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Applications of Nanometre Scale Particles as Pharmaceutical Delivery Vehicles in Medicine

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Abstract - Recent scientific advances have resulted in the emergence of the rapidly developing field of nanotechnology, which involves the rational design of materials and devices in the 1-100 nm range. At the nanometre scale, size, shape and surface morphology can have a profound influence on the chemical, physical, optical and electronic properties of a material, which is significantly different from their bulk counterparts. The novel size dependent properties at the nanometre scale, gives these materials their unique physiochemical properties, which have the potential to be used in a wide variety of applications. Nanomedicine is the term used to describe the use of nanotechnology in medicine, and includes its use in the development of diagnostic techniques and interventions, pharmaceuticals, regenerative medicine and tissue engineering. This brief review gives an overview of current developments in the use of nanometre scale particles that are specifically designed for the delivery of pharmaceuticals in medicine.

Keywords - Nanometre scale particles, Nanomedicine, Drug delivery

1. Introduction

Nanotechnology, an interdisciplinary research field involving chemistry, engineering, biotechnology and biomedical sciences, has been able to deliver a wide range of new and novel materials [1, 2]. By its nature, nanotechnology involves the design, characterization and application of materials and devices by controlling size and shape of materials in the nanometre scale range from 1 to 100 nm. Nanomaterials, with dimensions smaller than 100 nm have chemical, physical and biological properties that are different from their bulk counterparts. At the nanometre scale, the quantum mechanical properties resulting from atomic interactions, gives nanometre sized materials their unique properties, which have the potential to be tailored for specific applications [3]. Nanotechnology based techniques have already being used successfully in a number of fields such as biotechnology, material science, photonics and electronics.

From a biological perspective, the cells of most living organisms are in the micron-size range, with their internal organelles and other sub-cellular structures all in the sub-micron size range. The difference in size between a typical cell (around 10 μm or 1000 nm) and a nanometre scale particle (NP) can be in the order of at least a thousand times. This significant difference in size range between the cell and a NP gives the NP the potential to biophysically interact with biological molecules at a cell membrane and/or intracellular level [4]. For example, NPs smaller than 50 nm, can easily enter most cells and interact with intracellular molecules, which makes them an attractive platform technology in medical applications [5].

The merger of nanotechnology and medicine has resulted in the creation of the interdisciplinary field of nanomedicine, which encompasses molecular biology and nanotechnology. An important facet of nanomedicine is the development of pharmaceutical agents using nanotechnology-based techniqu es. The use of materials in the nanometre scale has the capacity to enhance the efficacy of existing medications and allow for the development of new and unique pharmacologic al products. Moreover, at the nanometre scale properties such as bioavailability, toxicity and side-effects can be potentially altered in order to improve the overall success of an intended therapy whilst minimizing associated adverse effects on the patient. For example, many chemotherapeutic used in the management of malignancy are limited due to their associated toxicities. This may result in the adjustment to dosing regimens or premature cessation of therapy in an attempt reduce the impact on quality of life which in turn may reduce the efficacy of the chemotherapeutic agent. Nanotechnology based drug delivery systems have the potential to change the landscape medicine through the development of nanometre scale therapeutic devices that can optimally deliver a sustained, controlled and targeted release of vaccines and therapeutic agents via a number of delivery routes as

presented in Figure 1. Currently there are several nanotechnology-based therapeutic products using previously authorised drugs approved by the Food and Drug Administration (FDA) for clinical use and many more pharmaceutical drugs, vaccines and therapeutic products being investigated [6-10].

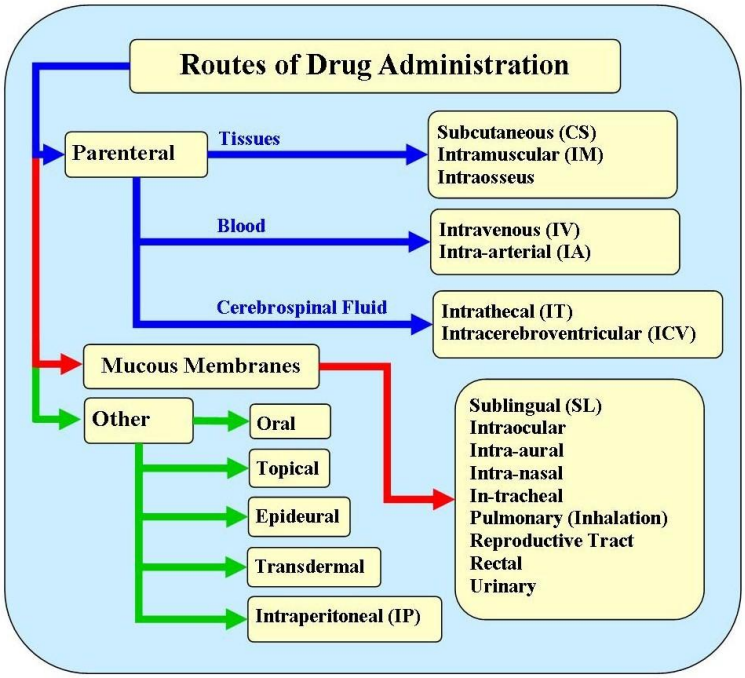

Fig. 1. Potential routes for nanometre scale particle delivery of pharmaceutical agents.

Another aspect of nanotechnology based techniques in medicine involves the development of new medical therapies in the fields of tissue engineering and reconstructive surgery. In the last few decades, nanotechnology based tissue engineering techniques have being able to produce a number of engineered implantable human tissues such as bone, cartilage and skin [11, 12]. This research has clearly demonstrated that to be an effective regenerative therapy, tissue engineering needs to create an environment starting from the nanometre scale that can promote productive and efficient cellular activity for a successful clinical outcome. The delivery of pharmaceutical agents by NPs incorporated into the regenerative medicine procedures has the potential to significantly improve clinical outcomes.

