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REVIEW ARTICLE

POLYMERIC BIODEGRADABLE NANOPARTICLE: A REVIEW

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Abstract

The nanoparticles are extensively studied due to their almost unlimited potential of providing drug targeting within areas non accessible by other means. Some of the newest findings in the domain of polymeric nanoparticles are presented, focusing on the synthesis and biomedical applications of polymer based nanoparticles. Use of polymeric biodegradable nanoparticles(NPs) for controlled drug delivery has shown significant therapeutic potential. Polymeric nanoparticles has been extensively studied as particulate carriers in the pharmaceutical and medical fields because they show promise as drug delivery system as a result of their controlled and sustained release properties, subcellular size, biocompatability with tissues & cells. Cocurrently, targeted delivery technologies are becoming increasingly important as a scientic area of investigation. They offer enhanced biocompatability, superior drug/vaccine encapsulation & convenient release profiles for a number of drugs, vaccines & biomolecules to be used in a variety of applications in the field of medicine.

Introduction

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymeric membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly(ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period of time targeting a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes (1-4).

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties (5,6).

Advantages of using nanoparticles as a drug delivery systems are:

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- 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.
- 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.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- 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 undergo clinical trials or made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, controlled release and potential applications of nanoparticles.

Polymeric biodegradable nanoparticles

Some general aspects on nanoparticles have been reviewed earlier (7,8–14). A majority of these reviews have dealt with the NPs of poly(D,L-lactide), poly(lactic acid) PLA, poly(D,L-glycolide) PLG, poly(lactide-co-glycolide), PLGA, and poly-(cyanoacrylate) PCA. The present review details the latest developments on the above mentioned polymers as well as NPs based on chitosan, gelatin, sodium alginate and other hydrophilic / biodegradable polymers. The PLA, PLG and PLGA polymers being tissue-compatible have been used earlier as CR formulations in parentral and implantation drug delivery applications (15–17).

In addition, poly(e-caprolactone), PCL, was first reported by Pitt et al. (18,19) for the CR of steroids and narcotic antagonists as well as to deliver opthalmic drugs (20), and poly(alkylcyanoacrylate), PACA, are now being developed as NPs. In addition, less frequently used polymers like poly(methylidene malonate), gelatin, chitosan and sodium alginate (24) are frequently studied polymeric materials(21-23).

Advantages of polymeric nanoparticles

- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

Polymers used in the preparation of nanoparticles

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible.

Natural polymers: The most commonly used natural polymers in preparation of polymeric nanoparticles are

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

There are many synthetic polymers like

1. Polylactides(PLA)
2. Polyglycolides(PGA)
3. Poly(lactide co-glycolides) (PLGA)
4. Polyanhydrides
5. Polyorthoesters
6. Polycyanoacrylates
7. Polycaprolactone
8. Poly glutamic acid
9. Poly malic acid
10. Poly(N-vinyl pyrrolidone)
11. Poly(methyl methacrylate)
12. Poly(vinyl alcohol)
13. Poly(acrylic acid)
14. Poly acrylamide
15. Poly(ethylene glycol) and
16. Poly(methacrylic acid)

Poly-D-L- lactide-co-glycolide (PLGA)

Poly-D-L- lactide-co-glycolide (PLGA) is one of the most successfully used biodegradable polymers. It undergoes hydrolysis in the body to produce biodegradable metabolite monomers such as lactic acid and glycolic acid. Since lactic acid and glycolic acids are normally found in the body and participate in a number of physiological and biochemical pathways, there is very minimal systemic toxicity associated with the use of PLGA for the drug delivery or biomaterial applications. PLGA NPs have been mostly prepared by the emulsification-diffusion, the solvent evaporation and the nanoprecipitation methods (kumari et al; 2010). PLGA nanoparticles have been used to develop protein and peptide based nanomedicines, nano-vaccines, and genes containing nanoparticles for in-vivodelivery systems (Carrasquillo KG et al; 2001).

