[Frontiers in Bioscience S2, 801-814, June 1, 2010]

Polymer- and liposome-based nanoparticles in targeted drug delivery

Subbu S.Venkatraman, Lwin Lwin Ma, Jayaganesh V. Natarajan, Sujay Chattopadhyay

School of Material Science and Engineering, Nanyang Technological University, 50, Nanyang Avenue, Singapore-639798

TABLE OF CONTENTS

1. ABSTRACT

This review focuses on polymer- and liposome-based nanoparticles used in targeted delivery of bioactive molecules, from drugs to siRNA to pDNA. The perspective centers around commercial and clinical successes, and a rationalization of these successes. Microparticulate systems are not covered, and only those applications that truly utilize the advantages of nano size are covered. “Stealth” systems dominate in this review, as most of the clinical successes are for passive targeting rather than for active targeting of tissue. The relevance of nano size to gene delivery is also discussed with relevant examples.

2. INTRODUCTION

In this review, we will focus on the status of research and development of polymer and liposome-based nanoparticles, as carriers for therapeutic drug delivery, and more specifically for targeted drug delivery. For the purposes of this review, a nanoparticle is defined as having dimensions below 1 micron; consequently, we will not discuss particles much larger than a micron. In addition, we will not discuss the use of ceramic or metallic particles, which have their own niche in this field. The review thus covers polymer- based nanoparticles including self-assembling systems such as micelles and liposomes.

We begin by noting that no polymeric nanoparticle, other than liposomes, has been approved by the FDA for therapeutic use. Particles in the nano range have been approved, but these are nanocrystalline drug molecules (1) or drug molecules conjugated to protein (2), which are not strictly polymeric carriers. Several liposomal carriers have in fact been approved (3), although it is not clear that all these are carriers in the nanometer range. A survey of the literature from 1990 onwards, shows that approximately 5000 articles feature nanoparticulate drug carriers, with approximately 700 of them being of polymeric origin: prolific by any yardstick. Similarly, a total of 1960 patents have been approved in the field of nanoparticulate drug delivery. So this is an extremely fertile field of research, and will continue to be fertile for the foreseeable future. Translation into products appears to be slow but steady.

Microparticulate systems have been around for a while, starting with the biodegradable Lupron-Depot® formulation of PLGA (Poly lactic acid and galaic acid copolymer) microspheres containing leuprolide acetate, for prostate cancer therapy. These are "solid" microparticles, in which the drug is dispersed in a matrix of a biodegradable polymer: the drug releases through a diffusional mechanism. In fact, such formulations are now common place, with the main advantage being the ability to carry reasonable amounts of drug, and being easily formulated into injectable (i.e., of acceptable viscosity) formulations.

In general, nanoparticles are touted to have advantages over microparticles. The most important advantage appears to be that of extravasation and of cellular penetration. There are two approaches to producing nanoparticles: communition of larger particles by milling or other grinding techniques; and by the conventional "synthetic" processes of emulsification/dialysis/lyophilization. Generally, processes such as spray-drying cannot produce particles in the nano range. For liposomal particles, ultrafiltration is frequently employed to weed out larger particles.

This review will focus on nanoparticulate systems in various stages of development: approved, in clinicals, in pre-clinicals and in an early research phase.

3. NANOPARTICULATES: STRUCTURE, PREPARATION AND CHARACTERIZATION

The different particle types are usually made by different processes, and have distinctive structures. In the following, we trace the broad outlines of these processes and structures.

3.1. Polymeric nanoparticles

3.1.1. Milling

Milling (which is basically a grinding process) in conjunction with filtration has been used to prepare polymeric nanoparticles. In the milling process, cryo-milling is generally needed if the polymer Tg (glass transition temperature) is below ambient. Spray-dried particles may be used as the starting particles, for drugs that are stable to spray-coating conditions. Otherwise, the starting particles may be from emulsion techniques. Milling generally leads to non-spherical particles, but this is acceptable from a drug delivery standpoint.

3.1.2. Emulsion methods

This is by far the preferred approach to obtain drug-incorporated polymeric nanocarriers. Hydrophobic drugs are incorporated by dissolving drug and polymer in an organic solvent, and then emulsifying this in water containing surfactant to obtain an oil-in-water emulsion. Evaporation of solvent followed by lyophilization is typically used to obtain drug-containing polymeric particles. Size ranges tend to be in the micron range, so further size reduction is indicated via milling or filtration methods.

To incorporate a hydrophilic drug, it is dissolved in water first, followed by emulsification into an organic polymer solution. This mixture is then emulsified into water containing surfactant to produce a water/oil/water (w/o/w) emulsion. Solvent drying followed by lyophilization leads again to micron-sized particles in general.

In both cases, controlling the homogenization during emulsification appears to be a key step. There is agreement that in the production of hydrophobic polymer particles, the size decreases with increasing homogenizer speeds (4) and with increasing amounts of surfactant (5). Speeds of approximately 10,000 rpm were required to bring sizes of PLGA (Poly (D,L-lactic acid and Glycolic acid copolymer) particles down to below 1000 nm, while PVA(Poly vinyl Alcohol) concentrations of greater than 5% did not further decrease PLA (Poly L-lactide) particle sizes below 120-150 nm.

Variations on the emulsion method have been proposed. For example, Quintanar-Guerrero *et. al* (5) have prepared both nanospheres and nanocapsules by the use of an oil in the organic phase. In this scheme, to prepare nanocapsules, a polymer, oil (miglyol) are first dissolved in ethyl acetate (EtAc)/water, and then emulsified into a PVA solution in water/EtAc mixture. When water is added to this emulsion, EtAc diffuses out into the water, forming nanosized structures, which are then solidified by solvent removal followed by ultracentrifugation. Nanospheres of about 400nm can be made by eliminating miglyol from the mixture, while nanocapsules of sizes 180-300 nm are made by addition of varying amounts of miglyol. Presumably the "skin" of the capsules was made of the polymer. This method allows for greater loading of hydrophobic drug in the core of the nanocapsule.

3.2. Polymeric Nanomicelles

Polymeric nanomicelles are made in an entirely different manner. Generally, an amphoteric molecule is first synthesized (typically, PLA-PEG copolymer), then its critical micellar concentration (CMC) is determined. Following this, the polymer and drug are dissolved first in an organic solvent (if drug is hydrophobic), then the mixture is dialyzed against water. The concentration of the polymer in the final amount of water is adjusted to be well

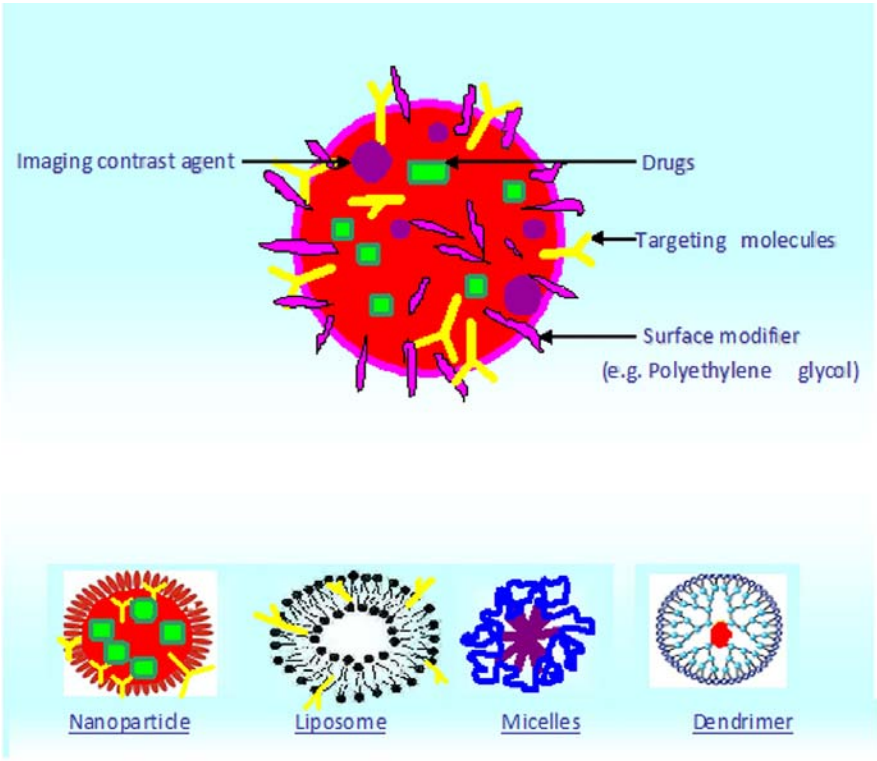

Figure 1. Depiction of different types of polymeric nanoparticles, and associated functionalities. Surface modifiers, targeting molecules (ligands) and incorporated agents are also shown.

above the CMC. Following dialysis, the "particles" may be isolated by centrifugation and/or freeze-drying. Reconstitution in water or saline should regenerate the micellar structure, as long as the micelles are stable in an ionic medium. Typically, the CMC is increased by the presence of ionic species.

