

## Nanotechnology in Therapeutics

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**Keywords:** nano-emulsion, nano-gel, solid lipid nanoparticle, liposome, micelles, dendrimer, hyperbranched polymer, carbon nanotube, carbon nanohorn, nano-sponge, thin film, nanoshell

## 1.1 Definition

For the purpose of this report, therapeutics are defined as drugs and their associated delivery systems used for the treatment of disease.

## 1.2 Short Description

The pharmaceutical industry is currently facing a crisis. The high increase in development costs, competition from generic manufacturers, complex patent management systems and increased failure rates are putting immense pressure on the profitability and future survival of the leading companies. There is also a shift away from the “one size fits all” blockbuster drug model towards personalised medicine, which will severely impact on the revenue of therapies.

New tools and techniques enabled by nanotechnology can help in better understanding intracellular functions. However it is the targeting of drugs, which can reduce toxicity and improve efficiency, where nanotechnology can make a huge impact. Nanotechnology-based delivery systems can also protect drugs from degradation. These properties can help reduce the number of doses required, make treatment a better experience and reduce treatment expenses. A number of nano-based systems allow delivery of insoluble drugs, allowing the use of previously rejected drugs or drugs which are difficult to administer e.g. paclitaxel. At present these systems are generally used for existing, fully developed off-patent drugs, the so-called “low-hanging fruit” of nanotechnology-based delivery. However, the success of these paves the way for new drugs being delivered in the same way.

The market for nanotechnology enabled drug delivery is set to have a cumulative growth rate of 48% over the next four years to increase to US \$20.1 billion by 2012, an 11% share of the global drug delivery market. In 2008, the nanotechnology-enabled drug delivery market is estimated at US \$4.1 billion, or 4% of the global drug delivery market<sup>1</sup>.

## 1.3 State of R&D

Currently, there are few commercially available therapeutic products based on nanotechnology. A number are in clinical development and are expected to appear on the market. Many of these are based on existing, off-patent drugs (e.g. paclitaxel), therefore eliminating part of the costly drug development process.

### 1.3.1 Polymer therapeutics

Polymer therapeutics is an umbrella term to describe polymeric drugs, polymer-drug conjugates, polymer-protein conjugates, polymeric micelles to which drug is covalently bound, and multi-component polyplexes being developed as non-viral vectors<sup>2</sup>.

One of the biggest advantages of using polymers in drug delivery is that it is possible to manipulate their properties (e.g. molecular weight, linkers etc.) to adapt to the drug delivery requirements. In addition, polymers are easy to scale up, provide high biocompatibility and increase the stability of volatile pharmaceutical agents. These particles and their conjugates can be used to deliver a higher concentration of pharmaceutical agents to a desired location with potential applications in antibiotics targeting and cancer therapy. Conjugating nanoparticles and other nanomaterials to polyethylene glycol (PEG), known as PEGylation, is used widely as it offers a number of advantages<sup>3</sup>. These include increased protein solubility and stability, reduced immunogenicity, prevention of clearance by the reticuloendothelial system (RES) and increased plasma half-life - leading to less frequent dosing.

#### 1.3.1.1 Polymer-protein conjugates

Polymers conjugated with proteins can then be administered parenterally can increase protein solubility and stability. PEGylation has been used to treat several diseases including hepatitis B and C (PEG-IFN $\alpha$  2a)<sup>4</sup>, acute lymphoblastic leukaemia (PEG-L-Asparaginase)<sup>5</sup>, neutropaenia associated with cancer chemotherapy (PEG-GCSF)<sup>6</sup> and different cancers [PEG-glutaminase combined with a glutamine anti-metabolite 6-diazo-5-oxo-norleucine (DON)]<sup>7</sup>.

#### 1.3.1.2 Polymer-drug conjugates

Polymer drug conjugates can improve the targeting ability, reduce the associated toxicity and overcome drug resistance. Linking drugs to polymers limits the cellular uptake to endocrine route and enhances the EPR Effect<sup>1</sup>. Hydrophilic polymers can be conjugated with hydrophobic drugs to increase their solubility.

Duncan outlined the major features required for the design of polymer-drug conjugates<sup>8</sup>. She suggests that it should be non-toxic and non-immunogenic, must be stable during transport, the drug should be released at an optimum rate on arrival within tumour cells and be able to carry an adequate drug payload in relation to its potency.

Different types of polymer have been proposed for drug conjugation. Kumazawa *et al.*<sup>9</sup> report the use of a dextran-linked camptothecin analogue to shrink tumours in mice. PEG has also been proposed as a suitable candidate to attach drugs to<sup>10</sup>. HPMA (N-(2-hydroxypropyl) methacrylamide) copolymer conjugates have also been used for targeted drug delivery in the inhibition of a number of tumour types<sup>11-17</sup>.

#### 1.3.1.3 Polyketal nanoparticles

Polyketals are readily-synthesized, biocompatible, hydrophobic polymers with biodegradable ketal linkages in their backbone. They can form nanoparticles for encapsulating hydrophobic drugs or proteins. They can undergo acid-catalysed hydrolysis to release their therapeutic payload in acidic environments such as tumours, inflammatory tissues etc. There are no acidic byproducts produced during degradation, unlike other polymers (e.g. polyester), that can cause inflammation.

Nanoparticles of poly(1,4-phenyleneacetone dimethylene ketal) (PPADK) have been used therapeutically<sup>18</sup>. Applications include the treatment of acute lung diseases<sup>19</sup>, the delivery and release of superoxide dismutase (SOD), *in vivo* imaging of hydrogen peroxide and its detection in atherosclerotic plaques<sup>20</sup>.