2. Nanometre Scale Particles (NPs) in Therapeutics

NPs can be made from a variety of materials such as metals, ceramics, polymers, organic materials

composites and come in wide range of morphologies. They range in size from 10 nm up to a few hundred nanometres and have diverse chemical structures, but share common properties such as a large surface area to volume ratio. There are many advantages of using a drug delivery platform based on NPs in therapeutic applications [13]. NPs can travel through biological barriers such as cell walls via mechanisms such as endocytosis and interaction with biological molecules such as proteins and nucleic acids. The NP based delivery platform could be a carrier, the active pharmaceutical itself or engineered to release drugs at a more specific half-life to maximize therapeutic impact whilst minimizing toxicity. For example longer circulation times, controlled release and prolonged drug half-life can reduce the frequency of administration, which may improve side-effect profiles and patient compliance [14]. The potential to alter pharmaceutical properties is of particular importance in anticancer drugs where targeted delivery of the agent will improve its effect on malignant cells whilst minimizing unwanted adverse side-effects such as hepatotoxicity, pancytopenia, fatigue and nausea which can in some cases have a greater impact on quality of life than the disease the medication was intended to treat. [15]. An attractive feature of a NP based drug delivery platform is that it can be engineered to improve the solubility of drugs that are normally hydrophobic or have low solubilities in water. Furthermore, the delivery platform also has the potential to carry two or more drugs for simultaneous release at the target. The multi-drug delivery platform has the potential to deliver a synergistic effect and reduce the effects of drug resistance.

The following section discusses the various types of NPs in current use or is undergoing preclinical testing or is undergoing development. NPs can be made using a variety materials including inorganic (metallic and ceramics), lipids

(liposome's), polymers (polymeric NPs, dendrimers, micelles), nanometre scale crystals, viruses and carbon based NPs. Among the various forms of NPs currently available, the two main types in common usage are liposomal drug carriers and polymers based drug delivery platforms [16].

2.1. Types of Nanoparticles

2.1.1. Inorganic NPs

Inorganic NPs composed of ceramics and metals have attracted considerable interest in recent years due to their potential therapeutic benefits. They can be engineered to evade the reticuloendothelial system by varying size and surface composition [17]. And in the case of ceramics they may have a porous structure that is capable of protecting an entrapped payload from degradation or denaturisation [18]. Metallic NPs that have attracted significant interest over the last decade are gold (AuNPs), silver (AgNPs) and iron oxide (FeONPs), see Figure 2 (e). Au NPs have been identified as potential platform structure for a number of biomedical applications such as biosensors [19], clinical chemistry [20], fluorescent labelling for immunoassays [21], tumour destruction via heating (hyperthermia) [22], targeted delivery of therapeutic drugs and genetic substances [23] and as antibacterial drugs [24-26]. For centuries, silver and silver compounds have been successfully used as an effective antimicrobial agent for the treatment of infections. Silver nanoparticles (Ag NPs), like its bulk counterpart have also been found to be an efficient antimicrobial agent capable of interacting with the cell membrane, interfering and damaging cellular nucleic acids [27]. Recent studies have shown that Ag NPs possess both anti-bacterial and anti-inflammatory that can promote faster wound healing. This combined anti-bacterial and anti-inflammatory property has resulted in Ag NPs being incorporated into a number of wound dressings, pharmaceutical preparations and implant coatings [28-30]. The main biological function of FeO NPs, after being coated with a suitable surfactant, phospholipids, or other compounds to improve their stability, is to be an effective delivery platform for biological agents or pharmaceuticals [31]. Another application of FeO NPs with super-paramagnetic properties, is their use as a contrast agent in magnetic resonance imaging (MRI) for the detection, diagnosis and monitoring the effectiveness of treatment protocols of patients with liver cancer andchronic kidney disease [32, 33]. Furthermore, super-paramagnetic FeO NPs or SPIONs have been used to delivery proteins, nucleric acids, plasmid DNA, small interfering RNA (SiRNA) and chemotherapy agents [34-39]. And recently, using magnetic field-induced excitatio n, aminosilane-coated super-paramagnetic FeO NPs were used in a thermotherapy procedure to treat brain tumours in a rat model. The results of the procedure were able to produce a 4.5-fold increase in the life expectancy of the treated rats compared to the untreated rats [40].

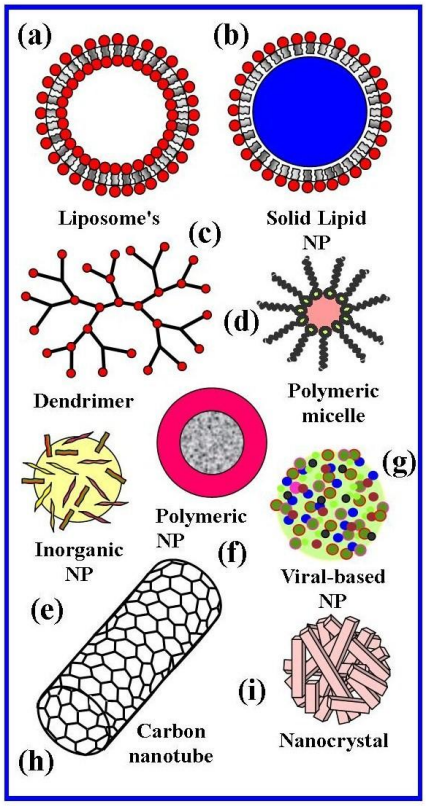

Fig. 2. Schematic illustrations of various therapeutic NPs delivery systems

Ceramics such as alumina, calcium phosphate, titania and silica are biologically compatible, biologically inert and importantly, have porous structures. These ceramics have been extensively used to form biocompatible surface coatings on artificial implants in a number of medical procedures [1, 41]. An attractive feature of these ceramics is their porous structure, which has the ability to provide a physical barrier capable of protecting an embedded molecular payload from degradation or denature within the fluid environment of the body. It is the porous nature of ceramics, particularly at the nanometre scale that has made them an ideal candidate for drug delivery vehicles. Ceramic NPs have the potential to be effective delivery platforms because of their biocompatibility, defined structures, surface chemistry and high stability *in vivo* [42, 43]. Currently, there are a number of ceramic NPs in preclinical development and a few in clinical trials. Silica based NPs are being examined as potential delivery platforms for photosensitizing anticancer drugs in photodynamic therapy [44] and for gene delivery platforms for various central nervous system (CNS) cell types [45]. While calcium phosphate NPs are being examined as a potential vaccine adjuvant [46].

