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Review Article

FORMULATION AND CHARACTERIZATION OF POLYMERIC NANOPARTICLES FOR EFFECTIVE TUMOR TARGETING STRATEGIES

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ABSTRACT

Most popular cancer targeted drug delivery system is polymeric nanoparticles, and it is used as a drug carrier which represents a marvellous path for cancer therapy. Polymeric nanoparticles have following important characteristics that are biodegradability, biocompatibility, nontoxicity, prolonged circulation and a wide payload spectrum of a therapeutic agent. Other outstanding characteristics is unique size and their shape properties for tissue penetration via an active and passive targeting, specific cellular/subcellular trafficking pathways and facile control of cargo release by sophisticated material engineering. The present review covered the preparation method, physico-chemical properties of polymeric nanoparticles, characterization, routes of administration and potential therapeutic applications.

Keywords: Drug delivery, Nanoparticles, physic-chemical characteristics, Targeting.

1. INTRODUCTION

Nanotechnology is creating a connection between biological and physical sciences by applying nano-structures and nanophases at various fields of science [1-3]. Nanomaterials can be well-defined as a material with sizes ranged between 1 and 100 nm that are using in biosensors, microfluidics, drug delivery, and microarray tests to tissue engineering [4-6]. The field of biomedicine together with nanobiotechnology, drug delivery, biosensors, and tissue engineering has been powered by nanoparticles [7]. As nanoparticles are planned at the atomic or molecular level, they are normally small sized nanospheres [8]. Therefore, they can move more freely in the human body as compared to bigger materials. Nanoscale sized particles show unique structural, chemical, mechanical, magnetic, electrical, and biological properties. Nanomedicines have become well acceptable in recent times due to the fact that nanostructures could be utilized as drug delivery agents via encapsulating drugs or attaching therapeutic drugs and deliver them to target tissues precisely with a controlled release [9]. It is a promising field implementing the use of knowledge and techniques of nanoscience in medical biology and disease prevention and remediation. A nanotechnique implicates the utilization of nanodimensional materials with nano-robots, nanosensors for diagnosis, delivery, and sensory purposes, and actuates materials in live cells (Fig. 1). For the example, a nanoparticle-based method has been developed which combined both the treatment and imaging modalities of cancer diagnosis [10]. The first generation formulation of nanoparticle-based therapy included lipid systems like liposomes and micelles, which are now FDA-approved [11]. These liposomes and micelles are having inorganic nanoparticles such as gold or magnetic nanoparticles [12]. These properties let to increase in the use of inorganic nanoparticles with an emphasis on drug delivery, imaging and therapeutics functions. Nanoparticles of drugs confirm higher oral bioavailability because they show typical uptake mechanisms of absorptive endocytosis.

Nanoparticles of drugs stay in the blood circulatory system for a long-lasting period and enable the release of amalgamated drugs as per the specific dose. Thus, they cause fewer plasma fluctuations [13]. Nanostructures drug easily enter in the tissue system, facilitate easy uptake of the drug by cells, allow an efficient drug delivery, and make sure action at the targeted location. The uptake of nanostructures by cells is much higher than that of large particles with size ranging between 1 and 10 μm [14]. Hence, they directly interact to treat the diseased cells with enhanced efficiency and reduced or negligible side effects.

Therefore, nanotechnology offers multiple advantages in treating chronic human diseases by site-specific and target

oriented delivery of medicines. However, insufficient knowledge about nanostructures toxicity is a major worry and undoubtedly warrants further research to improve the efficacy with higher safety to enable safer practical implementation of these medicines. Considering the above details, this review aims to information diverse nano based drug delivery systems, significant applications of natural compound-based nanomedicines, and bioavailability, targeting sites, and controlled release of nano-drugs, as well as other challenges related with nanomaterials in medicines.