#### 1.3.1.4 Nanogels

Nanogels are cross-linked nanoscale particles made of flexible hydrophilic polymers. They are soluble in water and allow spontaneous loading of drugs in aqueous media. The nanogel collapses to form dense nanoparticles after adding the drug molecules. Nanogels possess large surface area, tuneable size and a network to allow incorporation of molecules. They have been used to incorporate drugs, DNA/RNA and inorganic molecules such as quantum dots.

Nanogel particles comprised of PEG and polyethylenimine (>100nm) have been used to cross the blood-brain barrier (BBB) and deliver oligonucleotides to the brain<sup>21</sup>. Nanogels have also been used for pH-dependant release of doxorubicin<sup>22</sup> and incorporation of an insoluble small molecule anticancer drug, AQ10<sup>23</sup>.

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<sup>1</sup> EPR Effect: Enhanced Permeability and Retention (EPR) is the ability of drug conjugates to circulate within the blood for several hours more than the conventional low molecular weight drugs which leaves the blood stream within minutes. The higher drug availability facilitates better passive tumour adsorption through the permeability offered by tumour blood vessel.

To be used safely within the body they must be stable in blood and biodegrade sufficiently. For a recent review of synthetic methods and applications in nanogel-based drug delivery, see Oh *et al.*<sup>24</sup>.

### 1.3.1.5 Dendrimers & Hyperbranched Polymers

Dendrimers are unimolecular, monodisperse, micellar nanostructures with a well-defined, regularly branched symmetrical structure and a high density of functional end groups. They are robust, covalently fixed, 3D structures possessing both a solvent-filled interior core (nanoscale container) that can carry molecules, e.g. drugs, and a homogenous, defined, exterior surface functionality (nano-scaffold) that can be functionalised<sup>25</sup>. The first and most widely studied dendrimers are poly(amidoamine) (PAMAM) dendrimers.

Dendrimers can be created using a divergent method where the dendron originates from a central core and branches out. Alternatively, a convergent method where the dendrimers grow inwards to a focal point may be used. A review on dendrimers states that over one hundred compositionally different dendrimer families have been synthesized with over 1000 differentiated chemical surface modifications<sup>26</sup>. Recently alternative synthetic strategies have been utilised.

One of the advantages of dendrimers is that they are similar in size to many proteins and biomolecules like insulin, cytochrome C and haemoglobin. 2<sup>nd</sup> generation dendrimers have a width similar to that of DNA (2.4 nm), and 5<sup>th</sup> and 6<sup>th</sup> generation PAMAM dendrimers have similar widths to cellular lipid membranes (~5.5 nm).

The loading capacity of dendrimers can be manipulated by the addition of different guest molecules onto the surface of dendrimers. Diederich *et al.*<sup>27</sup> have developed dendrimers modified to bind hydrophobic molecules to the core, called dendrophanes, and those ones which bind to the polar bioactive compounds called dendroclefts<sup>28</sup>. Dendrophanes made with a cyclophane core have shown excellent steroid carrying properties and water solubility.

Several studies have demonstrated the use of dendrimers for targeted drug delivery, examples include cisplatin<sup>29</sup>, nifedipine<sup>30</sup>, doxorubicin<sup>31</sup> and methotrexate<sup>32</sup>. The size of the dendrimer, the type of functional groups and the pH of the medium all appear to play an important role in determining the amount of solubility. An antibody-dendrimer conjugate that targeted the prostate specific membrane antigen was reported for prostate cancer treatment<sup>33</sup>.

Dendrimers have also been used as for *in vitro* gene delivery as they can complex DNA<sup>34</sup>. Dendrimer-DNA complexes have been locally administered to eyes<sup>35</sup>, lungs<sup>36</sup>, heart<sup>37</sup> and tumours<sup>38</sup>. Generally larger dendrimers are more stable and better at binding DNA. However, one study<sup>39</sup> found that 2<sup>nd</sup> generation PAMAM dendrimers bind DNA better than 6<sup>th</sup> generation due to the more fluid structure of small dendrimers. Dendrimers can also accommodate and deliver large DNA constructs<sup>40,41</sup>.

Dendrimers have also been effective against bacterial and viral infection. Dendrimers hybridised with chitosan have useful antibacterial properties as well as potentially acting as drug delivery agents<sup>42</sup>. The ability of dendrimers modified with sulfonated naphthyl groups to inhibit viral activity has been shown by Bourne *et al.*<sup>43</sup>. They found that modified PAMAM dendrimers were highly effective *in vitro* against herpes simplex virus types 1 and 2.

The Australian company Starpharma ([www.starpharma.com](http://www.starpharma.com)) has produced Vivagel®, a dendrimer-based vaginal microbicide, for the prevention of STIs and also acts as a contraceptive. It effectively prevents transmission of HIV and HSV-2 with no inflammation or irritation<sup>44</sup>. Vivagel® is currently in clinical trials but has been granted Fast Track status by the FDA as a product for prevention of HIV infection, so is expected to enter the market in 2009-2010<sup>45</sup>. Starpharma is also developing Vivagel® for the prevention of HPV and as a condom coating in collaboration with SSL International, manufacturers of Durex® condoms.

Hyperbranched polymers (HBPs) are essentially irregular dendrimers. They are imperfectly branched, have an average (rather than precise) number of terminal functional groups and are chemically polydisperse. Due to their irregularity they can be prepared in a single step, making them much more cost effective than dendrimers. The large numbers of external groups are suitable for multifunctionalisation, advantageous for use as a drug carrier. Some HBPs are biocompatible and biodegradable with non-toxic degradation products.

