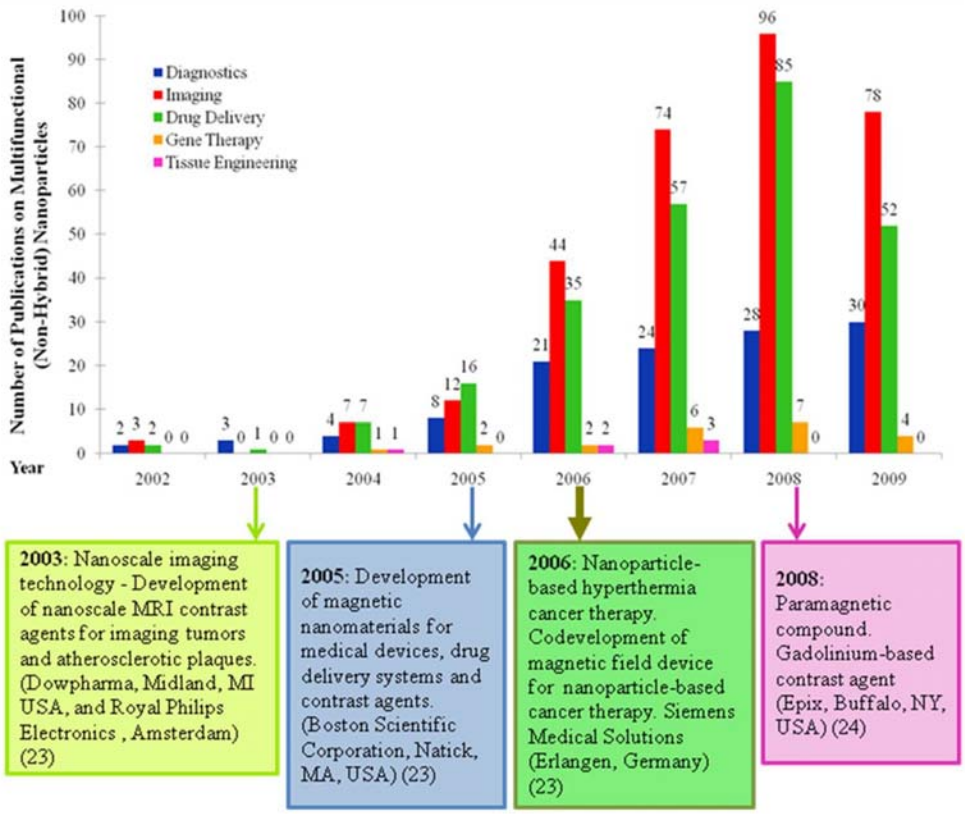

Figure 1. Advances in multifunctional nanoparticles from 2002-2009. Source. Science citation index. Drug delivery and imaging are the leading areas in academia and business (23), as shown in the upper and bottom part of the timeline. The bottom section includes some of the molecules entities approved by the US FDA's Center for Drug Evaluation and Research in 2008 (24).

creation of a new type of nanoparticle, referred to as hybrid nanoparticle. This new class of multifunctional nanoparticles combines physical and chemical features of the aforementioned nanoparticle constructs.

The advent of multifunctional hybrid systems has created the potential to improve upon the stability, encapsulation efficiency, and biocompatibility of non-hybrid systems. For instance, polymer-lipid hybrid nanoparticles have been shown to increase suspension or size stability of nanoparticles in biological conditions by modulating the molar ratio between polymer and lipid components (14). In addition, encapsulation of multiple chemotherapeutic drugs and chemosensitizers have been achieved using the properties of polymer-lipid hybrid nanoparticles by combining an anionic polymer with a cationic therapeutic agent, and stabilizing this complex with a lipid coating (15). Furthermore, biocompatibility of quantum dots was enhanced by coating the nanocrystal surface with a lipid layer (16).

Although hybrid nanoparticle systems may improve and expand the functionality of conventional nanoparticles systems, they also have limitations, including multiplicity of synthesis steps and incomplete understanding of the fundamentals of particle formation. In this review, we attempt to shed light on some currently available approaches for the synthesis of hybrid nanocarriers that can be useful in cancer therapy, while analyzing potential limitations.

3. CURRENT MULTIFUNCTIONAL HYBRID NANOPARTICLE PLATFORMS

3.1 Multifunctional non-hybrid nanoparticle systems

A multifunctional non-hybrid nanoparticle can be defined as a single construct composed primarily of a single component material which might be a polymer, lipid, metal, silica, etc. This type of nanoparticle is capable of performing several functions at a specific timescale (e.g., serving as an imaging agent or as a therapeutic agent carrier) (3). The physico-chemical characteristics of these particles such as size, surface charge, and solubility depend on composition and production method. Many of the current nanoparticle designs have core-shell configurations, as it is the case for di-block copolymer-based nanoparticles (17). Typically, the core serves as a container to store therapeutics or diagnostic payloads, while the shell serves as a protective "shield" that can provide stability to the particle in biological conditions. In addition, the surface of the particle is often functionalized with ligands, such as small molecules, proteins, enzymes, antibodies, or aptamers (18-22). These functionalities are particularly advantageous in three medical areas: imaging, diagnostics, and drug delivery. Figure 1 shows the number of scientific

publications in the development of multifunctional non-hybrid nanoparticles over the last 8 years in five different areas (diagnostics, imaging, drug delivery, gene therapy and tissue engineering). The greatest number of publications has been in the area of imaging. This is not surprising, since iron oxide nanoparticles and nanocrystals have shown remarkable imaging utility *in vitro* and *in vivo* from a research perspective. Additionally, one of the most profitable sectors related to nanotechnology is medical imaging and the development of new contrast agents (23-25).

3.2 Multifunctional hybrid nanoparticles (MHNPs)

Hybrid nanoparticles are new designs composed of 2 or more classes of materials such as polymers, lipids, ceramics, metals, etc., whose surface can be functionalized with a variety of moieties. Figure 2A-D shows some of the current designs of hybrid nanoparticles. Most of these designs contain more than one structural layer. In fact, the rich heterogeneity in physical structure and chemical composition is one unique feature of hybrid nanoparticles.

To date, the most common hybrid nanoparticle models can be broadly classified in four categories: Polymer-lipid (14, 26-28), Polymer-metal s (29, 30), Quantum dot-lipid (16, 31-33) and Polymer-silica (34-36) nanoparticles (Figure 2). For each of these particles, it is hypothesized that their diverse physical structure may improve or expand some of the functions displayed by non-hybrid multifunctional nanoparticles. Some of these functionalities include suspension and size stability of particles in biological conditions, drug/imaging agent encapsulation, controlled release of agent, and biocompatibility profiles.

3.3. Synthetic strategies of MHNPs

Most of the methods used in the synthesis of hybrid nanoparticles are modifications of methods already developed for the synthesis of non-hybrid systems. These modifications include multi-step synthesis or external energy inputs (e.g., mechanical agitation, heating, or sonication) for particle homogenization purposes. Some of the synthesis methods include nanoprecipitation (also known as solvent displacement) (14), emulsification-solvent evaporation (37), thin-film hydration (32), modified Stober method(38), layer-by-layer (LbL) synthesis (27), ionotropic gelification (39), ultrasonication (28, 30), polymer-monomer pair reaction system (29), and spray-drying (36). Methods developed to synthesize polymer-lipid, silica-metal, and quantum dot-lipid nanoparticles will be elaborated in the following paragraphs.