Hydrophilic drugs cannot be easily incorporated into simple spherical micellar structures. For this we need to go to another type of self-assembling system, the liposomes (Figure 1).

3.3. Liposomes

Generally, known amounts of lipids, with and without cholesterol, are dissolved in ethanol at 60^{o}C. Based on the desired concentration of the liposome formulation a certain amount of buffer is usually added at a temperature above the transition temperature of the lipid mixture. This allows for formation of multi lamellar vesicles (MLV); however, these have sizes in the range of 0.5 microns to 5 microns. These are downsized to about 70-500 nm (uni lamellar vesicle, or ULV) by extrusion through membrane filters at high pressures (6). Drugs are loaded either by passive or active loading methods. For passive loading, lipophilic drug is dissolved in the organic phase, while hydrophilic drug is dissolved in the aqueous phase. However, passive loading generally does not yield high encapsulation efficiency. For this reason, active methods such as a pH gradient (7) or an electrochemical gradient (8, 9) are employed for different drug types.

3.4. Dendrimers

These are a unique class of nanostructures that hold some promise for drug delivery. Specifically, these are hyperbranched structures (polymers), with sufficient packing space in their core to hold drug. However, control of release from such structures is not straight-forward, as we will see in the examples below. These dendrimers are typically prepared by a very specific reaction sequence, usually starting with an amine-terminated molecule. Such a molecule is reacted with an acrylate ester, and then subsequently with ethylene diamine to yield a 'full-generation" dendrimer (10). Repetition of the above reactions yields a highly branched structure with internal 'cavities' that may hold metal atoms or other guest molecules by virtue of the presence of amine groups. Molecules may be conjugated to the interior groups as well as the 'surface" groups and these molecules may be drug, peptide, antibody or PEG. This versatility opens up possibilities for targeted delivery (Figure 1), and sizes are controllable by reaction sequence and extent.

4. APPLICATIONS I: TARGETED CANCER CHEMOTHERAPY

We now review the applications of the above classes of nanopartculate carriers in different therapies. By far, the greatest attention has been paid to targeting tumor tissue, as nanoparticles have the ability to traverse easily out of blood vessels into tumor tissue in comparison to their microparticulate cousins. In addition, surface modification

of nanoparticles for evasion of the reticulo-endothelial system (RES) as well as for penetration of selected tissue, is no less feasible for nanoparticles. So in what follows, we discuss the relative successes of the different particle types in targeting tumor tissue.

4.1. Active and passive targeting

Two concepts have been used for targeting cancer tissue. Passive targeting involves injectable drug carriers that have been surface-modified (Figure 1) to evade the RES such that their blood lifetime is relatively long. Long-lived particles have a much greater chance of reaching the blood vessels surrounding solid tumors, and then extravasate by virtue of their size. Once in tumor tissue, the relative lack of lymphatic drainage allows for slow release of the payload into the surrounding tissue. The whole effect goes by the name of Enhanced Permeation and Retention (EPR).

Active targeting relies on conjugating a targeting ligand (usually an antibody) to the surface of the particle such that the ligand targets cancerous tissue only. Most of the work to date has focused on folate and transferrin receptor targeting; these two receptors are over-expressed in cancerous cells. Others have tried to exploit the presence of a tissue-specific antigen (TSA) whose antibody may be used for the targeting. In this approach, the nano size is not as critical, although it still helps in facile extravasation.

4.2. Liposomal Delivery

4.2.1. Doxil

Liposomal delivery systems have been by far the most successful of the nanoparticulate carriers. No less than 4 pharmaceutical products that use lipsomes as a carrier have been approved: of these, Doxil® was approved in 1995 for the treatment of ovarian and other cancers. Another two systems, one for lung cancer (Introgen) and another for solid tumours (Synergene Therapeutics) are in clinical trials.

Doxil is formulated as a “stealth” liposome for passive targeting (Figure 1), using a PEG molecule attached to one end of the liposome. The liposome is made from distearoyl-phosphatidyl-ethanolamine (DSPE) conjugated at its amine end to a 1900-Mw mPEG molecule (11). Doxorubicin is present at 20 mg or 50 mg loading in a 10mL or 30mL vial. Cholesterol is also added in the liposomal formulation to control the efflux of doxorubicin through the bilayers of lipid molecules. Most of the drug (90%) is claimed to be inside the liposomes. Such high loadings are generally only possible with what are called active loading methods. This uses a salt, such as ammonium sulfate, inside the core of the liposome, to bind to the drug molecule as drug-sulphate and precipitate which then drives the influx of drug to the liposome core (8).

The liposomal particles are claimed to be in the size range of 80-100 nm (12). It is further claimed that extravasation is possible only for particles less than 600 nm in size. The particles have a blood half-life of 15.2 h compared to about 3 hours for the particles without the PEG attached (12). Clinically, this is highly significant because, accumulation in liver and spleen is considerably reduced for the stealth particles, and this translates directly to tumor size reduction and its maintenance over 100 days, compared to free drug injections; also to a substantial improvement in the survival rate of mice with colon carcinoma.

Doxil® was a pioneer in the field of passive targeting. Its success prompted a frenzy of research into the concept of stealth particles, using polymeric and non-polymeric cores.

4.2.2. Liposomal systems in pre-clinical and clinical phases

Since the success of Doxil®, many liposomal systems have gone through animal testing and clinical trials. One such is for lung cancer targeting using the tumor suppressor p53 gene. The first paper on this approach was in 2001 by researchers from the M. D. Anderson cancer Center in Houston and it reported successful suppression of primary and metastatic lung tumor growth (13) in animal studies. Co-authors included employees of Introgen, which subsequently seemed to have abandoned liposomal delivery in favor of adenoviral carriers for delivering the gene (14). The liposome that was the optimum for gene transfection of the p53 and the FHIT genes [as well as the FUS1 gene, reported subsequently (15)] was the DOTAP:Cholesterol combination, which consisted of dioleyl trimethyl ammonium methyl sulfate (DOTAP) mixed in equimolar ratio with cholesterol. This liposome was extruded through filters to reach sub-micron sizes, then complexed with the plasmid DNA containing the appropriate gene to form particles in the 300 nm range (15). In mice bearing the experimental human lung metastatis A549, it was shown that the DOTAP:Chol liposomal vector with FUS1 gene suppressed tumor nodules and increased survival rates significantly. There is no mention of a targeting ligand attached to the liposomal vector, neither is there any PEGylation, hence this is not an example of either passive or active targeting. It success must be attribute then solely to fact that the only cells likely to be affected are the ones that lack the suppressor genes, namely the cancerous ones.

Although Phase I trials were announced, by November 2008, Introgen was bankrupt. It is not clear if the trials were successful or whether another company bought the patent rights.

Another company that had reported clinical trials with a liposomal vector was SynerGene Therapeutics based in Washington D.C., USA. As of May 2009, the Phase I trials have not been completed, although the trial started in 2007. From various company reports, this appears to be a liposomal vehicle with targeting ligands, presumably a folate receptor targeting ligand. The payload is likely to be a p53 gene also. Details are sketchy. A patent (16) describes an “immunoliposome” which is based on the liposome component DOPE (dioleyl phosphatidyl ethanolamine) incorporating a transferrin-receptor targeting ligand. The immunoliposome is complexed with p53 wild type genes and targets the transferrin receptor, which is over-expressed in many tumours.

To sum up, then, despite the success of Doxil for treating ovarian cancer, other lipsome-based products are still struggling to make it through the clinic.

4.3. Polymeric nanomicelles

Liposomes can be driven towards unilamellar (SUV) and multilamellar (MLV) structures with encapsulation of bioactives in the core of the vesicle. Polymeric nanomicelles in fact can spontaneously form under the appropriate conditions in aqueous or non-aqueous media depending on the molecular structure and extent of amphiphilic character.