HBPs have been conjugated to ibuprofen for delivery<sup>46</sup>. The conjugates gave rapid suppression of PGE<sub>2</sub> synthesis when localised in the cytosol compared with no activity using free ibuprofen. A novel class of HBP based on polyglycerol (PG) and PEG was synthesised and the blood compatibility of these polymers was assessed at various concentrations<sup>47</sup>. They had no significant effect on factors including complement activation, platelet activation, coagulation, erythrocyte aggregation and hemolysis compared to branched cationic polyethyleneimine (PEI). The polymers had high affinity for DNA and could condense it into highly compact, stable, water soluble nanoparticles in the range of 60-80 nm. The method offers potential applications in polymer therapeutics and could be used as a more biocompatible approach for high affinity DNA binding than a dendrimer based approach.

Dendrimers and HBPs have good potential as therapeutics as they are made from biocompatible materials and can have high drug loading. HBPs are cheaper to synthesise so may be more suited to these applications.

### 1.3.2 Lipids in Drug Delivery

Lipid-based structures and formulations have been used as delivery systems for many years. Liposomes and micelles are used in cosmetics (see 'Cosmetics subsector report) and foods. Lipid nanostructures are capable of protecting their contents from the conditions within the body that could potentially cause degradation. They can be used to deliver insoluble drugs and targeting ligands can be attached.

#### 1.3.2.1 Liposomes & Niosomes

Liposomes are vesicular structures with an aqueous core surrounded by a lipid bilayer. They were first prepared artificially in the mid 1960s<sup>48</sup> and their first practical application in drug delivery emerged in the 1970s. They are normally created by the extrusion of phospholipids. Solutes, such as a drug in the core, cannot pass through the hydrophobic bilayer although hydrophobic molecules can be adsorbed into the bilayer, enabling the liposome to carry both hydrophilic and hydrophobic molecules. They have also been used in the cosmetic industry (see Cosmetics subsector report). The size of the liposomes can vary from 15 nm to several  $\mu\text{m}$ . Liposomes with nanometre sized cavities are also called nanoliposomes. Solutions with high or low pH (e.g. dissolved drugs in solution) can be carried via the liposomes as the drugs get diffused out at the targeted site when the pH is neutralised in the body.

PEGylated liposomes that avoid clearance by the RES are known as "stealth liposomes"<sup>49</sup>. Liposomes exploit the leaky nature of tumour vesicles which allow particles of less than 400 nm to pass through. Early research in this area has shown that liposomes remain in the tumour interstitial fluid in close vicinity to tumour vessels<sup>50</sup>. Surface modification of liposomes with ligands like vitamins, antigens and antibodies for improved endocytosis by other cell types has also been proposed<sup>51</sup>.

A variety of biological and drug compounds have been delivered using liposomes. These include antibiotics, antioxidants (retinoids, carotenoids, tamoxifen, urate, glutathione etc.), vitamins (Vitamins A, C and E), haemoglobin, ATP, NSAIDs (indomethacin and naproxen) and genetic materials (plasmid DNA). Detailed lists of compounds that can be delivered via liposomes are mentioned elsewhere<sup>1</sup>. Several formulations including amphotericin B<sup>52</sup> and daunorubicin<sup>53</sup> have been successfully commercialised. Doxorubicin, a highly effective cancer drug with toxic side effects, has been encapsulated in liposomes and tested for treatment<sup>54</sup>. A good overview of the different methods of encapsulating doxorubicin into liposomes can be found in the review by Abraham *et al.*<sup>55</sup>.

Liposomes can also be modified to incorporate a magnetic element for use in monitoring their movement within the body using MRI. Such liposomes enhance the efficacy of their use by allowing more external control on its movement. De Cuyper *et al.*<sup>56</sup> have shown that magnetoliposomes with nanometre sized magnetite cores, enwrapped by a bilayer of phospholipid molecules can be used as biocompatible MRI agents with potential applications in combined imaging and drug delivery.

Liposomes can be used to entrap gases and drugs for ultrasound-controlled drug delivery and release, reviewed in this reference<sup>57</sup>. This technique has also been used to entrap photosensitisers. Ultrasound can enhance the pharmacological activity of certain drugs, improve drug transport through tissues and cell membranes, and can create a hyperthermic condition that promotes the destruction of cancerous tissue<sup>1</sup>. However, techniques using perflutren-containing ultrasound contrast agents have been given a 'black box' warning by FDA after 4 patient deaths. A detailed study of the side effects of microbubble-based delivery is necessary for the success of this novel approach. It is also important to find the optimum lipid composition for prolonged circulation and stable gas retention, methods of conjugating ligands to liposomes without compromising their echogenicity.

### Niosomes

Niosomes are non-ionic surfactant vesicles with a similar structure to liposomes. The name Niosome is a trademark of L'Oreal.

They can encapsulate aqueous solutes and act as drug carriers. Niosomes are formed by the self assembly of non-ionic amphiphiles in aqueous media. The application of heat or physical agitation helps the process to attain a closed bilayer structure<sup>58</sup>. Their uptake by organs such as the liver and spleen make niosomes best suited as drug delivery agents in diseases affecting these organs. They are also used in targeting cancer cells<sup>59</sup>. Since niosomal antigens are potent stimulators of the cellular and humoral immune responses they are also useful as adjuvants in vaccine delivery.

Niosomes for drug delivery have been reported since 1997. They are stable and retain their contents at low temperatures. Administration of methotrexate<sup>60</sup>, DOX<sup>61</sup> and DOX N(2-hydroxypropylmethacrylamide) copolymer conjugate<sup>62</sup> have been reported using niosomes. High levels of drugs were found in the target location when administered via niosomes compared to conventional routes. They have also been used with anti-inflammatory agents<sup>63</sup> and anti-infective agents<sup>64</sup>. PEGylated cationic niosomes have been used for the cellular delivery of oligonucleotides<sup>65</sup>.

