REVIEW ON: NANOPARTICLES AS TARGETED DRUG DELIVERY IN CANCER

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ABSTRACT
Cancer treatment involving chemotherapy is typically accompanied by toxic side effects, thereby limiting the amount of the drug that can be given to a patient. Conventional chemotherapeutic agents are distributed nonspecifically in the body where they affect both cancerous and normal cells, thereby limiting the dose achievable within the tumor and also resulting in suboptimal treatment due to excessive toxicities. Molecularly targeted therapy has emerged as one approach to overcome the lack of specificity of conventional chemotherapeutic agents. However, the development of resistance in cancer cells can evade the cytotoxicity not only of conventional chemotherapeutics but also of these newer molecularly targeted therapeutics. Nanoparticles, by using both passive and active targeting strategies, can enhance the intracellular concentration of drugs in cancer cells while avoiding toxicity in normal cells. Furthermore, when nanoparticles bind to specific receptors and then enter the cell, they are usually enveloped by endosomes via receptor-mediated endocytosis, thereby bypassing the recognition of P-glycoprotein, one of the main drug resistance mechanisms.

KEYWORDS: Chemotherapy, molecularly targeted therapy, passive targeting strategy, active targeting strategy.

1. INTRODUCTION
Nanotechnology deals with the design, production and characterization on ultra small particles which is extended to broad area in pharmaceutical, medical, chemical and
engineering application due to its unique properties. The development of technology occurs at the atomic, molecular or macromolecular range of approximately 1 nm - 100 nanometers (nm) to create and use structures that have novel properties. Nanoparticles (NPs) are defined as a small object that behaves as a whole unit in terms of transport and properties. They can be classified according to the size and diameter. Fine particles have the range of 100 to 2500 nm or ultrafine particles having the size of 1 to 100 nm.\cite{1}

There is significant interest in recent years in developing nanoparticles as a drug/gene delivery system. Nanoparticles are colloidal particles that are formulated using biodegradable polymers in which a therapeutic agent can be entrapped, adsorbed, or chemically coupled.\cite{2} NPs have a very high surface area to volume ratio and it allows many functional groups to be attached to a NPs which can bind to certain tumor cells. The smaller size of the NPs facilitates them to accumulate in tumor micro environment thus facilitating newer therapeutic strategies which may replace radiation and chemotherapy.\cite{1}

Nanoparticles applied as drug delivery systems are submicronized particles (3-200 nm), devices, or systems that can be made using a variety of materials including polymers nanoparticles, micelles, or dendrimers, lipids, viruses, and even organometallic compound.\cite{3}

![Types of nanocarriers for drug delivery](image)

Fig 1: Types of nanocarriers for drug delivery A. polymeric nanoparticles  B. polymeric micelles  C. Dendrimers  D. liposomes  E. viral-based nanoparticles  F. carbon nanotubes.
In recent years, scientists and engineers have been exploring different approaches to delivering multiple therapeutic agents with a single drug nanocarrier. Such efforts have been motivated by the fact that applying multiple drugs can suppress the notorious phenomenon known as cancer chemoresistance, which is accountable for most of the failed cases in cancer therapy. It has been frequently observed that cancer cells show diminishing response over the course of a chemotreatment as they acquire defense mechanisms by overexpressing drug efflux pumps, increasing drug metabolism, enhancing self-repairing ability or expressing altered drug targets. To reduce cancer drug resistance for better therapeutic effectiveness, combination chemotherapy has long been adopted in clinics as a primary cancer treatment regimen.

Drug resistance is one of the major obstacles in successful and effective treatment of cancer. The underlying causes of drug resistance are complex and multi-factorial providing the cancer cells with many ways to survive cancer chemotherapy. In general, the mechanisms of drug resistance can be classified into non-cellular resistance and cellular resistance. Resistance can be divided into two broad categories: Cellular resistance and non-cellular resistance.

![Factors leading to multi-drug resistance](image)

Fig 2: factors leading to drug résistance in cancer
A drug carrier should have as many of the following properties as possible

(1) Good biocompatibility profile
(2) Biodegradable with non-toxic degradation products
(3) Convenient, cost-effective, and reproducible preparation
(4) Ability to efficiently load the drug at high contents
(5) Controlled and tumor specific drug release kinetics
(6) Optimum size and circulation half-life
(7) Passive and/or active tumor targeting capabilities
(8) “Upgradability”, i.e., allowing further surface modifications.