In terms of papers and patents, nanomicellar drug delivery has been a fertile field. For polymeric molecules to self-assemble to spherical micelles or lamellar structures, amphipathic structures are needed. For this purpose, block copolymers are ideal, with one block being hydrophilic and the other hydrophobic. Tri-blocks, diblocks and even 4-armed blocks have been used (17, 18). Relative to liposomes, success in the clinic has been harder to come by, but, there are some notable exceptions, as we will see in the next section.

4.3.1. Clinical candidates

Years of exploratory work has led to the development of three promising nanomicellar formulations, containing paclitaxel (NK105), doxorobucin (NK911) and cisplatin (NC-6004). The work was spearheaded by the National Cancer Center Hospital/Research Institute East, in Chiba, Japan, in conjunction with University of Tokyo researchers, and some companies.

4.3.1.1. NK105

The polymer is a diblock copolymer of PEG and modified polyaspartate (19) where half of the aspartate groups are converted to 4-phenyl 1-butanolate. Presumably this modification is required to bring down the CMC. The overall Mw of the polymer used was 20,000, of which the PEG block was 12,000 and the aspartate block was 8000. Approximately 20% of the polymer weight can be loaded with paclitaxel, held in the micellar core by hydrophobic self-association. The micelles obtained in water or aqueous media were lyophilized to obtain particles, which upon re-constitution yielded micelles of average diameter about 85nm, with size ranging from 20 to 430 nm.

Paclitaxel is a potent anti-cancer drug that suffers from poor bioavailability due to its low solubility. In addition, systemic injection with a solubilizer such as Cremaphore EL induces hypersensitivity reactions. Moreover, peripheral neuropathic reactions as well neutropenia have been reported with repeated use of paclitaxel (20). For all these reasons, a better delivery system that can at least partially target tumours is highly desirable for paclitaxel.

In a (colon 26 tumour bearing) CDF1 mice study, NK105 particles were detected in blood 72 hours after injection (19). The half-lives of NK105 were ~ 5-6 hours in comparison to paclitaxel drug whose half-life was around 1 hour. This differential in half life was enough to enhance tumour concentrations by a factor 2-3 while tumour half-lives were enhanced by a factor of 10. Tumor suppression was notably more efficient with NK 105 compared to paclitaxel drug, using a BALB/1 mouse model bearing a colonic tumour.

Following these promising animal data, a Phase 1 trial was conducted in 2007 on 19 patients (21), who had solid tumors refractory to conventional chemotherapy. Escalating doses of NK105 were administered every 3 weeks, and clinical parameters including neuropathy and PK parameters, were evaluated. NK105 showed slow clearance, and comparable $t_{1/2}$, indicating retention in tumors. Neuropathy was not observed. Hypersensitivity was also negligible, even without co-administration of steroids.

The maximum tolerated dose was determined from the study and suggested for a Phase 2 study, for which recruitment is currently under way. The encouraging consequence of the tolerability and pharmacokinetics (PK) study was the response seen in a patient with metastatic pancreatic tumor, where a 90% reduction in liver metastasis size was seen. Clearly these are highly promising signs for a partial targeting model for NK105.

4.3.1.2. NC-6004

Cisplatin (a Pt (II) complex with cis-dichlorodiamine) is another anti-cancer drug that suffers from various limitations, including nephrotoxicity and neuropathy, with repeated use (22). A novel micellar system using PEG and poly(glutamate) has been developed to incorporate cisplatin via complexation of the glutamate unit to Pt(II) (23). This is a novel way to improve cisplatin loading, and control its release. In fact *in vitro* release of cisplatin into saline is triggered by chloride substitution for glutamate in the complex, and shows no burst, but sustained release over 150h. The sizes of the particles remained steady, at about 30 nm over 72 hours, and presumably decreases beyond that due to micellar dissociation into unimers.

Clearance and tissue distribution are superior for the micellar formulation of cisplatin compared to free cisplatin. In the mouse study (23), tumour accumulation over 24 hours is roughly 5-10 fold higher than free cisplatin, depending on tumour location. Tumour inhibition was demonstrably superior with the micellar formulation, for mice bearing colon adenocarcinoma.

Based on these animal results, a Phase I study was carried out in 2008 (24), with more encouraging results. Although the patient size was small 17, the study results clearly indicated that the micellar formulation of cisplatin was much better tolerated, with very few adverse effects reported, including very little nausea (no anti-emetics were administered). The best patient response was stabilization of disease; however, a Phase II study is planned with recommended dosing for mono and combined therapy.

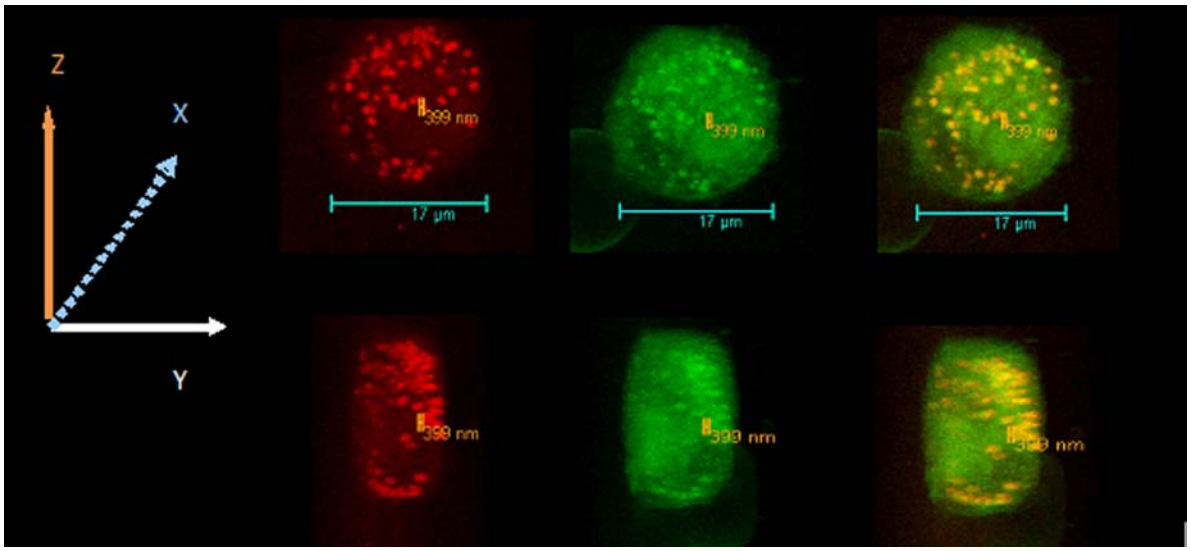

Figure 2. These 3D confocal images show the effect of size of block copolymer particles on uptake by the chief phagocytic cell of the RES, the monocyte. The larger-sized particles or micelles made from P(dl)LA-PEG (250-350nm) are tagged with a red fluorescent dye, while the smaller-sized particles (90-150nm) are tagged with a green dye. The top and bottom sets are from different positions of a cell collection, after incubating the particles with human monocyte cells for several hours. The leftmost image shows only the red (larger) particles, the middle one only the green, and the right image shows the "overlay" of red and green. Clearly the red dots are more numerous relative to the green dots, confirming that larger particles (in this range) are taken up more readily by phagocytic cells, and therefore will have shorter blood lifetimes.

4.3.1.3. NK911

A similar micellar system was developed for doxorubicin (25). Based on PEG-polyaspartate copolymer (PEG Mw = 5000; polyaspartate has 30 repeats), Doxorubicin (dox) was conjugated to the aspartate moiety for greater loading efficiency. The conjugated drug confers sufficient hydrophobicity to enable formation of stable micelles. This enhanced hydrophobicity allows more packing of "incorporated' dox (as distinct from 'conjugated" dox). The conjugated dox is not active, and is not released from the micelle, while the incorporated dox is released slowly over 24 hours. The mean diameter of the micellar particles is 42 nm, after freeze drying of the micellar solution.

Using mice models of various cancers, efficacy of treatment and accumulation was greatly enhanced for the micellar dox compared to free dox. A Phase I study was initiated in 2003 with this micellar (26) formulation. A total of 23 patients with various cancers were enrolled, with pancreatic cancers being actively recruited. Patients were infused (over 1 to 12 min) with the micellar dox. Escalating doses starting from 6mg were administered over 6 cycles. The clearance times, half-lives and volume of distribution all seemed to confirm that the micellar dox circulates longer in blood and accumulates preferentially in tumor tissue. Patients showed disease stabilization with cycle 4, and one patient showed a therapeutic response after cycle 6.