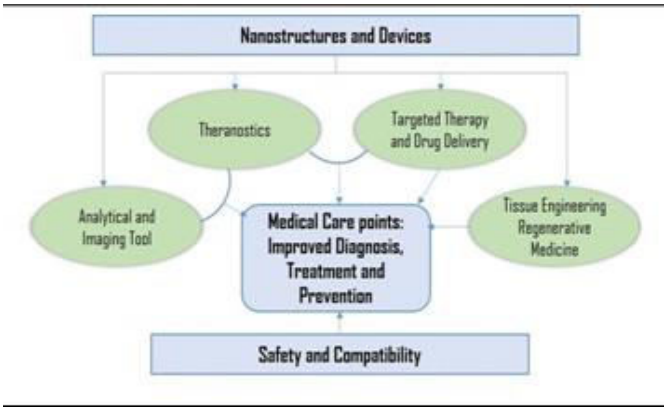

Fig. 1: Nano structures and devices

1.1. Important concepts in nanoparticle drug delivery for cancer

There are numerous common concepts that are important in nanoparticle drug delivery. These include the enhanced permeability and retention (EPR) effect, nanoparticle clearance by the mononuclear phagocyte system (MPS) and desirable nanoparticle characteristics for cancer applications.

1.2. Enhanced permeability and retention effect

Tumour vasculatures are usually abnormal, with aberrant branching and leaky walls [15]. This leakiness is due to the reduced number of pericytes and quick proliferation of endothelial cells. These characteristics result in big pores in the tumour vasculatures, ranging from 100 nm to numerous hundred nanometers in diameter, as compared to normal vessel junctions of 5-10 nm [16]. These large pores permit higher vascular permeability and hydraulic conductivity in tumours, enabling macromolecules like nanoparticles to pass into tumours [17]. In normal tissue, lymphatic system clears the macromolecules. However, solid tumours are commonly characterized by impaired lymphatics. Proliferating tumour cells reduce lymphatic vessels and collapse most of the vessels, particularly at the centre of tumours. The impaired lymphatic system coupled with enhances permeability of tumour vasculature results in the EPR effect. Nanoparticles, and macromolecules, have good extended retention times in tumour, which results in higher concentrations than in plasma or in other tissues. Thus, nanoparticles can get passive targeting to tumours through the EPR effect.

1.3. Nanoparticle clearance by the mono-nuclear phagocyte system

To fully take benefit of the enhanced permeability and retention (EPR) effect, nanoparticles should remain in circulation long enough for tumour accumulation. However, nanoparticles are horizontal to clearance by the mononuclear phagocyte system, previously called the reticuloendothelial system. The myeloproliferative neoplasms (MPS) is part of the immune system that is chiefly responsible for clearing macromolecules from circulation, and immunotoxin therapy of cancer [18]. The myeloproliferative neoplasm (MPS) comprises bone marrow progenitors, blood monocytes and tissue macrophages, and it also contains the Kupffer cells of the liver and macrophages of the spleen, which are account-table for clearance of macromolecules from circulation. Nanoparticles of drug interact with myeloproliferative neoplasm (MPS) cells and lead to their opsonization. Since premature elimination from circulation will stop nanoparticles from accumulating in tumours, much effort has been devoted to creating "stealth" nanoparticles.

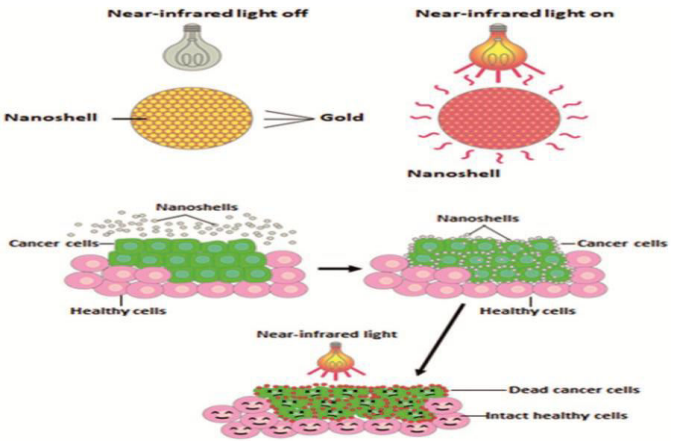

Fig. 2: Light-triggered drug delivery systems

The most common strategy has been grafting polye-thylene glycol (PEG) or other macromolecules such as polysaccharides onto the nanoparticle surface [19]. The presence of PEG or other molecules enables steric

stabilization, preventing protein adsorption, interactions among particles and interactions with immune cells.

