CANCER GENOMICS & PROTEOMICS *3*: 147-158 (2006)

Review

Design of Nanoparticles as Drug Carriers for Cancer Therapy

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Abstract. *This review explores the recent chemotherapeutic work on drug delivery using nanoparticles as carriers for the targeted treatment of cancer. Compared to direct drug delivery, delivery through a carrier can increase the efficacy of a drug, but decrease the side-effects by utilizing the enhanced permeability and retention (EPR) effect and tumor-specific targeting. The search for efficient and safe transport vehicles (carriers) to achieve better drug availability at the target site has been a challenging yet exciting area of research. Current interest focuses on the colloidal nanoparticles (diameter <500 nm), including the biodegradable polymer- and liposome-systems, bioconjugating with antitumor drugs. These biocompatible nanoparticles, with an enlarged surface area-volume ratio can overcome non-cellular and cellular-based mechanisms of resistance and increase the selectivity of drugs towards cancer cells, while reducing their toxicity towards normal tissues. This review focuses on the evolution of nanoparticles as carriers for anticancer drug delivery, with emphasis on the biocompatible magnetic nanohybrids.*

Cancer is caused by the uncontrolled growth and spread of abnormal cells. It is estimated that approximately 11 million people were diagnosed with cancer worldwide in 2003 and an additional 1.3 million new cases were diagnosed in 2004 (1). More than half a million deaths are caused annually by cancer, *i.e.*, one in every four deaths. The overall costs for cancer treatment in the United States in 2004 were approximately $190 billion (2).

Effective treatments include surgery, radiotherapy, chemotherapy, hormone therapy and immunotherapy. Each of these treatment modalities has advantages and disadvantages and a combination is usually needed to produce the most effective results. Because most human cancers (>85%) are related to solid tumors, current cancer therapy usually involves intrusive processes including the application of catheters for chemotherapy, with initial chemotherapy to shrink any cancer present, surgery to remove the tumor(s) if possible, followed by chemotherapy and radiation to kill the tumor cells. Research efforts to improve chemotherapy over the past 25 years have led to an improvement in patient survival. However, the efficacy of the therapy and the possible side-effects vary among different agents. Some drugs may have excellent efficacy, but also serious side-effects affecting the quality of life. In addition, they may be in limited supply and, therefore, very expensive. More effective and less expensive anticancer drugs have been under development. A typical example is dEpoB, which was synthesized based on the mechanism of action of paclitaxel and is reportedly 30 times more effective than paclitaxel (3). However, it normally takes at least 10 years and billions of dollars to discover a new drug. The development of new anticancer drug delivery systems or new administration schedules offer less expensive, but more effective, treatment with negligible side-effects.

Usually, a pharmaceutical agent will distribute evenly within the body. However, ideal chemotherapeutics require a high local concentration of the drug at the disease site(s), while the concentration in other non-target organs and tissues should be below a certain minimal level to prevent any negative side-effects. The concept of drug targeting, also called the "magic bullet", comes from the experience of the 19th century German chemist, Paul Ehrlich, who selectively stained bacteria for histological examination. The "magic bullet" as an entity comprises two components: one should recognize and bind the target, while the other should provide a therapeutic action in this target. In the clinical setting, anticancer payloads, such as radionucleotides, toxins and

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Key Words: Drug delivery, cancer chemotherapy, nanoparticles, polymer- and liposome-systems, magnetic nanohybrids, review.

chemotherapeutic agents, have been delivered to tumors through monoclonal antibodies (4).

Different pharmaceutical carriers, including soluble polymers, microcapsules, microparticles, cells, cell ghosts, lipoproteins, liposomes and micelles, have been recently used to multiply the number of drug molecules per single targeting. All of them can assist targeting in one way or another (5-7). For instance, encapsulating anticancer drugs in liposomes enables targeted drug delivery to tumor tissues and prevents damage to the normal surrounding tissues. In addition, anticancer drugs bound with magnetic beads have been injected into the arterial circulation and guided to the tumor by a magnetic field for targeted drug delivery. There has been intensive research into the development of biodegradable and biocompatible nanoparticles (diameter <100 nm) as effective drug delivery systems, especially for chemotherapy and gene delivery. In passive targeting, liposomes, or macromolecular and nanoparticle carriers exploit the enhanced permeability and retention (EPR) effect, which is a consequence of the increased vasculature and decreased lymphatic function of tumors, to target the drug to the tumor. Pharmaceutical carriers, therefore, provide an approach which is more time- and cost-effective than new drug development (8, 9). Progress in nanoparticle technology, cellular and molecular physiology and pathology have contributed to advancements in chemotherapy and the gene therapy of cancer and other diseases, hopefully avoiding the near-toxic doses of non-specific agents.

This review primarily addresses new methods for delivering therapies with a focus on nanoparticle (NP) formulations and others that specifically target tumors. The growth of tumor tissues and the abnormal structure of tumors in comparison to normal tissues are overviewed. The challenge of efficiently transporting drugs to tumor tissues is discussed, as are the advantages of a strategy using NPs as carriers in drug delivery. The interaction between biomolecules and NPs of different types (*i.e.*, organic and inorganic NPs) and the mechanisms of transport and release of the nanoparticles in tumors are discussed. Finally, current research into drug delivery using hybrid magnetic NPs is summarized.