The synthesis of polymer-lipid hybrid nanoparticles involves mixing polymeric nanoparticles with liposomes through mechanical agitation or ultrasonication to form polymer-lipid complexes. These are formed by the fusion of lipid multilayer on the surface of polymeric nanoparticle (40-42). Our group developed a single-step preparation of polymer-lipid nanoparticles, where the polymer in one solvent was mixed with a lipid suspended in an anti-solvent. Such mixtures result in the self-assembly of the hybrid construct after solvent displacement (14). Here, intermediate steps such as heating and vortexing are implemented. Finally, we have also developed a microfluidic system where polymer-lipid hybrid nanoparticles are prepared in a single step through rapid mixing without further processing (43). Rapid mixing has also been used to prepare other types of hybrid nanoparticles such as polymer-coated gold nanoparticles (44).

In terms of particles made from silica, the Stober method was originally developed for the preparation of silica microspheres in 1968(45). In recent years a modified Stober method was developed to prepared silica-coated silver hybrid nanoparticles(38). In this method, silver nanoparticles are coated with silica through a seeded polymerization technique with a sol-gel reaction. Silica hybrid nanoparticles have also been prepared through the emulsification and spray-drying methods(36).

In case of quantum dot-lipid nanoparticles, a thin-film evaporation method has been implemented. In this strategy, lipid and quantum dots (together or separately) are dissolved in a solvent and the resulting solution is dried until a thin film of solvent remains. Subsequently, an antisolvent (e.g. water or buffer) is added above the lipid's critical micelle concentration while the whole mixture is heated simultaneously (16, 32). The result is quantum-dots encapsulated in a lipid envelope. A modification of this procedure can be implemented to encapsulate more than one imaging agent in a single construct. For instance, Park *et al.* incorporated magnetic nanoparticles as well as quantum dots in a lipid envelope forming a "magnetofluorescent" hybrid nanoparticle (46). Finally, a microfluidic platform used for the preparation of polymer-lipid nanoparticles was also utilized for the preparation of quantum dot-lipid hybrid constructs in a single step (43). The number of quantum dots inside the lipid envelope can be regulated by controlling the lipid-to-quantum dot ratio.

3.4. Stability of MHNPs

The suspension stability (i.e., ability to remain in solution indefinitely) of hybrid nanoparticles in physiological conditions is one of the most important prerequisites for their use in biomedical applications (47). This property will depend on the physical and chemical composition of the nanoparticles, in particular the charge (48). Hybrid nanoparticles possess the advantage that properties from each constituent can be regulated to yield a nanocarrier with a desired suspension stability profile. In this respect, scientists working with very different hybrid nanoparticles have observed improvements in particle size stability and have implemented design techniques that allow for fine-tuning of the system (49). When discussing nanoparticle size stability, colloidal stability (i.e. the ability of particles to resist aggregation) in physiological conditions is a key factor that determines whether a particle has the potential to move forward to the clinic. The following section discusses colloidal stability and size stability of hybrid nanoparticles.

Colloidal stability of nanocarrier systems is crucial for delivery of the payload (50). As nanoparticles

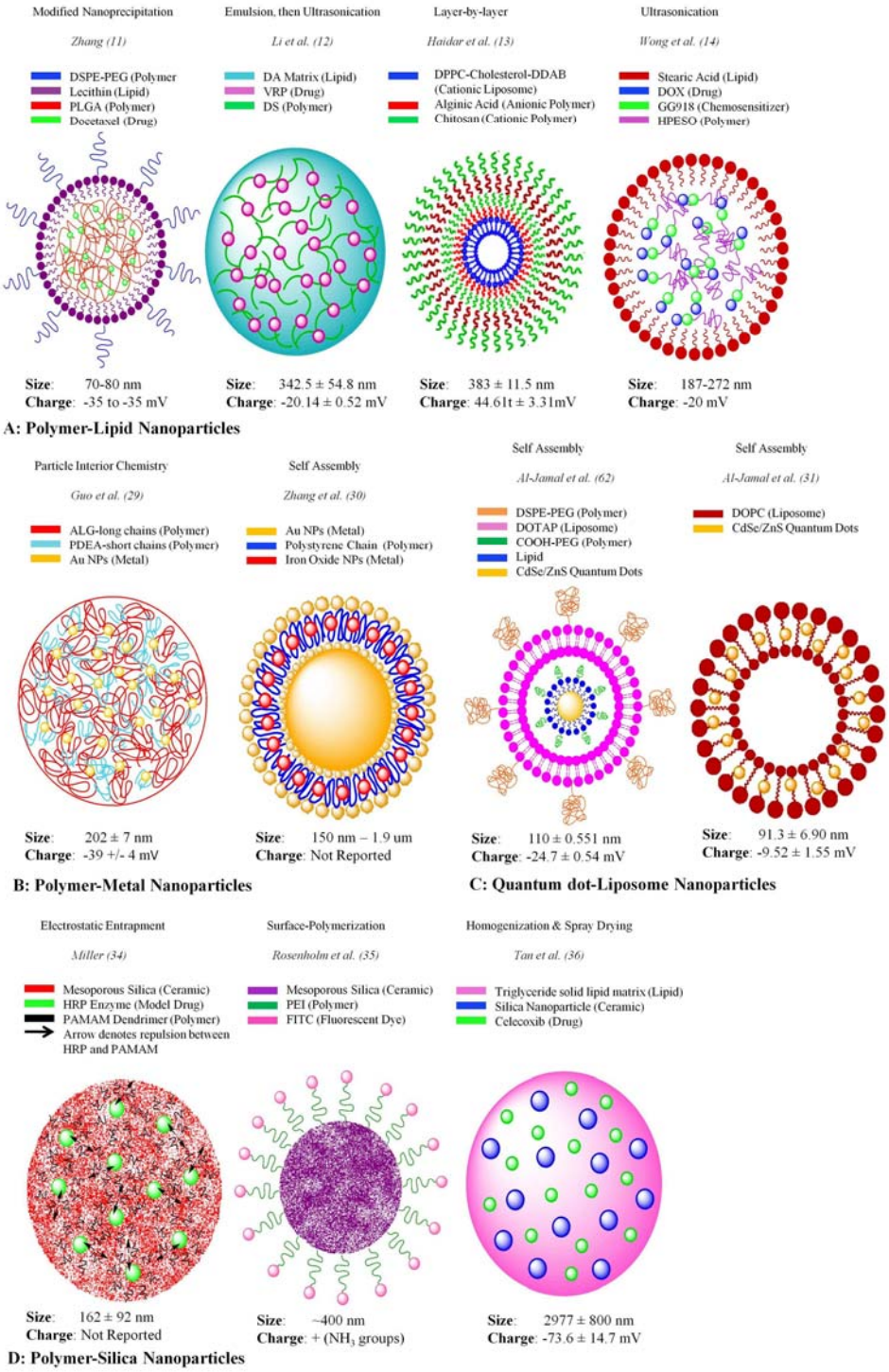

Figure 2. Synthesis and physico-chemical composition of hybrid nanoparticle designs. Hybrid nanoparticles can be broadly classified in four main groups: Polymer-lipid, Polymer-metals, Quantum dots-lipid and Polymer-silica nanoparticles. They can be synthesized by several methods including modified nanoprecipitation, ultrasonication, self-assembly, layer-by layer self assembly, and spray-drying methods. Hybrid architectures combine features from polymers, liposomes, metals and mesoporous silica. Their size ranges from nanometers to micrometers with a positive or negative charge.