2.1.2. Lipid Based NPs

Liposomes are spherical self-closed structures composed of concentric lipid bi-layers consisting natural or synthetic amphiphilic lipid molecules surrounded by a phospholipid membrane as graphically presented in Figure 2 (a). The lipid

bi-layers surround a central aqueous interior that can encapsulate hydrophilic compounds, or hydrophobic compounds, which tend to diffuse out of the phospholipid membrane [47]. Historically liposomes were first described in the early 1960s as a potential delivery platform for proteins and therapeutic drugs [48]. Despite being able to protect the pharmaceutical payload from degradation and having less toxicity than conventional therapies, liposomes suffered from leakage of therapeutic agents from the aqueous core when in the blood stream which significantly reduced their encapsulation efficiency and *in vivo* circulation life. However, recent studies to improve the solubility and encapsulation of hydrophobic compounds have revealed that incorporating inert and biocompatible polymers such as polyethylene glycol (PEG) with liposomes increased the circulation half-life and permitted better control of payload release in vivo [49, 50]. In the last decade, liposomes have been extensively used as pharmaceutical delivery platforms due to improvements in encapsulation of both hydrophilic and hydrophobic therapeutic agents, protective coatings to prolong circulation half-life in vivo and functionalizing liposomes with target specific ligands that guide the liposome to particular cells, tissues and organs [51-53]. For example, liposomes have been used to deliver genes into the brain of vertebrates to achieve a successful transfection of neurons [54] and a liposomal complex carrying a plasmid encoding of tyrosine hydroxylase (TH) was used to alleviate the effects of Parkinson's disease in animal models [55, 56].

Solid lipid NPs are lipid-based colloidal carriers ranging in size from 50nm up to 1000 nm and were introduced in the early 1990's as an alternative to emulsions, liposomes and polymeric NPs. To improve their stability, the liquid lipid central core was replaced by hydrophobic lipids that are solid at room temperature, which significantly improves their stability in vivo as seen in Figure 2 (b) [57]. The stability is further enhanced by the inclusion of high levels of surfactants, which assists in engineering the hydrophobic cores to carry larger pharmaceutical loads and improve drug delivery performance. Solid lipid NPs can be used to deliver pharmaceuticals orally, intravenously, topically, or via inhalation. Also due to their favourable biodegradation properties, they are considered less toxic than ceramic or polymers based NPs and are considered safe delivery platforms [58]. In addition, when solid lipid NPs are treated with molecules such as modified fatty acids, polysorbates and poloxamers to improve their stability properties, they can be used to carry pharmaceuticals across the blood brain barrier [59, 60]. And they have also been used as carriers to deliver pharmaceuticals to CNS cells in both in vitro and in vivo studies [58, 61, 62].

2.1.3. Polymeric NPs, Polymeric Micelles and Dendrimers

NPs derived from polymeric materials are composed of natural or synthesized macro-molecules that are biodegradable and decompose into by-products that can easily be excreted from the body. The basic principle of using a polymeric matrix as potential delivery platform lies in the ability of the matrix to carry sufficient a sufficient therapeutic drug payload to specific cells, tissues or organs. The drug payload can be dissolved into, entrapped, encapsulated or attached to the matrix structure as presented in Figure 2 (f) [63]. A typical polymeric NP is a sphere or a capsule. The sphere based NP consisting of a polymeric matrix in which the drug is physically and uniformly dispersed throughout the matrix. The capsule configuration (nano-capsules), are vesicular systems which consist of a polymeric shell and an inner core where the drug payload is confined. The most attractive features of using polymeric NPs are their biocompatibility, ease of formulation, and biodegradability which provides an effective delivery platform for the sustained release of pharmaceutical preparations such as vaccines, anticancer drugs, proteins, peptides and gene therapies [64-68]. The most important properties that need to be considered when designing a polymeric NP based drug delivery platform are: 1) the size of the matrix structure and interior porosity; 2) size distribution of particles; 3) drug diffusivity; 4) drug encapsulation efficiency; 5) drug release kinetics; 6) drug stability; 7) hemodynamic properties; 8) surface adhesion; 9) surface chemistry; 10) surface charge; 11) surface erosion, and 12) surface morphology [69].

Polymeric NPs including both natural and synthetic have some distinct advantages compared to other forms of NPs, which makes them attractive for drug delivery platforms. These advantages include: 1) The ability to protect the pharmaceutical payload that is normally encapsulated or entrapped within the biodegradable polymer matrix; 2) being able to control the particle size and surface properties of the nanoparticles; 3) the controlled and sustained release of pharmaceuticals at the target cells or tissues to maximize the therapeutic efficiency and reduce side effects; 4) the controlled degradation of the platform by selecting the matrix composition; 5) designing the matrix with specific surface ligands to enhance target uptake; and 5) the delivery system can be administered through a number of different delivery routes such as oral, nasal, ocular, transdermal and intravenous.

Natural polymers such as albumin, chitosan and heparin have been used as delivery platforms for various pharmaceuticals [16]. For example, albumin has been clinically investigated as a delivery platform for the anticancer drug paclitaxel, for the treatment of metastatic breast cancer [70]. Historically, synthetic polymers such as Poly (*lactide*) (PLA), poly (*d,l-lactide-co-glycolide*) (PLGA) a copolymer of PLA and Poly (*glycolide*) (PGA) and poly(*Ɛ-caprolactone*) (PCL) were all originally synthesized in the 1950s for non-drug delivery applications such as surgical sutures, textile grafts and implants. However, subsequent studies of these polymeric materials revealed that they had some significant advantages over other therapeutic agent carriers. For example, polymeric NPs can be engineered to release their pharmaceutical payload in a controlled and sustained manner, which promotes drug stability, prolongs the

delivery period and overall enhances the therapeutic efficiency [71, 72]. Moreover, these polymers are the most widely researched Federal Drug and Food Authority (FDA) approved and investigated biodegradable polymers in the literature for a number of drug delivery platform applications [73]. Other polymers such as poly (*vinyl-pyridine*) (PVP) along with poly (ethylene) glycol (PEG), PCL, PLA and PLGA have been found suitable for DNA, protein and drug delivery systems [16]. While poly (ethylene mine) (PEI) NPs have been used for gene delivery [74] and poly (butyl cyanoacryalate) (PBCA) NPs have been used as non-viral vectors for therapeutic treatment of the brain [75, 76]. The advantage of using these polymers comes from their chemical composition, total molecular weight and their block length ratios which can be adjusted to control the size and morphology of the delivery platform [6]. Furthermore, the degradation time of the delivery platform can be synchronized to the release kinetics of the pharmaceutical payload. And in the case of PLGA, the controlled degradation results in ester hydrolysis, which forms biocompatible bi-products in the body environment [6, 77 and 78].