Tumor Structures and Properties

A tumor consists of some normal tissue plus cancer cells that are descendants of one cell. The single cancer cell surrounded by healthy tissue replicates at a higher rate than healthy cells, placing a strain on the nutrient supply and elimination of metabolic waste products. Tumor cells require the normal cell building blocks, such as oxygen, glucose and amino acids for division and progress to malignancy, while apoptosis is typically not initiated in a low nutrient environment. Once a small tumor mass has formed, the healthy tissue is not able to compete with the cancer cells for the nutrient supply from the blood stream. In addition, tumor cells displace healthy cells until the tumor reaches a diffusion-limited maximal size. There is often a steady state tumor size at the beginning of a tumor's growth when the rate of proliferation is equal to the rate of cell death. This diffusion-limited maximal size of most tumors is around 2 mm^3 (10, 11). To grow beyond this size, the tumor must recruit the formation of blood vessels to provide the nutrients necessary to fuel its continued expansion. Angiogenesis is a vital process to the continued development of a tumor mass. During angiogenesis, new blood vessels, sprouting from mature blood vessels in the surrounding normal tissues, grow towards the tumor cells. Tumor cells are capable of secreting molecules that initiate the angiogenic process, in which an imbalance of positive to negative regulators cause endothelial cell proliferation and migration. These endothelial cells form a vessel which extends towards the tumor and provides nutrients to sustain cell proliferation. A fully vascularized tumor is capable of continued growth with metastatic potential due to its proximity to the blood stream. The exact molecular mechanisms that initiate angiogenesis at a tumor site are not known and could be unique to the site of origin, but more information about what factors play a role is being discovered. Most research is focused on developing treatments to slow angiogenesis and limit tumor growth and dissemination.

The tumor blood supply plays a key role in the delivery of therapeutic agents to solid tumors. The tumor vasculature differs, both functionally and morphologically, from the vasculature in normal tissues. Tumor blood vessels are generally more heterogeneous in distribution, larger in size and more permeable. For instance, Yamaura and Sato reported that the volume of the blood vasculature in a transplanted rat hepatoma tumor was at least twice that of the normal subcutaneous tissues (12). Their study also showed the near absence of vessels in the necrotic region of the tumor. It have been reported that the microvessels in rat colon tumors showed larger capillary diameters (5–20 *vs.* 5–8 μm) and venules (15–70 *vs.* 12–50 μm) in comparison to normal colon tissues (13). The resulting enhanced permeability of the tumor vasculature is thought to be regulated by various mediators, such as vascular endothelium growth factor (VEGF), bradykinin, nitric oxide, prostaglandins and matrix metalloproteinases (14). The abnormalities of tumor blood vessels also include a high proportion of proliferating endothelial cells, a deficiency in pericytes, an aberrant basement membrane formation and hyperpermeability (15-17). Compared to normal tissues, tumor tissues show similar arterial pressure but a lower venous pressure (18). Most blood vessels in the internal regions of a tumor are veins or venuoles, whereas the peripheral regions of tumors have a

few arteries and/or arterioles. The arteriolevenuole pressure difference as a driving force for blood flow is negligible in the central region of a tumor, but is greater in the periphery. Tumor cells at the outer edge of a mass have the best access to nutrients, while cells on the inside die, creating a necrotic core within tumors that relies on diffusion to deliver nutrients and eliminate waste products. This explains, in part, the heterogeneous blood flow within a solid tumor; blood flow is lower in the center but higher in the periphery of the tumor relative to the blood flow in the surrounding normal tissues.

Transporting Anticancer Drugs into Tumors

Generally, anticancer drugs can be delivered to cells in solid tumors by transport within a vessel, *e.g.*, blood circulation. Other alternative processes include transport across the vasculature walls into surrounding tissues and transport through the interstitial space within a tumor. Macromolecular transport across tumor microvessels can occur *via* interendothelial junctions and transendothelial channels, vesicular vacuolar organelles and fenestrations. In addition, the major pathway of drug transport across the tumor microvascular wall is by extravasation *via* diffusion and/or convection through the discontinuous endothelial junctions. Furthermore, the tumor interstitial compartment is predominantly composed of a collagen and elastic fiber network (19). Interdispersed within this cross-linked structure are the interstitial fluid and macromolecular constituents (hyaluronate and proteoglycans), which form a hydrophilic gel. The interstitium, unlike most normal tissues, is also characterized by a high interstitial pressure leading to an outward convective interstitial fluid flow, as well as the absence of an anatomically well-defined functioning lymphatic network. Hence, the transport of an anticancer drug in the interstitium will be governed by the physiological (*i.e.*, pressure) and physicochemical (*i.e.*, composition, structure, charge) properties of the interstitium and by the physicochemical properties of the molecule (size, configuration, charge, hydrophobicity) itself.

On the other hand, cellular mechanisms such as alterations of specific enzyme activities or apoptosis regulation, and transport-based mechanisms such as the P-glycoprotein-related multidrug resistance (MDR) may also contribute to the resistance of tumors to therapeutic drugs. MDR is mainly due to the overexpression of the plasma membrane P-glycoprotein (Pgp), which is capable of extruding various, generally positively-charged, xenobiotics, including some anticancer drugs, out of the cell. Thus, to deliver therapeutic agents to tumor cells *in vivo*, the drug resistance problem must be solved at the vascular, interstitial and cellular levels (20).