An interesting comparison with Doxil (the liposomal dox) shows the micellar dox to be cleared 400 times faster than Doxil (26). However, it is not clear that this longer blood lifetime translates into greater accumulation in tumour tissue, as the volume of distribution for doxil appears higher than that for micellar dox. Further studies are required to compare the efficacies of micellar dox and Doxil.

4.3.2. Other nanomicelle systems and studies

There is an excellent review of the large amount of research in the field of micellar nanocarriers (27). The major polymeric systems studied appear to be block copolymers of hydrolysable polyesters with PEG. The hydrolyzable polyesters include poly (caprolactone) (28,29), poly(L-lactide) (17,18) and Poly(L-lactide-co-glycolides) (30). In addition, 'stealth" entities other than PEG have been studied as micelles, including Poly(vinyl pyrrolidone) or PVP and poly(vinyl alcohol) or PVA. However, no other entity has been shown to work as well as PEG.

There are works on the optimum PEG length and configuration needed for stealthiness (31). In our study, we synthesized triblock PLA-PEG-PLA copolymers (ABA type) as well PEG-PLA-PEG copolymers (BAB type), with varying lengths of PEG and PLA, and measured uptake by blood cells *in vitro*. We also compared the uptake behavior of triblocks with diblocks of PEG-PLA. The lower the uptake greater is the stealthiness" or longer the blood lifetime. The confocal image of Figure 2 shows that as the size of the nano particle increases (marked red) the phagocytic uptake increases compared to the smaller particle (marked green). In general, uptake was greater for ABA triblocks compared to diblocks and BAB triblocks of similar PEG and PLA lengths; in addition, it was found that

the PEG segment length had to be above 5000 Mw in order for any effect to be seen for uptake reduction by blood cells. Surface concentration of PEG was not the only determinant factor for reducing uptake; the surface conformation of the PEG was important as well. More free PEG molecules (i.e. with conformational freedom) exerted a bigger effect compared to more constrained molecules. We have attempted to correlate the *in vitro* results to *in vivo* blood lifetimes. Generally, the correlation is good, with some notable exceptions, which we plan to explain in a forthcoming paper.

In general, loading of hydrophobic drug is easier for these micellar constructs. However, even for hydrophobic drugs, loadings are typically in the range of 5-20%. To increase loading further, specific interactions with the core molecule is needed. This approach has been successfully tried out with core molecule modification (32). In this case, PEG was copolymerized with PCL, or glycidyl methacrylate (PGMA) or a diisopropylamino ethyl methacrylate (PDPA). Indomethacin (and its derivatives) contains a carboxylic group; hence, by acid-base interaction with the amine group, loadings of 100% were achieved using the PEG-PDPA copolymer. It follows that the drug release is also a function of pH: at pH=7.2, release is by diffusion kinetics with almost 100% of drug released in 24 hours. At lower pH, release is instantaneous, as the protonated core molecules force disassociation of the micelle. So in practical terms, although high loadings are achievable, release rates are also too high.

A slightly different situation exists for 17 β-estradiol loading in PCL-PEG micelles. In this case, loadings of 200% were achievable by simple micellization in the presence of the drug (33). Here the authors attribute the high loading is due to hydrophobic interactions, as well as the relatively large number of molecules/micelle (reported to be 125 for PCL_{20}-b-PEO_{44}). This large number of PCL entities ties up about 4000 17 β -estradiol molecules, based on a 190% loading. This sort of association is unusual, and not expected for the large majority of drug molecules.

Loading of more hydrophilic drug and control of its release requires special techniques, including drug conjugation. Fortunately, most of the anti-cancer drugs tend to be fairly hydrophobic. This is in fact the main reason that these aqueous micelles have been predominantly studied for anti-cancer therapy. Loading of antibiotics, for example, will not be high, and loaded drug may release quicker than other drug types.

One example of hydrophilic drug loading is in the example shown above, for cisplatin, using poly glutamate as a conjugating agent (NC6004). Cisplatin is moderately hydrophilic, and the loading can be increased to 35-40% by conjugation. Although other approaches have been proposed, including the so-called "core surface-crosslinked" micelles (34), it is not clear that these increase loading to any higher amount without the accompanying increase in release rate.

4.3.3. A comparison of liposomes and nanomicelles: areas for future research

In comparing these systems, we can ask the following questions:

- Which system has greater blood lifetimes?
- Does this translate to greater tumour tissue accumulation?
- Are drug loading and drug release well-controlled and optimum?
- Is bioactivity enhanced by liposomal and micellar encapsulation?

To answer these questions, we note first of all that only liposomal drug delivery systems have been successfully introduced for cancer therapy to date. Nanomicellar systems are in advanced clinical stages, but yet to be submitted for regulatory approval in the major economies. What is the reason for this discrepancy? One possible explanation is the relative "stability" of liposomal formulations compared to micellar ones. Lasic, one of the pioneers in the liposome field, argues (35) that liposomes do not form unilamellar or multilamellar structures spontaneously, unlike micelle formation from block copolymers. This means that the lamellar structures are not thermodynamically favored. Yet, paradoxically, once they are formed (by extrusion process *i.e.* external energy input), these structures tend to be more stable, due to what Lasic calls "kinetic trapping". This kinetic trapping is essentially caused by hydration of the polar heads, and thus it results in lack of penetration of the lipid cores by water. In spite of this meta-stability, liposomal preparations are still freeze-dried (with cryoprotectants), stored at cold temperatures and reconstituted just before use. This is to prevent excessive loss of trapped drug.

Micelles are thermodynamically favored structures for block copolymers in solvent (i.e. good solvent for one of the constituents). Thus an amphipathic molecule can form micelles in water as well as so-called "reverse micelles" in organic solvents. However, all of them have a critical micelle concentration (CMC). The lower the CMC, the greater is the tendency for these micelles to form, and of course, greater the resistance to structure breakdown upon dilution. Another complication is that added salts generally increase CMCs, and hence break down existing micelles structures. Since, micellar formulations are naturally diluted upon injection into the bloodstream, it is speculated that they tend to become monomeric entities upon injection; this leads to immediate drug release, which is undesirable for targeted drug delivery. Liposomes are not susceptible to this, as the kinetic entrapment ensures that dilution has very little effect on the liposome. In other words, water penetration into the core of a liposome is not enhanced by adding more water, hence the apparent stability of liposomes following injection. Incorporation of cholesterol in liposomal

formulations is also believed to enhance this kinetic stability.

The comparison of cisplatin formulations of liposome and micelles, however, is very interesting in this regard. SPI-077 is a cisplatin-encapsulating liposomal formulation, which is also PEGylated. It contains 14 μg of cisplatin per mg of liposome in 100nm particles (36). A Phase I-II study in 18 patients with inoperable head and neck cancer (for whom free cisplatin is effective), showed no efficacy of liposomal cisplatin. This was in contrast to mice studies which showed superior *tumors* size reduction and prolonged blood lifetimes for the liposomal cisplatin (37). The authors of the clinical study attributed the lack of efficacy to much slower release of cisplatin from the liposome, resulting in concentrations in the tumour that were below the minimum effective concentration required. Here, the enhanced stability of the liposome appears responsible for decreased efficacy! As noted earlier, micellar formulations of cisplatin (NC6044), release cisplatin rather rapidly in comparison, by a novel mechanism of chloride displacement of conjugated polymer. Thus at least for cisplatin a relatively rapid release may be a prerequisite for efficacy.

In general, (while noting specific deviations such as cisplatin) the answer to the 4 questions posed above is this: whichever entity has greater stability in blood, will probably have enhanced blood lifetimes, greater tumor tissue accumulation (although this one can be influenced by other factors such as size and distribution in specific tissues), better control of drug release and enhanced bioactivity. Thus, in our opinion, research into micelles should focus on introducing "kinetic trapping"; for example, by means of cross linking of the core polymer post-micelle formation. How feasible this is remains to be seen. This kinetic entrapment refers mainly to stabilization of the micellar structure and should not result in slow release of entrapped drug. It may turn out that these are mutually incompatible requirements, and that micelles may work for a certain class of drugs while liposomes may work for another.

4.4. Nanoparticles with dispersed drug

We now turn to drugs dispersed in solid nanoparticles or dissolved in nanocapsules. Solid nanoparticles are defined as matrix formulations whereby the drug is either dissolved or dispersed in a polymer that is processed into a nanoparticulate form (other than vis self-assembly), whereas nanocapsules are core-shell particles, made with two different types of polymers or with lipids as cores and polymeric shells. Of the two, it appears that nanocapsules are rare, as most core-shell particles tend to have micron dimensions. Thus most of the discussion below relates to matrix-type nanoparticles.