The disadvantages of using NPs in general stems from their small size and large surface area that causes the particles to agglomerate in liquid and dry forms, which makes them difficult to handle. In addition, there is also a limited amount of pharmaceutical loading capacity that each nanoparticle can carry due to its size and structure. And in the case of polymeric NPs being used as drug delivery platforms, the mechanical strong matrix structure with its slow degradation rate can result in slow drug release profiles. These slow drug release profiles often fail to deliver the required drug concentration to the targets cells or tissues to be effective. Also, there are a number of issues associated with the bioactivity of proteins and peptides when they are encapsulated in the polymer matrix. This arises from the polymers hydrophobic nature which tends to break down the proteins and peptides to produce acidic breakdown products (lactic and glycolic acid end groups). This situation is further exacerbated by water not being able to enter the matrix. The highly hydrophobic nature of the polymer matrix also makes it incompatible with hydrophilic drugs and hydrophilic molecular probes used for targeting, which can lead to complications in the drug preparation technology being used. It is due to these disadvantages, that there is considerable ongoing research into developing new biodegradable polymers and copolymer delivery platform systems for administering pharmaceuticals [8, 79-81].

Nanometre scale spherical polymeric micelles are formed by the self-assembly of amphiphilic block copolymers into a core-shell structure in an aqueous medium. The structure consists of two or more polymer chains with different hydrophobicity. The hydrophobic polymer chains form the core structure to reduce their exposure to the aqueous surroundings. This creates a reservoir for hydrophobic pharmaceuticals, while the hydrophilic polymer chains form the shell structure, which stabilizes the hydrophobic core in the aqueous environment [82]. The hydrophilic shell also makes the micelle water soluble and in the process creates a drug delivery platform with promising therapeutic potential and an ideal candidate for administration [83-86]. Polymeric micelle delivery platforms have the potential to be multifunctional; they could co-deliver a number of therapeutic drugs with imagining agents and targeting ligands [87, 88]. Also under investigation is the possibility of differentially targeting cells by selectively modifying the surface of the polymeric micelle with ligands such as antibodies, carbohydrates and small molecules [89]. The most commonly used biodegradable polymers to form polymeric micelles for drug delivery are PLGA, PLA, PCL and poly (*ethylene glycol*) (PEG) [90, 91].

Dendrimers are polymer-based macromolecules formed from monomer units that branch out from a central core in a radial direction. The symmetrical structure consists of a core composed of a single atom or group of atoms which are surrounded by a branching structure composed of repeating units that form multiple concentric layers as shown graphically in Figure 2 (c). The degree of branching has the advantage of creating voids within the structure that can be used for pharmaceutical encapsulation [16, 85]. The exterior of the branching structure terminates with surface ligands, which promote interactions with other molecular groups, solvents and biological surfaces. Despite having a central core and branch structure, dendrimers can have significantly different chemical properties. Some of these properties can include adjustable surface functionality, water solubility and mono-dispersible size, which enhances the ability of dendrimers to cross biological barriers carryingpharmaceutic al payloads. For example, poly (*amidoamine*) (PAMAM) dendrimers has been found to be an effective drug delivery platform for the transport for anti-cancer agents [92]. And because their adjustable surface properties and structure they have been modified with thiamine to improve the delivery of pharmaceuticals across the blood brain barrier via intravenous administration [93]. Furthermore, PAMAM dendrimers have been modified with Angiopep-conjugated poly (*ethylene-glycol*) [94] or lactoferrin to improve the delivery of genetic material to brain tissues [95].

2.1.4. Nanocrystals, Viral NPs and Carbon Nanometres Scale Tubes

Many pharmaceutical formulations developed over the years suffer from poor water solubility. Studies have shown that poor water solubility correlates to poor bioavailability of administered drug formulations [96, 97]. Nanocrystals are an attractive method of delivering drug formulations with poor solubility profiles and overcome the bioavailability problems normally associated with their administration [98]. Nanocrystals are nanometre scale particles composed of a combination of molecules in a crystalline form which forms the bulk of the preparation as presented in Figure 2 (i). Higher dosages can be delivered in this type of formulation compared to polymeric nanoparticles, which have a significant carrier

material component. The advantage of the nanocrystals formulation is the smaller particle size, which leads to a larger surface area and a much improved saturation solubility [99]. This results in a significant increase in the dissolution velocity and as a consequence greater bioavailability. However, excessively high dissolution rates are undesirable since they produce high plasma peaks and significantly reduced circulation times. Therefore, for many applications nanocrystals are combined with conventional controlled drug release mechanisms such as coated pellets to avoid rapid dissolution rates and short circulation life [98]. In addition, nanocrystals are usually coated with a thin hydrophilic layer, which prevents aggregation of the crystalline drug formulation and assists in the circulation, distribution and bioavailability. The effective delivery of nanocrystal based pharmaceutical formulations needs to take into account the type of administration route used to deliver the drug payload and the stability of the pharmaceutical to maintain the necessary concentration levels during circulation to be effective [100].