Meanwhile, a major obstacle for successful chemotherapy is the resistance of cancer cells to effective anticancer drugs and the destructive action of these drugs on normal cells, tissues and organs. For example, the commonly used anticancer drugs, including paclitaxel, doxorubicin, fluorouracil (5-FU), cisplatin and Tamoxifen, are toxic to both tumor and normal cells, so the efficacy of chemotherapy is often limited by severe side-effects (21).

In summary, the problems frequently occurring with many drugs include: i) poor solubility; ii) insufficient *in vitro* stability (shelf-life); iii) low bioavailability; iv) short *in vivo* stability (half-life); v) strong side-effects; vi) regulatory issue/hurdles; and vii) lack of large scale production. These problems may preclude the clinical use of certain drugs. To overcome the problems, a biotechnologically-produced drug needs to be combined with a smart drug delivery system and/or delivery technology to make it applicable for the treatment of patients. The poor solubility of certain drugs makes an appropriate delivery system necessary, while undesirable side-effects require the technology for site-specific delivery (*i.e.*, drug targeting). In addition, one of the main challenges in chemotherapy is the dosage, which for the most effective schedule is determined by the toxicity of the anticancer drugs used. Therefore smart delivery systems are required.

Generally, two approaches have been used for anticancer drug delivery, namely affinity targeting and passive targeting. The affinity targeting approach attempts to take advantage of overexpressed tumor-associated antigens or receptors to selectively target the drug and an affinity-targeting carrier such as a peptide, antibody, or antibody fragment. In passive targeting, liposomes, NPs or macromelecular carriers exploit the EPR effect, which is a consequence of the increased vasculature permeability and decreased lymphatic function of tumors, to target the drug to the tumor (22).

As discussed above, the tumor blood supply plays an important role in the delivery of therapeutic agents to solid tumors. The differences in vascular permeability between tumor and normal tissues partly explain passive tumor targeting, *i.e.*, the tumor-selective delivery of macromolecules such as liposomes and drug-conjugated high molecular-weight polymers. The approach for passive targeting and target-specific carriers in blood vessels is illustrated in Figure 1; incoporation of the drug into a particular carrier can protect it against degradation *in vitro* and *in vivo*. Once the drug carrier has concentrated at the target, the drug can be released either *via* enzymatic activity or changes in physiological conditions, such as pH or temperature, and be taken up by the tumor cells. One of the unique features of tumor microvessels is their leakiness as a result of the discontinuity of the endothelium, which facilitates the extravasation of the carrier ingredient and, subsequently, the release of the medication (23, 24). Particles, such as micelles and liposomes, ranging from 10 to 500 nm in size, can extravasate and accumulate inside the

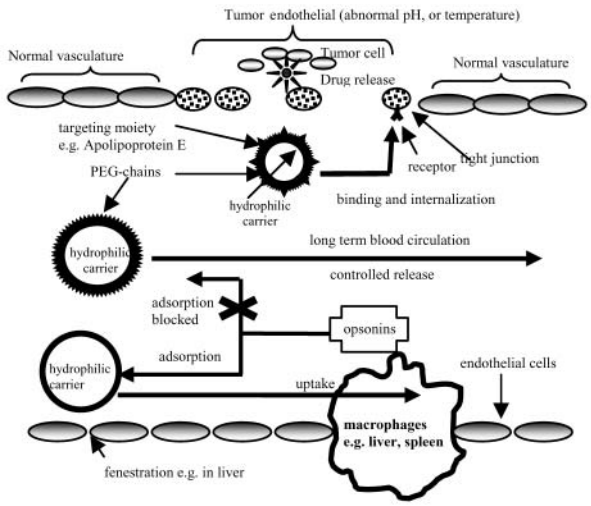

Figure 1. *Illustration of passive targeting in blood vessel of a long-circulating carrier made of a biocompatible polymer, or liposome, which diffuses into a tumor site through "leaking" and releases drugs due to pH, or temperature sensitivity.*

interstitial space due to the increased vascular permeability in this area. If these particles are loaded with a certain drug, they can bring this drug into the "leaky" zone where it can be released as a result of normal carrier degradation. Since the "cut-off" size of the permeabilized vasculature can vary from case to case, the size of a drug-carrying particle may be used to control the efficacy of such spontaneous or "passive" drug delivery by utilizing the EPR effect.

Innovative Carriers in Drug Delivery

It has been well documented that the pore cut-off size of several tumor models is in the range of 380 and 780 nm (25, 26). *In vivo* fluorescence microscopy measurements showed that the extravasation of sterically stabilized liposomes into solid tumor tissue has a cut-off pore size of around 400 nm (27). This limits the distribution of molecules/particles larger than 1 μm across the tumor vasculature. Therefore, one strategy is to associate antitumor drugs with colloidal NPs, with the aim of overcoming non-cellular and cellular-based mechanisms of resistance and to increase the selectivity of drugs towards cancer cells, while reducing their toxicity towards normal tissues. NPs generally are defined as submicronic (<500 nm) colloidal systems, which refer to a broad spectrum of materials including lipid, polymer and inorganic materials. NPs can provide a controlled and targeted way to deliver the encapsulated anticancer drugs, resulting high efficacy and few side-effects (28, 29).