Niosomes can enhance transdermal drug delivery. Vanhal *et al.*<sup>66</sup> reported that niosome encapsulated drugs have been delivered through the stratum corneum which is normally considered as highly impermeable. Niosomes made from a novel surfactant (Bola surfactant), have been found highly effective for percutaneous drug delivery applications<sup>67</sup>. Studies have shown that they improve percutaneous passage of 5-fluorouracil (5-FU) through human stratum corneum and epidermis, and are non-toxic<sup>68</sup>. Niosomes of frusamide have been reported, that increased skin permeability and sustained drug levels<sup>69</sup>.

### 1.3.2.2 Micelles

Micelles are also spherical lipid nanostructures but they do not have a bilayer or inner cavity. The hydrophobic ends of the phospholipids point inwards and the hydrophilic ends face the outside, forming a spherical structure. Reverse micelles have this polarity the opposite way. The typical size of micelles for pharmaceutical applications ranges from 10- 80 nm.

Compared to liposomes, micelles have a short circulation time within the body due to their smaller size. However, this gives them the advantage of being able to enter tumour cells more easily, because of the EPR effect.

Micelles can also be made from polymers. Polymeric micelles are formed by block-copolymers consisting of hydrophilic (e.g. PEG) and hydrophobic monomer units with longer hydrophilic blocks and shorter hydrophobic blocks<sup>70</sup>. They have a hydrophobic core stabilised by hydrophilic units. These micelles are more stable than conventional micelles and are preferred for drug delivery applications as the circulation time is longer and they offer better biodistribution<sup>71</sup>.

Lipid-polymer conjugate micelles can also be made. They can carry different types of chemicals like paclitaxel<sup>72</sup>, diazepam<sup>73</sup> and captothecin<sup>74</sup>. They also exhibit good longevity and stability. Micelles with improved solubility and intracellular delivery have been prepared using PEG-phosphatidylethanolamine (PEG-PE) conjugates. In recent studies PEG-PE micelles were targeted to tumours in mice<sup>75</sup> and to damaged heart cells in rabbits with myocardial infarction<sup>76</sup>. Micelles with shorter PEG have been found to be better and more efficient carriers of soluble drugs due to their high hydrophobic to hydrophilic phase ratio.

Conjugating polymer micelles with ligands has been used for efficient delivery. Micelles conjugated with transferrin can target cancer cells and deliver DNA<sup>77</sup>. Similarly folate residues attached to micelles have been used to deliver adriamycin to cancer cells<sup>78</sup>. Positively charged nanoparticles have been shown to enhance drug uptake by cancer cells. However, many of the PEG-PE micelles carry a net negative charge, so positively charged lipids have been attached to these micelles to enhance uptake.

Micelles of thermal or pH sensitive polymers have been prepared. pH-sensitive micelles have been proposed for oral delivery applications. Temperature dependent micelles are reported to have increased drug release capacity<sup>79</sup>. Ultrasound has been suggested as a non-invasive stimulus to micelles for triggering drug release<sup>80</sup>.

Micelles have also been proposed as contrast agents in diagnostic applications to be used along with drug delivery. Paramagnetic metals, such as gadolinium (Gd) or manganese (Mn), normally used in contrast agents can easily be incorporated into micelles for imaging applications. The advantage of such agents is enhanced target penetration due to the smaller size and easy movement to the target location. Chelated Gd in PEG-PE based micelles has been used for experimental percutaneous lymphography in rabbits by gamma-scintigraphy and MRI<sup>81</sup>.

### 1.3.2.3 Lipid Nanoparticles (SLNs, NLCs & lipid drug conjugates)

#### *Solid Lipid Nanoparticles*

Solid lipid nanoparticles (SLNs) are particles of nanometre dimensions with a solid lipid matrix. They are oily droplets made from lipids which are solid at room temperature and stabilised by surfactants. The advantage of SLNs is that there is no need for organic solvents in the preparation, they provide protection from water and can be used for controlled drug release. They also have applications in cosmetics (see Cosmetics subsector report). Stealth and non-stealth SLNs have been used to deliver paclitaxel<sup>82</sup>. The drug accumulated up to 2.8 %, did not crystallise and was stable over time. Sustained release of doxorubicin has been reported using SLCs<sup>83</sup>.

Although SLNs are promising they suffer some drawbacks. Their loading capacity is low and there is a tendency to expel the contents during storage. These problems are caused by the tendency for the particle matrix to form a perfect crystal lattice when solid lipids are used. The high water content of SLN dispersions can also be problematic.

#### *Nanostructured lipid carriers (NLCs)*

In order to overcome some of the drawbacks of SLNs, a second generation of lipid particles have been developed by mixing solid lipids with liquid lipids. They are called nanostructured lipid carriers (NLCs). Compared to SLNs, NLCs usually have a distorted structure which makes the matrix structure imperfect, creating spaces to accommodate active compounds. They have been investigated for the topical delivery of drugs, including anti-fungals and non-steroidal anti-inflammatories and have been recently reviewed<sup>84</sup>. These structures also have applications in cosmetics and are discussed in the Cosmetics subsector report.

#### *Lipid-drug conjugate nanoparticles*

In order to overcome the limitations of SLNs, drug-lipid conjugates have been developed with an observed loading capacity of up to 33 %<sup>85</sup>.

Mehnert *et al.*<sup>86</sup> investigated the structure of lipid based nanoparticles and reported that SLNs and other nanostructured lipid carriers did not show any advantage with respect to incorporation rate compared to conventional nanoemulsions.

#### *1.3.2.4 Nanoemulsions*

Nanoemulsions are dispersions of nanoscale droplets of one liquid within another. There are a number of high and low-energy methods of formation<sup>87</sup>.

Nanoemulsions have a number of advantages over larger scale emulsions. They can be stabilised to increase the time before creaming occurs<sup>88</sup>. They are transparent or translucent, and have a larger surface area due to the small particle size. They are currently used in commercially available cosmetics (see Cosmetics subsector report) and have been used for imaging and delivery of poorly soluble drugs.