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begin to aggregate, homogenous suspension becomes impossible and the system is bound for failure. For instance, aggregation is a concern when designing metal nanoparticles, which are generally prone to precipitation once critical concentrations of metal are exceeded. Papaphilippou *et al.* created polymer-metal nanoparticles through co-precipitation of Fe(III)/Fe(II) in the presence of di-block copolymers, resulting in the formation of hybrid micelles with magnetic nanoparticles enclosed in the core (51). Of note, stable micelle-entrapped iron oxide nanoparticles could be stably formed, even at high initial iron/salt concentrations, by virtue of the polymeric "coating" around the magnetic nanoparticles. Guo et al. (29) had similar results with alginic acid-enveloped gold nanoparticles, crediting the polymer with stabilizing the system. Unlike Papaphilippou et al. (51), Guo et al. found a critical concentration of gold seeding that prevented formation of stable nanoparticles. They hypothesized that above the critical gold:polymer ratio of 1:5, colloidal stability is prevented due to the protonation of the alginic acid component from the acidic $HAuCl_4$. They then proceeded to further analyze the particle, highlighting the importance of full characterization of newly designed nanoparticles in allowing a smooth transition to the clinic.

The colloidal stability and photostability of quantum dot nanoparticles have raised concern in the past, but the use of lipids can reduce such pitfalls (52-54). Lipid encasement of quantum dots has resulted in stable physiochemical properties after 7 days of storage, which is a substantial improvement from previous reports on quantum dots (31). To further provide *in vivo* stability, cholesterol has been incorporated into the lipid bilayer. Furthermore, optical stability was improved with the incorporation of the lipid bilayer in quantum dot nanoparticle design. This hybrid system renders a high fluorescent signal emission and reduces the required dose to achieve *in vivo* detection.

An approach taken to further enhance the colloidal stability of the hybrid quantum dot-lipid nanoparticles is to add polyethylene glycol (PEG) to the surface of the particles. The benefits of PEG on the surface of nanoparticles in increasing circulation time via nanoparticle stability and "stealth" are well known and widely used (55, 56). Specifically, Al-Jamal *et al.* (32, 33) reported the preparation of a "PEGylated" quantum dot-lipid hybrid nanoparticle. They show that the addition of PEG to the quantum dot-lipid nanoparticles resulted in far less turbidity and decreased aggregation, as evidenced by size stability.

In addition, to protect nanoparticles with specific components like polymers and PEG, colloidal stability can be further adjusted by manipulating charge. For example, Haidar *et al.* (27) used layer-by-layer assembly to construct hybrid liposomes with alternating alginate and chitosan surfaces (Figure 2). With the addition of each layer, the ζ-potential was seen to invert, fluctuating from approximately -40mV to +40mV. Furthermore, the hybrid particles were lyophilized with sucrose, which is thought to provide stability to the size and surface charge of the nanoparticle by acting as a spacer (57).

Fatty acid micelles are another type of nanoparticles with desirable characteristics such as the ability to encapsulate hydrophobic agents. However, dissolution of these micelles at low concentrations has posed a challenge in obtaining stable constructs. With high concentrations needed to prevent dissolution, fatty acid micelles are unlikely to translate to the clinic because intravenous administration would immediately dilute the nanocarriers to the point of instability. In order to capture the beneficial characteristics of fatty acid micelles while improving colloidal stability, Ishihara *et al.* (58) mixed fatty acid calcium salts with a diblock copolymer forming another type of lipid-polymer hybrid nanoparticles. These hybrid constructs have a core composed of fatty acids, calcium and poly(lactic acid) (PLA) and a shell composed of PEG. The stabilization of the micelle core with calcium increased the solubility of a water-insoluble drug in the fatty acid, with PEG providing greater colloidal stability as a result of steric repulsion. The addition of fatty acid calcium salts allowed Ishihara *et al.* (58) to take advantage of the unique features of the hybrid system, creating a drug carrier with high stability and a neutral charge.

Polymer-lipid hybrid nanoparticle has shown stability upon encapsulation of multiple drugs as well. As will be discussed in section 4, Wong *et al.* (28, 59) attempted to prevent multidrug resistance with the simultaneous delivery of both Doxorubicin (Dox) and GG918 in a single polymer-lipid hybrid nanoparticle to resistant breast cancer cells (28, 59). Their formulation has proven stable for encapsulation of Dox and GG918 both alone and in combination, with mean particle size and ζ-potential remaining constant regardless of drug incorporation. Similar versatility has been seen in other polymer-lipid nanoparticle designs. Li *et al.* (26) described a polymer-lipid carrier rationally crafted by characterizing solid-state properties and interactions between potential lipid carriers and the model drug Verapamil (VRP), a calcium channel blocker, combined with dextran sulfate (DS), an anionic polymer. Through the optimization process based on solubility parameters and partitioning coefficients, dodecanoic acid (DA) was identified as the best lipid carrier for the VRP-DS complex. Due to their stepwise discovery of the most advantageous lipid carrier, Li *et al.* (60) later hypothesized that the amorphous state of the VRP-DS complex in the lipid phase inhibited crystallization and increased stability.

In another class of hybrid nanoparticles, ceramic-lipid combinations take advantage of the solubility properties of lipids with the stabilizing effects of ceramic material. Using celecoxib as a model fat-soluble drug, Tan *et al.*(36) produced silica-lipid hybrid nanoparticles with stable physiochemical properties. The particles were also found to readily redisperse in aqueous solution. Linking silica to lipid drug carriers shows the potential for stable long-term storage and dependable suspension upon reconstitution. The ceramic-polymer approach can afford fine-tuning abilities as well. In a triple layered design of a core-shell-shell nanoparticle, Rosenholm *et al.* (35) constructed a hybrid carrier system utilizing the enhanced cargo protection of a mesoporous silica core, suspension

stability in a poly(ethylene imine) (PEI) shell, and further modification possibilities of bioconjugating targeting moieties to the outer surface. In addition, succinylation of the outer surface of the ceramic-polymer construct resulted in a particle with the desired charge to prevent aggregation at physiological pH.