Polylactic acid (PLA)

PLA is a biocompatible and biodegradable polymer which is broken down to monomeric units of lactic acid in the body. Lactic acid is a natural intermediate/by product of anaerobic respiration, which is converted into glucose by the liver during the Cori cycle. Glucose then is used as an energy source in the body. The use of PLA nanoparticles is therefore safe and devoid of any major toxicity. PLA nanoparticles have been mostly prepared by the solvent evaporation, solvent displacement, salting out and solvent diffusion methods (Choi C et al; 2006). The salting out procedure is based on the separation of a water- miscible solvent from aqueous solution by adding a salting out agent like magnesium chloride or calcium chloride. The main advantage of the salting out procedure is that it minimizes stress to protein encapsulants.

Poly-ε-caprolactone (PCL)
poly-ε-caprolactone is degraded by hydrolysis of its ester linkages under the normal physiological conditions in the human body and has minimal or no toxicity. Therefore, PCL has grabbed the attention of researchers as a candidate of choice for use in drug delivery and long-term implantable devices. PCL's slower rate of degradation compared to polylactides has made it better candidate for making long-term implantable devices. PCL nanoparticles have been prepared mostly by nanoprecipitation, solvent displacement and solvent evaporation(Kim SY et al; 2001).

Chitosan
Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of the crustacean-derived natural biopolymer chitin. There are at least four methods reported for the preparation of chitosan nanoparticles. The four methods are ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex formation (Gan Q et al; 2007).

Gelatin
Gelatin is extensively used in food and medical products and is a nontoxic alternative. Gelatin NPs are very efficient in delivery and controlled release of the drugs. They are nontoxic, biodegradable, bioactive and inexpensive. Gelatin is a poly-ampholyte consisting of both cationic and anionic groups along with a hydrophilic group. It is known that the mechanical properties such as swelling behavior and thermal properties of gelatin NPs depend significantly on the degree of cross-linking between cationic and anionic groups. These properties of gelatin can be manipulated to prepare desired type of NPs from gelatin. Gelatin nanoparticles can be prepared by the desolvation/coacervation or emulsion methods (Ofokansi K et al; 2008).

Poly-alkyl-cyano-acrylates (PAC)
The biodegradable as well as biocompatible poly-alkylcyanoacrylates are degraded by enzyme esterases found in the body. On degradation they produce some toxic products that may stimulate or damage the central nervous system. Thus this polymer is not authorized for application in humans. PAC nanoparticles are prepared mostly by emulsion polymerization, interfacial polymerization and nanoprecipitation(Kumari A et al; 2010).

Preparation of Nanoparticles
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 (25): (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 and (f) Antigenicity of the final product.

Nanoparticles have been prepared most frequently by three methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or coacervation of hydrophilic polymers. However, other methods such as supercritical fluid technology and particle replication in non-wetting templates (PRINT) are also used for production of nanoparticles.

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)(26-28).

This technique can be used in various ways as described below.

a) **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 (29). In order to produce small particle size, often a high-speed homogenization

or ultrasonication may be employed (30).

b) **Spontaneous emulsification or solvent diffusion method**:

This is a modified version of solvent evaporation method (31). 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 in the internal aqueous phase.

c) **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 ultra-centrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles (32,33). Nanocapsule formation and their particle size depends on the concentration of the surfactants and stabilizers (34).

d) **Coacervation or ionic gelation method**

Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation (35,36). 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 due to ionic interaction conditions at room temperature.

e) **Production of NPs using supercritical fluid technology**

Production of NPs with the desired physicochemical properties to facilitate the targeted drug delivery has been a topic of renewed interest in pharmaceutical industries. Conventional methods like solvent evaporation, coacervation and in situ polymerization often require the use of toxic solvents and / or surfactants. Therefore, research efforts have been directed to develop the environmentally safer en-capsulation methods to produce the drug-loaded micron and submicron size particles. If solvent impurities remain in the drug-loaded NPs, then these become toxic and may degrade the pharmaceuticals within the polymer matrix. Supercritical fluids have now became the attractive alternatives because these are environmentally friendly solvents and the method can be profitably used to process particles in high purity and without any trace amount of the organic solvent. Literature on the production of drug-loaded microparticles using supercritical fluids is enormous (37–43).