The main advantage for using these particles in preference to micelles and liposomes are simplicity of manufacture, greater stability and better control over release of drug. Disadvantages are relative difficulty in attaching PEG or ligands to the particle. As mentioned above, matrix microparticulate drug delivery systems have been around for a long time, but nanoparticulate ones are rare. As far as we are aware, none of the FDA-approved nanosystems are based on solid nanoparticles (Table 1).

Most of the work in this area has focused on biodegradable nanoparticles, particularly Poly (lactide-co-glycolide) or PLGA particles. This focus, at least partially, is due to the success of PLGA microparticles in drug delivery with products such as Lupron-Depot ® (TAP Pharmaceuticals) having enjoyed success for at least 2 decades as an approved product; another product using similar micron-sized particles was Nutropin Depot®, which was approved in 1999 but removed from the market in 2004, with the company citing expensive manufacturing as the reason.

PLGA particles can be made using a variety of ways, from emulsion techniques to spray-drying. More usually, however, the emulsion technique is preferred with the use of special dispersion techniques such as ultrasound. In a variation of the emulsion technique, called the spontaneous emulsification solvent diffusion (SESD) method, a mixed solvent system is used for the polymer solution, which is then emulsified into an aqueous medium containing a surfactant. To minimize agglomeration of the particles, a low-M_w PVA surfactant is used, and two completely water-miscible solvents can be used for the polymer phase (38). This technique produces non-aggregating particles in the size range 100-200 nm that remains stable even after freeze-drying.

Such particles can incorporate a wide range of drug molecules, from low-Mw hydrophobic molecules (39) to proteins (40) and even plasmid DNA (41). As mentioned earlier, such nanoparticles may again be surface modified, but it requires special techniques to do so. For example, a surfactant called Polysorbate 80 (the hydrophilic part is essentially PEG, while the lipophilic part is an oleic acid derivative), has been used to coat polycyanoacrylate particles (42), and one assumes that this method may be applicable to other polymeric particles. PLGA has also been coated with PEG and PEG-like molecules, including the surfactant molecule, PEG-PLA-PEG (43). Grafting of PEG molecules to surfaces of already-formed nanoparticles is seldom reported, most of the "chemically-bound" PEG nanoparticles relying instead on copolymerization with PLA (44) or PLGA (45) or even poly cyanoacrylate (46).

So are particles coated with PEG as good as micellar particles of PEG block copolymers? In an interesting study, Mosquiera *et. al.* (47) found that if a PEO/PPO copolymer is coated on to a PLA nanoparticle, the mean residence time in blood (after intravenous injection into mice) increases to 70 minutes from a few seconds for the uncoated PLA particle. When a diblock copolymer of PLA and PEG is injected (presumably at concentrations well above CMC), the mean plasma residence time increases to about 140 minutes. A triblock (PLA-PEG-PLA) on the other hand, shows a reduced residence time, presumably because of configurational constraints on the PEG block, in agreement with our own *in vitro* results (35).

Table 1. List of nanoparticle-based products at various stages of research and development

Lipid systems				
Product	Type of nanoparticles or drug	Indication	FDA	Company
AmBisome®	Liposomal/amphotericin B	Fungal infections	Approved 1997	Gilead Sciences
Doxil®	PEGylated liposome/doxorubicin hydrochloride	Ovarian cancer, AIDS-related Kaposi sarcoma	Approved 1995	Ortho Biotech
Diprivan®	Lipid emulsions/Propofol	Anesthetic	Approved 1989	Astra Zeneca Pharmaceuticals
INGN-401	Liposomal/FUS1	Lung cancer	Phase I study terminated	Introgen (company is no longer listed)
SGT-53	Liposome/p53 gene	Solid tumors	Phase II in progress	SynerGene Therapeutics
Dendrimer systems				
Vivagel®	Dendrimer/microbicide (not clear that nanoparticles are involved/needed)	Topical microbicide for sexually-transmitted diseases	FDA fast track(Phase II)	StarPharma Holdings
Nano micellar systems				
NK105	Diblock copolymer micelles/ paclitaxel (PEG-modified Poly aspartate)	Solid tumours (pancreatic, gastric, bile)	Phase 1 completed	National Cancer Centre East, Japan
NC 6004	Diblock copolymer micelles/ cisplatin (PEG-poly(glutamate))	Solid tumours (pancreatic, esophageal)	Phase 1 completed	NCC East, Japan NanoCarrier Ltd, Japan
NK 911	Diblock copolymer micelles/ Doxorubicin ((PEG-Poly aspartate)	Metastatic solid tumours	Phase 1 completed	NCC East, Japan Nippon Kayaku Co, Japan
Conjugated/Bound/Coated Nanoparticles				
Abraxane®	Nanoparticulate albumin/paclitaxel	Various cancers	Approved in 2005	American Pharmaceutical Partners
Cyclosert®	PEG-lated β-cyclodextrin conjugated to camptothecin	Solid tumors	Phase I/II	Insert Therapeutics/ Calando Pharmaceuticals
Lipid systems				
Product	Type of nanoparticles or drug	Indication	FDA	Company
AmBisome®	Liposomal/amphotericin B	Fungal infections	Approved 1997	Gilead Sciences
Doxil®	PEGylated liposome/doxorubicin hydrochloride	Ovarian cancer, AIDS-related Kaposi sarcoma	Approved 1995	Ortho Biotech
Diprivan®	Lipid emulsions/Propofol	Anesthetic	Approved 1989	Astra Zeneca Pharmaceuticals
INGN-401	Liposomal/FUS1	Lung cancer	Phase I study terminated	Introgen (company is no longer listed)
SGT-53	Liposome/p53 gene	Solid tumors	Phase II in progress	SynerGene Therapeutics
Dendrimer systems				
Vivagel®	Dendrimer/microbicide (not clear that nanoparticles are involved/needed)	Topical microbicide for sexually-transmitted diseases	FDA fast track(Phase II)	StarPharma Holdings
Nano micellar systems				
NK105	Diblock copolymer micelles/ paclitaxel (PEG-modified Poly aspartate)	Solid tumours (pancreatic, gastric, bile)	Phase 1 completed	National Cancer Centre East, Japan
NC 6004	Diblock copolymer micelles/ cisplatin (PEG-poly(glutamate))	Solid tumours (pancreatic, esophageal)	Phase 1 completed	NCC East, Japan NanoCarrier Ltd, Japan
NK 911	Diblock copolymer micelles/ Doxorubicin ((PEG-Poly aspartate)	Metastatic solid tumours	Phase 1 completed	NCC East, Japan Nippon Kayaku Co, Japan
Conjugated/Bound/Coated Nanoparticles				
Abraxane®	Nanoparticulate albumin/paclitaxel	Various cancers	Approved in 2005	American Pharmaceutical Partners
Cyclosert®	PEG-lated β-cyclodextrin conjugated to camptothecin	Solid tumors	Phase I/II	Insert Therapeutics/ Calando Pharmaceuticals

So in terms of stealth properties, the micellar particles appear superior. However, coatings of nanoparticles with PEG copolymers appear to work as well: coated particles have the advantage that the core polymer type may be changed to accommodate various drug types. Nevertheless, we find few reports of solid, coated nanoparticles making it to the clinic for passive targeting. Liposomal and micellar particles are the particles of choice in such applications.

In what follows, we focus on those applications where stealthiness is not that important an attribute.

5. APPLICATIONS II: INTRACELLULAR DELIVERY

The other application where nano size becomes important is in delivering into cells. These include delivery of low-M_W drugs (for anti-restenosis or anti-thrombotic effects), as well as genes for gene therapy and siRNA for anti-sense therapy. Here the blood lifetime is no longer a predominant requirement, as the mode of administration may be via infusors at the site of delivery.

One such important application is in treating thrombus formation and restenosis following angioplasty. Several infusors, such as Dispatch® and Infiltrator®, have been approved by the FDA for catheter-based drug delivery. The Dispatch® device, for example, was given a 501K approval in 1996 for delivery of anti-thrombotic agents such as heparin and urokinase following angioplasty. Since then the device has been used in a variety of preclinical and clinical studies. The interesting feature of this indwelling catheter is that it is designed to deliver drug intra-arterially for up to 4 hours without

blocking blood flow. This makes it an ideal delivery mechanism for various bioactive molecules from drugs to proteins to plasmid DNA.