Not only is colloidal stability crucial to creating a hybrid nanoparticle that will prove useful *in vivo*, but size stability is also important for retaining therapeutic characteristics. A great benefit of using nanoparticles for drug delivery is that their size allows for passive targeting of solid tumors due to the leaky vasculature of tumors (61). This is known as the Enhanced Permeability and Retention (EPR) effect. Fortunately, hybrid systems have been shown to have tunable sizes that are stable for long periods of time. Specifically, our group has shown that poly($_{D,L}$-lactide-co-glycolide)-lipid-poly(ethylene glycol)(PLGA-lipid-PEG)hybrid nanoparticles do not undergo size increase under physiological conditions simulated with human plasma incubation (14). In contrast, PLGA nanoparticles undergo a size increase of approximately 200 nm (from 90 nm to 300 nm) after ten minutes of incubation. Moreover, the size of the polymer-lipid nanoparticles can be tuned by varying the lipid/polymer weight ratio, polymer molecular weight, and initial polymer concentration in organic solution, providing a platform for size adjustment, while maintaining stability (43, 49). As mentioned above, Al-Jamal *et al.* (33, 62) also utilized the stabilizing effects of PEG in their quantum dot-lipid nanoparticles, which prevented size increases due to aggregation in their quantum dot-lipid nanoparticles. Size stability was also achieved through the layer-by-layer synthesis of hybrid nanoparticles reported by Haidar *et al.* (27). In this case, the particle size did not change after 12 months in room temperature solution, or after rehydration following lyophilization. Finally, Guo *et al.* (29) discovered for gold-polymer nanoparticles that even when the gold-loading level was very high, gold nanoparticles were only found in the interior of the nanospheres. The fact that the nanospheres are formed from the inside out results in a hybrid system with a size that depends on the polymer used, making another tunable system with stable size.

3.5. Drug and imaging agent encapsulation in MHNPs

Efficient drug encapsulation and controlled drug release profiles are essential characteristics for nanoparticle platforms that are envisaged to be used in therapeutic applications. In order for hybrid nanoparticles to move forward to the clinic they need to show comparable or improved performance to well-developed non-hybrid delivery nanovehicles. As of now, new hybrid constructs offer opportunities to enhance drug entrapment, retention and controlled release by utilizing different materials and synthetic approaches. To improve drug entrapment and retention, various efforts in finding new synthetic strategies have been made and depend on the nature of the core of the nanoparticles. For example, for micelles, drug encapsulation obstacles have been minimized by using physical crosslinking in the core of micelles, chemical modifications of the hydrophobic segments and blending hydrophobic polymers, lipid and different amphiphilic block copolymers (58). In addition, the nature of the encapsulated molecule plays an important role in the drug encapsulation. Many potential pharmaceuticals with high biological activity are never used clinically because of their low bioavailability and unanticipated side effects (63, 64). Hybrid nanoparticles have the potential to minimize this problem by using several heterogeneous strategies to potentially solubilize and control the biodistribution profiles of such pharmaceuticals. We will discuss some of the challenges in drug encapsulation, and how the hybrid models can possibly address them.

3.5.1. Encapsulation of drug and imaging agents

A wide range of chemotherapeutics can be incorporated into non-hybrid nanoparticles using either the "incorporation" method or the "absorption method" (4, 65). In the incorporation method the drug is incorporated during the synthesis of the nanoparticles whereas in the absorption method, once the particles are formed, the drug is added and it is absorbed by the particle during a period of incubation. Although, both methods have advantages and disadvantages for specific applications, the incorporation method it is more widely used than the absorption method.

In general, drug loading not only depends on the loading method but also on the physico-chemical characteristics of the nanoparticle constituents. In the case of polymer nanoparticles, drug encapsulation depends on the nature of the polymers (66), the type of surface-active materials and stabilizers used in the synthesis, and the physico-chemical characteristics of the encapsulated molecule (67). For instance, ionizable drugs, which are generally less hydrophobic, have been encapsulated by using several techniques: chemical conjugation with polymers, ion complex formation with polymers, and formation of complexes with metals.

In terms of hybrid systems, silica-lipid hybrid nanoparticles have demonstrated an ability to overcome the encapsulation problems of poorly soluble drugs (36). This has been achieved due to the synergy between lecithin-based emulsion systems and the stabilizer effect of the hydrophilic silica. In dendrimer silica nanocomposites, the rapid and efficient encapsulation of molecules such as enzymes has been possible using the water-soluble biomimetic template PAMAM dendrimer. In this particular case, dendrimers act as catalytic moieties that cause the condensation of silica (34).

An example of how increased encapsulation and retention of agents can be achieved by adding to the construct different carefully chosen materials is presented by Guo *et al.* (9) for polymer-metal hybrid nanoparticles—specifically alginic acid enveloping gold nanoparticles. Alginate functions as a stabilizer in reactions where noble metal ions are present and can also act as a reductant (68), while PDEA is a cationic, hydrophilic polymer and has an especially high affinity towards anionic $AuCl_4^-$ (69). Under specific conditions, ALG and PDEA form nanospheres; when aurochloric acid ($HAuCl_4$) is added, the PDEA

attracts the anionic Cl_4 ions to the core of the nanoparticle. Simultaneously, the reducing property of ALG causes the aurochloric acid to reduce into Au-NPs, ensuring 100% encapsulation of Au-NPs at the core of the nanospheres.

Other approaches to increase encapsulation involve conjugating therapeutic or imaging agent to nanoparticle core or surface. Zhang et. al. (70) report the conjugation of polystyrene-gold nanoparticle conjugates (PS-AuNPs) and polystyrene-coated ferrous oxide nanoparticles (PS-coated Fe_3O_4) using the nanoprecipitation method. The amphiphilicity of the PS-AuNPs and the hydrophobicity of the PS-coated Fe_3O_4 cause nanoparticles to form through nanoprecipitation in which the hydrophobic PS of PS-AuNPs and the PS-coated Fe_3O_4 particles form the inner nanoparticle wall, while the hydrophilic gold nanoparticles form the outer shell of the vesicles (70). While this method also causes nanoparticle formation, it is not as efficient as the Guo *et al.* (29) method in which the Au-NP formation occurs *in situ*, thus guaranteeing homogeneity of nanoparticles. Due to the homogeneity in formation of the ALG-PDEA-Au model, it is useful in cell imaging and drug tracking as the Au-NPs elicit useful photonic properties (29). Apart from this function, Guo *et al.* (29) have used Dox as a model drug to evaluate the ability of ALG-PDEA-Au hybrid NPs to delivery drugs. In their studies, it was found that these nanoparticles afford high drug encapsulation efficiency (96.5%) due to the interaction of the amino group in Dox and the carboxyl group of ALG (29). Overall, this model has been proven to have the dual-capability of diagnostic imaging/tracking as well as drug delivery. The model presented by Zhang is still in the experimental phase to assess its efficacy for therapeutic uses. Since the gold nanoparticles form the outer hydrophilic shell of this nanosystem, there are predicted uses in cancer imaging and drug delivery tracking (70).

An interesting approach to efficiently encapsulate different hydrophobic agents in a polymer envelope uses a method called flash nanoprecipitation (71). Using an engineered vortex mixer, Gindy *et al.* controlled the mixing of precursors, the nucleation time of hydrophobic agents, and the self-assembly of the polymer envelope (44). With this mixer they prepared polymeric nanoparticles that can simultaneously carry gold nanoparticles (a model imaging agent) and beta carotene(a model hydrophobic therapeutic agent) in their core (44).