1) In the rapid expansion of supercritical solution (RESS) method the solute of interest is solubilized in a supercritical fluid and the solution is expanded through a nozzle. Thus, the solvent power of super-critical fluid dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitated solute is completely solvent free. Unfortunately, most polymers exhibit little or no solubility in supercritical fluids, thus making the technique less of practical interest. RESS was very popular in the late 80s and early 90s for particle production of bioerodible drug-loaded polymers like PLA. A uniform distribution of drug inside the polymer matrix can be achieved by this method for low molecular mass (,10 000) polymers. However, the RESS method cannot be used for high molecular mass polymers due to their limited solubility in supercritical fluids. For these reasons, much less information is found in the literature over the past 6–7 years on this technique.

2) In the supercritical anti-solvent (SAS) method, the solution is charged with the supercritical fluid in the precipitation vessel containing solute of interest in an organic solvent. At high pressures, enough anti-solvent will enter into the liquid phase so that the solvent power will be lowered and the solute precipitates. After precipitation, when the final operating pressure is reached, the anti-solvent flows through the vessel so as to strip the residual solvent. When the solvent content has been reduced to the desired level, the vessel is depressurized and the solid product is collected. In a modified version of the SAS technique, the solid of interest is first dissolved in a suitable solvent and then this solution is rapidly introduced into the supercritical fluid through a narrow nozzle. The supercritical fluid completely extracts the solvent, causing the super-critical fluid insoluble solid to precipitate as fine particles. This method, also called as gas anti-solvent (GAS) technique, has been successfully used to produce microparticles as well as NPs.

Low and high molecular weight polymeric bioderadable nanoparticles

High molecular weight biodegradable polymer (HMWBP) based nanoparticles have been used as suitable carriers for nucleic acids, proteins and drugs since last several years. Conventional HMWBP based nanoparticles, which are used for therapeutics, include especially chitosan, alginate, heparin, polyacrylate, dextran, pullulan, hyaluronic acid etc (44). But conventional HMWBP based nanoparticles (>100 kDa), which have already been used in therapeutics (via oral, nasal and pulmonary routes), stimulate effective immunologic inflammatory responses (45-47). It is well known that the HMWBP based nanoparticles degraded slowly in vivo, and there is a consequential risk of accumulation in the tissues in a long period of administration (48). To this end, these major limitations of HMWBP based nanoparticles regarding lower biodegradability and higher cytotoxicity are driving intense research towards Low Molecular Weight Biodegradable Polymer (LMWBP) based nanoparticles as potential delivery systems. LMWBP based nanoparticles are attractive future delivery systems for nucleic acids, proteins and drugs due to their smaller particle size, higher solubility, higher permeability, higher association efficiency,better biodegradability, higher release efficiency, nonhaemolytic nature and lower cytotoxicity at normal physiological condition (49). However, the short chain LMWBP based nanoparticles are easily subsequently excreted as carbon dioxide (50). Even majority of research works have shown that LMWBP based nanoparticles reveal negligible cytotoxic effect in various cell lines (51). As a result, the current trend is shifting towards the LMWBP based nanoparticles as better delivery systems for drugs, proteins and nucleic acids. Chitosan, gelatin polylatides and polyglycolides etc. comes under category of low molecular weight biodegradable nanoparticles(Ghosh et al; 2010).

Hydrophilic Polymeric Nanoparticles

Some of the most commonly used hydrophilic polymers are **chitosan, gelatin and sodium alginate**. Some methods of nanoparticle formation were described until now. Hydrophilic drug carriers with a limited capacity of loading proteins were described by Greff et al; 1994 (52).

Calvo et al; 1997, 98 (53-56) presented a method to obtain nanoparticles of chitosan by ionic gelation. This method involves two phases: one of the phases contains chitosan and a diblock copolymer and the second one a polyanion – sodium tripolyphosphate (STPP). The positively charged amino moieties of the chitosan interact with STPP ions. The average size of the nanoparticles can be varied between 200 and 1000 nm, depending on the chitosan and PEO-PPO copolymer percentage. These nanoparticles present a good capacity of bonding bovine serum albumin,(53),(54) insulin,(55) tetanus and diphtheria toxins and nucleotides (56).

Mao et al; 1999 have prepared chitosan-DNA nanoparticles by a complex coacervation technique. That can be used for oral gene delivery (57). The same coacervation technique was used to prepare gelatine -DNA nanoparticles but with a lower loading capacity for antineoplazic proteins than chitosan-DNA nanoparticles (58).