1.4. Limitations

The nanoparticles of drug have several limitations that are due to their smaller size and large surface area. Due to particle-particle aggregation makes this physical handling of nanoparticles is difficult in liquid and dry form.

2. NANO BASED DRUG DELIVERY SYSTEMS

At present, there has been an enormous development in this field. By using nano based drug delivery systems is the drug is delivered in targeted site. This technology is suitable for all therapeutic agents or natural based bio-active compounds, and it is perfect for treatment of various ailments [20, 21]. There are a number of drug delivery systems effectively in use in the recent times, however there are still certain challenges that need to be addresses and an advanced technology need to be developed for successful release of drugs to its target sites. Consequently the nano based drug delivery systems that are presently been studied that will make more therapeutic effective, and increase patient efficacy.

2.1. Types of nanoparticles

There are two types of nanoparticles depending on the preparation processes:

- Nanospheres
- Nanocapsules

The term nanoparticles are a combined name of nano-spheres and nanocapsules. Nanospheres have a monolithic type structure (matrix) in which drugs are distributed or adsorbed onto their surfaces or encapsulated within the particles.

Nanocarriers are the vesicular system in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane. In this case the active substance is usually dissolved in the inner core, but may also be adsorbed to the capsule surface [Figure 1] [22].

2.2. Fundamentals of nanotechnology based techniques in designing of drug

Nano-drugs is the branch of drug formulation that utilizes the science for the treatment various diseases, and using there nanoscale materials, such as bio-compatible nanoparticles [23] and nanorobots [24], for various applications including, diagnosis [25], delivery [26], sensory [27], or actuation purposes in a living organism [28]. Drugs having very low solubility property have different biopharmaceutical properties. These type of drugs can be formulated with limited bio accessibility after intake through mouth, less diffusion capability into the outer membrane, need to more quantity for intravenous intake and unwanted after-side effects preceding traditional formulated vaccination process.

All these limitations could be defeated by the application of nanotechnology approaches in the drug delivery mechanism. Drug designing at the nano-scale has been studied by various researchers. It is the most advanced technology in the area of nano-particle applications because of its possible advantages such as the possibility to change properties like solubility, drug release profiles, diffusivity, bioavailability and immunogenicity. This can as a result lead to the improvement and development of convenient administration routes, lesser toxicity, less side effects, enhanced bio-distribution and extended drug life cycle [29]. Nanotechnology drug delivery systems are either targeted to a particular location or are intended for the controlled release of therapeutic agents at a particular site. Nano-drug dosage formulation involves self-assembly where in well-defined structures or patterns are spontaneously formed from building blocks [30]. In-addition they need to overcome barriers like opsonization/sequestration by the mononuclear phagocyte system [31].

Nano-drug dosage formulation, deliver the drug through two ways such as passive and self-delivery. In the former, drugs are incorporated in the inner cavity of the structure of polymers mainly. When the nanostructure materials are need to targeted at particular sites, the planned quantity of the drug is released in low dose of the drugs which is encapsulated in a hydrophobic environment of polymer or drug carrier [32]. Conversely, in the latter, the drugs intended for release are directly conjugated to the carrier nanostructure polymer material for easy delivery. In this technique, the timing of release is vital as the drug will not reach the target site and it dissociates from the carrier very quickly, and conversely, its bioactivity and efficacy will be reduced if it is released from its nano-carrier system at the correct time [33].