Quantum dot-lipid hybrid nanoparticles is another construct in which imaging agents are encapsulated inside a lipid. Functionalized and non functionalized quantum dots have been encapsulated in the hollow part of a liposome (32, 62) and in the lipid bilayer of such vesicles (31), respectively. In the first scenario, the encapsulation efficiency depends on functionalized quantum dots to lipid molar ratio whereas in the second case such efficiency relies on the hydrophobic self-association of quantum dots with the biomembranes. The most recent development in this hybrid model, reports that encapsulated functionalized quantum dots in the hollow part of liposomes can remain in the tumor for 24 hrs (62). However, in all studies (31-33, 62), there is a consensus that biocompatibility needs to be addressed and further investigated, especially when the route of administration is intravenous. In Section 4.4 we will present a detailed discussion of the biocompatibility aspect of several hybrid nanoparticle systems.

3.5.2. Controlled release of therapeutic and imaging agents

Once the therapeutic and imaging agents are encapsulated in the hybrid architectures, their controlled release is the next challenge of the nanocarrier. Currently, diffusion and degradation are the main factors that govern drug release. In non-hybrid nanoparticles, the release rates depend upon several factors (65): 1) desorption of the surface-bound/adsorbed drug, 2) diffusion through the nanoparticle matrix, 3) diffusion (in the case of nanoparticles through the polymer wall, 4) nanoparticle matrix erosion and 5) a combined erosion/diffusion process. These factors are closely related with the formulation of the nanoparticles as well as the drug encapsulation method.

In the case of matrix-based particles, drug is uniformly distributed/dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix degradation, the mechanism of drug release occurs mainly by diffusion, otherwise it depends upon degradation or both. Some common methods to control drug release are layer-by-layer self-assembly (LbL), various emulsions, nanoprecipitation, surface polymerization, and crosslinking (27, 29, 35, 36, 42, 60, 72). Certain methods are more efficient in exhibiting controlled drug release than other methods due to their means of drug encapsulation. For example, in layer-by-layer self-assembly, several layers of different materials self-assemble onto a core thereby enabling small amounts of drug to be encapsulated in each layer (27). In this nanoparticle design moderate controlled release was achieved by loading small amounts of BSA into each layer. Furthermore, nanocarriers synthesized by the emulsion method offer longer controlled release due to the homogeneous distribution of drug during synthesis which forms a drug-laden matrix (42, 60, 72). In fact, Wong's group reports an increased level of cytotoxicity against multi-drug resistant breast cancer cells due to the systematic controlled release of Dox (28).

Finally, crosslinking has been identified as another method of reducing the burst "effect"of encapsulated agents (29, 72). The key feature in the crosslinking method is the addition of a substance to create an additional barrier to moderate drug release and prevent drug leakage. For example, in Guo's polymer-metal hybrid nanoparticle system (ALG-PDEA-Au) loaded with Dox, nanoparticles crosslinked with Ca^{2+} exhibited greater controlled released as compared to uncrosslinked nanoparticles. While uncrosslinked nanoparticles showed 30% initial burst drug release and 100% release within 24 hours, Ca^{2+} crosslinked nanoparticles only showed 15%

initial burst drug release, and 80% release within 24 hours (29).

Poorly encapsulated drugs are generally released quickly through an "initial burst," leading to problems such as uncontrollable drug targeting, and unsustainable toxicity to tumors which lead to multi-drug resistance (72). Hybrid nanoparticles engineered to exhibit controlled drug release can eliminate the problem of initial rapid bursts. For instance Zhang *et al.* (14), reported that the release of drug from polymer-lipid nanoparticles can be controlled by varying lipid to polymer ratio. In fact, these nanoparticles loaded with Docetaxel, a model hydrophobic drug, exhibited drug-release over 120 hours, with 50% released in the first 20 hours – this rate is much slower due to the lipid monolayer serving as a "nanofence" to control release and leakage of the drug from the polymeric core. In addition, Haidar et. al. (27)showed that polymer-lipid hybrid nanoparticles overcome the problem of initial burst drug release via proper encapsulation in which less than 50% of the encapsulated model protein, bovine serum albumin (BSA), was released over 30 days.

3.5.3. Drug degradation

In situ drug degradation is another important aspect to assess. Various drugs may undergo oxidative degradation as well as enzymatic degradation if not adequately protected. Certain materials such as silica enhance the stability of encapsulated drugs/biomolecules and protect them from biodegradation, mainly because of the mild conditions in which the encapsulation takes place. In addition, the formulation strategy for silica carriers plays a key role for improving encapsulation efficiency and preventing degradation. For example, Miller *et al.* (34) utilize a water-soluble biomimetic template, PAMAM dendrimers, to encapsulate enzymes. They report no degradation, and the encapsulation efficiency was improved by 20% relative to the typical 0.1-5% obtained with other methods. Rosenholm *et al.* (35) report the synthesis of hybrid PEI-mesoporous silica particles, where hyper-branched PEI is covalently linked to the silica surface. Payload stability and absence of degradation were observed using this method. Also, Guo *et al.* (29) report no degradation as well as enhanced cytotoxicity of Dox when encapsulated within hybrid ALG-PDEA-Au nanoparticles as compared to free Dox. Finally, Haidar *et al.* (27) report a method that involves dual-polymeric complexation and ionic interaction to increase drug stability. Specifically, they used alginate complexed to chitosan to prevent BSA degradation. Through this method, the ionic interactions between amine groups in chitosan and carboxyl groups in alginate demonstrated enhanced stability of BSA against degradation (27).

Drug encapsulation, drug release, and drug degradation are extremely important in evaluating the efficacy of the therapeutic effect of the hybrid nanoparticle systems. We believe that novel strategies to improve stability, drug encapsulation and drug release, and to reduce drug degradation are some of the major potential contributions of the hybrid systems in the drug delivery field.

4. MEDICAL APPLICATIONS OF HYBRID NANOPARTICLE PLATFORMS

4.1. Introduction to medical applications of MHNPs

Since their inception, multifunctional nanoparticles have been hypothesized to become efficacious cancer therapeutics due to their physical and chemical properties. The size and multimodal functionalization of the nanoparticles allows one to take advantage of passive and active targeting of solid tumors. To date, multi-drug resistance is the major barrier for effective cancer treatment using multifunctional nanoparticles. The following sections are devoted to the most important clinical aspects that must be considered when designing new hybrid multifunctional nanoparticle systems: multidrug resistance in solid tumors and blood-compatibility. The latter includes a discussion of the protein binding adsorption phenomenon at the nano-interface of hybrid nanoparticles.

