# Nanoparticle-based targeted drug delivery

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## Abstract

Nanotechnology could be defined as the technology that has allowed for the control, manipulation, study, and manufacture of structures and devices in the "nanometer" size range. These nano-sized objects, e.g., "nanoparticles", take on novel properties and functions that differ markedly from those seen from items made of identical materials. The small size, customized surface, improved solubility, and multi-functionality of nanoparticles will continue to open many doors and create new biomedical applications. Indeed, the novel properties of nanoparticles offer the ability to interact with complex cellular functions in new ways. This rapidly growing field requires cross-disciplinary research and provides opportunities to design and develop multifunctional devices that can target, diagnose, and treat devastating diseases such as cancer. This article presents an overview of nanotechnology for the biologist and discusses the attributes of our novel XPclad© nanoparticle formulation that has shown efficacy in treating solid tumors, for single dose vaccination, and oral delivery of therapeutic proteins.

## Keywords

nanoparticles; drug delivery; cancer therapy; quantum dots; XPclad© nanoparticles

## Introduction

The development of a wide spectrum of nanoscale technologies is beginning to change the scientific landscape in terms of disease diagnosis, treatment, and prevention. These technological innovations, referred to as nanomedicines by the National Institutes of Health, have the potential to turn molecular discoveries arising from genomics and proteomics into widespread benefit for patients. Nanoparticles can mimic or alter biological processes (e.g., infection, tissue engineering, *de novo* synthesis, etc.). These devices include, but are not limited to, functionalized carbon nanotubes, nanomachines (e.g., constructed from interchangeable DNA parts and DNA scaffolds), nanofibers, self-assembling polymeric nanoconstructs, nanomembranes, and nano-sized silicon chips for drug, protein, nucleic acid, or peptide delivery and release, and biosensors and laboratory diagnostics.

Biodegradable polymers have been studied extensively over the past few decades for the fabrication of drug delivery systems. Considerable research is being directed towards developing biodegradable polymeric nanoparticles for drug delivery and tissue engineering, in view of their applications in controlling the release of drugs, stabilizing labile molecules (e.g., proteins, peptides, or DNA) from degradation, and site-specific drug targeting. The late 1960's and early 1970's saw the advent of polymer microparticles based on acrylamide micelle polymerization (Kreuter, 1994a). Since then, along with different polymerization methods, preformed polymers also have been developed and studied (Barratt, 2000; Kreuter, 1994a; Pitt et al., 1981). The majority of studies on nanoparticles reported to date have dealt with microparticles created from poly(D,L lactide), poly(lactic acid) [PLA], poly(D,L glycolide) [PLG], poly(lactide-co-glycolide) [PLGA], and poly-cyanoacrylate [PCA] (Pitt et al., 1981).

## Nanoparticle Delivery Systems

Nanocapsules are vesicular systems in which a drug is confined to a cavity surrounded by a polymer membrane, whereas nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Nanoparticles are solid, colloidal particles consisting of macromolecular substances that vary in size from 10 nm to 1000 nm (Kreuter, 1994a). However, particles >200 nm are not heavily pursued and nanomedicine often refers to devices <200 nm (i.e., the width of microcapillaries). Typically, the drug of interest is dissolved, entrapped, adsorbed, attached and/or encapsulated into or onto a nano-matrix. Depending on the method of preparation nanoparticles, nanospheres, or nanocapsules can be constructed to possess different properties and release characteristics for the best delivery or encapsulation of the therapeutic agent (Barratt, 2000; Couvreur et al., 1995; Pitt et al., 1981).

## Applications and Advantages of Nanoparticle Drug Carriers

Polymeric nanoparticles made from natural and synthetic polymers have received the majority of attention due to their stability and ease of surface modification (Herrero-Vanrell et al., 2005; Vauthier et al., 2003). They can be tailor-made to achieve both controlled drug release and disease-specific localization by tuning the polymer characteristics and surface chemistry (Kreuter, 1994b; Moghimi et al., 2001; Panyam and Labhasetwar, 2003; Panyam et al., 2003b). It has been established that nanocarriers can become concentrated preferentially to tumors, inflammatory sites, and at antigen sampling sites by virtue of the enhanced permeability and retention (EPR) effect of the vasculature. Once accumulated at the target site, hydrophobic biodegradable polymeric nanoparticles can act as a local drug depot depending on the make-up of the carrier, providing a source for a continuous supply of encapsulated therapeutic compound(s) at the disease site, e.g., solid tumors.