Biocompatible nanoparticles in drug deliveries. Bangham, who first provided a description of phospholipid vesicles in the early 1900's, rediscovered liposome-based pharmaceutical products (30). Liposomes entered the market in 1986. The use of liposomes for drug delivery across the brain capillaries has been documented and examined. Small unilamellar vesicles (SUVs), as well as cationic liposomes coupled with brain drug transport vectors, may be transported through the blood-brain barrier (BBB) by receptor-mediated or absorptive-mediated transcytosis (31-34). However, because liposome-spherical vesicles made of phospholipids are particles, they can be taken up by macrophages. High levels of liposomes can be found in the liver and spleen. In addition, liposomes have other side-effects, such as extravasation, in which the liposome moves from the blood vessel into tissue where it is not wanted. Antibodies, meanwhile, have the disadvantage that most receptors on tumor cells are also present on normal cells, making it hard to find ones that are unique to cancer. These colloidal carriers are subjected to conductive opsonization and subsequent opsonic phagocytosis by circulating phagocytes (monocytes and neutrophils) and by macrophages of the liver and spleen (35, 36). In some cases, solid lipid nanoparticles (SLNs), with diameters in the range of 50-100 nm, have been shown to be more efficient drug carriers than liposomes, due to their better stability (37). This is one of reasons why, in recent decades, many drugs have been associated to NPs (*e.g.*, antibiotics, antiviral and antiparasitic drugs, cytostatics, protein and peptides). Recently, Gasco *et al.* produced SLNs and studied them in cultures of macrophages, and also after loading them with paclitaxel *in vivo* (38, 39). It was indicated that the SLNs led to higher and prolonged plasma levels of paclitaxel. Interestingly the SLNs showed a low uptake by the liver and the spleen macrophages. Studies on SLNs demonstrated the advantages of the traditional systems with the avoidance of some of their major disadvantages, such as toxicity/tolerability and long-term stability (40-42).

Compared to liposomes, polymers with a smaller particle size (<100 nm) and a hydrophilic surface, or appropriate surface coating, not only allow intracapillary or transcapillary passage, but also reduce opsonization reactions and subsequent clearance by macrophages (43). Solid polymeric NPs, first developed by Speiser and co-workers in the mid-1970s (44), are vesicular systems in which biodegradable polymeric nanospheres are matrix systems, while the drug is dispersed throughout the NPs. A number of US FDA-approved biodegradable polymers have been employed to make NPs for controlled delivery of various effective anticancer agents to avoid the use of toxic adjuvants, to realize the desired pharmacokinetics and to enhance their uptake by cancer cells (45-47). Biocompatible polymer NPs are usually taken up by the liver, spleen and other parts of the reticuloendothelial system (RES), depending on their surface characteristics. It has been reported that particles with more hydrophobic surfaces will preferentially be taken up by the liver, followed by the spleen and lungs (48, 49). In contrast,

hydrophilic particles (35 nm diameter), such as those produced from poly(vinyl pyrrolidone), show less than 1% uptake by the spleen and liver and 8 h after injection show 5-10% still circulating in the bloodstream (50).

Tumor blood vessels are more permeable than blood vessels in other tissue, so drugs enter the tumor tissue fairly easily. Particles with longer circulation times and, hence, greater ability to target the site of interest, should usually be 100 nm or less in diameter and have a hydrophilic surface in order to reduce clearance by macrophages (51). To further increase the circulation time of a drug in the bloodstream, coatings of hydrophilic polymers can create a cloud of chains at the particle surface which will repel plasma proteins. Work in this area began by adsorbing surfactants to the NP surface. Other routes include forming the particles from branched or block copolymers with hydrophilic and hydrophobic domains.

In addition, Duncan has worked with modified water-soluble polymers to avoid the liver and the spleen (52). For instance, uncharged hydrophilic polymers, such as polyethylene glycol (PEG) and *N*-(2-hydroxypropyl) methacrylamide, were bound with an anticancer agent using a peptide linkage that was designed to be specifically clipped at the tumor tissue; they can circulate in the blood for periods of up to about 24 h. Duncan used the fact that new blood vessels in tumors are "leaky" to passively target tumors. A review by Jain described the experimental methods to ascertain the transport into solid tumors from the bloodstream (53). However, most of the intelligent polymer carriers have regulatory issues and scaling-up problems. Inorganic NPs as new non-viral carriers, due to their substantially decreased size, can be modified for better efficiency to facilitate their application in different fields such as bioscience and medicine.

Inorganic NPs generally possess versatile properties suitable for cellular delivery, including wide availability, rich functionality, good biocompatibility, potential capability for targeted delivery (*e.g.*, selectively destroying cancer cells but sparing normal tissues) and controlled release of the carried drugs. This is why increasing efforts in research and development worldwide in the last decade have been devoted to various inorganic materials such as novel non-viral carriers (54-56). Many inorganic materials, such as calcium phosphate, gold, carbon materials, silicon oxide, iron oxide and layered double hydroxide (LDH), have been studied (57, 58).