Viruses can be considered as nature's most highly efficient nanometre scale biological material with an in-built core-shell structure capable of injecting nucleic acids into host cells. The shell structure is protein based, naturally biocompatible and their ability to carry a payload makes them an ideal model for developing virus-like nanometre scale particles (VNPs) for the delivery of pharmaceutical molecules (Figure 2 (g)) and imaging reagents [101]. Targeting molecules can be attached to the external surface of VNPs in a biologically functional form using either chemical or genetic engineering techniques [16, 102].The development of targeted VNPs for therapeutic protocols has been extensively studied and used as gene delivery carriers due to their high transfection efficiency and effective targeting of cancer cells [103-105]. Effective targeting of specific cells significantly reduces the quantities of therapeutic molecules needed by delivering the required concentration to the targeted cellsand in the process reducing adverse side effects of the therapeutic molecules. Recently, a variety of VNP including cowpea mosaic virus (CPMV), cowpea chlorotic mottle virus (CCMV) and bacteriophages such as MS2, M13 and HK97 have been developed to target cancer cells [106-109]. For example, a CPMV formulation was effectively used to target tumours, pass through the endothelial layer and accumulate within the tumour [110].

Carbon nanometre scale cylindrical tubular structures or carbon nanotubes (CNTs) are formed by self-assembling sheets of grapheme. They can have a small radius, typically starting from a few nanometres and lengths of varying length, usually less than 1 μm. The tubular structure has a high aspect ratio which can be composed of one (single-walled CNTs) or several (multi-walled CNTs) walls that create large internal volumes as seen in Figure 2 (h).. CNTs are completely insoluble in all solvents and have a strong tendency to agglomerate into micrometres scale structures. However, the external surface can be chemically modified to make them water-soluble and functionalized to promote bonding with a variety of molecules such as proteins, nucleic acids and pharmaceutical agents [111]. CNTS have been investigated for a number of potential applications such as biomedical sensors [112, 113], neural growth and neural signalling [113, 114, 115], delivery of genetic material [116, 117] and drug delivery [118]. In spite of being potentially advantageous for biomedical and pharmaceutical applications, recent studies have shown that the needle-like shape of CNTs can be extremely toxic to human tissues [119]. Toxicity can result from surface charge and modification [120], while structural characteristics such as shape [121], length [122], agglomeration [123] and number of layers [124, 125] can significantly reduce the biocompatibility of the CNTs. The influence of chemical and physical properties of CNTs, and the mechanisms causing toxicity are the focus of ongoing research.

3. Properties and Characteristics of Nanoparticles on the Delivery of Pharmaceutical Agents

3.1. Particle Size and Size Distribution

Both particle size and size distribution are important characteristics that influence the pharmaceutical loading capacity of the nanoparticles, which has a direct bearing on the efficient delivery of the pharmaceuticals to the targeted diseased and/or cancerous cells and the therapeutic benefit delivered to the patient receiving the treatment. On advantage of using nanoparticles for drug delivery over micrometre sized particles is the larger surface area for a given amount of weight or volume. This means in the case of nanoparticles, the bulk of the pharmaceutical agents are located near or close to the surface of the particle, leading to fast surface diffusion or surface erosion and rapid drug release. This is unlike the case for the larger micrometre sized particle, where their larger core can encapsulate the pharmaceutical agent and promote a reduced diffusion rate [126]. In addition; there is a great tendency of nanoparticles to aggregate during storage, it is necessary to make nanoparticle formulations that promote non-aggregation and enhance particle stability.

Another factor that needs to be taken into consideration with polymeric nanoparticles is the polymer degradation with time. Currently there are conflicting studies of degradation rate for PLGA; *Dunne et al.* [127] has reported an increases degradation rate with increasing particle size *in vitro*, while Panyam *et al.* has reported that different size PLGA particles had similar degradation rates *in vitro* [128]. Further studies are needed to investigate the degradation rates for polymeric nanoparticles to determine the exact mechanisms behind the degradation behaviour of particles both *in vitro* and *in vivo*. After the manufacturing process, the particle size, in most cases is determined using laser light scattering, photon-correlation spectroscopy or dynamic light scattering [129]. The results of particle size analysis determination are

usually confirmed by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy. The surface chemistry is analyzed using x-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). While the surface charge properties of the nanoparticles are measured via the Zeta potential.

The size and size distribution of nanoparticles within the body makes them attractive for a wide range of cells and cellular tissues, with a greater interaction with cells compared to micrometre sized particles. Studies have shown that the 100 nm nanoparticle uptake is 2.5 times greater than 1 μm particles and 6 times greater than 10 μm particles in a Caco-2 cell line [130, 131]. An important property of nanoparticles is their ability to penetrate drug barriers such as the gastrointestinal barrier (GI) and blood brain barrier (BB). For example, murine study revealed that nanoparticles were able to penetrate and diffuse throughout the submucosal layers of an intestinal loop model, while the larger micrometre sized particles were generally found in the epithelial lining [132]. The evidence suggests that size selection is important, since some cell lines will only allow nanoparticles and submicron particles to enter the cell [133]. In similar studies of the BB barrier, nanoparticles have been able to penetrate through the barrier and in some cases a surfactant [polysorbate 80 (Tween 80)] was used to coat the nanoparticles to assist their passage through the barrier [134, 135]. This type of nanoparticle application makes it possible to deliver a sustained release of therapeutic pharmaceutical agents to treat difficult to get to brain cancers.

3.2. Surface Chemistry and Charge of Nanoparticles

When nanoparticle based pharmaceutical agents are administered by intravenous injection or infusion, the body's immune system immediately identifies the foreign materials and immediately responds. During circulation with the blood the nanoparticles interact and adsorb opsonins onto their surface, which forms a bridge between the nanoparticles and the phagocytes [136, 137]. As the opsonised nanoparticles pass through mononuclear phagocyte system (MPS); which consists of organs such as bone marrow, lymph nodes, liver and spleen, they are removed by the macrophages [138].

To improve the delivery of therapeutic pharmaceuticals to a specific target the nanoparticle drug carrier needs to minimize the effects of opsonisation and extend the circulation life of the carrier within the body environment. The surface chemistry of the nanoparticles is also important in determining the interaction and adhesion of with target cells and/or tumours.