These systems in general can be used to provide targeted (cellular or tissue) delivery of drugs, improve bioavailability, sustain release of drugs or solubilize drugs for systemic delivery. This process can be adapted to protect therapeutic agents against enzymatic degradation (i.e., nucleases and proteases) (Haixiong Ge, 2002). Thus, the advantages of using nanoparticles for drug delivery are a result of two main basic properties: small size and use of biodegradable materials. Nanoparticles, because of their small size, can extravasate through the endothelium in inflammatory sites, epithelium (e.g., intestinal tract and liver), tumors, or penetrate microcapillaries. In general, the nanosize of these particles allows for efficient uptake by a variety of cell types and selective drug accumulation at target sites (Desai et al., 1997; Panyam and Labhasetwar, 2003; Panyam et al., 2003b). Many studies have demonstrated that nanoparticles have a number of advantages over microparticles (>1 μm) as a drug delivery system (Linhardt, 1989). Nanoparticles have

another advantage over larger microparticles because they are better suited for intravenous delivery. The smallest capillaries in the body are 5–6 μm in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 μm, without forming aggregates, to ensure that the particles do not cause an embolism.

The use of biodegradable materials for nanoparticle preparation allows for sustained drug release within the target site over a period of days or even weeks. Biodegradable nanoparticles formulated from PLGA and PLA have been developed for sustained drug delivery and are especially effective for drugs with an intracellular target (Barrera et al., 1993; Davda and Labhasetwar, 2002; Panyam and Labhasetwar, 2003). Rapid escape of hydrophobic PCL-coated nanoparticles from endo-lysosomes to the cytoplasm has been demonstrated (Barrera et al., 1993; Woodward et al., 1985). Greater and sustained anti-proliferative activity was observed in vascular smooth muscle cells that were treated with dexamethasone-loaded nanoparticles and then compared to cells given drug in solution (Redhead et al., 2001). Hence, nanoparticles can be effective in delivering their contents to intracellular targets.

## Characteristics Important for Drug Delivery using Nanoparticles

### Particle size

Currently, the fastest and most routine method of determining nanoparticle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties (Swarbrick and Boylan, 2002). The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

Particle size and size distribution are the most important characteristics of nanoparticles. They determine the *in vivo* distribution, biological fate, toxicity, and targeting ability of these delivery systems. In addition, they can influence drug loading, drug release, and stability of nanoparticles. Many studies have demonstrated that nanoparticles have a number of advantages over microparticles (Panyam and Labhasetwar, 2003). Generally nanoparticles have relatively high cell uptake when compared to microparticles and are available to a wider range of cellular and intracellular targets due to their small size and mobility. Nanoparticles can cross the blood-brain barrier following the opening of endothelium tight junctions by hyper-osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors (Kroll et al., 1998). Tween 80-coated nanoparticles have been shown to cross the blood-brain barrier as well (Kreuter et al., 2003). Submicron nanoparticles, but not larger microparticles, are taken up by the majority of cell types (Zauner et al., 2001). Indeed, 100 nm nanoparticles had a 2.5-fold greater uptake rate than 1 μm microparticles, and a 6-fold greater uptake than 10 μm microparticles by Caco-2 cells (Desai et al., 1997). In a similar study, nanoparticles were shown to penetrate throughout the submucosal layers of a rat intestinal loop model, while microparticles were predominantly localized in the epithelial lining (Redhead et al., 2001). This indicates that particle distribution can, in part at least, be tuned by controlling particle size.

Drug release also is affected by particle size. Smaller particles have a larger surface area-to-volume ratio; therefore, most of the drug associated with small particles would be at or near the particle surface, leading to faster drug release. In contrast, larger particles have large cores, which allow more drug to be encapsulated per particle and give slower release (Redhead et al., 2001). Thus, control of particle size provides a means of tuning drug release rates.

Smaller particles also have a greater risk of aggregation during storage, transport, and dispersion. Polymer degradation also can be affected by particle size. For instance, the rate of PLGA degradation was found to increase as the particle made from this polymer increased in size (Dunne et al., 2000). This process is believed to be due to PLGA degradation products which can more easily diffuse through shorter distances in smaller nanoparticles, while the polymer matrix of larger particles increases the time of release due to the greater distance and may also cause autocatalytic degradation of the polymer material (Panyam et al., 2003a). Therefore, it was hypothesized that larger particles will contribute to faster polymer degradation as well as the drug release. However, additional studies will be required to confirm and better elucidate the mechanisms of this paradox.

## Surface properties of nanoparticles

The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocyte system (MPS) such as liver, spleen, lungs and bone marrow. Nanoparticles can be recognized by the host immune system when intravenously administered and cleared by phagocytes from the circulation (Muller et al., 1996). Apart from the size of nanoparticles, nanoparticle hydrophobicity determines the level of blood components (e.g., opsonins) that bind this surface. Hence, hydrophobicity influences the *in vivo* fate of nanoparticles (Brigger et al., 2002; Muller et al., 1996). Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the MPS (Grislain et al., 1983).

To increase the likelihood of success in drug targeting, it is necessary to minimize the opsonization and prolong the circulation of nanoparticles *in vivo*. This can be achieved by coating nanoparticles with hydrophilic polymers/surfactants or formulating nanoparticles with biodegradable copolymers with hydrophilic characteristics, e.g., polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine, and polysorbate 80 (Tween 80). Studies show that PEG on nanoparticle surfaces prevents opsonization by complement and other serum factors. PEG molecules with brush-like and intermediate configurations reduced phagocytosis and complement activation, whereas surfaces comprised of PEG with mushroom-like structures were potent complement activators and favored phagocytosis (Bhadra et al., 2002; Olivier, 2005).