Cytotoxic drugs generally do not discriminate between cancer cells and healthy cells in the body hence ideal drug delivery system should exhibit minimal non-specific drug release while in the circulation followed by increased release rate upon accumulation in the tumor. Size, blood circulation time, and colloidal stability of the nanoparticles are all important characteristics of the drug carrier. Nanoparticles which are smaller than 10 nm are rapidly cleared by the renal route preventing adequate time for tumor accumulation, while passive targeting by the EPR effect is significantly reduced with particles larger than 300 nm. The size range of 50-200 nm has found to be optimal in promoting the passive targeting of the nano-carriers to the tumor site. However, it has to be pointed out that the range of acceptable nanoparticle sizes for optimized chemotherapy is highly material dependent and will change from polymeric to inorganic to lipid based formulations.[4,5,6]

The advantages of using nanoparticles as a drug delivery system include the following:

a) Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
b) They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
c) Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
d) Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
h) The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. \(^7\)

2. Preparation of Nanoparticles

Drug nanoparticle formulations are usually created in one of two ways. Particles may be precipitated out of solution (bottom-up), or they are milled from larger particles (top-down). In both mechanisms, the total surface area increases which increases the free energy of the particles. The system compensates for this increase in free energy by dissolving crystalline nuclei and precipitating onto other particles in a process known as Ostwald Ripening or by agglomerating smaller particles. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers.

The selection of matrix materials is dependent on many factors including:
(a) Size of nanoparticles required
(b) Inherent properties of the drug, e.g., aqueous solubility and stability
(c) Surface characteristics such as charge and permeability
(d) Degree of biodegradability, biocompatibility and toxicity
(e) Drug release profile desired
(f) Antigenicity of the final product. \(^7\)

Nanoparticles have been prepared most frequency by three methods:
(1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or coacervation of hydrophilic polymers

2.1 Dispersion of preformed polymers

Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D,L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA). This technique can be used in various ways as described below.
2.1.1 Solvent evaporation method

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

2.1.2 Spontaneous emulsification or solvent diffusion method

This is a modified version of solvent evaporation method. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved.

Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

2.2 Polymerization method

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium.

2.3 Coacervation or ionic gelation method

The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in
the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel.

2.4 Production of nanoparticles using supercritical fluid technology

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO2 (SC CO2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1 °C, Pc = 73.8 bars), nontoxicity, non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles.[7]

2.5 Spray Drying to Produce Pure Drug Nanoparticles

Spray drying is a process that uses jets of dissolved or suspended drug in an aqueous or other fluid phase that is forced through high pressure nozzles to produce a fine mist. Often, a bulking agent such as lactose will be added to the fluid as well. The aqueous or other liquid contents of the mist evaporate, leaving behind a fine powder. A new modification of spray drying, called air nebulization spray drying, uses two wedge-shaped nozzles through which compressed air passes and liquid solutions pass at high velocity. The wedge-shaped nozzle acts as a fluid acceleration zone where the four streams collide at high velocity, producing a shock wave that generates fine droplets. The droplets then descend into a column while being dried into a solid powder by heated air before being collected.[8]

3. Drug loading methods: Drug incorporation into polymeric nanoparticles can be categorized into entrapment during nanoparticle formulation, drug conjugation to polymer strands before or after nanoparticle formulation, and drug absorption in preformed
nanoparticles. Drug entrapment is by far the most commonly used strategy. Common examples of this involve water-in-oil-in-water (W/O/W) and oil-in-water (O/W) emulsion nanoparticles. With this traditional strategy, drug molecules are either dissolved or suspended in the inner aqueous or oil phase of emulsion-based nanoparticles; as nanoparticles form, drugs are entrapped in the polymer matrix or may even partition into the polymer matrix in the case of hydrophobic drugs to promote drug loading. Conjugation drug loading is less common. This involves either chemical conjugation of drug and polymer and then use of these conjugates to form nanoparticles or chemical conjugation of drugs to preformulated blank nanoparticles. This method has not been widely adopted mainly because of the small number of reactive sites available on nanoparticles for drug loading as well as finding suitable conjugation reactions that will not degrade any components. Absorption loading, or in certain cases, adsorption, is also less commonly used. Here, preformulated nanoparticles are suspended in an aqueous drug solution in rare cases, certain organic solvents may also be used for drug dissolution as long as nanoparticle integrity is not compromised, and incubation conditions may be tailored/altered to produce desirable results.\textsuperscript{[9]}

4. Release mechanisms/stages: A very important consideration for polymeric nanoparticles is drug release rate. In a perfect world, drug release from polymeric nanoparticles, or any nanoparticle formulation for that matter, would be zero-order with continuous drug release over days, weeks, or months depending on the drug and indication of interest. Unfortunately, due to the small diameter of nanoparticles, surface area-to-volume ratio is very large and can lead to burst release of the majority of encapsulated drug over the first few hours and little release afterwards. Drugs in the surface layer of nanoparticles may be released from pores near the surface (hydrophilic drugs) or partition through the polymer phase to the bulk aqueous phase (hydrophobic drugs). Next, drugs in the core of nanoparticle matrix diffuse out, with hydrophilic drugs released through aqueous channels while hydrophobic drugs diffuse out through the hydrophobic polymeric matrix. Also during this stage, the polymer may become increasingly swollen due to water absorption, further facilitating drug diffusion through the polymer matrix.\textsuperscript{[9]}

5. Drug Accumulation at Target Sites\textsuperscript{[10,11]}

Drug delivery carriers remain in the circulation via the reticuloendothelial system (RES), e.g., liver and spleen, depending on their size and surface characteristics. To overcome their drawbacks, various parameters, including size and surface modification, have been studied. Among surface modification strategies, PEG as hydrophilic polymers can increase the half-life due to their high water solubility by hydrogen bonding between their oxygen atoms and water, as well as they can reduce the uptake by the RES through protection against degrading enzymes.