Extending the operational life of the nanoparticle based pharmaceutical delivery platform can be accomplished by: 1) Controlling the surface charge (zeta potential) of the nanoparticles, this would prevent any aggregation of the particles in the blood and it would also be important at the target cells whose surface membrane is generally negatively charged. If the surface charge of the nanoparticles is not compatible with the cell membrane then there will not be any interaction or adhesion between the two, which will in turn inhibit drug delivery; 2) Adding a surface coating of nanoparticles with hydrophilic polymers or surfactants which will have a significant effect on drug encapsulation and assist in controlled drug release; and 3) formulation of nanoparticles with biodegradable copolymer surfaces with hydrophilic brush like surface projections which repel opsonins and reduce phagocytosia [139]. For example, poly (ethylene glycol) (PEG) is a widely used synthetic polymer used *in vivo* applications because of its biocompatible, hydrophobicity, protein-resistance and high surface mobility, which results in a high steric exclusion [140].

3.3. Drug Loading and Release Properties

Drug load and encapsulation efficiency are important parameters when designing a drug delivery platform since a high pharmaceutical load results in lower matrix materials being administered to the patient. The pharmaceutical agents can either be integrated at the time of nanoparticle formulation or be incorporated using an absorption/adsorption method after the nanoparticle matrix is pre-formed. The loading and encapsulation efficiency of the matrix material is dependent on the solubility of the pharmaceutical agent, composition of the matrix, molecular weight, matrix/agent interaction and the presence of any degradation end products of the matrix such as carboxyl or esters [141-143]. The delivery of pharmaceutical preparations from drug delivery platforms faces many difficulties, from the drug loading and encapsulation point of view the molecular size play an important role. In the case of small molecular based therapeutic agents, studies have shown that the ionic interaction between the platform matrix structure and the agent is an effective method of inducing a larger agent carrying capacity of the matrix [144-146]. Larger macromolecules and proteins have the greatest loading capacity at the isoelectric point where their solubility and adsorption is at its maximum [147, 148].

The controlled release of pharmaceutical agents incorporated in a nanometres scale particulate delivery platform matrix is important because it delivers an effective therapeutic effect for a desired period of time. To develop an optimum nanometre scale particulate delivery system many factors have to be considered since each factor can have a significant effect on the degradation process and the release of pharmaceutical agents from polymeric matrix. In general, the solubility of the pharmaceutical agent, desorption of the agents from the surface attached, agent diffusion through the matrix and the erosion and degradation of the matrix influence the release of pharmaceuticals. In particular, the factors the influence the degradation and erosion of the polymer matrix include: 1) particle size, composition, crystallinity and molecular weight [149-151]; 2) porosity, permeability and size of matrix material [152, 153]; 3) presence of additives such as plasticisers and residual solvents [154, 155]; 4) flow rate, pH and temperature stability [156-158]; 5) strength and

strain properties of matrix [159, 160]; and 6) sterilisation of the matrix [161, 162]. All these factors must be considered when developing a controlled degradation and pharmaceutical release process to deliver a therapeutic effect to a specifically targeted disease or cancerous tumour.

When the matrix is composed of nanospheres, with the pharmaceutical agent uniformly distributed throughout the matrix, the resulting release of agents is by diffusion and erosion. If the diffusion process releases pharmaceutical agents faster than the erosion rate, then the release mechanism is diffusion controlled. Under these conditions it is possible to produce a short-term burst of pharmaceuticals from the weakly bound or adsorbed agents at/or in close proximity to the surface [163]. It has been shown that the method of pharmaceutical inclusion, the chemical properties of the pharmaceutical agent and its interaction with the polymeric matrix can significantly influence the degradation of the matrix which in turn affects the release pattern [164]. In addition, the ionic interaction between the pharmaceutical and additives such as plasticisers and residual solvents can form low soluble complexes which tend to slow down the release rate [165]. On the other hand using the block copolymer ethylene oxide-propylene oxide with chitosan induces a strong ionic interaction between them, to the detriment of bonds formed between bovine serum albumin (BSA) and the chitosan matrix [166]. When the pharmaceutical is incorporated into the polymeric matrix, there is a relatively small initial burst of agents followed by an improved sustained release profile [167]. The pharmaceutical release from the inner regions of the matrix is then controlled by the diffusion of the pharmaceutical across the outer annular region which acts as a regulating barrier. This is also the case if the nanoparticle matrix is encapsulated by a polymer membrane which effectively controls the diffusivity of the pharmaceuticals from the inner matrix.

4. Nanoparticle Based Drug Delivery Platform Applications

4.1. Targeted Delivery of Pharmaceuticals

Currently, most pharmaceutical agents are not target orientated and are administered systematically to disperse throughout the body. This non targeted delivery method is inefficient, requires larger doses of the pharmaceutical to be evenly distributed and the larger doses also produces serious side effects. In addition, there are many barriers such as the GI and BB that need to be overcome to deliver the targeted therapeutics. Targeted delivery of nanoparticle based pharmaceutical delivery systems has the potential to deliver a significant therapeutic benefit to the patient. Targeting is an attractive option because it reduces the amount of pharmaceuticals used and also reduces the negative side effects. Targeting is possible because cells, tissues and organs all exhibit distinctive markers that can greatly assist in the targeting of specific therapeutics. In particular, the presence of target orientated ligands can be placed on the delivery system which permits the delivery system to target specific cells and tumours. The ligands have the potential to greatly enhance cellular uptake and retention of the pharmaceuticals, which leads to increased concentration levels and in turn improves the therapeutic efficiency [168-170]. In vivo mice studies have shown that drugs delivered in nanoparticle formulation are found in higher concentrations in the mononuclear phagocytic system (MPS), liver and spleen than the free drug agent that is normally injected into the patient. Factors that can contribute to the drug uptake include: ligand biocompatibility; ligand surface density and arrangement; cell type; polymer matrix hydrophobicity, composition, pharmaceutical loading and biodegradability profile; and period of time the delivery platform is actively in the circulation system [171-173]. For example, bio-distribution studies of nanoparticle delivery platforms have shown that with the passage of time pharmaceutical concentrations in the heart, lungs, kidneys and blood decrease, while concentrations in the liver, spleen, targeted cells such as tumours steadily increase. After the initial injection, approximately 56 % of the pharmaceutical ends up in the liver and as little as 1.6 % collects in the tumour after 48 hours [174, 175]. The percentage accumulation of pharmaceuticals in the liver and the tumour indicate that target orientated delivery is important. On one hand an efficient targeted delivery system can reduced the level of pharmaceuticals needed and the subsequent decrease in the side effects associated with the treatment. On the other hand, the delivery system must carry sufficient pharmaceuticals to overcome the filtering and accumulation effects of the MPS, in particular the liver, during the nanoparticles travel through circulatory system as graphically presented in Figure 3. The larger accumulation of pharmaceuticals in the MPS makes it possible treat tumours in the organs and tissues of the MPS more efficiently. A mice study using a nanoparticle platform delivery system loaded with doxorubicin was found to be more effective in reducing the hepatic metastasis than a conventional free drug injection treatment [176, 177].