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles (Couvreur et al., 2002). It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above $\pm$ 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential also can be used to determine whether a charged active material is encapsulated within the center of the nanoparticle or on the surface.

## Drug loading

A successful nanodelivery system should have a high drug-loading capacity, thereby reducing the quantity of matrix materials for administration. Drug loading can be accomplished by two methods. The incorporation method requires the drug to be incorporated at the time of nanoparticle formulation. The adsorption/absorption methods calls for absorption of the drug after nanoparticle formation; this is achieved by incubating the nano-carrier with a concentrated drug solution. Drug loading and entrapment efficiency depend on drug solubility in the excipient matrix material (solid polymer or liquid dispersion agent), which is related to the matrix composition, molecular weight, drug-polymer interactions, and the presence of end functional groups (i.e., ester or carboxyl) in either the

drug or matrix (Govender et al., 1999; Govender et al., 2000; Panyam et al., 2004). A polymer of choice for some nanoparticle formulations is PEG, which has little or no effect on drug-loading and interactions (Peracchia et al., 1997). In addition, the macromolecules, drugs or protein encapsulated in nanoparticles show the greatest loading efficiency when they are loaded at or near their isoelectric point (pI) (Calvo et al., 1997). For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be very effective in increasing drug-loading (Chen et al., 1994; Chen et al., 2003).

## Drug release

It is important to consider both drug release and polymer biodegradation when developing a nanoparticulate delivery system. In general, the drug release rate depends on: (1) drug solubility; (2) desorption of the surface-bound or adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion or degradation; and (5) the combination of erosion and diffusion processes. Hence, solubility, diffusion, and biodegradation of the particle matrix govern the release process.

In the case of nanospheres, where the drug is uniformly distributed, drug release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix erosion, then the mechanism of release is largely controlled by a diffusion process. The rapid, initial release, or 'burst', is mainly attributed to weakly bound or adsorbed drug to the relatively large surface of nanoparticles (Magenheim et al., 1993). It is evident that the method of incorporation has an effect on the release profile. If the drug is loaded by the incorporation method, then the system has a relatively small burst effect and sustained release characteristics (Fresta et al., 1995). If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the polymeric membrane.

Membrane coating acts as a drug release barrier; therefore, drug solubility and diffusion in or across the polymer membrane becomes a determining factor in drug release. Furthermore, the release rate also can be affected by ionic interactions between the drug and auxiliary ingredients. When the entrapped drug interacts with auxiliary ingredients, a less water soluble complex can form, which can slow the drug release – having almost no burst release effect (Chen et al., 1994). Whereas if the addition of auxiliary ingredients, e.g., ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the drug with the matrix material due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be achieved (Calvo et al., 1997). Various methods can be used to study the release of drug from the nanoparticle: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion; (3) reverse dialysis bag diffusion; (4) agitation followed by ultracentrifugation/centrifugation; or (5) ultra-filtration. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred. However, these methods prove difficult to replicate and scale-up for industrial use.

## Targeted drug delivery

The development of nanoparticle delivery systems for targeted drug delivery has been recently reviewed (Moghimi et al., 2001). Targeted delivery can be actively or passively achieved. Active targeting requires the therapeutic agent to be achieved by conjugating the therapeutic agent or carrier system to a tissue or cell-specific ligand (Lamprecht et al., 2001). Passive targeting is achieved by incorporating the therapeutic agent into a macromolecule or nanoparticle that passively reaches the target organ. Drugs encapsulated in nanoparticles or drugs coupled to macromolecules can passively target tumors through the

EPR effect. Alternatively, catheters can be used to infuse nanoparticles to the target organ or tissues. For example, localized delivery of drug-bearing nanoparticles to sites of vascular restenosis may be useful for providing sustained drug release at specific sites on the arterial wall (Maeda, 2001; Sahoo et al., 2002).

Liposomes have been demonstrated to be useful for delivering pharmaceutical agents. These systems use 'contact-facilitated drug delivery', which involves binding or interaction with the targeted cell membrane. This permits enhanced lipid-lipid exchange with the lipid monolayer of the nanoparticle, which accelerates the convective flux of lipophilic drugs (e.g., paclitaxel) to dissolve through the outer lipid membrane of the nanoparticles to targeted cells (Guzman et al., 1996). Such nanosystems can serve as drug depots exhibiting prolonged release kinetics and long persistence at the target site.