When used for drug delivery, nanoemulsions have significant advantages over traditional formulations for poorly soluble drugs. They eliminate the need for harmful co-solvents and can be administered at a much higher dose (approx. 10-fold), cutting down on the administration volume and time. The components of nanoemulsions are usually GRAS compounds (generally recognised as safe), therefore they are considered relatively safe systems which can break down to their safe components.

Cornerstone Pharmaceuticals ([www.cornerstonepharma.com](http://www.cornerstonepharma.com)), have developed a stable oil water lipid nanoemulsion called Emulsiphan for tumour targeting. Their lead product, an Emulsiphan nanoemulsion containing paclitaxel (EmPAC), showed increased efficacy and uptake by tumour cells compared to Taxol® and is currently in clinical development.

Nanoemulsions allow transport through membranes so have been suggested for use in patches for administering drugs across the skin.

#### *1.3.2.5 Lipid nanocapsules (LNCs)*

These systems can be thought of as a cross between liposomes and nanoemulsion particles. Their outer wall is thicker than a traditional nanoemulsion particle allowing functionalisation and more controlled delivery. LNCs are composed of a liquid, oily core (medium-chain triglycerides) surrounded by hydrophilic and lipophilic surfactants. They were patented in 2001 for therapeutic applications<sup>89</sup> and an efficient, solvent free phase-inversion method for their preparation has been developed<sup>90</sup>. Stealth LNCs have also been synthesised using PEG to improve circulation time. LNCs have been used to deliver anticancer drugs<sup>91,92,93</sup>. LNCs have been used to deliver therapeutic molecules and radionuclides across the blood brain barrier by conjugation of antibodies or antibody fragments<sup>94,95,96</sup>.

### 1.3.3 Nanoparticles in Drug Delivery

The interest in using nanoparticles for drug delivery has increased at an exponential rate in the past few years. Nanoparticles can offer significant advantages over the traditional delivery mechanisms in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different types of drug administration and the capability to transport both hydrophilic and hydrophobic molecules.

The drugs may be enclosed inside the sphere of the nanoparticle or linked to the surface. Once they are at the target site, the drug payload may be released from the nanoparticle by diffusion, swelling, erosion or degradation. Active systems are also possible, e.g. drug release in response to the input of external energy such as targeted ultrasound, light or magnetic field.

#### 1.3.3.1 Protein nanoparticles

##### *Albumin nanoparticles*

The protein albumin has been modified to create novel nanostructures for applications in drug delivery. The surface of albumin has several groups available for covalent conjugation of biomolecules and drugs. Albumin-DNA-polyethylenimine (PEI) conjugates have been used for gene delivery, with reduced irritation, damage and toxicity<sup>97</sup>.

Albumin can also form nanoparticles which can be modified to alter size, polydispersity, surface charge, drug loading and release. Functionalised albumin nanoparticles have been shown to cross the BBB<sup>98</sup>. Similarly, bovine serum albumin nanoparticles loaded with sodium ferulate have been targeted to the liver<sup>99</sup>. These experiments showed that cross-linking with varying amounts of glutaraldehyde can alter the drug release rate.

The most advanced use of albumin nanoparticles has been Abraxane®, a solvent-free formulation of paclitaxel (Abraxis Biosciences Ltd.)<sup>100</sup>, which has been approved for breast cancer treatment<sup>101</sup> in 36 countries across Europe, North America, Asia and Australia. The drug is conjugated to the nanoparticles (130 nm) and administered as a saline suspension at up to 10-fold higher concentration than previous formulations. It eliminates the solvent-derived side effects and the need for pre-medication against these, and shows reduced toxicity. The increased dose means there is a much lower administration time. There are a number of clinical trials planned to demonstrate the use of Abraxane® in other types of cancer<sup>102</sup>.

##### *Chitosan and Lectin nanoparticles*

Chitosan is a natural linear polysaccharide derived from the shells of crustaceans. Chitosan has the ability to clot blood and is used in bandages and other haemostatic agents. Its derivatives such as trimethylchitosan are used in non-viral gene delivery. It has also been used for the production of nanoparticles by ionotropic gelation with tripolyphosphate<sup>103</sup>. Nanoparticles of chitosan and egg phosphatidylcholine (ePC) have been reported for the delivery of anticancer drug paclitaxel<sup>104</sup>. The chitosan-ePC structure was found to be highly stable and biocompatible with these properties dependent on the ratio of the two materials. The activity of this system was confirmed in mice<sup>105</sup> but an accumulation of paclitaxel was seen in the heart which may cause complications<sup>106</sup>.

Lecithin is a lipid mixture of phospholipids mainly comprising phosphatidylcholine which is normally extracted from egg yolk or soy beans, and is widely used as a food additive. It is also used for liposome and micelle formation<sup>107</sup>. Chitosan is normally coated on the surface of lipid based nanostructures to improve the adhesive properties and increase stability.

Self-assembled spherical nanoparticles were formed by injection of an alcoholic lecithin solution into an aqueous chitosan solution<sup>108</sup>. The particles could encapsulate progesterone with an efficiency of ~60 %, however they had poor loading capabilities with hydrophilic drugs.

### 1.3.3.2 Gold nanoparticles

Colloidal gold nanoparticles have been used for a relatively long time for the treatment of diseases including cancer, rheumatoid arthritis, multiple sclerosis and neurodegenerative conditions such as Alzheimer's disease. The advantages of gold nanoparticles are their ease of preparation in a range of sizes, good biocompatibility, easily functionalised and their ability to conjugate with other biomolecules without altering their biological properties<sup>109</sup>. They also have unique optical properties, making them suitable for various spectroscopic applications and as photo-thermal agents in hyperthermia. Gold nanoparticles with diameters  $\leq 50$  nm have been shown to cross the BBB<sup>110</sup>.