4.2. Long Circulating Drug Delivery Platforms

Polymeric pharmaceutical delivery platforms have been extensively investigated for use as intravascular delivery devices. Unfortunately the delivery devices are rapidly cleared from the circulation system by the bodies MPS, which prevents them from providing and maintaining a sustained drug concentration in the blood for long period of time. There have been attempts to improve vascular circulation by coating the delivery devices with hydrophilic polymer brushes such as polyethylene glycol, polyoxamines, polyoamers and polysaccharides which are not detected by either macrophages or phagocytes and avoid opsononization [178, 179]. The cloud of hydrophilic brushes repel proteins in the blood, which results in the MPS not being able to detect the presence of the surface treated delivery platform and in turn promotes longer circulation times [180-182]. The hydrophilic

polymer brushes can be set up on the surface of the delivery carrier using two methods; the first is by adsorption of surfactants and second is the use of block or branched copolymers [183, 184]. In addition to their surface characteristics, the size of pharmaceutical delivery platforms is also an important factor in determining their effectiveness and ultimate fate. The delivery platform should be large enough to prevent its outflow into capillaries and small enough to avoid detection and capture by macrophages and phagocytes. Furthermore, the size of cellular structures such as the sinusoid in the spleen and fenestra of the Kuffer cells in the liver ranges from 150 to 200 nm [185]; while the size in the gap junction between endothelial cells of the leaky tumor vasculature range from 100 to 600 nm [186]. Because of these cellular structures the size of the delivery platform, including hydrophilic surface treatment should be less than 100 nm to reduce the effects of opsonisation and subsequent clearance by macrophages [187].

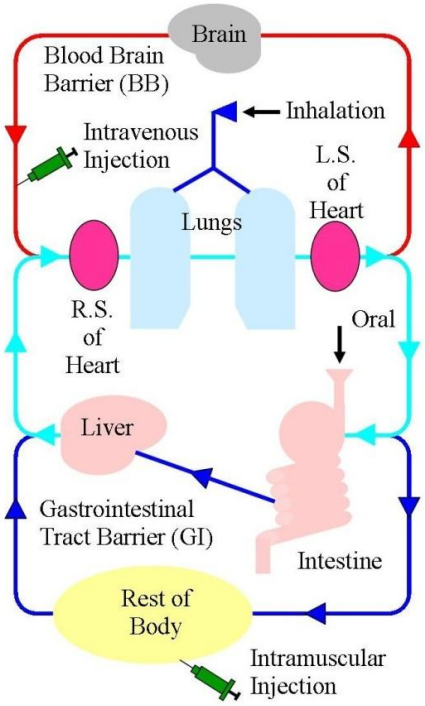

Fig. 3. Schematic of the circulatory system that a NP based deliverysystem must travel once introduced into the body

4.3. Potential Application of Nanoparticles to Overcome Drug Resistance in Cancerous Cells

A chemotherapeutic agent can be delivered into the interstitium of a tumour, but the therapeutic benefits may be severely limited due to the drug resistance mechanisms developed by the cancer cells [188]. Drug resistance has turned out to be the most important factor in preventing an effective therapeutic treatment and render a once successful medication ineffectual against the tumour. The resistance mechanisms developed by the cancer cells is called multi-drug resistance (MDR) and it permits tumours to avoid chemotherapeutic agents. The best known and most studied drug resistance mechanism is P-glycoprotein (Pgp), which is the result of an over expression of the plasma membrane with positively charged xenobiotics [189]. Nanoparticle based drug delivery platforms have the potential to avoid recognition and enter the targeted cell. The delivery platform can be encased in an endosome, which is not recognised by the Pgp efflux pump and permits the entrance of the delivery platform into the targeted cell [190-192]. Another potential technique to overcome drug resistance is use receptor-targeting ligands which are normally internalised via receptor-mediated endocytosis. Recent studies have shown that a folate receptor-targeted polymeric micelle containing doxorubicin was able to inhibit drug resistant MCF-7 cells and compared to a conventional non targeted free drug delivery [193].

4.4. Nanoparticle Systems for Oral Delivery

There have been significant advances in developing pharmaceutical and chemotherapeutic medications with excellent properties and therapeutic effects, however, a major problem still remains, that of being able to deliver these agents and improve their bioavailability. Oral treatment has the potential to deliver and maintain an appropriate concentration of the agents in the circulation to attain a prolonged exposure period. This form of treatment will improve the efficiency of the treatment and will reduce the negative side effects. It also has the advantage of providing the opportunity for the patient to carry out the treatment away from the hospital environment and maintain their quality of life. For this to take place their needs to be an effective delivery platform that can make the pharmaceutical agents reach their desired targets. Unfortunately, the oral route means the pharmaceutical agents must be able to cross the epithelial barriers of the gastrointestinal tract and also be able to resist degradation by the digestive enzymes. Encapsulation of pharmaceutical agents with a polymeric nanoparticle based drug delivery platform has the potential to provide the protection needed to prevent both enzymatic and hydrolytic degradation.

The surface area of human mucosa is 200 times greater than that of the skin and is composed of a number of physiological and morphological barriers designed to prevent the passage of particular matter across the gastrointestinal tract (GI) [194]. Therefore, to make an effective oral nanoparticle based drug delivery platform it is necessary encourage the interaction between the epithelia cells lining the gastrointestinal system and the delivery platform [195]. This interaction can be enhanced by using: smaller nanoparticle sizes; biodegradable and bio-adhesive materials; and coating the nanoparticles with bio-adhesive materials that are more biocompatible [196]. Targeting becomes an important factor to be considered, since the interaction of the drug delivery platform can be engineered with binding ligands or receptors to interact with specific cell types in the gastrointestinal system.