Nanoparticles also can be formulated to deliver drugs across several biological barriers (Fisher and Ho, 2002; Lockman et al., 2002). Anti-neoplastics, anti-viral drugs, and several other types of drugs are markedly hindered because of inability of these molecules to cross the blood-brain barrier (BBB). The application of nanoparticles to deliver across this barrier is extremely promising. It has been reported that nanoparticles can cross the BBB following the opening of tight junctions by hyper-osmotic mannitol, which also may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors (Avgoustakis et al., 2002). Tween 80-coated nanoparticles also have been shown to cross the BBB (Beletsi et al., 1999).

## Nanotechnology-based Drug Delivery in Cancer

Drug delivery in cancer is important for optimizing the effect of drugs and reducing toxic side effects. Several nanotechnologies, mostly based on nanoparticles, can facilitate drug delivery to tumors.

### Hydrogels

Hydrogel-nanoparticles are based on proprietary technology that uses hydrophobic polysaccharides for encapsulation and delivery of drug, therapeutic protein, or vaccine antigen. A novel system using cholesterol pullulan shows great promise. In this regard, four cholesterol molecules gather to form a self-aggregating hydrophobic core with pullulan outside. The resulting cholesterol nanoparticles stabilize entrapped proteins by forming this hybrid complex. These particles stimulate the immune system and are readily taken up by dendritic cells. Alternatively, larger hydrogels can encapsulate and release monoclonal antibodies.

Curcumin, a substance found in the cooking spice turmeric, has long been known to have anti-cancer properties. Nevertheless, widespread clinical application of this relatively efficacious agent has been limited due to its poor solubility and minimal systemic bioavailability. This problem has been resolved by encapsulating curcumin in a polymeric nanoparticle, creating "nanocurcumin" (Bisht et al., 2007). Further, the mechanism of action of nanocurcumin on pancreatic cancer cells mirrors that of free curcumin, including induction of apoptosis, blockade of nuclear factor kappa B (NFκB) activation, and downregulation of pro-inflammatory cytokines (i.e., IL-6, IL-8 and TNF-α). Nanocurcumin provides an opportunity to expand the clinical repertoire of this efficacious agent by enabling soluble dispersion. Future studies utilizing nanocurcumin are warranted in preclinical *in vivo* models of cancer and other diseases that might benefit from the effects of curcumin.

## Micelles and liposomes

Block-copolymer micelles are spherical super-molecular assemblies of amphiphilic copolymer. The core of micelles can accommodate hydrophobic drugs, and the shell is a hydrophilic brush-like corona that makes the micelle water soluble, thereby allowing delivery of the poorly soluble contents. Camptothecin (CPT) is a topoisomerase I inhibitor that is effective against cancer, but clinical application of CPT is limited by its poor solubility, instability, and toxicity. Biocompatible, targeted sterically stabilized micelles (SSM) have been used as nanocarriers for CPT (CPT-SSM). CPT solubilization in SSM is expensive yet reproducible and is attributed to avoidance of drug aggregate formation. Furthermore, SSM composed of PEGylated phospholipids are attractive nanocarriers for CPT delivery because of their size (14 nm) and ability to extravasate through the leaky microvasculature of tumors and inflamed tissues. This passive targeting results in high drug concentration in tumors and reduced drug toxicity to the normal tissues (Koo et al., 2006).

Stealth micelle formulations have stabilizing PEG coronas to minimize opsonization of the micelles and maximize serum half-life. Currently, SP1049C, NK911, and Genexol-PM have been approved for clinical use (Sutton et al., 2007). SP1049C is formulated as doxorubicin (DOX)-encapsulated pluronic micelles. NK911 is DOX-encapsulated micelles from a copolymer of PEG-DOX-conjugated poly(aspartic acid), and Genexol-PM is a paclitaxel-encapsulated PEG-PLA micelle formulation. Polymer micelles have several advantages over other drug delivery systems, including increased drug solubility, prolonged circulation half-life, selective accumulation at tumor sites, and lower toxicity. However, at the present time this technology lacks tumor specificity and the ability to control the release of the entrapped agents. Indeed, the focus of nano-therapy has gradually shifted from passive targeting systems (e.g., micelles) to active targeting.

Super paramagnetic iron oxide particles can be used in conjunction with magnetic resonance imaging (MRI) to localize the tumor as well as for subsequent thermal ablation. This has been used, for example, to target glioblastoma multiforme (GBM), a primary malignant tumor of the brain with few effective therapeutic options. The primary difficulty in treating GBM lies in the difficulty of delivering drugs across the BBB. However, nanoscale liposomal iron oxide preparations were recently shown to improve passage across the BBB (Jain, 2007).

## Nanomaterial formulation

Nanomaterials have been successfully manipulated to create a new drug-delivery system that can solve the problem of poor water solubility of most promising currently available anticancer drugs and, thereby, increase their effectiveness. The poorly soluble anticancer drugs require the addition of solvents in order for them to be easily absorbed into cancer cells. Unfortunately, these solvents not only dilute the potency of the drugs but create toxicity. Researchers from the University of California Los Angeles California Nanosystem Institute have devised a novel approach using silica-based nanoparticles to deliver the anticancer drug CPT and other water insoluble drugs to cancer cells (Lu et al., 2007). The method incorporates the hydrophobic anticancer drug CPT into the pores of fluorescent mesoporous silica nanoparticles and delivers the particles into a variety of human cancer cells to induce cell death. The results suggest that the mesoporous silica nanoparticles might be used as a vehicle to overcome the insolubility of many anticancer drugs.