4.2. Multidrug resistance: a challenge to overcome

One of the greatest challenges in treating solid tumors is multidrug resistance, which refers to a tumor's lack of response to multiple structurally unrelated drugs (73, 74). Most solid tumors are either resistant to drugs at the start of chemotherapy, or gain intrinsic and acquired resistance during administration (75). Intrinsic resistance occurs as a result of the tumor microenvironment applying survival and selective pressures on the cancer cells. Conversely, acquired resistance is thought to be a result of inadequate drug concentration, brief exposure to therapeutic agents, poor diffusion and penetration in the interstitial space of the tumor, and selective pressures provided by the drugs (76). Areas of hypoxia, nutrient deprivation, and repeated insufficient toxic insults apply selective and survival pressures on the cancer cells resulting in deregulated expression of numerous genes. Five major mechanisms of drug resistance initiated by these cellular stresses include increased drug efflux, decreased drug influx, upregulation of DNA repair pathways, apoptotic inhibition, and increased drug metabolism. Although it is possible to shrink adult solid tumors, chemotherapy rarely provides a cure and is of little effectiveness due to multidrug resistance. As a last resort, anticancer agents are often combined and doses are increased. Toxic side effects ensue and chemotherapy courses are interrupted because of intensified therapeutic regimens, contributing to and exacerbating multidrug resistance (74). New nanotechnology approaches, specifically the use of multifunctional hybrid nanoparticles, seem to be promising tools to help reduce these problems, as will be discussed in the following section.

4.3. Utilizing MHNPs to combat multidrug resistance

The failure of current chemotherapeutic strategies due to multidrug resistant cancer has led to a search for alternative drug delivery approaches. The implementation of nanocarriers to encapsulate and deliver common chemotherapeutic agents has shown encouraging

results. Hybrid nanocarriers have the potential to overcome some of the obstacles needed to defeat multidrug resistance. Specifically, by adding different functionalities to a single construct one can "tackle" a problem from "different angles" at the same time. In the next paragraphs we discuss some of the early attempts to accomplish this goal.

One of the most prominent and well-characterized mechanisms of the multidrug resistance phenotype is an increase in drug efflux caused by the ATP-Binding Casette (77) transporters. P-glycoprotein (P-gp) is the most extensively studied member of the ABC-transporters and has become a popular target for combating multidrug resistance as it actively expels many substrates, including multiple cytotoxic drugs. Using a polymer-lipid hybrid nanoparticle (PLN) system to encapsulate Doxorubicin (Dox), Wong *et al.* (42) showed increased efficacy against multidrug resistant breast cancer cells overexpressing P-gp. Complexing Dox to the anionic polymer dextran sulfate allowed for increased loading of a water-soluble drug into a lipid nanoparticle formulation, as well as efficient release with a gradual liberation of 60% of the drug within the first 16 hours.

The Dox-PLN system achieved greater drug retention within the multidrug resistant P-gp overexpressing cells as well as up to 8-fold increases in cell death rates. Wong *et al.* (42) further demonstrated that these effects were a result of drug encapsulation within the hybrid system and not from the PLN itself, as free Dox with empty PLN administration resulted in no benefit over free Dox alone. These findings are encouraging as Dox is a known substrate of P-gp and the hybrid delivery system is able to circumvent drug efflux.

Knowing that the enhanced anticancer activity of their Dox-PLN complex was not due to the inactive components of the nanoparticles, Wong *et al.* (42) hypothesized that the increased efficacy was due to more efficient delivery of Dox by the hybrid system. This spurred the group to perform a mechanistic study of cellular Dox delivery with Dox-PLNs. Endocytosis inhibition experiments revealed that phagocytosis was a major Dox-PLN uptake pathway, which may allow the drug to physically bypass P-gp. Furthermore, the group discovered that the hybrid nanoparticles greatly improved drug delivery to the nucleus (59). Many chemotherapeutic agents, including Dox, require nuclear localization to exert their cytotoxic effects, and these findings suggest that hybrid nanoparticles are a platform that can achieve delivery to the proper target. In addition, Wong *et al.* (59). have exhibited a novel pathway to overcome drug efflux by P-gp overexpression. In the past, it has been shown that the polymer or lipid itself conferred the greater cytotoxicity in multidrug resistant cells by directly inhibiting the P-gp transporter (78) (79-84). More specifically, the Brij 78 surfactant used in liposomal particles inhibits P-gp and deplete ATP levels alone (85). However, the phagocytic bypass of the drug efflux pathway by Wong *et al.* (59) may be promising, as simply inhibiting the transporter with the lipid or polymer carrier could result in further overexpression of the transporter. Moreover, the Dox-PLN system may result in intracellular stores of drug complexed with nanoparticulate carriers that are unable to be transported by P-gp, and can continually discharge anticancer activity and prevent multidrug resistant clonal expansion.

An alternative approach to potentially overcoming multidrug resistance has been combinatorial therapy. Simply administering P-gp inhibitors, or "chemosensitizers", with chemotherapies in solution has proven disappointing in the clinic due to increased toxicity from the inhibitors and/or altered biodistribution from co-administration of the two drugs (86, 87). Recognizing the pitfalls inherent to administration of two toxic substances in solution, there have been numerous studies investigating combinatorial therapies delivered with nanoparticles. Delivery of P-gp inhibitors with anticancer drugs in nanocarriers has been a popular strategy. Wong *et al.* (28) co-encapsulated GG918, a chemosensitizer, with Dox in a PLN complex, which they termed (DG)n, for treatment of multidrug resistant breast cancer cells. The hybrid system allowed encapsulation of both the water-soluble Dox and the lipophilic GG918 while retaining the beneficial properties of the single-drug loaded Dox-PLN discussed above. This demonstrates the versatility of hybrid nanocarriers, as any combination of drugs could theoretically be formed for delivery despite differing solubility requirements. The Dox-chemosensitizer combined hybrid nanoparticle validated their hypotheses by providing significantly improved drug retention and cellular toxicity when compared to all other treatments, including the previously studied single-loaded Dox-polymer-lipid nanoparticles. Interestingly, co-encapsulated nanoparticles produced more effective drug retention than co-administration of Dox and GG918 single-loaded nanoparticles. Wong *et al.* (28) believe this effect may be due to the intimate spatial relationship obtained when co-delivered in the same nanoparticle, as the enhancement of the anticancer drug is highly dependent on the simultaneous presence of the chemosensitizer (28).

In addition to using anticancer drugs synchronously with drug efflux inhibitors, other groups have attacked different crutches of the multidrug resistant phenotype akin to Saad *et al.* (88). Antiangiogenic therapy for cancer has gained popularity, but its full potential has yet to be realized. Complications with the timing of antiangiogenic treatment arise as chemotherapies cannot be adequately delivered to regions of tumor that have lost blood supply. A novel polymer-lipid nanoparticle was developed by Sengupta *et al.* (89). to combat this predicament. An anti-angiogenesis agent, Combrestatin, was entrapped in a lipid envelope that surrounded the polymeric nanoparticle conjugated to the chemotherapeutic agent Dox. In theory, the anti-angiogenic agent can be released first, shutting down the tumor's blood supply and trapping the anticancer nanoparticles inside to destroy the mass. The Dox and Combrestatin hybrid nanoparticle showed decreased lung carcinoma and melanoma tumor volumes *in vivo* when compared to PBS, single-loaded Dox nanoparticles, single-loaded Combrestatin liposomes, co-

administration of the single loaded particles, or a simple Dox and Combrestatin co-encapsulated liposome. Furthermore, the hybrid combination system had the least systemic toxicity owing to the targeted release of Dox once it was trapped inside the tumor by anti-angiogenic therapy (88).