In normal drug administration, the molecules will distribute to targeted and untargeted cells *via* the blood circulation without preference. Therefore, vector molecules possessing high specific affinity towards the affected zone are required. Surface modification of inorganic NPs and biomolecules can improve their interaction. The organic molecules used to functionalize inorganic NPs usually have two feature groups: the anchoring group and the charging group. The former anchors itself onto the NP surface, while the charging group

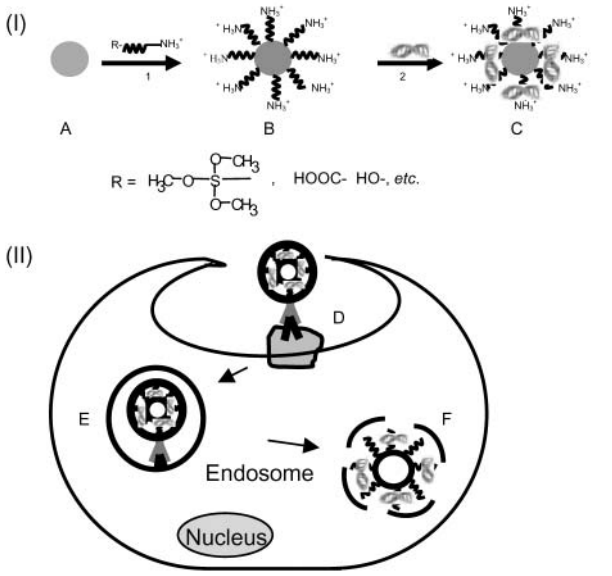

Figure 2. *(I) Schematic representation of surface-functionalization of inorganic nanoparticles and the loading of biomolecules; (II) possible interaction models of nanohybrids with cell membrane and possible endocytosis pathways of nanohybrids. A: Inorganic nanoparticle; B: organically-modified inorganic nanoparticle; C: DNA loaded nanohybrid; D: cell recognition of nanohybrid via 'Y' and 'V' interconnection between the loaded protein and membrane protein; E: nanohybrid endocytosed into cell; F: nanohybrid-endosome breaking down and releasing DNA.*

binds with the biomolecules and also impacts the positive charge to the hybrid NPs. Much effort has been put into the surface modification of biocompatible inorganic particles. For example, *N*-(2-aminoethyl)-3-aminopropyl-trimethoxysilane is a suitable modifier for silica NPs (59), in which methoxysilane ($Si\text{-}OCH_3$) acts as the anchoring group and the amino ($\text{-}NH_2$) group as the charging group, as shown in Figure 2. When amino groups are protonated at pH=7.4, the modified NPs are positively-charged and bind with the DNA chain *via* electrostatic interactions. In fact, the charging group, usually amino ($\text{-}NH_2$), imino (-NH-) and /or =N-, is readily protonated under the physiological conditions (pH=7.4) and, thus, positively charges the NPs.

Generally, the interactions between modified NPs and biomolecules are primarily electrostatic, while hydrophilic and hydrophobic interactions do exist among the organic chains. For example, the negatively-charged DNA chains interact with positive amino groups ($\text{-}NH_3^+$), resulting in adsorption (loading) of DNA on the modified NPs, as shown in Figure 2 (IC). This physical interaction means that biomolecule loading is dependent on the charge density, the modified structure and the chain length. It should also be noted that this interaction keeps biomolecules, *e.g.*, DNA, from being degraded by enzymes in the plasma due to the steric effect and electrostatic repulsion from the modifiers. This is especially important in

Table I. *Various inorganic nanoparticles used as drug carriers.*

Type	Size (nm)	Functional group	Anchoring group
Gold	1-100	Au	HS-
CNT	1-10	-COOH	H_2N-
$C_{60, 70, 80}$	~1	-COOH	H_2N-
SiO_2	5-100	-Si-O-	-O-Si-
Fe_3O_4	1-50	Fe-OH	-O-Si-

gene therapy since nucleic acids are easily attacked by enzymes in the plasma.

A large number of inorganic NPs have been studied as carriers for the cellular delivery of various drugs including genes and proteins (60-65). The functionalization or modification depends on the types of NPs that provide specific functional groups on the surface, as summarized in Table I. For instance, silica NPs are usually modified with silane species; gold NPs can be modified with thiol groups for gene transfection due to the strong chemical affinity between gold and the -SH group. Our group assembled Au NPs on a carbon film with 2D-array through surface modification with the capping agent dodecanethiol ($C_{12}H_{25}SH$), as shown in Figure 3. The Au NPs, with an average particle size 5 nm and a small size distribution, were produced by laser-based synthesis (63).

Other biocompatible materials include various formations of carbon, which can be functionalized by either -COOH or -NH_2 to anchor the organic modifier. MgAl-LDA is another example with the anionic exchange property making it is easy to directly load the biomolecules into the interlayer space. Further modification of these NPs involves the grafting of some other functional groups for specific cells or targeted sites. For example, Zhang *et al.* reported that silane-modified magnetic nanoparticles (MNPs), when grafted with poly (ethylene glycol)-folic acid (PEG-FA), demonstrated a 5-fold increase in cellular uptake by BT20 breast cancer cells as compared with those only grafted with PEG (66, 67). Similarly, many specific antibody proteins can also be used to target their corresponding cells, as demonstrated in Figure 2D denoted by the "Y" and "V" interaction.