## Nanosystems

Novel nanosystems can be pre-programmed to alter their structure and properties during the drug delivery process, allowing for more effective extra- and intra-cellular delivery of encapsulated drug (Wagner, 2007). This is achieved by the incorporation of molecular

sensors that respond to physical or biological stimuli, including changes in pH, redox potential, or enzymes. Tumor-targeting principles include systemic passive targeting and active receptor targeting. Physical forces (e.g., electric or magnetic fields, ultrasound, hyperthermia, or light) may contribute to focusing and triggering activation of nano systems. Biological drugs delivered with programmed nanosystems also include plasmid DNA, siRNA, and other therapeutic nucleic acids.

Using a degradable, polyamine ester polymer, polybutanediol diacrylate co amino pentanol (C32), a diptheria toxin suicide gene (DT-A) driven by a prostate-specific promoter was directly injected into normal prostate and prostate tumors in mice (Peng et al., 2007). This C32/DT-A system resulted in significant size reduction, apoptosis in 50% of normal prostate. However, a single injection of C32/DT-A triggered apoptosis in 80% of tumor cells present in the tissue. It is expected that multiple nanoparticle injection would trigger a great percentage of prostate tumor cells to undergo apoptosis. These results suggest that local delivery of polymer/DT-A nanoparticles may have application in the treatment of benign prostatic hypertrophy and prostate cancer.

Multidrug resistance (MDR) of tumor cells is known to develop through a variety of molecular mechanisms. Glucosylceramide synthase (GCS) is responsible for the activation of the pro-apoptotic mediator, ceramide, to a nonfunctional moiety, glucosylceramide. This molecule is over-expressed by many MDR tumor types and has been implicated in cell survival in the presence of chemotherapy. A study has investigated the therapeutic strategy of co-administering ceramide with paclitaxel in an attempt to restore apoptotic signaling and overcome MDR in a human ovarian cancer cell line using modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles to encapsulate and deliver the therapeutic agents for enhanced efficacy (van Vlerken and Amiji, 2006). Results show that MDR cancer cells can be completely eradicated by this approach. Using this approach, MDR cells can be resensitized to a dose of paclitaxel near the IC50 of non-MDR cells. Molecular analysis of activity verified the hypothesis that the efficacy of this therapeutic approach is due to a restoration in apoptotic signaling, showing the promising potential for clinical use of this therapeutic strategy to overcome MDR.

## Nanocells

Indiscriminate drug distribution and severe toxicity of systemic administration of chemotherapeutic agents can be overcome through encapsulation and cancer cell targeting of chemotherapeutics in 400 nm nanocells, which can be packaged with significant concentrations of chemotherapeutics of different charge, hydrophobicity, and solubility (MacDiarmid et al., 2007). Targeting of nanocells via bispecific antibodies to receptors on cancer cell membranes results in endocytosis, intracellular degradation, and drug release. Doses of drugs delivered via nanocells are ~1,000 times less than the dose of the free drug required for equivalent tumor regression. It produces significant tumor growth inhibition and regression in mouse xenografts and lymphoma in dogs, despite administration of minute amounts of drug and antibody. Indeed, reduced dosage is a critical factor for limiting systemic toxicity. Clinical trials are planned for testing this method of drug delivery.

## Dendrimers

In early studies, dendrimer-based drug delivery systems focused on encapsulating drugs. However, it was difficult to control the release of drugs associated with dendrimers. Recent developments in polymer and dendrimer chemistry have provided a new class of molecules called dendronized polymers, which are linear polymers that bear dendrons at each repeat unit. Their behavior differs from that of linear polymers and provides drug delivery advantages because of their enhanced circulation time. Another approach is to synthesize or

conjugate the drug to the dendrimers so that incorporating a degradable link can be further used to control the release of the drug.

DOX was conjugated to a biodegradable dendrimer with optimized blood circulation time through the careful design of size and molecular architecture (Lee et al., 2006). Specifically, the DOX-dendrimer controlled drug-loading through multiple attachment sites, solubility through PEGylation, and drug release through the use of pH-sensitive hydrazone dendrimer linkages. In culture, DOX-dendrimers were >10 times less toxic than free DOX toward colon carcinoma cells. Upon intravenous administration to tumor bearing mice, tumor uptake of DOX-dendrimers were nine-fold higher than intravenous free DOX and caused complete tumor regression and 100% survival of the mice after 60 days.