4.4. Blood-compatibility of MHNPs

The biocompatibility profile of hybrid nanoparticles becomes a critical requisite for clinical application. The term biocompatibility is used to describe the ability of a material to interact with an appropriate host response in a specific application without causing damage or adverse reactions (90). The biocompatibility aspect of any nanomaterial must be studied in human organs, tissues, cells, and biochemical systems. To date, the biocompatibility of hybrid and non-hybrid nanoparticle systems has been assessed mainly by cellular based assays (29), (51), (49), (58-59). The Methylthiazol Tetrazolium Assay (MTT) is the most common method used to assess cellular toxicity. However, cellular assays do not give any information on blood compatibility which involves the assessment of the biocompatibility aspect of human serum or plasma with a biomaterial. To our knowledge, only one paper has investigated the biocompatibility of polymer-lipid hybrid nanoparticles in human serum and plasma (91). More research in this area is needed to use novel hybrid nanoparticles in biomedical applications. The majority of medical devices make contact with blood either upon implantation or when functioning. Some examples of blood-contact applications are blood plumps, oxygenerators, and hemodialyzers. Stents, vascular grafts, miniature pumps, sensors, pacemakers, and heart valves are permanently in contact with blood. Hybrid nanoparticles envisaged to form a building block for these types of devices will sooner or later interact with blood and there is a great risk that a synthetic material will provoke an undesirable response through complement activation. This section discusses the implications of the complement system in the biocompatibility of several hybrid systems.

4.4.1. The complement system cascade

The assessment of complement system activation is of great relevance to intravenous administration of nanocarriers because such activation can generate hypersensitivity reactions, also referred to as infusion reactions (92-94). A substantial percentage (11-25%) of patients treated intravenously with nanomaterials such as liposomes have shown hypersensitivity reactions (95). Cardiovascular distress, which includes tachypnea, tachycardia, hypotension, hypertension, chest pain and back pain are among the most common reactions (95).

The complement system is a group of approximately 35 soluble and cell-surface proteins in blood that interact to recognize, opsonize, and clear or kill invading microorganisms, altered host cells (e.g., apoptotic or necrotic cells), and other foreign materials (96). Thus, the major role of the complement system is to recognize and promote clearance (by phagocytosis) of foreign materials and damaged or altered host cells. The complement system is activated by a wide range of materials including immune complexes and non-immunoglobulin activators such as gram-positive and gram-negative bacteria, viruses, virus-infected cells, yeasts, fungi, and by-products of tissue damage. It can also be activated by synthetic materials such as polystyrene (97). Activation may occur by any of 3 pathways: the classical, lectin, and alternative pathways.

Recently, our group reported the immunocompatibility properties of PLGA-Lecithin-PEG nanoparticles functionalized with amine, carboxyl and methoxyl functional groups (91). A major contribution of this work was to demonstrate the modulation of the complement system through several functional groups. These nanoparticles can be less immunogenic or more immunogenic depending on the functional groups on the surface of the nanoparticle. PLGA-Lecithin-PEG-OCH_3 nanoparticles show negligible levels of complement activation, while the PLGA-Lecithin-PEG-NH_2 nanoparticles induce the highest complement activation among carboxylic and methoxyl functional groups. None of these nanoparticles activated the coagulation cascade. In general, PLGA-Lecithin-PEG nanoparticles functionalized with these three functional groups present good biocompatibility profiles in comparison to zymosan, a well known activator of the complement system.

Among the category of polymer-metal hybrid systems, gold nanoparticles deserve close examination and discussion of their biocompatibility properties due to the increasing number of publications in the literature, and their potential applications in catalysis and biosensoring areas. Few papers report the biocompatibility properties of gold nanoparticles by assessing their ability to activate the complement system. Recently, Dobrovolskaia *et al.* (98) have reported the inactivation of the complement system and coagulation cascade by different sizes of gold nanoparticles. Surprisingly, 30 nm and 50 nm non-pegylated gold nanoparticles do not activate the complement system cascade. On the contrary, Hulander *et al.* (99) have recently reported that gold metal activates both the complement system and coagulation cascades. The discrepancy of these findings might encourage the evaluation of the complement system and coagulation cascade via complementary and alternative methods.

Complementary techniques for such assessments are particularly important for hybrid systems that involve the pH dependent reversible aggregation of gold nanoparticles (100). Regardless of the fact that encapsulated gold nanoparticles in polymeric nanoparticles seem harmless, engineering gold nanoparticles that do not remain in the body for a long period of time is essential to prevent adverse immune responses such as the formation of granulomas. In addition, it is worth keeping in mind that metal with high atomic numbers deposited in organs would interfere with medical treatments such as positron emission tomography (PET) and computed tomography (101).

Similarly, the biocompatibility of other hybrid systems remains to be elucidated. This is the case of hybrid nanoparticles that utilize silica. Recently, *in vivo* studies

conducted by Hudson *et al.* (102) report considerable systemic toxicity in rats of non-functionalized mesoporous silicates of particles sizes between 150 nm and 4000 nm, but benign local biocompatibility. To our knowledge this is the only paper in this area that reports *in vitro* and *in vivo* toxicity studies. Most of the silica hybrid nanoparticles (35) report only cellular toxicity. However, cellular toxicity is insufficient to assess the biocompatibility performance of new hybrid nanoparticle designs as mentioned above, especially when the designs involve key functional groups that are known to activate the complement system. This is the case of mesoporous silica nanoparticles functionalized by surface hyperbranching polymerization of poly(ethylene imine)(PEI) (35). These nanoparticles are decorated with dendrimers which have a great number of amine functional groups which are known to activate the alternative pathway as reported by Salvador-Morales *et al.* (91) and others (103). Other dendrimer silica nanocomposite and silica-lipid hybrid microcapsules did not report any toxicity studies (34, 36).

The toxicity of quantum dot-lipid nanoparticles has not been addressed in depth. One of the major concerns of these hybrid systems is the clearance of functionalized or non-functionalized quantum dots as intact nanoparticles after *in vivo* performance. Although renal filtration and urinary excretion of inorganic and metal-containing nanoparticles have been reported (104), there are still concerns. In the particular case of quantum dots, renal filtration will occur whenever the quantum dots are in the range of 3- 5 nm in size (104). The surface charge and size of the quantum dots are main determinants of renal clearance (104). In current quantum dot-lipid hybrid systems, the route of clearance still unknown. These vesicles have sizes of 100 nm or more. Wafa *et al.* (32) have shown that the size and charge of the nanoparticles determine the vesicles'half-life in the human body as well as tissue biodistribution profile after intravenous administration. It is not surprising that cationic functionalized quantum dot-lipid nanoparticles showed faster blood clearance compared to zwitterionic systems, as it is likely that the complement system was activated via the alternative pathway.