4.5. Nanoparticles Systems for Drug Delivery into the Brain

The blood brain (BB) barrier exists between the blood and the central nervous system, which is formed of the brain and the spinal cord. The function of the BB barrier is to: 1) to deliver nutritional requirements to the neurons; 2) permit the transfer of ions and chemicals needed to maintain the central nervous system; and 3) isolate the central nervous system from toxic materials in the blood. It is in the role of isolating the central nervous system from the blood that the BB barrier becomes an important factor in limiting the development of new pharmaceuticals for the delivery of therapeutic drugs for the treatment of diseases and cancers in central nervous system. The BB barrier is a semi-impervious layer composed of endothelial cells with tight junctions, which restricts the passage of water-soluble molecules contained in the blood circulation system from entering the central nervous system. The barrier is also involved in reducing the concentration level of lipid-soluble molecules by via enzymatic activity and active efflux transport systems [197]. Because of the BB barriers functionality, only a very selective range of molecules can pass across the barrier into the central nervous system. Therefore, any potential pharmaceutical delivery system designed for the central nervous system it must first be able to cross the BB barrier. The advantage of using nanoparticles is their extremely small size which would allow them to cross the endothelial lining. In addition, nanoparticle polymer matrix, type of surfactant coating, attached ligands and specific receptor-mediation can all potentially assist in drug delivery across the BB barrier [198-201].

5. Health and Safety Assessment of NP for Potential Use in Medicine.

The development of novel pharmaceutical agents using nanotechnology-based techniques has the potential to produce delivery platforms with the ability to transport smart drugs effectively without the side effects normally associated with more conventional drug therapies. The extremely small size of NPs gives them their distinctive chemical and physical properties that can be significantly different from their bulk form. Materials at the bulk scale that are inert may not be so at the nanometre scale. For example, the bulk form of Au is inert, but Au NPs are far from inert and their non-inert properties have enhanced their use in medical imaging and drug delivery. These unexpected differences in material properties also raise the possibility of unwanted biological toxicological reactivity and concerns of potential hazards to humans when NP based pharmaceuticals are used in therapeutic applications [119, 202-204]. Furthermore, there have also been concerns expressed about the potential undesirable effects and risks posed by engineered NPs used in industrial products. And as a result there have been a number of studies recently carried out to investigate the environmental impact and exposure of these materials to susceptible sections of the population [205, 206]. Compared with conventional bulk materials, NPs can easily gain access, travel, accumulate or be rapidly cleared from the circulation system. For instance, inhaled NPs can pass through all respiratory or gastrointestinal tissues and end up in the blood circulatory system [207, 208]. They can even move along olfactory nerves and travel into brain tissues [209]. These advantageous properties allow NPs to be used as smart drug delivery platforms to carry pharmaceuticals to targeted body sites. However, it is also possible for NPs with undesirable properties to have the same ability to rapidly travel through the circulation system and accumulate in tissues and organs. The accumulation of undesirable NPs would then trigger immunological and inflammatory responses [210, 211]. Moreover, NPs accumulate a surface covering of proteins when they are exposed to biological fluids and it is this protein layer that is believed to influence the way cells see and react with these NPs [212].

Additionally, cell toxicity thresholds and cell response to undesirable materials varies between different cell types. It is for this reason that many studies have been carried out and many more are currently underway to evaluate cytotoxicity and estimate toxicity levels [213-218]. The general consensus of *in vitro* studies is that NP based preparations at small dosages will not induce significant cytotoxicity. There are fewer articles in the literature reporting *in vivo* and clinical toxicity studies, and as a result, this area remains an active field of study. However, as in the case of most drugs and pharmaceuticals, increasing concentrations or longer exposure times will ultimately lead to cytotoxicity [219]. Nevertheless, a balance between efficient medical protocols and potential toxicity effects needs to be clarified when developing new therapeutic treatments. For example, the use of localized hyperthermia therapy to target and destroy cancerous tissues surrounded by normal healthy tissues using magnetic NPs. During this procedure magnetic NPs are predominantly loaded into affected tissue or organs with cancerous growths,(due to their high rate of metabolism) to enhance heating relative to the surrounding tissues. Tissues are then subjected to time-varying electromagnetic fields that induce mild inductive heating. During the therapy, the temperature within the NP loaded cancerous growths will rise from 37 ℃ up to around 45 ℃. The local temperature increase triggers cell death due to the disruption of cell functions within the cancerous tissues and leaves the surrounding normal tissues unaffected [220, 221]. Another NP based procedure currently under development is the use of biologically degradable magnesium oxide NPs to improve tumor thermal conductivity prior to cryosurgery. During the procedure the excellent cooling conductivity of the NPs is used to promote rapid freezing of the tumor and induce cell apoptosis [222, 223]. Both these developing NP based procedures highlight the obstacles that need to be overcome before any new NP based therapy can undergo clinical trials. In particular, these techniques highlight the importance of targeting specific cells, tissues and organs, thus reducing the effects of systemic toxicity. The delivery of pharmaceuticals

is a very important and integral part of medicine. In the future new NP based pharmaceuticals and administrative protocols will be commercially available. In the meantime, more research is needed in this field to ensure these new products together with their respective manufacturing processes result in optimally designed NP based pharmaceuticals that are safe and serve as effective treatments.

6. Conclusion

Nanotechnology is an exciting discovery which is at the leading edge of current medical developments. A variety of nanometre scale particle delivery platforms have already been approved for the delivery of pharmaceuticals [187]. The pharmacokinetic properties of many of these delivery platforms have improved the efficiency of the pharmaceuticals by improving their bioavailability, reducing drug toxicity and altering rates and timing of delivery, which will not only serve to create more effective therapies, but may also improve patient compliance and decrease costs associated with administration. In addition, effective design of delivery platforms has the potential to breach biological barriers, target specific cell types and improve accumulation of pharmaceuticals in targeted cells. However, further studies are needed to understand the biological response of cells and tissues to new nanometre scale delivery platforms and pharmaceuticals for safe administration in the future.

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