Once NPs have adhered to the surface of a cell, they may be internalized into the cell. One possible pathway for such nanohybrids (<500 nm) to transfect into the cells is through the process of endocytosis. Microscopic observations indicated the changes in cell morphology and cytoskeleton structure during the process (68). Figure 2 (II) shows that the nanohybrid is gradually embedded by deforming the membrane and is endocytosed into the cell. The endocytosis may be a receptor-mediated or non-receptor-mediated process. It has been reported that the endocytosis process can be promoted by certain functionalities on the NP surface (68). When the biomolecule-loaded NPs enter cells by endocytosis,

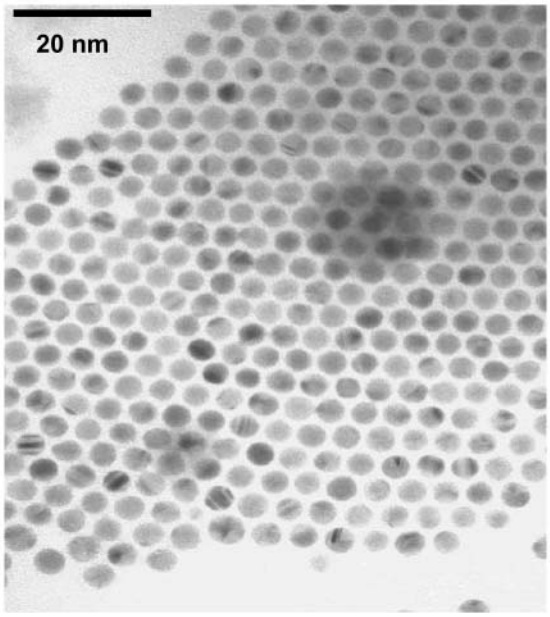

Figure 3. *2-D array of Au nanoparticles driven by capping agent of dodecanethiol on carbon film.*

an endosomal compartment is formed, as shown in Figure 2 (II). The nanohybrids containing DNA or biomolecules may be released from this endosome into the cytoplasm *via* disruption of the endosomal membrane (69).

Driving forces for cellular transport of nanohybrids. As discussed above, modified NPs as carriers of drugs and biomolecules have several particular advantages in driving loaded drugs to the target site. First, modified NPs with positive charges can drive the particles to approach the normal negatively-charged cell membrane *via* electrostatic interactions. In addition, inorganic NPs are modified so as to adjust the adhesion of organic/inorganic nanohybrids to the cell membrane. For example, NPs coated with PEG can improve their non-specific cellular uptake. Meanwhile, grafting specific biomolecules onto NPs can provide site-specific delivery into cells. For example, the folic acid (FA) segment can recognize the folate receptor expressed on the cancer cell surface and, thus, be used as a targeting agent.

On the other hand, physical targeting is another promising method to drive drug molecules to recognize targeted cells. It is either based on the abnormal environment (*e.g.*, pH, temperature) in a target zone, *e.g.*, tumor or inflammation (pH- and temperature-sensitive drug carriers); or magnetically targeting certain cells by driving drug-loaded biocompatible magnetic nanoparticles (MNPs). External magnetic forces can be applied to concentrate the organic/inorganic nanohybrids to a targeted region and thus increase their chance of interacting with the target cells (70, 71). This intelligent carrier

can decrease the concentration of the drug at non-target sites, thus reducing side-effects and increase the concentration of the drug at the target site, thus promoting its efficacy.

Magnetic Drug Targeting

It has been noted that the disadvantage of most chemotherapies is the relatively non-specific and induced side-effects in healthy tissue. One of the strategies to overcome this problem is to magnetize the drug-loaded carrier (*e.g.*, Fe_3O_4) so that it can be retained at or guided to the target site with the help of an external magnetic field of appropriate strength.

Magnetic nanoparticles as carriers in anticancer-drug delivery. Historically, the concept of injection of magnetic fluid compounds arose in the 1970's. Widder *et al.* (72), and Morimoto *et al.* (73) developed magnetic albumin microspheres encasing anticancer drugs. Stabilization of the albumin microsphere matrix was accomplished by either heat denaturation at various temperatures in the range of 110˚C to 190˚C, or crosslinking with carbonyl compounds in an ether phase reaction. The degree of stabilization controls the rate of drug diffusion out of the carrier, as well as the extent of carrier degradation. The magnetic particles injected into an animal were retained at the target site and apparently delayed the reticuloendothelial clearance and neodisposition of the drug-containing carrier. External high-gradient magnetic fields are used to concentrate the complex at a specific target site within the body. In a rat subcutaneous tumor, the uptake ratio using a magnet was 70.0% of the total magnetic particles (1< diameter <7 μm) injected, while in the case where no magnet was used the uptake was only 27.4% (74). Therefore, the magnetic force enhances the uptake of magnetic particles by tumor cells. In most cases, the magnetic field gradient is generated by a strong permanent magnet, such as NdFeB, fixed outside the body on the target site. Preclinical models utilizing magnetic drug delivery systems have demonstrated increased tumor selectivity and efficacy in different types of tumors, including brain (75, 76).