4.4.2. The protein binding adsorption phenomenon

The adsorption of protein on hybrid nanoparticles is also of relevance to the biocompatibility of nanomaterials, as all proteins possess an inherent tendency to deposit very rapidly on surfaces. The protein binds tightly with the biomaterial surface, which strongly influences subsequent interactions of many different cell types with the surface. Due to this effect, inert materials interacting with plasma proteins may become thrombogenic and unsuitable for the design of blood contact devices. The adsorbed proteins can initiate thrombosis on the foreign surfaces via platelet interactions or intrinsic clotting cascade proteins (105). The sensitivity of platelet-surface interactions to adsorbed proteins is fundamentally due to the presence of adhesion receptors in the platelet membrane that bind to certain plasma proteins (105). There are only a few proteins in plasma that bind to platelet adhesion receptors. The selective adsorption of these proteins to synthetic materials, in competition with the many non-adhesive proteins that also tend to adsorb, is believed to mediate platelet adhesion to these surfaces (106). In addition, the adsorption of plasma proteins on the surface of the nanoparticles can influence cellular uptake, organ accumulation, and route of clearance.

4.4.3. Protein binding adsorption on multifunctional hybrid interface

To date, the focus of multifunctional hybrid nanoparticles has largely been on their physical and chemical properties due to the early stages of development. However, understanding the interaction of the hybrid systems with the immune system will be important in the next few years due to its relevance in clinical settings.

The adsorption of biomolecules, including proteins, enzymes, antibodies, aptamers, etc. on the surface of hybrid nanoparticles depends on properties such as size, shape, surface area, surface charge, energy, roughness, porosity, valency, conductance states, functional groups, ligands, crystallinity, defects, hydrophobicity, and hydrophilicity of the nanomaterial as reported by Net *et al.* (107). Particularly, size, surface charge and hydrophobicity of a nanomaterial will have the strongest effect on their biocompatibility and toxicity (107). The influence of human serum and plasma proteins on the surface of metal hybrid systems is significant, as it can determine the fate of these nanoparticles in the blood stream. For instance, when quantum dots of about 5 nm are coated with serum proteins, their 4 nm size increase prevents them from being removed from the body via renal filtration (104). Preventing the coating of nanoparticles with human serum and plasma proteins can be achieved by inserting units of PEG on the surface of the nanoparticles. However, while dense PEGylation may increase blood half-life, it also may reduce elimination from the body (104). This strategy has been widely used for polymer nanoparticles, and it has been particularly effective when the polymers are biodegradable. Recently, Salvador Morales *et al.* (91) have reported the binding of human serum and plasma proteins on the surface on PEGylated functionalized polymer-lipid hybrid nanoparticles. These nanoparticles were modified with methoxyl, carboxyl and amine functional groups. Due to the chemical composition of the functional groups, differences in the protein binding profile of the nanoparticles were observed. Interestingly, PLGA-Lecithin-PEG-amine nanoparticles bind to the key complement regulator proteins, Factor H and C3b. The manipulation of these functional groups allows greater control of the complement system by the sequestration of factor H and C3b. The protein binding adsorption of other polymer-lipid nanoparticle systems, such as liposome cores with alternating layers of alginic acid polymer and chitosan polymer can compromise their biocompatibility (27) because the cationic polymer nature of chitosan can potentially activate the complement system via both the classical and alternative pathways.

For hybrid mesoporous nanoparticles, the protein binding adsorption phenomenon has not been addressed in

depth, although there are some reports about protein/enzyme binding on the mesoporous material (108). These studies have concluded that the immobilization of proteins and enzymes on mesoporous silica will vary depending on several factors, including pH value, ionic strength, pore diameter, pore volume, surface characteristics, isoelectric point, morphology, particle size, and adsorption conditions. As the use of functionalized mesoporous material becomes ubiquitous in several applications, it is important to note that such functionalization is capable of altering isoelectric point. This will lead to the alteration of electrostatic interactions with proteins or other biomolecules. Silica hybrid nanoparticles also raise increased concern with regard to biocompatibility and toxicity, since other foreign constituents might activate the complement system. Illustrating this point, dendrimer-silica hybrid nanoparticles activate the complement system not only through their functionalized surface groups, but also through their physical structure (35).

5. PHARMACEUTICAL PERSPECTIVE OF MHNPs

5.1. Opportunities and challenges

To date, heart disease, malignant neoplasms (cancer) and cerebrovascular disease are among the ten leading causes of death in United States. Hybrid and non-hybrid multifunctional nanoparticles represent promising tools for the effective treatment of these diseases, particularly for the treatment of several types of cancers. The small size, biodegradable nature, and multi-targeting platform are some of the characteristics of nanoparticles that are urgently needed in several medical areas, such as drug delivery, imaging, tissue engineering, and gene therapy. Nevertheless, from a commercial perspective, these novel emerging technologies may face several challenges. In the case of hybrid systems, their complexity could be one of the primary challenges because they are formed from multiple materials. This means that several types of technology will be needed to convert hybrid nanoparticle platforms into feasible technologies. Another challenge that may emerge is large-scale production of the hybrid systems. Hybrid nanoparticles prepared on a small-scale in a laboratory setting may be difficult to prepared reproducibly with the same physicochemical properties in a large-scale industrial setting—primarily due to the complexity of the particles and multiplicity in synthetic steps. Biomedical applications could require milligram or gram quantities, apart from demanding excellent batch reproducibility, and hybrid systems must comply with this requirement. In addition, from an investment standpoint, multiple synthetic steps are unattractive because they usually increase manufacturing costs. Another important aspect of hybrid systems is their potential hazardous effect on the human body. Today, biocompatibility is one the major barriers to reaching clinical trials for multifunctional non-hybrid systems; however, these concerns might be amplified for hybrid systems. For example, targeting moieties on the surface, core, and intercalated layers of nanoparticles might have individual and synergistic toxic effects. Among the several chemical features involved in the synthesis of hybrid nanoparticles, charge is a key element and plays a pivotal role for the manipulation of the biocompatibility and toxicity profile of these nanomaterials.

Furthermore, adoption of emerging technologies may create a marketing barrier for hybrid nanoparticle systems, as the end-users might be hesitant to use new technology. Competition derived from emerging technologies and the crowded intellectual property landscape should not be ignored, especially for the use of hybrid nanoparticle models in the medical field where intellectual property of third parties will most likely be required.

6. CONCLUSIONS AND PERSPECTIVES

There is special interest on the future of hybrid nanoparticles as platforms that bring potentially unforeseen benefits to the medical field, and in particular to cancer therapy. Here, we present a snapshot of some of the early technologies in hybrid constructs that have the potential to further advance several medical fields including drug delivery and imaging. Some of these hybrid constructs include polymer-lipid, silica-metal, polymer-metal, and lipid-quantum dot nanoparticles, among others. With a variety of synthesis approaches and material choices, hybrid nanoparticles provide opportunities to overcome medical challenges such as blood compatibility and multi-drug resistance. However, several limitations exist, including the complexity of technology and regulatory issues. We believe that the present limitations in the synthesis of hybrid nanoparticles can be overcome to create robust systems with effective multi-functionality for the treatment of several diseases.

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