Since the infused drug usually is in a liquid (either suspended or dissolved), the delivery of nanocrystalline drugs and particles becomes easy, without having to worry about agglomeration effects or rapid clearance that usually accompanies intravenous administration. Sustained and localized delivery is possible, but surface modification of particles appears to be critical for cellular uptake. Song *et. al.* have developed an *ex vivo* model for measuring arterial uptake, using an explanted canine femoral artery prefused with the drug solution for 30 seconds at 37°C (48).

In a study of PLGA nanoparticles (49), whose surfaces were modified via chemical and physical means, it was found that a cationic detergent (didodecyldimethylammonium bromide, DMAB) adsorbed at 5% onto the PLGA particle, yielded the highest arterial uptake, approximately 40 times that of the unmodified PLGA particle. Other cationic adsorbents similarly increased arterial uptake, presumably due to the charge and size combination. The sizes of these particles were reported to be about 100 nm. These findings were translated into positive results in a rat study, to confirm cellular delivery of dexamethasone incorporated in PLGA particles (50). The study involved an artery injury model, with "local" infusion of the drug-containing nanoparticles, over a 3-minute period in the carotid artery. The local infusion is accomplished by closing off the arterial segment, creating a closed arterial space into which the drug suspension is infused. The study, clearly demonstrates that these nanoparticles (without any surface modification) were able to penetrate the luminal, the medial and the adventitial layers, with sufficient dexmethasone being delivered. Although the authors claim that the infused drug inside the nanoparticles is able to reduce stenosis by 31% compared to intraperitoneally delivered dexamethasone. It has to be admitted that this is a non-conventional animal model for restenosis, and such studies need to be extended to the standard porcine models.

Larger animal studies of this concept do not appear in the literature, neither is there any first-in-man (FIM) study reported. We suspect that the concept has been superseded by the emergence of the drug-eluting stents, since stenting is accepted for arterial stenosis. Overcoming the roughly 20-30% restenosis caused by the use of bare metal stents was demonstrated to be possible by the localized delivery of two anti-proliferative drugs, rapamycin (51) and paclitaxel (52). Although other issues have cropped up with the use of drug-eluting stents, notably late-stage thrombosis, these are being addressed with the use of fully biodegradable stents (53) and another concept, drug-eluting balloons. However, it is possible that the use of nanoparticles in localized delivery will see other applications, including siRNA and plasmid DNA delivery, which we discuss below.

5.1. Gene Delivery

Non-viral vectors for delivering genes has been of interest for some time, since the reports of leukemia being induced in children undergoing viral gene therapy for severe combined immune deficiency syndrome, or SCID (54). Such reports have fuelled research into alternative non-viral vectors, but with limited success to date.

The accepted "gold standard" in transfection vectors (non-viral) is a liposome-based vector, Lipofectamine®, sold by Invitrogen as a reagent for laboratory studies. Although the composition of the liposome is proprietary, it is believed to be a mixture of two cationic lipid molecules, dioleyl phosphatidyl ethanolamine (DOPE) and 2,3-dioleoyloxy-*N*-[2(sperminecarboxamido)ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate (DOSPA). These lipids form lipsomes believed to be multilamellar. The multi-lamellarity in this case appears essential for complexing with plasmid DNA, with the supercoiled plasmid DNA being sandwiched between lipid lamellae via electrostatic interactions of the cations with the anionic DNA segments (55).

The size of this complex is reportedly around 300-400 nm, via light-scattering analyses, but the size depends on the medium as well. Neutralization of the excess positive charge (due to the cationic liposomes being present in molar excess) leads to aggregation which must be minimized for transfection. Nevertheless, these so-called lipoplexes are excellent transfection reagents, mostly due to the ease of entry into cells via the positive charge and the small size, as well as to some degree of survival in the cytoplasm due to the protection by the liposomal cover on the complexed pDNA.

Cryo-TEM has shown that these structures (the complexes) are essentially circular in diameter, but perhaps with an elongated tail in some cases (56). The pDNA (in super-coiled form) is sandwiched between lipid bilayers, such that there is almost complete coverage of the pDNA by the lipid bilayers. The size is a function of the lipid-to-pDNA ratios (56), going through a maximum at a lipid/pDNA ratio of ~5 (weight to weight, quoted for a mixed neutral/cationic lipid system). Sizes on either side of the maximum are around 100-250 nm. siRNA is supposed to behave in similar fashion, but with some notable differences. Principally, the difference is due to the much smaller size of siRNA (21-23 nucleotide units) compared to the minimum pDNA size of 400 nucleotides required for condensation (57). A study of liposomal "condensation" of siRNA using a cationic diamine (CDAN) mixed with DOPE showed that the size of the siRNA lipoplex is essentially insensitive to the lipid to siRNA ratio over a range of about 7-15 (58). This size at neutral pH is about 300 nm, and decreases at lower pH. Transfection efficiency depends on the ratio of CDAN to DOPE, but it appears difficult to separate "non-specific" transfection from siRNA-induced knockdown, in this model of a galactose protein expression/down regulation. (Non-specificity in this context refers to random knockdown of protein production in the cell, which is not desirable).

As mentioned in Sec 4.2.2., tumor suppressor genes have been successfully encapsulated in liposomal complexes and delivered via intravenous injection. That liposome was based on DOTAP-Cholesterol, and the data in a mouse model of disseminated human lung cancer was extremely promising. The promise does not appear to have been fulfilled in human studies, however.

Other cationic agents used for plasmid DNA complexation include poly (L-lysine), although it suffers from cellular toxicity at levels needed for efficient transfection (59). Other cationic polymers have been tried, including chitosan, with limited success. Encapsulated pDNA, on the other hand, appears to show greater promise for sustained delivery. In an interesting study of pDNA encapsulated in PLGA nanoparticles of size approximately 500-600 nm, it was found (41) that these particles sustained the release of pDNA in intact form over 28 days *in vitro*. The particles were prepared by a double emulsion method, starting with emulsification of the pDNA in aqueous solution into a PLGA organic solution, followed by emulsification into a PVA-containing aqueous medium. Addition of Ca^{2+} ions in the second aqueous medium increased the pDNA loading in the particles.

In vitro analysis showed sustained release into buffer of the pDNA, with more than 50% being released on day 1. *In vivo* studies were carried out on rats using intra muscular injection of the particles and of naked pDNA into the tibialis muscle. Efficiency of transfection was followed by monitoring alkaline phosphatase expression in the muscle tissue. Up to day 7 following intramuscular administration, the injected pDNA showed better transfection than the NP-DNA. However, on day 28, the NPs showed significantly higher transfection. This could be due either to better "protection" of the pDNA by the particle from DNAses (most likely) and subsequent release of the pDNA extracellularly. It is unlikely that the enhanced transfection on day 28 alone is due to enhanced cellular penetration as this would have manifested itself on days 3 and 7 as well. So the role of the nanoparticle in this instance is merely to encapsulate the pDNA, protect it, and sustain its release over time. As such, perhaps microspheres would have worked just as well.

In the same paper, it is claimed that liposomal complexation of pDNA does not work as well as polymeric encapsulation, based on comparing a liposomal injection at the same site into the rats (both DOTAP/DOPE and DOSPA/DOPE liposomes were used, but *in vivo* transfection on day 7 was markedly inferior to either naked pDNA or pDNA-NP. Reasons for this are not addressed by the authors, who do claim that in general, liposomal transfection efficiencies are usually much higher *in vitro* than *in vivo*.

We have studied the sustained release of lipoplexes (60), polyplexes (61) and naked pDNA *in vitro* from biodegradable matrices. It was found that bioactivity (as measured by transfection) was reduced with time of release, possibly due to aggregation of the released complexes. In the case of fast-degrading polymers (62), the released pDNA or compelxed pDNA loses its activity much sooner, possibly due to co-released negatively-charged oligomeric PLGA.

5.1.1. Status

In general, it must be admitted that non-viral vectors have not proven to be as efficient as viral vectors. Given the less stringent demands made of these vectors for siRNA delivery, it is more likely that non-virals would enjoy more success for this application than for pDNA delivery. We expect liposomes and solid particles to be studied more intensively in the coming years with siRNA delivery in view. Again, current understanding of the effects of liposomal or synthetic polymer encapsulation of genetic material does not allow us to forecast sustained pDNA or siRNA delivery from a single injection or implantation. That area is still in its infancy, as more attention has been paid to improve transfection efficiencies rather than sustaining it over time.