PEGylated gold nanoparticles conjugated with TNF (tumour necrosis factor) can enter tumour cells through their leaky vasculature. The treatment, called Aurimune™, is being developed by CytImmune Sciences Inc. ([www.cytimmune.com](http://www.cytimmune.com)) and is currently in clinical trials. Similar systems which also have a chemotherapeutic drug attached are being developed. Functionalised gold nanoparticles have been used for effective oral and intranasal insulin delivery in a rat diabetes model<sup>111</sup>.

### 1.3.3.3 Magnetic nanoparticles

Magnetic nanoparticles have become one of the most studied and applied nanotechnology in the past few years. Applications involving magnetic nanoparticles include targeted drug delivery, as contrast agents in MRI (e.g. Feridex), gene delivery and cell separation/cell labelling. Iron oxide nanoparticles are widely studied due to their biodegradable nature, biocompatibility and superparamagnetic properties suited for MRI applications<sup>112</sup>.

Magforce Nanotechnologies ([www.magforce.de](http://www.magforce.de)) are currently undertaking phase II clinical trials for a magnetic nanoparticle-based cancer therapy (Nano-Cancer® therapy). Iron oxide nanoparticles coated with aminosilane are injected into a tumour and an alternating magnetic field is applied. The particles oscillate and can produce a range of temperatures, from 41 °C to 70 °C. At temperatures up to 46 °C (hyperthermia) the effect of radiation or chemotherapy is enhanced. At temperatures over 46 °C the tumour cells are destroyed (thermoablation). Current clinical trials are being undertaken for a number of cancers<sup>113</sup>.

Magnetic nanoparticles that can be loaded with drugs and still retain their MRI properties have been reported<sup>114</sup>. The iron oxide nanoparticles were coated with oleic acid and loaded with anticancer agents doxorubicin and paclitaxel with a loading efficiency of up to 95%. Experiments in breast cancer cells showed high antiproliferative activity, greater sensitivity in T2 weighted imaging and a higher circulation half life than Feridex. The drug release can be enabled by inducing hypothermia through an applied magnetic field.

### 1.3.3.4 Ceramic nanoparticles

Nanoparticles of silica, titania, alumina etc. are normally classified under the heading ceramic nanoparticles. One of the advantages of these particles is that their preparation is very simple. They are unaffected by changes in pH or temperature. It is possible to manipulate many features of these nanoparticles, including size, shape, porosity, inertness etc., and they can easily be modified to attach different biomolecules. Their typical size is around 50 nm.

Ceramic nanoparticles have been used to encapsulate hydrophobic drug molecules<sup>115</sup>, the acid-labile model enzyme, serratiopeptidase<sup>116</sup> and increase the transfection efficiency of DNA (used with a DNA-dendrimer conjugate)<sup>117</sup>.

### 1.3.3.5 Nanoshells

A nanoshell comprises a spherical core made from silica or other similar materials, surrounded by a coating a few nms thick. Typically the coatings comprise a metal such as gold or silver. One of the advantages of gold-coated nanoshells is that they possess plasmon resonance. This is the property of the electrons to oscillate collectively at a particular frequency when the incident light wave vector matches that of the electrons. By changing the thickness of the gold layers, the surface plasmon resonance can be tuned for various frequencies. This property has also been utilised in biosensing applications.

In cancer applications, antibodies or other biomolecules are attached to the gold surface to target it to the tumour site. By applying moderately low levels of near infra-red radiation *in vitro*, an appropriate dose of heat can be applied to kill the cancer cells (thermoablation). This technique has been used to kill prostate cancer cells<sup>118</sup>. The nanoshell-based heating mechanism can also be used for increasing tumour perfusion or to shut down perfusion completely for thermal ablative therapy. Recently, Zasadzinski *et al.*<sup>119</sup> showed that hollow gold nanoshells tethered to liposomes achieved near complete liposome release using a near infra-red (NIR) pulsed laser.

### 1.3.3.6 Aptamer-nanoparticle conjugates

Aptamers are nucleic acid ligands (single-stranded DNA or RNA) with high affinity and specificity towards target receptors or molecules like phospholipids, sugars and proteins. They offer a number of advantages over antibodies, although many of these are also offered by other ligands e.g. peptides. Aptamers have been reported for many targets and can be used as inhibitors themselves. An aptamer-based therapeutic is available (known as pegaptanib or Macugen®) for the treatment of age-related macular degeneration (AMD). Others are also in clinical trials.

Recently, there has been increasing interest in using aptamer-nanoparticle conjugates for targeted drug delivery and other therapeutic applications. The first example of this was reported in 2004 using rhodamine-labelled dextran as a model drug<sup>120</sup>. RNA aptamers specific to the prostate-specific membrane antigen (PSMA), a protein over-expressed in prostate cancer epithelial cells, were attached to polymer nanoparticles. The conjugate delivered drugs selectively to cells expressing the marker. This system was extended to deliver the anti-cancer drug docetaxel in an *in vivo* model of prostate carcinoma and reduced tumour size effectively following a single intratumour injection<sup>121</sup>.

Aptamer have also been conjugated to gold nanoparticles, liposomes, quantum dots and carbon nanotube field-effect transistors for delivery, imaging and sensing applications. A recent review has been published reviewing the use of aptamers<sup>122</sup>.