### Nanotubes

Even though it was previously possible to attach drug molecules directly to antibodies, attaching more than a handful of drug molecules to an antibody significantly limits its targeting ability because the chemical bonds that are used tend to impede antibody activity. A number of nanoparticles have been investigated to overcome this limitation. Tumor targeting single-walled carbon nano-tube (SWCNT) have been synthesized by covalently attaching multiple copies of tumor-specific monoclonal antibodies (MAbs), radiation ion chelates and fluorescent probes (McDevitt et al., 2007). A new class of anticancer compound was created that contains both tumor-targeting antibodies and nanoparticles called fullerenes (C60). This delivery system can be loaded with several molecules of an anticancer drug, e.g., Taxol[®] (Ashcroft et al., 2006). It is possible to load as many as 40 fullerenes onto a single skin cancer antibody called ZME-108, which can be used to deliver drugs directly into melanomas. Certain binding sites on the antibody are hydrophobic (water repelling) and attract the hydrophobic fullerenes in large numbers so multiple drugs can be loaded into a single antibody in a spontaneous manner. No covalent bonds are required, so the increased payload does not significantly change the targeting ability of the antibody. The real advantage of fullerene-based therapies vs. other targeted therapeutic agents is likely to be fullerene's potential to carry multiple drug payloads, such as taxol plus other chemotherapeutic drugs. Cancer cells can become drug resistant, and one can cut down on the possibility of their escaping treatment by attacking them with more than one kind of drug at a time. The first fullerene immuno-conjugates have been prepared and characterized as an initial step toward the development of fullerene immunotherapy.

### Polymersomes

Polymersomes, hollow shell nanoparticles, have unique properties that allow delivery of distinct drugs. Loading, delivery and cytosolic uptake of drug mixtures from degradable polymersomes were shown to exploit the thick membrane of these block copolymer vesicles, their aqueous lumen, and pH-triggered release within endolysosomes. Polymersomes break down in the acidic environments for targeted release of these drugs within tumor cell endosomes. While cell membranes and liposomes are created from a double layer of phospholipids, a polymersome is comprised of two layers of synthetic polymers. The individual polymers are considerably larger than individual phospholipids but have many of the same chemical features.

Polymersomes have been used to encapsulate paclitaxel and DOX for passive delivery to tumor-bearing mice (Ahmed et al., 2006). The large polymers making up the polymersome allows paclitaxel, which is water insoluble, to embed within the shell. DOX is water-soluble and stays within the interior of the polymersome until it degrades. The polymersome and drug combination spontaneously self-assembles when mixed together. Recently, studies have shown that cocktails of paclitaxel and DOX lead to better tumor regression that either

drug alone, but previously there was no carrier system that could carry both drugs as efficiently to a tumor. Hence, this approach shows great promise.

## Quantum dots

Single-particle quantum dots conjugated to tumor-targeting anti-human epidermal growth factor receptor 2 (HER2) MAb have been used to locate tumors using high-speed confocal microscopy (Tada et al., 2007). Following injection of quantum dot-MAb conjugate, six distinct stop-and-go steps were identified in the process as the particles traveled from the injection site to the tumor where they bound HER2. These blood-borne conjugates extravasated into the tumor, bound HER2 on cell membranes, entered the tumor cells and migrated to the perinuclear region. The image analysis of the delivery processes of single particles *in vivo* provided valuable information on MAb-conjugated therapeutic particles, which will be useful in increasing their anticancer therapeutic efficacy. However, the therapeutic utility of quantum dots remains undetermined.

## XPclad® nanoparticles

The poor aqueous solubility of many drug candidates presents a significant problem in drug delivery and related requirements such as bioavailability and absorption. Recently, our laboratory has developed XPclad® nanoparticles that represent a novel formulation method that uses planetary ball milling to generate particles of uniform size (Figure 1), 100% loading efficiency of hydrophobic or hydrophilic drugs, subsequent coating for targeted delivery, and control of LogP for systemic, cutaneous, or oral administration of cancer drugs, vaccines, or therapeutic proteins (Figure 2).

The method for making XPclad® nanoparticles uses a novel and relatively inexpensive preparation technique (i.e., planetary ball milling), which allows for controlling the size of the particles (100 nm to 50 μm; ± 10% of mean size) with >99% loading efficiency, polymer- or ligand-coating for controlled-, protected-, and targeted-release and delivery of their contents. The nanoparticles produced thereby contain the desired biologically active agent(s) in a biopolymer excipient such as alginate, cellulose, starch or collagen and biologically active agents. Generally, there are two types of mills that have been employed for making particles: vibratory or planetary ball mills. The vibratory ball milling grinds powders by high velocity impact while planetary ball milling employs a grinding motion. Typically, planetary ball milling has been used only to generate micron-sized particles, while vibratory milling can yield nano-particles. However, the high impact resulting from the vibratory milling technique makes incorporating biologicals difficult. Planetary ball mills pulverize and mix materials ranging from soft and medium to extremely hard, brittle and fibrous materials. Both wet and dry grinding can be carried out. Minerals, ores, alloys, chemicals, glass, ceramics, plant materials, soil samples, sewage sludge, household and industrial waste and many other substances can be reduced in size simply, quickly and without loss. Planetary ball mills have been successfully used in many industrial and research sectors, particularly wherever there is high demand for purity, speed, fineness and reproducibility. The planetary ball mills produce extremely high centrifugal forces with very high pulverization energies and short grinding times. Because of the extreme forces exerted, the use of vibratory and planetary ball mills to formulate therapeutics has not been practiced until now. In general, XPclad® particle size can be engineered to range from 5 to 30 nm up to 10 to 60 μm by controlling the size and number of planetary balls, grinding speed, milling cycles, and centrifugal force by varying the revolutions per second and planetary jar velocity.