6. OTHER APPLICATIONS AND PROGNOSIS

Nanoparticles have been intensively studied for penetration of the blood-brain barrier (42, 63) with varying degrees of success. This is an important hurdle for drug delivery products to cross. It appears that active ligand-mediated transport may be essential for success here (64). In our opinion, future successes for nanoparticle-based delivery systems will come in these 4 areas:

a. Passive and active targeting of solid tumours
b. SiRNA delivery
c. Ligand-mediated delivery of anti-infectives and low-MW drugs across the BBB and to the back of the eye
d. Localized infusion therapy involving intracellular delivery

8. ACKNOWLEDGEMENTS

We acknowledge support from the School of Materials Science and Engineering, Nanyang Technological University for part of this work.

9. REFERENCES

1. Rapamune®, an immunosuppressant, approved in 1999; Emend®, an anti-emetic, approved in 2003.

2. Abraxane®, albumin-bound paclitaxel, approved 2005; IT-101, a camptothecin bound cyclodextrin polymer, in clinical trials currently

3. Ambisome®, approved in 1997, for fungal infections; Diprivan®, an anaesthetic approved in 1989; and Doxil® approved in 1995 for ovarian cancer.

4. M Cegnar, J Kos & J Kristl: Cystatin incorporated in poly(lactide-co-glycolide) nanoparticles: development and fundamental studies on preservation of its activity. *Eur J Pharm Sci.* 22, 357-364 (2004)

5. D Quintanar-Guerreor, E Alleman, E Doelker & H Fessi: Preparation & characterization of nanocapsules from preformed polymers by a new process based on emulsification-diffusion technique. *Pharm Res*. 15, 1056-1062 (1998)

6. F Olson, C A Hunt, F C Szoka , W J Vail & D Papahadjopoulos : Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. *Biochim Biophys Acta*. 557, 9-23 (1979)

7. L D Mayer, M B & P R Cullis: Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. *Biochim Biophys Acta.* 857, 123-126 (1986)

8. G Haran, R Cohen, L K Bar & Y Barenholz: Transmembrane ammonium sulfate gradients in liposomes to produces efficient and stable entrapment of amphipathic weak bases, *Biochim Biophys Acta.* 1151, 201-215 (1993)

9. S Clerc & Y Barenholz: Loading of amphipathic weak acids into liposomes in response to transmembrane calcium acetate gradients. *Biochim Biophys Acta.* 1240, 257-265 (1995)

10. A K Patri, I J Majoros, & J R Baker Jr: Dendritic polymer macromolecular carriers for drug delivery, *Curent opinionin Chemical Biology.* 6, 466-471 (2002)

11. J Vaage, D Donovan, E Mayhew, R Abra & A Huang: Therapy of human ovarian carcinoma xenografts using doxorubicin encapsulated in sterically stabilized liposomes. *Cancer.* 72, 3671-3675 (1993)

12. D Papahadjopoulos, T M Allen, A Gabizon E Mayhew, K Matthay, S K Huang, K D Lee, M C Woodle, D D Lasic, C Redemann, et. al**.:** Sterically stabilized liposomes: improvements in pharmacokinetics and anti-tumor therapeutic efficiency. *Proc Natl Acad Sci USA.* 88, 11460-11464 (1991)

13. R Ramesh, T Saeki, N S Templeton L Ji, L C Stephens, I Ito, D R Wilson, Z Wu, C D Branch, J D Minna & J A Roth: Successful treatment of primary and disseminated human lung cancers by systemic delivery of tumor suppressor genes using an improved liposome vector. *Mol Ther.* 3, 337-350 (2001)

14. http://seekingalpha.com/article/33114-introgen-therapeutics-empty-promises-for-cancer-patients-&-shareholders (entry is dated April 23, 2007)

15. I Ito, L Ji, F Tanaka, Y Saito, B Gopalan, C D Branch, K Xu, E N Atkinson, B N Bekele, L C Stephens, J D Minna, J A Roth & R Ramesh: Liposomal vector mediated delivery of the 3p FUS1 gene demonstrates potent anti-tumor activity of human lung cancer *in vivo*. *Cancer Gene Ther.* 11, 733-739 (2004)

16. L Xu, C C Huang, W Alexander, W Tang & E H Chang: US patent 7,479,276 (2009)

17. P Jie, S Venkatraman, F Min, F Boey & L H Gan : Micelle-like nanoparticles of star-branched PEO-PLA copolymers as Chemotherapeutic Carrier**.** *J Control Release.* 110. 20-33 (2005)

18. SS Venkatraman, P Jie, F Min, F Boey & L H Gan: Micelle-like nanoparticles of PLA-PEG-PLA triblock copolymers as chemotherapeutic carrier**.** *Int J Pharm.* 298, 219-232 (2005)

19. T Hamaguchi, Y Matsumura, M Suzuki, K Shimizu, R Goda, I Nakamura, I Nakatomi, M Yokoyama, K Kataoka & T Kakizoe: NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend *in vivo* antitumour activity and reduce the neurotoxicity of paclitaxel. *Br J Cancer.* 92, 1240-1246 (2005)

20. E K Rowlinsky & R C Donehower: Paclitaxel (taxol) *N Engl J Med* 332. 1004-1014 (1995)

21. T Hamaguchi, K Kato, H Yasui, C Morizane, M Ikeda, H Ueno, K Muro, Y Yamada, T Okusaka, K Shirao, Y Shimada, H Nakahama & Y Matsumura: A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation. *Brit J Cancer* 97. 170-176 (2007)

22. V Pinzani, F Bressolle, I J Haug, M Galtier , J P Blayac & P Balmes : Cisplatin induced renal toxicity & toxicity-modulating strategies: a review. *Cancer Chemother Pharmacol.* 35, 1-9 (1994)

23. N Nishiyama, S Okazaki, H Cabral, M Miyamoto, Y Kato, Y Sugiyama, K Nishio, Y Matsumura, & K Kataoka: Novel Cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice, *Cancer Res.* 63, 8977-8983 (2003)

24. R H Wilson, R Plummer, J Adam, M M Eatock, A V Boddy, M Griffin et. al. Phase I and pharmacokinetic study of NC-6004, a new platinum entity of cisplatin-conjugated polymer micelles, *Am Soc Clin Oncol* abstract #2573 (2008)

25. T Nakanishi, S Fukushima, K Okamoto, M Suzuki, Y Matsumura, M Yokoyama, T Okano, Y Sakurai, & K Kataoka: Development of the polymeric micelle carrier system for doxorubicin. *J Control Release*. 74, 295-302 (2001)

26. Y Matsumura, T Hamaguchi, T Ura, K Muro, Y Yamada, Y Shimada, K Shirao, T Okusaka, H Ueno, M Ikeda & N Watanabe: Phase I and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *Br J Cancer.* 91, 1775-1781 (2004)

27. V P Torchilin: Micellar nanocarriers: pharmaceutical perspectives. *Pharm Res.* 24, 1-16 (2007)

28. S Y Kim, I G Shin, Y M Lee, C G Cho & Y K Sung: Methoxypoly (ethylene glycol) and e-capolactone amphiphilic block copolymeric micelle containing indomethacin.II. Micelle formation and drug release behaviours. *J Control Release.* 51, 13-22 (1998)

29. C Allen, Y Yu, D Maysinger & A Eisenberg: Polycaprolactone-b-poly(ethylene oxide) block copolymer micelles as a novel drug delivery vehicle for neurotropic

agents FK506 and L-685, 818. *Bioconjugate Chemistry.* 9, 564-572 (1998)

30. R Gref, M Luck, P Quellec, M Marchand, E Dellacherie, S Harnisch, T Blunk & R H Muller: "Stealth" corona-core nanoparticles surface modified by polyethylene glycol (PEG):influence of the corona (PEG chain length and density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids Surf B Biointerfaces.* 18, 301-313 (2000)

31. L L Ma, P Jie & S Venkatraman: Block copolymer 'stealth" nanoparticles for chemotherapy: Interactions with blood cells *in vitro*. *Adv Functional Mat.* 18 , 716-725 (2008)

32. C. Giacomelli, V Schmidt & R Borsali: Specific interactions improve the loading capacity of block copolymer micelles in aqueous media. *Langmuir.* 23, 6947-6955 (2007)

33. P L Soo, J Lovric, P Davidson, D Maysinger, A Eisenberg: Polycaprolactone-block-poly(ethylene oxide) micelles: a nanodelivery system for 17β-estradiol. *Mol Pharm*. 2, 519-527 (2005)