Along with magnetic systems utilizing uncharged particles, bioadhesive cationic microspheres have been shown to increase particle retention and tumor uptake (77, 78). The therapeutic efficacy was dependent upon the intact and constant bond of both substances to the outer protein layer. However, the size of these albumin spheres with a dimension >1 μm has several limitations, including: i) the possibility of embolization of blood vessels in the target region due to the accumulation of magnetic carriers; ii) difficulties in scaling up from animal models due to the larger distances between the target site and the magnet; iii) once the drug has been released, the large particle is no longer attracted to the magnetic field; and iv) toxic responses to the large magnetic carriers. These limitations were not overcome until 1996, when starch-coated magnetic nanoparticles (ferrofluids) were ionically and reversibly bound to a chemotherapeutic agent by Lübbe *et al.* (79, 80). Active "drug targeting", employing MNPs, along with their transported substances, are held in position by an external magnetic field at the target region to prevent their flow with the blood stream. The magnetic field characteristics (magnetic field strength and gradient) are important factors in this type of application and the active manipulation of the NPs is mainly dependent upon it. Alexiou *et al.* showed that this can be accomplished using a strong electromagnet with a field of 1.7 Tesla (81). Consequently, the MNPs have been studied with the intention of applying them in bioscience. Furthermore, the controlled release of the magnetic-coupled cytostatic agent on a cellular or subcellular level makes it possible to minimize the toxicity in normal tissue and endothelial cells. This minimal toxicity is in contrast to the adverse side-effects often seen in the systemic administration of chemotherapeutic agents (82).

However, most inorganic MNPs have to be subjected to chemical and/or biological modification to meet the stringent requirements for cellular delivery, such as good biocompatibility, strong affinity between the carriers and biomolecules, high charge density of the nanohybrids, site-specificity, *etc.* Thus, the resulting organic/inorganic nanohybrids can be directly delivered into cells. For example, MNPs modified with dextran showed a strong adhesion to the membrane of human fibroblasts, which retained the MNPs just outside the cells (83). To date, the synthesis of polymer-coated magnetite, including those containing derivatives of PEG, has been achieved by forming magnetite particles in an aqueous phase in the presence of a polymer species such as dextran, or v-dicarboxymethyl-PEG (84-86). In addition, magnetic liposomes have been investigated for targeted drug carrying potential (87-89).

Magnetic nanohybrids used in target organic brain. Targeting of the drugs to the brain using these biocompatible magnetic nanohybrids remains an unfulfilled goal because of the stern limitations imposed by the impervious capillaries that supply the brain. These capillaries are the major constituents of the BBB. Even liposomes with a diameter as small as 100 nm fail to penetrate the BBB by free diffusion (90). The principal basis of the BBB is thought to be specialized endothelial cells in the brain microvasculature, which are aided, at least in part, by interactions with glia. Among the unique properties of these endothelial cells is the presence of tight junctions between the cells, with gaps between the junctions of approximately 4 nm (91). To gain entry to the brain, water-soluble MNPs should pass through the cell membrane of the endothelial cells of the brain, rather than between the endothelial cells. The penetration of molecules into the brain is largely related to their lipid solubility and to their ability to pass through the plasma membranes of the cells forming the

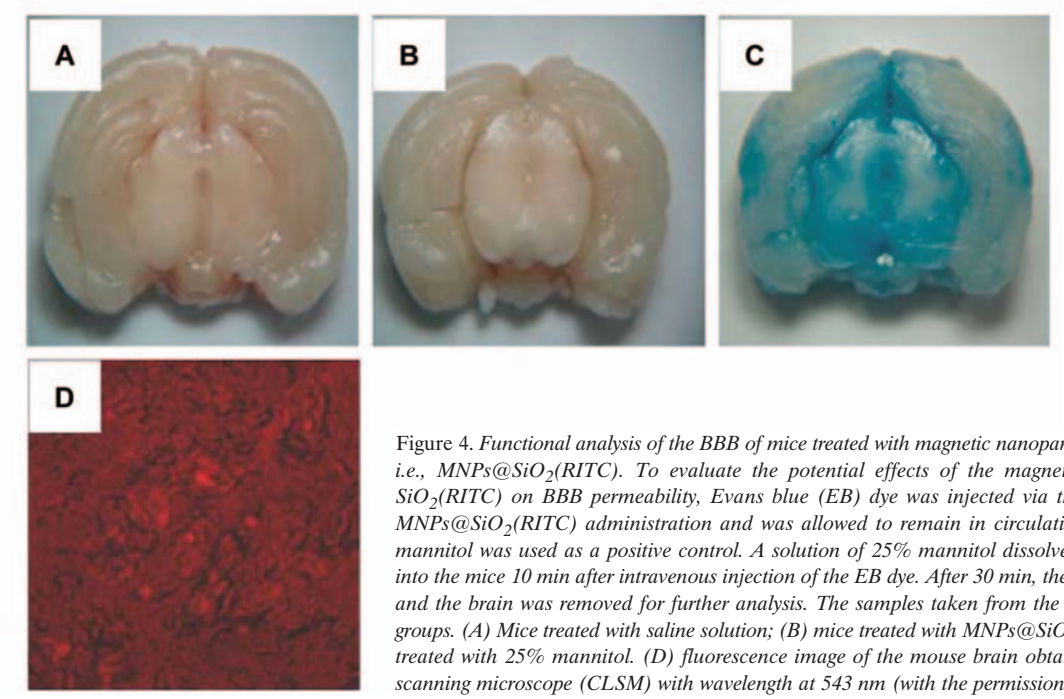

Figure 4. *Functional analysis of the BBB of mice treated with magnetic nanoparticles coated with SiO_2(RITC), i.e., MNPs@SiO_2(RITC). To evaluate the potential effects of the magnetic nanoparticles coated with SiO_2(RITC) on BBB permeability, Evans blue (EB) dye was injected via the tail vein at 5 min prior to MNPs@SiO_2(RITC) administration and was allowed to remain in circulation for 30 min. Hyperosmolar mannitol was used as a positive control. A solution of 25% mannitol dissolved in 0.9% saline was injected into the mice 10 min after intravenous injection of the EB dye. After 30 min, the mice were sacrificed, perfused and the brain was removed for further analysis. The samples taken from the brains were divided into three groups. (A) Mice treated with saline solution; (B) mice treated with MNPs@SiO_2(RITC) (10 mg/kg); (C) mice treated with 25% mannitol. (D) fluorescence image of the mouse brain obtained by using a confocal laser scanning microscope (CLSM) with wavelength at 543 nm (with the permission of Toxicological Sciences).*