The surface of XPclad® nanoparticles can be modified with hydrophilic (e.g., PEG) and/or hydrophobic (e.g., PCL) polymers to precisely control LogP values. Surface polymers can

be modified through the conjugation of targeting molecules (e.g., antibodies, folate, etc.) to active delivery of encapsulated agents. The interior core can entrap hydrophobic or hydrophilic molecules (e.g., drug, immune adjuvant, nucleic acid, metal ion, fluorophore, therapeutic protein, and/or peptide). For example, PC3 tumor-bearing mice that received folic acid-coated XPclad® nanoparticles containing Texas red plus cisplatin showed significant tumor regression compared to similar control mice (Figure 2). Moreover, XPclad® nanoparticles selectively induced PC3 cell death but did not kill normal epithelial cells of similar origin (RWPE-1 cells). Mice receiving dendritic cell-binding peptide-coated XPclad® nanoparticles containing *Streptococcus pneumonia* pneumococcal surface protein A (PspA) peptide and TLR7/8 agonist as adjuvant showed significant reduction in bacterial load after challenge compared to similarly challenged naïve animals or control mice that did not receive nanovaccines with PspA peptide or toll-like receptor (TLR)7/8 adjuvant. Therapeutic proteins (e.g., antibody) also can be encapsulated in XPclad® particles (4-12 μm) for oral delivery. Passive immunity can also be afforded by oral delivery of anti-protective antigen MAbs using XPclad® nanoparticles to neutralize anthrax toxin after systemic, oral, or respiratory exposure.

## Conclusions

Nano delivery systems hold great potential to overcome some of the obstacles to efficiently target a number of diverse cell types. This represents an exciting possibility to overcome problems of drug resistance in target cells and to facilitate the movement of drugs across barriers (e.g., BBB). The challenge, however, remains the precise characterization of molecular targets and ensuring that these molecules only affect targeted organs. Furthermore, it is important to understand the fate of the drugs once delivered to the nucleus and other sensitive cells organelles.

## Acknowledgments

## Abbreviations

| | |
|---|---|
| **BBB** | blood-brain barrier |
| **CPT** | camptothecin |
| **DT-A** | diphtheria toxin suicide gene |
| **DOX** | doxorubicin |
| **EPR** | enhanced permeability and retention |
| **PEO-PPO** | ethylene oxide-propylene oxide block copolymer |
| **C60** | fullerenes |
| **GBM** | glioblastoma multiforme |
| **GCS** | glucosylceramide synthase |
| **HER2** | anti-human epidermal growth factor receptor 2 |

| **MRI** | magnetic resonance imaging |
| **MAbs** | monoclonal antibodies |
| **MPS** | mononuclear phagocyte system |
| **MDR** | multidrug resistance |
| **PspA** | pneumococcal surface protein A |
| **C32** | polybutane diol diacrylate co amino pentanol |
| **PCA** | poly cyanoacrylate |
| **PLG** | poly(D,L glycolide) |
| **PEO-PCL** | poly epsilon-caprolactone |
| **PEG** | polyethylene glycol |
| **PLA** | poly lactic acid |
| **PLGA** | poly lactide-co-glycolide |
| **Tween 80** | polysorbate 80 |
| **SWCNT** | single-walled carbon nano-tube |
| **SSM** | sterically-stabilized micelles |
| **TLR** | toll-like receptor |