## 1.3.4 Nanosuspensions & Nanocrystals

Nanosuspensions are colloidal dispersions of nanoparticles of an insoluble molecule, which are stabilized by surfactants. Nanosuspensions can be used to maintain these drugs in a preferred crystalline state of sufficiently small size for intravenous administration<sup>123</sup>. Their advantages are similar to those of nanoemulsions. They can also achieve even higher levels of drug loading because the drug is in the solid state. Several studies have demonstrated the use of nanosuspensions for drug delivery with improved efficacy and release<sup>124,125</sup>. A good review of the recent developments in this field is available<sup>126</sup>.

Nanocrystals are aggregates comprising several hundred to tens of thousands of atoms that combine into a "cluster". Typical sizes of these aggregates are between 10-400 nm and they exhibit physical and chemical properties somewhere between that of bulk solids and molecules. By controlling the size and surface area, other properties such as bandgap, charge conductivity, crystalline structure and melting temperature can be altered. The crystals must be stabilised to prevent larger aggregates from forming.

Nanocrystals are produced by nanosonication. First, a nanosuspension is formed by high speed stirring, followed by wet milling, high pressure homogenisation, nanocrystallisation and spray drying to create nanosized crystals<sup>127</sup>. The advantages of nanocrystallisation are the ability to solubilise poorly soluble drugs, high bioavailability, major decrease in dosage volume (up to 4-fold), and an increase in tolerated dose (up to 10-fold for one cancer compound).

Elan Corporation (<http://www.elan.com>) have successfully commercialised and licensed their NanoCrystal® technology to produce nanocrystals of drug molecules that can be used in a number of dosage forms. Four products using this technology are currently available and another nine are in clinical development<sup>128</sup>.

Baxter have also developed a similar technology called Nanoedge ([www.baxterbiopharmasolutions.com](http://www.baxterbiopharmasolutions.com)) to solve drug formulation problems by reducing the size of particles to 100 nm and coating them with an excipient to increase the solubility<sup>129</sup>.

### 1.3.5 Carbon Nanostructures in Drug Delivery

#### 1.3.5.1 Carbon nanotubes

CNTs have the ability to transport drug molecules, proteins and nucleotides. Due to their size and shape, carbon nanotubes can enter living cells without causing cell death or obvious damage. Molecules can be covalently or non-covalently attached to the surface. The hollow structure of CNTs allows encapsulation of molecules but as yet there are very few examples of this for drug delivery<sup>130</sup>.

For biological applications CNTs require covalent<sup>131-137</sup> or non-covalent<sup>138-146</sup> functionalisation to prevent aggregation and increase their solubility. Several drugs have been successfully delivered, including amphotericin B<sup>147</sup>, which is normally insoluble and toxic due to its tendency to aggregate. When delivered using CNTs there was increased solubility, low aggregation (and therefore lower toxicity) and increased anti-fungal action.

A number of therapeutic applications of CNTs have been reported, including boron neutron capture therapy (BNCT)<sup>148</sup>, inducing immunoresponse<sup>149</sup>, gene<sup>150</sup> and siRNA delivery<sup>151</sup>. A recent review details biological applications of CNTs<sup>152</sup>.

Recently a 'smart bio nanotube' was reported which has ends that can open and close through altering the charge<sup>153</sup>. This opens up possibilities for controlled encapsulation and release.

Functionalisation needs to be efficient and cost effective. However, effective procedures for the production and/or purification to isolate CNTs of uniform size and shape are required before they can be used for medical purposes. There is debate about the toxicity of CNTs and much research is required in this area before medical applications can move from the lab to the clinic.

#### 1.3.5.2 Carbon nanohorns

Carbon nanohorns have a structure similar to CNTs except they are closed at one end, forming a cone-shaped cap, or 'horn'. They have a tendency to form spherical dahlia-flowerlike aggregates, roughly <100 nm. They have been used to deliver cisplatin<sup>154</sup> and dexamethasone<sup>155</sup>. Of the different carbon structures used in delivery, single walled nanohorns have been suggested as the most suitable due to their size, ease of synthesis (no metal catalyst required) and initial results showing that functionalised nanohorns are non-toxic to cells<sup>156</sup>.

### 1.3.5.3 Nanodiamonds

Diamond nanoparticles, or nanodiamonds have the capability for surface functionalisation. This has been used to immobilise proteins and deliver drug molecules<sup>157</sup>. Recently, nanodiamonds bound to doxorubicin were embedded into a polymer microfilm to achieve slow release of the drug over one month<sup>158</sup>. This system could potentially be used for tumour patches. Fluorescent nanodiamonds can enter cells, and may have applications in cell tracking and imaging. Currently, functionalised nanodiamonds are considered as biocompatible but very few studies have been conducted on this.

A recent review<sup>159</sup> on carbon-based nanomaterials for cancer therapy highlights excellently the pros and cons of using such materials. In summary, all of the materials show promise as drug delivery systems. However, there are a number of toxicity issues, particularly with CNTs, that have not been fully investigated and remain an area of concern. There is currently a lot of research ongoing in this area but so far there have been no conclusive studies on the effects of CNTs<sup>160-164</sup>. Carbon nanohorns and nanodiamonds are relatively new discoveries and have even less safety data.

Due to these issues, at present carbon nanostructures do not seem ideal candidates for a general delivery system. Other systems, such as nanoemulsions, do not contain components with these toxicity issues and at present appear more likely to be accepted. This may change if conclusive safety data is produced and made available although it may prove difficult to predict the long-term effects of allowing these materials to enter the body.

## 1.3.6 Other Nanotechnologies for Drug Delivery

### 1.3.6.1 Cyclodextrin Nanosponges

Cyclodextrin nanosponges are complex networks of cross-linked cyclodextrins cross-linked and formed into a roughly spherical structure, about the size of a protein, with channels and pores inside. The surface charge density, porosity and pore sizes of sponges can be controlled to attach different molecules. Nanosponges have been used for removal of organic impurities in water<sup>165</sup>. Few studies have been conducted on their drug delivery capabilities, but in 2006 Trotta *et al.* showed that lipophilic and hydrophilic drugs can be solubilised and carried<sup>166</sup>. Researchers at Vanderbilt University are developing nanosponges attached to dendrimers for drug delivery<sup>167</sup>.