Drug targeting approach is another significant aspect that is classified into active and passive form. In active targeting, moieties, such as antibodies and peptides are attached with drug delivery system to anchor of polymer them to the receptor structures expressed at the target site. In passive targeting, the prepared drug carrier complex circulates through the bloodstream, and it is driven to the target site by affinity or binding influenced

by using following properties of drug like pH, temperature, molecular site and shape. The chief targets in the body receptors are cell membranes, lipid components of the cell membrane and antigens or proteins on the cell surfaces [34]. Currently, most nanotechnology-mediated drug delivery system is targeted towards the cancer disease and its cure.

2.3. Targeted delivery of nanoparticles

Ideally, for the effectiveness of anticancer drugs in cancer treatment, they should have following properties like first, after administration, be able to penetrate through the barriers in the body and reach the wanted tumour tissues with minimum loss of their volume or activity in the blood circulation of body. Second, after reaching the desired site, drugs should have the ability to selectively kill tumour cells without affecting normal cells. These are the two basic approaches that associated with improvements in patient survival and quality of life by raising the intracellular concentration of drugs and reducing dose-limiting toxicities simultaneously. Increasingly, nanoparticles seem to have the possible to satisfy both of these desires for effective drug carrier systems.

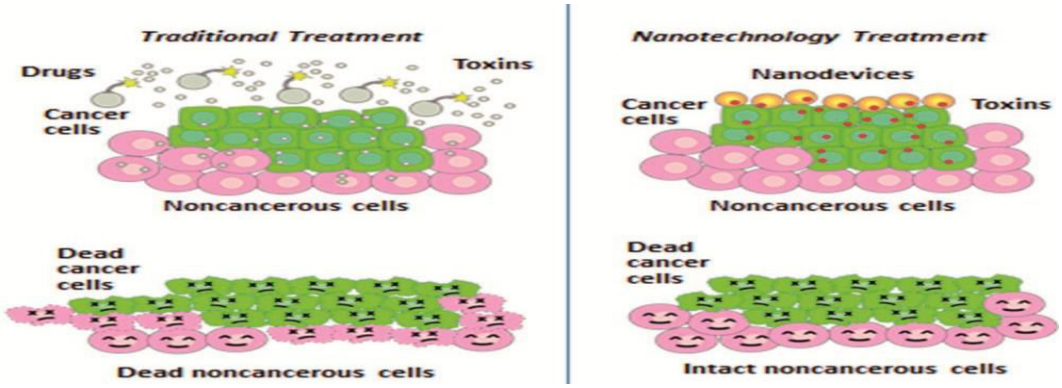

Fig.1: Improving cancer treatment.

2.4. Criteria for ideal polymeric carrier for nanoparticles

The ideal criteria for polymeric cancer drug carrier of nanoparticles are [35] as follows:

- Easy to synthesise and characterize
- Inexpensive
- Biocompatible
- Biodegradable
- Non-immunogenic
- Non-toxic
- Water-soluble

2.5. Carriers used in the preparation of nanopaticles

The polymers are used for preparation of nanoparticles are as follows:

- Natural hydrophilic polymer
- Synthetic hydrophobic polymer [36].

2.5.1. Natural hydrophilic polymer

Natural hydrophilic polymers that are proteins (gelatine,albumin, lecithin, legumin and vicillin) and polysaccharides (alginate, dextran, chitosan, agarose and pullulan) are commonly used. But these polymers have certain disadvantages such as poor batch-to-batch reproductivity, the definite conditions for their degradation and potential antigenicity.

2.5.2. Synthetic hydrophobic polymer

These polymers are separated into two groups: The first group have polyesters (poly (Є-caprolactone), poly (lactic acid), poly (lactide-co-glycolide), polystyrene) and the second group includes poly (alkyl cyanoacrylates) (poly (isobutyl cyanoacrylates), poly (butylcyanoacrylates), poly methyl (methcyanoa-crylates)) [37-39].