Chitosan nanoparticles were also obtained by the emulsion-coacervation technique (59). Chitosan and the bioactive drug were dissolved into water. A water in liquid paraffin emulsion was stabilized in the presence of a surfactant. A NaOH solution in liquid paraffin emulsion was added onto the first emulsion. Chitosan forms nanoparticles in contact with NaOH, by coacervation.

Biodegradable polyesters with short polylactone chains grafted on the PVA backbone were synthesized by bulk polymerization of monomers in the presence of polyol groups. By changing the composition, branched biodegradable polyesters can be obtained. These copolymers undergo self assembling processes, generating stable nanoparticles that can complex proteins (human serum albumin, cytochrome C, tetanus toxin). The advantage of

these copolymers is that no surfactants and no solvents are required (60, 61).

Applications of biodegradale polymeric nanoparticles

Corticoids release

Corticoids are anti-inflamatory drugs with high efficiency in the treatment of posterior segment eye diseases such as uveitis. It has also been proved that corticoids can improve the wound healing and they may be effective in the case of fibrosis (proliferative vitreoretinopathy and subretinal neovascularization).

Systemic administration of corticoids determines a series of side effects, topic administration being preferred. In the eventuality of topic administration, only a small amount of the drug reaches the posterior segment of the eye. Direct injections in the vitreous can increase the therapeutic efficiency but usually repeated injections are required, generating a great discomfort for the patient (62-63). Some risks are also associated with this technique, such as vitreous haemorrhage or retinal detachment. Some local toxic effects have been also observed.

Another modality to insure a therapeutic concentration of the drug is to use drug releasing implants (62). There are also disadvantages associated with this technique: a large surgical incision is required to install the implant; (64) which is very difficult to remove and it exists the possibility that the implant would migrate, endomaging the epithelium (65).

An alternative to these rather complicated methods is to use corticoids loaded nanoparticles. Some of the most promising polymers are PLGA due to their very low toxicity (66).

Gomez et al; 2001 (67) presented the synthesis of dexamethasone loaded PLGA nanoparticles. Dexamethasone is a poorly soluble crystalline corticoid generally used in the treatment of diabetic macular edema (as an implantable device).

Blood brain barrier

It is known that between the blood streamline and the central nervous system there is a barrier known as the blood-brain barrier (BBB). BBB allows only the exchange of ions in order to maintain a constant osmotic pressure and the passage of nutrients. Its role is to protect the brain and the spinal axis from any chemical or bacteriological threats. The protection offered by the BBB comes at a certain price: it is impossible to get drugs through the barrier, so the therapy for the central nervous system is very difficult. In the brain, endothelial cells are packed more tightly together, due to the existence of tight zonulae occludentes junctions between them.

The blood-brain barrier recognizes therapeutic agents as foreign particles and doesn't allow their passage. Because of the blood-brain barrier, finding a way to deliver bioactive substances to brain has become a real challenge.

One of the methods to achieve drug delivery to the central nervous system is to entrap the drugs into nanoparticles. Because of their reduced size, nanoparticles are able to pass through the vascular endothelium of BBB. There are several studies that showed good result in the treatment of brain tumors by drug loaded nanoparticles (68-71).

Vaccine and gene therapy

Another field where nanoparticles are very important is gene delivery. By encapsulating the genes into nanoparticles it is possible to protect them from degradation in the presence of certain factors (pH, bile, proteolytic enzymes). The entrapment of genes into nanoparticles has encountered some problems regarding the stability of the synthesized structures during the preparation as well as after administration. One method to ensure the stability is to bind the genes to the surface of nanoparticles or nanocapsules. The binding must be reversible in order to allow the cleavage of the complex once the target has been reached.

A method to obtain nanoparticles without using a surfactant was proposed by Castadello et al; 2006 (72). The nanoparticles have a core and a shell of PEG and positively charged groups. The PEG chains are both biocompatible and biodegradable and provide steric stability while the positively charged groups bind DNA. By using these complex structures, the risk of physical desorbtion is greatly decreased. Such a vaccine is also stable and non toxic while it is possible to administer it orally.