### 1.3.6.2 Drug Carrying Implantable Thin films

These are nanoscale thin films that can be precisely controlled to release chemical agents by applying an electrostatic field. Hammond *et al.*<sup>168</sup> reported the development of a thin film of approx. 150 nm thickness using a layer by layer approach. It is made up of the negatively charged material Prussian Blue and a positively charged drug molecule, or a positively charged molecule enclosing a drug. The pigment sandwiches the drug molecules and holds them in place. When a voltage is applied the pigment disintegrates and delivers the drugs. A very small voltage (in the range of 1.25 V) can be used to release multiple doses from one or more films in a single solution. By changing the voltage, the time for delivery and quantity of drug can be tuned.

The advantages of the layer by layer approach include ease of preparation, versatility, capability of incorporating high loading of biomolecules into films, fine control over the structure, and robustness of the products under ambient and physiological conditions<sup>169</sup>. The film can be implanted in the body and can carry discrete packets of drugs that can be released separately, which could be particularly useful for chemotherapy. This electrostatic system can be used to deliver drugs for a variety of diseases like diabetes, cancer, epilepsy etc. In addition, it has potential applications in tissue engineering, diagnostics and chemical detection. The films are easy to mass-produce using a variety of techniques, and can be directly applied or patterned onto surfaces irrespective of size, shape or chemical composition

Lynn *et al.*<sup>170</sup> made thin films for the localized delivery of fDNA into cells. Multilayered polyelectrolyte films of thickness 100nm were created by alternative layer by layer deposition of plasmid DNA and a synthetic degradable cationic polymer. Quartz crystals coated with the thin films were put in contact with COS-7 cells. The introduction of quartz resulted in localised gene expression in cells under the film, without the aid of a secondary transfection agent. By incorporating plasmid DNA into thin films, it is possible to control the location and distribution of these molecules from implantable materials or other biodevices.

#### 1.4 Additional Demands for Research:

- Understanding the mechanism of nanostructure transport across cell membranes and the interactions between these novel structures and cells, particularly at the sub-cellular level.
- Tumour targeting strategies other than EPR effect.
- Development of self assembling polymers to create novel structures for drug encapsulation and delivery.
- Development of novel structures that can release drugs in response to change (pH, temperature, enzyme interaction etc).
- Development of coatings that can enable nanostructures to respond to change.
- Novel triggers for the controlled release of drugs from nanostructures.
- Development of implantable nanodevices for drug delivery.
- Incorporation of nanosensors in implantable drug delivery devices.
- Virus-like particles for gene delivery<sup>171</sup>.
- Methods to improve the drug loading efficiency of nanostructures.
- Methods to prevent the accumulation of drugs in organs when used with nanostructures for delivery (seen in many studies)<sup>172,173</sup>.
- Development of new nanoformulations which can be administered orally.
- Development of new structures that can cross the blood brain barrier for treatment of CNS diseases.
- Development of methods to cross specialized neural barriers (eye, ear, and peripheral nerves etc.).
- Developing effective scalable processes to attach small drug molecules, proteins, peptides and genes inside and onto carbon nanotubes.
- Manufacturing and scale up of nanostructures.
- Studies on the fate of delivery systems and their degradation pathways (including environmental fate).

- Toxicity studies (*in vitro* and *in vivo*) of the nanomaterials used in drug delivery.
- Development of new risk management plan for nanotechnology based medical products.

## 1.5 Applications and Perspectives

The technologies reviewed in this document underline the importance of nanotechnology in therapeutics and the role played by nanotechnology in combating some of the chronic diseases, such as cancer. Areas in drug delivery where nanotechnology can make a difference include<sup>1</sup>:

- developing systems that improve the solubility and bioavailability of hydrophobic drugs
- designing delivery vehicles that can improve the circulatory presence of drugs
- eliminating or minimising toxicity
- increasing specificity
- targeting drugs to specific cells or tissues
- developing delivery systems for slow release
- improving vaccine adjuvants and delivery
- developing novel nanostructures that can be used in specific applications, e.g. ocular, cancer therapy, neurology, orthopaedics

Nanotechnology-based therapeutics have the potential to significantly impact on the pharmaceutical industry. Smaller companies can afford to develop novel formulations of off-patent drugs, but may not have the capital to invest in a costly and lengthy drug discovery and approval process. This may encourage more partnerships, licensing and co-development of products. Also, until relatively recently the pharmaceutical industry generated revenue from huge blockbuster drugs that sold many millions of units worldwide. With a trend towards personalised medicine emerging, in part enabled through nanotechnology, this huge market for a single product may no longer be there. Therefore, a new approach to the drug discovery and development process may be required. However, many novel nanotechnology-based delivery systems enable the delivery of insoluble drugs, opening up larger regions of 'chemical space' for exploration. Lead compounds previously rejected due to solubility issues may now be feasible and useful drug candidates.

However, the novel properties and characteristics that bring new benefits in drug delivery also bring new challenges in risk management and toxicity. Some of these novel characteristics are poorly understood or studied. Presently, systems made from natural or GRAS components, such as liposomes or albumin nanoparticles, appear to have the most potential. This is both due to their minimal toxicity concerns and the precedent set by existing products. Other systems such as CNTs, while promising, raise a number of toxicity issues. Until these are addressed CNT-based delivery systems are not likely to appear as therapies.

In order to take nanotechnology-based medical products forward to the clinic, it is necessary to address the risk issues simultaneously, including any novel risks resulting from the nanoscale properties of the materials used.

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