34. P Xu, E A Van Kirk, S Li, W J Murdoch, J Ren, M D Hussen, M Radosz & Y Shen: Highly stable core-surface-crosslinked nanoparticles as cisplatin carriers for cancer chemotherapy. *Colloids Surf B Biointerfaces.* 48, 50-57 (2006)

35. D D Lasic: On the thermodynamic stability of liposomes. *J Colloid Interface Sci.* 140, 302-304 (1990)

36. K J Harrington, C R Lewanski, A D Northcote, J Whittaker, H Wellbank, R G Vile, A M Peters & J S Stewart: Phase I-II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. *Anals of Oncology.* 2, 493-496 (2001)

37. M S Newman, G T Colbern, K Working, C Engbers & M A Amantea: Comparative pharmacokinetics, tissue distribution and therapeutic effectiveness of cisplatin encapsulated in long-circulating, pegylated liposome (SPI-077) in tumour-bearing mice. *Cancer Chemother Pharmacol.* 43, 1-7 (1999)

38. H Murakami, M Kobayashi, H Takeuchi & Y Kamwashima: Preparation of poly (DL-lactide-co-glycolide) nanoparticles by the modified spontaneous emulsification solvent diffusion method. *Int J Pharm.* 187, 143-152 (1999)

39. A Beletsi, P Klepetsanis, D S Ithakissios, S Kounias, A Stavropoulos & K Avgoustaki: Simultaneous optimization of cisplatin-loaded PLGA-mPEG nanoparticles with respect to their size and drug encapsulation. *Curr Nanoscience.* 4, 173-178 (2008)

40. M Desai, J Hilfinger, G Amidon, R J Levy & V Labhasetwar: Immune response with biodegradable nanospheres and alum: studies in rabbits using staphylococcal enteroxin B-toxoid. *J Microencapsulation* 17. 215-225 (1999)

41. H Cohen, R J Levy, J Gao, I Fishbein, V Kousaev, S Sosnowski, S Slomkovski & G Golomb: Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. *Gene Ther.* 7. 1896-1905 (2000)

42. R N Alyautdin, V E Petrov, K Langer, A Berthold, D A Kharkevitch & J Kreuter: Delivery of Loperamide Across the Blood-Brain Barrier with Polysorbate 80-Coated Polybutylcyanoacrylate Nanoparticles. *Pharm Res.14,* 325-328 (1997)

43. S E Dunn, A G A Coombes, M C Garnett, S S Davies, M C Davies & L Illum: *In vitro* cell interaction and *in vivo* biodistribution of poly(lactide-co-glycolide) nanospheres surface modified by polaxamer and polaxamine copolymers. *J Control Release.* 44, 65-76 (1997)

44. F D Jaeghere, E Allemann, J C Leroux, W Stevels, J Feijen, E Doelker & R Gurny: Formualtion and lyoprotection of poly (lactic acid-co-ethylene oxide) nanoparticles: influence on physical stability and *in vitro* cell uptake. *Pharm Res*. 16, 859-866 (1999)

45. R Gref, Y Minamitake, M T Peracchia, V Trubetskoy, V Torchilin & R Langer: Biodegradable long-circulating polymeric nanospheres. *Science.* 263. 1600-1603 (1994)

46. M T Peracchia, C Vauthier, C Pasirani, P Couvreur & D Labarre: Complement consumption by poly (ethylene glycol) in different conformations chemically coupled to poly (isobutyl 2-cyanoacrylate) nanoparticles. *Life Sciences.* 61, 749-761 (1997)

47. V C F Mosqueira, P Legrand, J L Morgat, M Vert, E Mysiakine, R Gref, J P Devissaguet & G Barratt: Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density. *Pharm Res*. 18, 1411-1419 (2001)

48. C X Song, V Labhasetwar, H Murphy, X Qu, W R Humphrey, R J Shebuski & R J Levy: Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery. *J Control Release*. 43, 197-212 (1997)

49. V Labhasetwar, C Song, W Humphrey, R Shebuski & R J Levy: Arterial uptake of biodegradable nanoparticles: effect of surface modifications. *J Pharm Sci*. 87, 1229-1234 (1998)

50. L A Guzman, V Labhasetwar, C X Song, Y Jang, A M Lincoff, R Levy & E J Topol : Local intraluminal infusion of biodegradable polymeric nanopartilcles. *Circulation.* 94, 1441-1448 (1996)

51. T Suzuki, G Kopia, SI Hayashi, L R Bailey, G Llanos, R Wilensky, B D Klugherz, G Papandreou, P Narayan, M B Leon, A C Yeung, F Tio, P S Tsao, R Falotico & A J

Carter: Stent-based Delivery of Sirolimus reduces neointimal formation in a porcine coronary model. *Circulation.* 104, 1188-1193 (2001)

52. E Grube, S Silber, K E Hauptmann, R Mueller L Buellesfeld, U Gerckens & M E Russell: TAXUS 1: Six- and twelve-month results from a randomized double-blind trial on a slow-release paclitaxel-eluting stent for de novo coronary lesions. *Circulation.* 107, 38-42 (2003)

53. S Venkatraman & F Y C Boey: Release Profiles in Drug-eluting Stents: Issues and Uncertainties. *J Control Release.* 120, 149-160 (2007)

54. C E Thomas, A Ehrhardt & M Kay: Progress and problems with the use of viral vectors for gene therapy. *Nat. Rev. Genetics*. 4. 346-358 (2003)

55. J O Radler, I Koltover, T Salditt & C R Safiny: Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. *Science.* 275, 810-814 (1997)

56. N S Templeton, D D Lasic, P M Frederik, H H Strey, D D Roberts & G N Pavlakis: Improved DNA: liposome complexes for increased systemic delivery and gene expression. *Nature Biotech.* 15, 647-652 (1997)

57. V A Bloomfield: Condensation of DNA by polyvalent cations: considerations on mechanism, *Biopolymers.* 31, 1471-1481 (1991)

58. S Spagnou, A D Miller & M Keller: Lipidic carriers of siRNA; differences in the formulation, cellular uptake and delivery of plasmid DNA. *Biochemistry.* 43, 13348-13356 (2004)

59. S C W Richardson, H J V Kolbe & R Duncan: Potential of low molecular mass chitosan as a DNA delivery system: biocompatibility, body distribution & ability to complex and protect DNA. *Int J Pharm.* 78, 231-243 (1999)

60. Y Ramgopal, D Mondal, W T Godbey & S.Venkatraman: Sustained Release of Complexed and Naked pDNA from Polymer Films. *J Biomed Mat. Res B: Applied Biomaterials.* 85B (2), 496-503 (2007)

61. Y Ramgopal, D Mondal, S S Venkatraman, W T Godbey & G Y Yuen: Controlled release of complexed DNA from polycaprolactone film: Comparison of lipoplex & polyplex release, *J Biomed Mat Res B: Applied Biomaterials.* 89, 439-447 (2009)

62. Y Ramgopal, S S Venkatraman & W T Godbey**:** *In vitro* release of complexed pDNA from biodegradable polymer films. *J App Polym Sci.* 108, 659-664 (2008)

63. A Agarwal, N Lariya, G Saraogi, N Dubey, H Agarwal & G P Agarwal: Nanoparticles as novel carrier for brain delivery: a review. *Current Pharm Design.* 15, 917-925 (2009)

64. L Liu, S S Venkatraman, Y-Y Yang, K Guo, J Lu, B He, S Moochhala & L Kan: Polymeric micelles anchored with TAT for delivery of antibiotics across the blood-brain barrier. *Biopolymers (Peptide Science)* 90,617-623 (2008)

Abbreviations: siRNA: small interfering RNA, used to denote spliced RNA molecules that interfere with specific protein production in the ribosome of the cell, dox: short for doxorubicin, used in cancer chemotherapy, pDNA: plasmid DNA, DOTAP: dioleyl trimethyl ammonium methyl sulfate, DOPE: dioleyl phosphatidyl ethanolamine, NPs: short for nanoparticles

Key Words: Polymeric nanomicelles, Dendrimer, Liposome, siRNA, pDNA, Doxorubicin, Cisplatin, Gene delivery, Paclitaxel, Cisplatin, Targeted Delivery, Pegylated-liposome, Review

Send correspondence to: Subbu S.Venkatraman, School of Material Science and Engineering, Nanyang Technological University, 50, Nanyang Avenue, Singapore, 639798, Tel: 0065-6790-4259, Fax: 0065-6790-9081, E-mail: ASSubbu@ntu.edu.sg

http://www.bioscience.org/current/vol2S.htm