5.1 Passive Targeting by EPR Effects
Tumors can present an increased production of several mediators and enzymes, which altogether enhance the permeability of tumor vessels with respect to those of normal tissues, because of the rapid vascularization. In addition, little or no lymphatic drainage in tumors leads to passive accumulation and retention of nanoparticles with prolonged circulation times in the tumor resulting in an enhanced permeation and retentions (EPR) effect. In order to reach the target solid tumor site, the circulation time of drug carriers in the blood should be increased, which can be achieved by incorporating well-characterized macromolecules, e.g., PEG, polyacrylic acid, polyvinyl alcohol, dextran, chitosan and polyethyleneimine.

![Enhanced Permeability & Retention Effect (EPR)](image)

**Figure 3:** Passive targeting of the tumor by EPR. The EPR effect is the balance of enhanced tumor permeability with poor tumor interstitial fluid drainage, resulting in the selective uptake and retention of nanoparticles in the tumor tissue.
Figure 4: Drug loaded nanoparticles can overcome MDR cancer cells. Endocytosis of the drug loaded nanoparticles in membrane bound vesicles protects the drug from the action of the membrane efflux pumps. The nanoparticles release the drug deep inside the cell and the drug can gain access to its cellular target site (e.g. DNA).

5.2 Active Targeting by Molecular Binding Receptor
Selectively delivering drugs to target tumors can serve to improve the therapeutic efficiency in cancer treatment, while reducing side effects in normal tissues. Drug delivery carriers that are modified by specific surface markers (targeting moieties) enable their specific recognition by target cells, which facilitates effective delivery to target tumor tissues. As targeting moieties, antibodies, peptides (arginine-glycine-aspartate), nucleic acids, polysaccharides (hyaluronic acid [HA]), glycoproteins (transferrin) and small molecules (folate) are extensively employed.

5.3 Active Targeting by Magnetic Guidance
In this case, drug delivery carriers containing magnetic nanoparticles have been utilized, in which magnetic particles were guided along an externally placed magnet, and thus, drug in company of magnetic nanoparticles could be delivered to the desired site. Therefore, this approach achieved significantly high localization and retention in the target region with low unwanted effects of chemotherapy.
6. Ongoing Advances in Drug Delivery Systems

Stimuli responsive drug delivery systems are investigated for remotely controlled drug release by specific external or internal stimuli, including light, magnetic field, ultrasound, pH and specific enzymes’ activity. These systems allow the drug concentration to be maintained within its therapeutic window to target sites and to release the drug by changing the structures of their components.

7. Surface properties of Nanoparticles\(^7\)

When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the \textit{in vivo} fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) such as liver, spleen, lungs and bone marrow. Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the macrophages of MPS rich organs. Generally, it is IgG, compliment C3 components that are used for recognition of foreign substances, especially foreign macromolecules. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles \textit{in vivo}.

This can be achieved by (a) surface coating of nanoparticles with hydrophilic polymers/surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80). Studies show that PEG conformation at the nanoparticle surface is of utmost importance for the opsonin repelling function of the PEG layer. PEG surfaces in brush-like and intermediate configurations reduced phagocytosis. The zeta potential of a nanoparticle is commonly used to characterise the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above \((+/−) 30\) mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine
whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface.

8. Characterization of Nanoparticles

1. Physical characterization
   Particle size and size distribution
   Particle morphology
   Zeta potential

2. Drug loading, release, and stability:
   Encapsulation efficiency
   In vitro drug loading and release
   Time-, temperature-, pH-dependent formulation stability
   Fusion and contents leakage
   Custom 2D-LC-MS/MS assay development and validation

3. In vitro bioactivity of nanoparticles
   Cell binding and uptake
   Optimization of targeting ligands
   Hemolysis and complement activation
   Plasma stability and protein binding
   Cytotoxicity (necrosis and apoptosis)
   Custom cell-based potency assays

Various methods which can be used to study the in vitro release of the drug are:

a. Side-by-side diffusion cells with artificial or biological membranes;
b. Dialysis bag diffusion technique;
c. Reverse dialysis bag technique;
d. Agitation followed by ultracentrifugation/centrifugation;
e. Ultra-filtration or centrifugal ultra-filtration techniques.

Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.
REFERENCES