barrier (92). Recently, Jain *et al.* studied negatively-charged magnetic liposomes, which were prepared using soya lecithin (Soya PC), cholesterol and phosphatidyl serine (PS), for their preferential presentation to circulating blood phagocytes (monocytes and neutrophils). The arginine–glycine–aspartic acid (RGD)-containing synthetic peptide was subsequently coated on the magnetic liposome through covalent coupling. RGD-coated magnetic liposomes were 9.1-, 6.62- and 1.5-fold more potent compared to the free drug solution, non-magnetic RGD-coated liposomes and uncoated magnetic liposomes, respectively. This study suggested the potential of negatively-charged and RGD-coated magnetic liposomes for monocyte/neutrophil-mediated active delivery of drugs to relatively inaccessible inflammatory sites, *i.e.,* brain. It may open a new perspective in the active delivery of drugs for possible treatment of cerebrovascular diseases (93).

In addition, the design of MNPs coated with a biocompatible layer (*i.e.*, core-shell MNPs) has attracted much interest recently in the field of drug delivery, due to the enhanced biocompatibility and ideal magnetic properties. For instance, magnetic Co ferrite NPs were coated with a shell of amorphous silica, which contained luminescent organic dyes, such as rhodamine B isothiocyanate (RITC, orange, λmax(em) = 555 nm) or fluorescein isothiocyanate (FITC, green, λmax(em) = 518 nm) on the inside of the silica shell and biocompatible PEG on the outside. The amorphous silica shell is a stable and biocompatible material, which can avoid potential toxic effects on cells (94). Recently, Cho *et al.* determined the distribution pattern and potential toxicity of nanomaterials using MNPs@SiO_2(RITC) (95). The core-shell MNPs were found in almost all organs in a time-dependent manner. The results showed that core-shell MNPs are rapidly and widely redistributed in the body, except in the case of the lungs. Interestingly, the MNPs were also found in the brain and testes, which indicated that they could penetrate the BBB as well as the blood-testis barrier (Figure 4).

To evaluate the potential effects of MNPs@SiO_2(RITC) on BBB permeability, Evans blue (EB) dye was injected intravenously as a BBB permeability tracer. Their results on spectrophotometrical analysis also demonstrated that the BBB permeability was intact. This work demonstrated that MNPs@SiO_2(RITC) could penetrate the BBB without affecting its function. Further, the toxicity of MNPs was also investigated through the *Salmonella* mutagenesis test because of its initial promise of high qualitative predictivity for cancer in rodents and, by extension, in humans. The results of Cho's work clearly demonstrated that MNPs measuring approximately 50 nm did not cause toxicity under the current conditions. These results suggest that MNPs@SiO_2(RITC) can penetrate the BBB and persist in the body for a long time without causing toxicity. In addition, another type of core-shell MNP, *i.e.*, magnetic Fe NPs coated with a layer of gold (Au), has been studied due to the high magnetization of Fe and biocompatibility of Au, that can act as a versatile platform for application in gene transfer and gene/drug delivery (96). Therefore, extensive studies are required to provide the basis for a new class of nanomaterials for drug, protein and gene delivery applications.

Conclusion

Current research is actively aimed towards achieving specific and targeted delivery of anticancer agents through new avenues of directing drugs to tumors, as well as new types of drugs. It is evident that the versatility of NPs, including polymer and lipidic NPs, makes them very suitable as potential delivery carriers, particularly due to their small size and enlarged surface area: volume ratio. Recently, an increasing number of inorganic NPs have been studied as anticancer drug (gene) delivery carriers, because of their versatile physicochemical properties. NPs are readily available, can be easily functionalized, and possess good biocompatibility and low cytotoxicity. A better understanding of the interaction between NPs and biomolecules and the driving forces in the transfection process would lead to cell-specific target delivery with high efficiency. Furthermore, it was pointed out that magnetic nanohybrid particles could be the next generation of drug carriers for successful chemotherapy, since the transfection efficiency can be enhanced dramatically by using external magnetic fields to overcome the effect of the BBB. The most significant challenge is target (directing) delivery to a specific cell. The difficulty lies in the recognition of the targeted cell among thousands of untargeted cells, as well as suitable stability of the nanohybrids during the delivery process. In conclusion, the key to cancer therapy is to treat it as early as possible. This requires superior detection and targeting methods combined with nanotechnology. Research in the field continues.

Acknowledgements

We thank Mr. Dashan Wang, from the Institute for Chemical Process and Environmental Technology (ICPET), National Research Council Canada, for the work on TEM measurements.

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Received March 16, 2006
Revised April 27, 2006
Accepted May 8, 2006