3. PREPARATION OF NANOPARTICLES

3.1. Ionic gelation

In this method, chitosan is firstly dissolved in an aqueous acidic solution to obtain the cation of chitosan. Then after it is then added dropwise under constant stirring to a poly anionic tripolyphosphate (TPP) solution. Due to complexation between of oppositely charged species, chitosan change in ionic gelation and precipitates to

form spherical particles. Three kinds of phenomena were seen such as solution, aggregation and opalescent suspension. The resulting chitosan particle suspension was later centrifuged and dried.

3.2. Nanoprecipitation

Nanoparticle formation is immediate and the whole procedure is carried out in only one step. Briefly, it needs two solvents that are miscible. Ideally, both the polymer and the drug must dissolve in the first one (the solvent) but not in the second system (the non-solvent). Nanoprecipitation occurs by rapid desolvation of the polymer when the polymer solution is added to the non-solvent [41-42].

3.3. Emulsion cross-linking method

In this method, water-in-oil (w/o) emulsion is prepared by using emulsifying the chitosan solution in the oil phase. Aqueous droplets are stabilised by using an appropriate surfactant; the stable emulsion. It is cross-linked by a suitable cross-linking agent such as glutaraldehyde to harden the droplets, and the particles are filtered and washed repeatedly. By using this technique particle size can be controlled by controlling the size of the aqueous droplets. Still, the particle size of the final product is depends upon the extent of the cross-linking agent. It is used while hardening in addition to the speed of stirring during the formation of emulsion. The disadvantage of this technique is tedious procedure as well as use of harsh cross-linking agents, which might possibly induce chemical reactions with the agents; however, complete elimination of un-reacted cross-linking agent may be difficult in this process [40].

3.4. Spray-drying

In Spraying and drying method, chitosan is first dissolved in acetic acid; then a suitable cross-linking agent is added; this solution or dispersion is then atomised in a stream of hot air. Atomisation leads to the formation of small droplets, from which the solvent evaporates, leading to the formation of free-flowing powders. Particle size depends upon size of the nozzle, spray flow rate, atomisation pressure and inlet air temperature, and extent of cross-linking [41].

3.5. Salting-out method

In this method acetone is selected as the water-miscible organic solvent because of its pharmaceutical acceptance with regard to toxicity. The method contains of addition of water-soluble polyvinyl alcohol (PVA) in a highly concentrated salt solution in water (aqueous phase) to a polymer solution in acetone (organic phase). Although acetone solvent is miscible with pure water in all ratios, the high salt concentration of the aqueous phase prevents mixing of the phase. After the emulsification, addition of pure water in a sufficient quantity causes acetone to diffuse into the aqueous phase, ensuing in the formation of nanoparticles [42].

4. PHYSICOCHEMICAL CHARACTERISTICS

The physicochemical methods for characterisation of nanoparticles are tabulated [Table1].

Table 1: Physicochemical methods for characterisation of nanoparticles

Parameter	Method
Particle size	Photon-correlation spectroscopy
	Transmission electron microscopy
	Scanning electron microscopy
	Scanned probed microscope
	Fraunhofer diffraction
	Freeze-fracture electron microscopy
Molecular weight	Gel chromatography
Density	Helium compression pycnometry
Crystallinity	X-ray diffraction
	Differential scanning calorimetry
Surface charge	Zeta potential measurement
	Electrophoresis
	Laser droplet anemometry
	Amplitude-weighed phase structure
Hydrophobicity	Determination
	Hydrophobic interaction chromatography
	Contact angle measurement
Surface properties	Rose Bengal binding

Surface element analysis	Static secondary-ion mass spectroscopy X-ray photon spectroscopy Molecular magnetic resonance Fourier transform infrared spectroscopy

4.1. Drug loading

The loading of drug in the nano-particulate system is evaluated by two methods such as

- Incorporation method: Incorporating at the time of nano-particle formulation.
- Incubation method: Adsorbing the drug molecule after the formation of nano-particles by incubating the polymer carrier with the concentrated drug solution.