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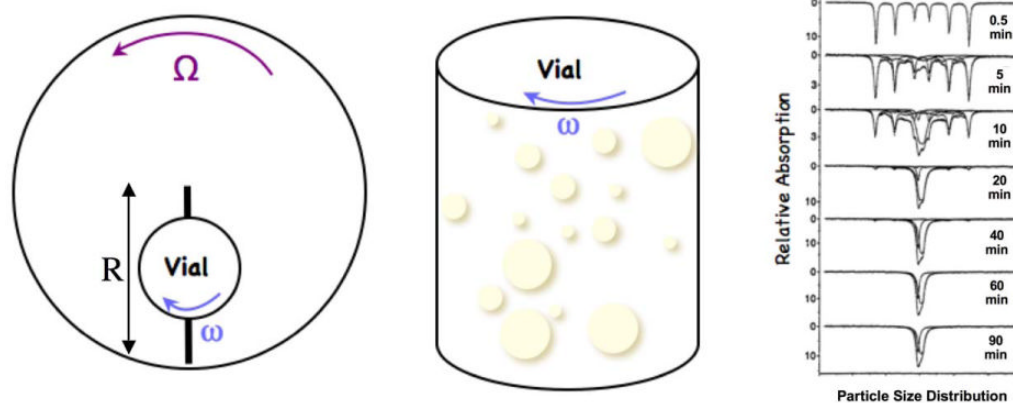
**Figure 1. XPclad® nanoparticle formulation**
The milling jar holds heat-absorbent zirconium oxide planetary milling balls, rotates about its own axis as well as in the opposite direction, around a common axis of the chamber wheel. This produces the rotation of planetary balls and enables the milling of particles from macroparticles containing materials such as starch, polyethylene glycol, Texas red, and/or drug. By controlling the centrifugal force, varying the revolutions/sec of $\Omega$, jar velocity ($\omega$), radius (R), the size as well as number of the zirconium planetary balls, duration and number of cycles, the size of the core can be controlled to generate 5-30 nm up to 10-60 µm particles. In this system, the combination of impact and frictional forces cause the planetary balls to mill the contents in the jar.
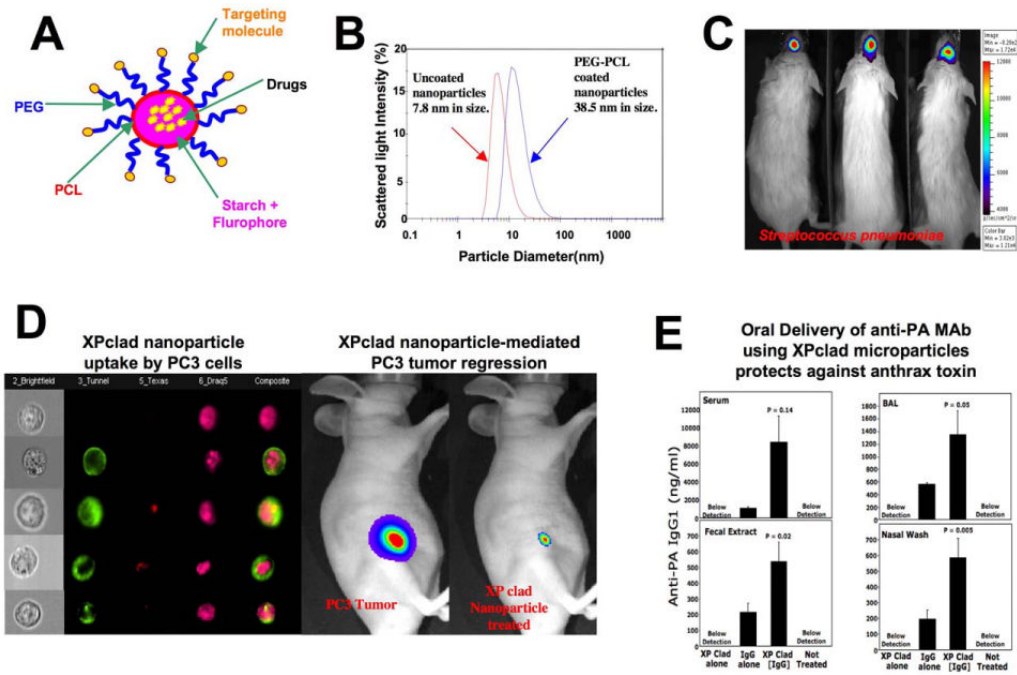
**Figure 2. Overview of XPclad® nanoparticle applications**
(*Panel A*) XPclad® nanoparticles produced by planetary ball milling contain the desired biologically active agent(s) in a biopolymer excipient such as alginate, cellulose, starch or collagen and biologically active agents with >99% loading efficiency. XPclad particles are coated with polymers and/or ligands for controlled, protected, and targeted delivery of their contents. (*Panel B*) XPclad® particle size can be engineered to various size ranges. (*Panel C*) Mice receiving dendritic cell-binding peptide-coated XPclad® nanoparticles containing *Streptococcus pneumonia* pneumococcal surface protein A (PspA) peptide plus TLR7/8 agonist as adjuvant caused a significant reduction in viable bacteria after challenge compared to similarly challenged naïve animals or control mice. (*Panel D*) XPclad® nanoparticles selectively target prostate cancer (PC3) cells but do not kill normal prostate epithelial cells (RWPE-1). Similarly, PC3 tumor-bearing mice that received folic acid-coated XPclad® nanoparticles containing cisplatin showed significant tumor regression compared to similar control mice. (*Panel E*) Passive immunity to anthrax toxins was effected by oral delivery of anti-protective antigen (PA) MAbs using XPclad® nanoparticles to neutralize anthrax toxin after systemic, oral, or respiratory exposure.