Gene therapy is a potential method for treating neurodegenerative diseases such as Parkinson. The controlled delivery of genes responsible for GNDF (Glial Cell Line-Derived Neurotrophic Factor) formation stops the disease evolution and maintains a constant level of dopamine despite the cells lost because of the disease. Also the delivery of genes involved in the tyrosine hydroxylase has shown good results.

Initially viral vectors were used (recombining adenoviruses or retroviruses). A more efficient method is to entrap the genes into nanoparticles. One of the preferred polymers is poly(ethyleneimine) . Polyethyleneimine (PEI) has been already used to deliver genes inside the neurons with promising results (73). It protects the DNA inside the nanoparticle while the ionic character favours the membrane penetration by binding to the negatively charged heparan sulphate, expressed on the cell surface.

Diagnostic

Sun et al; 2006 (74) presented the modality to obtain copper chlorophyl labeled nanoparticles. These nanoparticles can be directly traced in vivo by analytical electron microscopy (AEM). Nanoparticles covered with Polisorbate T-80 have been detected in the brain, what proves the existence of endocytosis and/or transcytosis at some extent. Therefore this type of nanoparticles can be used in the functional exploration of the brain.

Conclusion

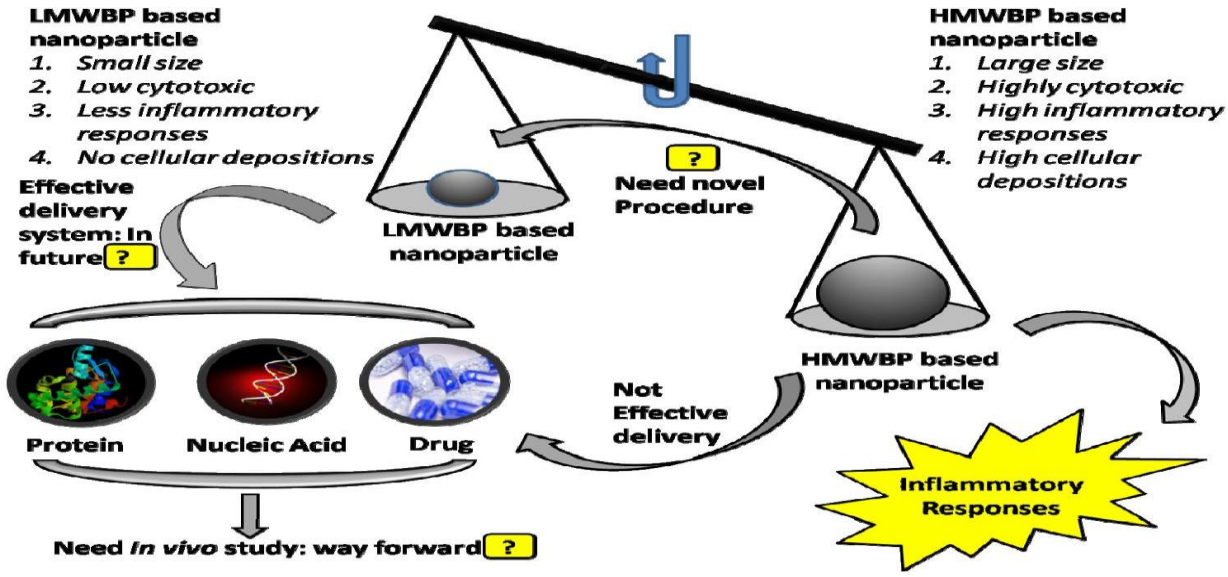

Fig. 1: Conceptualization on LMWBP based nanoparticles as efficient delivery systems

The use of biodegradable polymer especially low molecular weight biodegradable nanoparticles for the controlled release of therapeutic agents is now well established. Although currently there are only a small number of commercially available products that utilize this technology (e.g., Lupron Depot), these polymers have great utility for the controlled release of several drugs like vaccines, human growth hormone, insulin, anti-tumor agents, contraceptives and also vaccines. Long circulation of drugs in the body is the key in successful drug delivery and drug targeting to the site of action. Many polymeric NPs have been developed for this purpose. Certainly, surface modification is useful in achieving these goals. From the polymer chemistry viewpoint, it is important to synthesize newer polymers and copolymers to match the hydrophilic and hydrophobic properties of drugs.

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