Both methods result in:

- A solid solution of the drug molecule in the polymers and Solid dispersion of the drug in the polymer [41-43].
- Surface adsorption of the drug.
- Chemical bonding of the drug in the polymer.

In these systems the drug is physically implanted into the matrix or adsorbed onto the surface. Various methods of loading have been developed to improve the efficiency of loading, which largely depend upon the method of preparation as well as the physiochemical properties of the drug and the polymer. Maximum loading can be achieved by incorporating the drug during the time of formation of particles, but it may get affected by process parameters such as method of preparation, presence of additives, etc.

4.2. Drug release and release kinetics

Drug releases from the surface of carrier-based particulate system, the release rate of drug depends upon following factors such as cross-linking, morphology, size, density of the particulate system and the physiochemical properties of the drug, as well as presence of adjuvant.[39, 43] In-addition, *in-vitro* drug release also depends upon pH, polarity and presence of enzymes in the dissolution medium.

The release of drug from the particulate systems which depends upon three different mechanisms

- By release from the surface of particles.
- By diffusion through the swollen rubbery matrix.
- By release due to erosion.

Mechanism of drug release through the particle system, when it comes in contact with the release medium, the drug immediately dissolves, as a result affecting its release from the surface. Drug entrapped in the surface layers of the particles, and follows this mechanism. This type of drug release leads to a burst effect.

Drug release through diffusion mechanism that involves three steps

- By penetration into the particulate system, which produce swelling of the matrix.
- By alteration of the glassy polymer into a rubbery matrix.
- By diffusion of the drug from the rubbery matrix.

Evaluation methods, by using in-vitro release of drug, are as follows:

- By side-by-side diffusion cells with an artificial or biological membrane.
- By dialysis bag method.
- By ultracentrifugation.
- By centrifugal ultra-filtration.

5. APPLICATIONS OF NANOTECHNOLOGY

The various therapeutic applications of nanoparticles are shown in Table 2.

Table 2: Therapeutic applications of nanoparticles

Application	Material
Cancer therapy	Poly (alkyl cyanoacrylate) nanoparticlesbwith anticancer agents, oligonucleotides
Intracellular-Targeting	Poly (alkyl cyanoacrylate) polyesters nanoparticles with anti-parasitic or antiviral agents
Prolonged systemic circulation	Polyesters with adsorbed poly ethylene glycols or pluronics
Vaccine adjuvant	Poly (methyl methacrylate) nanoparticles with vaccines (oral and IM immunisation)
Peroral absorption	Poly (methyl methacrylate) nanoparticles with proteins and

	therapeutic agents
Ocular delivery	Poly (alkyl cyanoacrylate) nanoparticles with steroids, anti-inflammatory agents, anti-bacterial agents for glaucoma
DNA delivery	DNA-gelatine nanoparticles, DNA-chitosan nanoparticles, PDNA-poly (D, L-lactide-co-glycolide) nanoparticles
Oligonucleotides delivery	Alginate nanoparticles, poly (D, L-lactic acid) nanoparticles Poly (alkyl cyanoacrylate) nanoparticles with peptides
Other applications	

6. CONCLUSION

In the last few decades, many researchers have studied, and found alternative drug delivery systems which can improve the efficacy of different drugs. Nanotechnology is a current novel technology, and it hopes for improvements in wide variety of uses in cancer drug delivery in pharmaceutical research. Various methods are used for cancer drug targeted delivery, most popular is polymeric nanoparticles. It offer a new avenue to achieve drug delivery and drug targeting with newly discovered disease definite site of drugs and existing poorly soluble drugs. Overcoming the obstacles in conventional drug delivery systems, polymeric nanoparticles are expected for better application and efficient drug delivery, and would finally enhance treatment and patient compliance.

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