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Low Molecular Weight Biodegradable Polymer Based Nanoparticles as Potential Delivery Systems for Therapeutics: The Way Forward?

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ABSTRACT

A significant effort is underway to develop low molecular weight biodegradable polymer based nanoparticles as potential alternates for conventional high molecular weight biodegradable polymer based nanoparticles as delivery systems. Among the various options, low molecular weight biodegradable polymer 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, non haemolytic nature and lower cytotoxicity at normal physiological condition. In this current scenario, recent progresses on preparation techniques of Low Molecular Weight Biodegradable Polymer based nanoparticles and their therapeutic applications are focused in this review. However, there are significant remaining issues to practical applications, such as specific interactions of these low molecular weight biodegradable polymer based nanoparticles with human organs, tissue cells, resistant microbial cells, biomolecules, and metabolic fate. Hence, further deep research for overcoming these practical barriers may be pioneered a new avenue in the near future.

Keywords: Nanoparticles, biodegradable polymer, low molecular weight, delivery system, therapeutic application.

INTRODUCTION

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. [1] 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. [2-4] 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. [5] 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 (Fig. 1) 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. [6] However, the short chain LMWBP based nanoparticles are easily subsequently excreted as carbon dioxide. [7] Even majority of research works have shown that LMWBP based nanoparticles reveal negligible cytotoxic effect in various cell lines. [8] As a result, the current trend is shifting towards the LMWBP based nanoparticles as better delivery systems for drugs, proteins and nucleic acids. In this current scenario, we have discussed the recent progresses on preparation techniques of LMWBP based nanoparticles with their therapeutic applications as nucleic acids, proteins and drug delivery systems in this review.

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LMWBP BASED NANOPARTICLES AND THERAPEUTIC APPLICATIONS

Chitosan as LMWBP based nanoparticle

Chitosan is the most promising biodegradable polymer for LMWBP based nanoparticle preparation for therapeutic applications. According to the literature, Low molecular weight chitosan-poly-γ-glutamic acid (LMW-CPGA) nanoparticles were prepared by a simple ionic-gelation method for oral insulin delivery. The average molecular weights (M_W) of chitosan and γ-glutamic acid were used 80 kDa and 60 kDa, respectively. The diameters of the prepared nanoparticles were in range of 110-150 nm depending upon

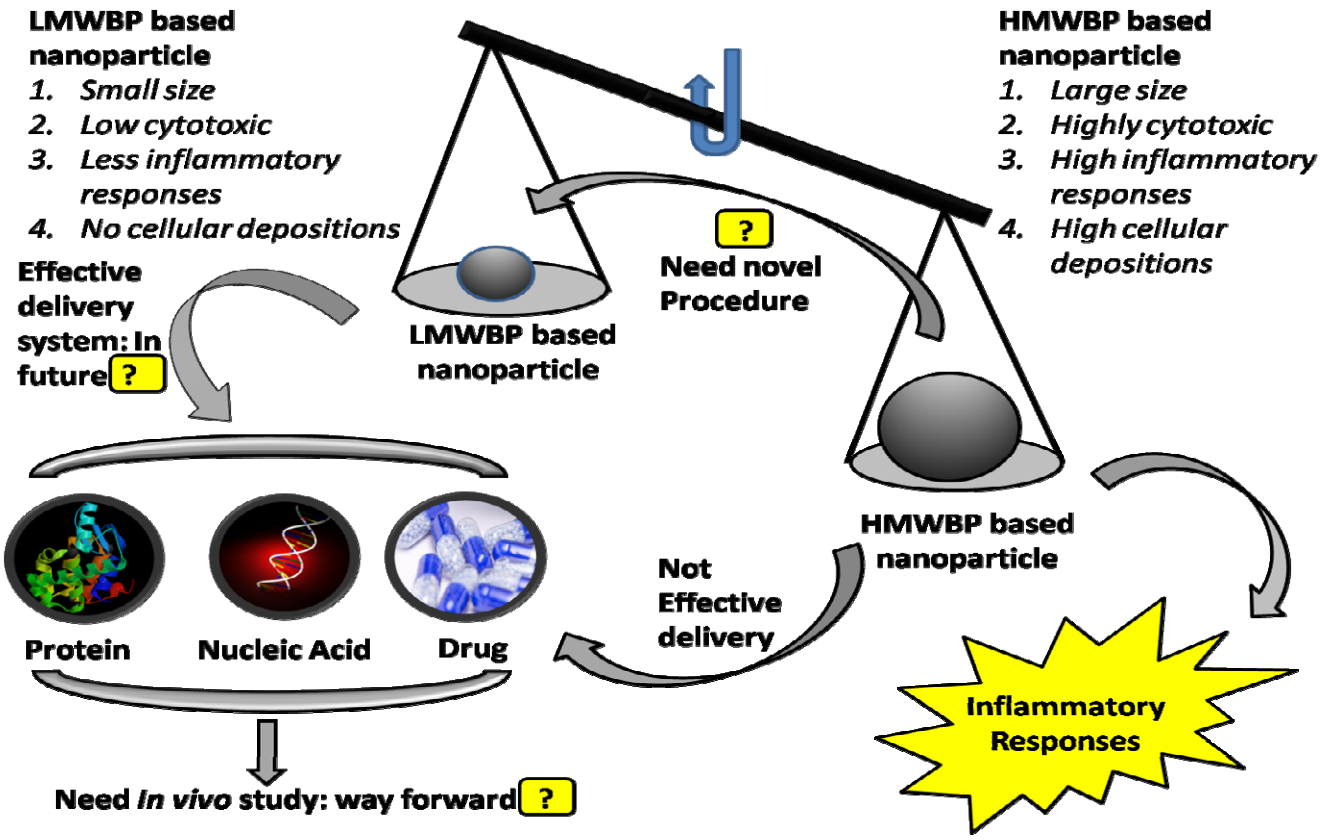

Fig. 1: Conceptualization on LMWBP based nanoparticles as efficient delivery systems

the relative concentrations of chitosan and poly (γ-glutamic acid) used. Zetapotential of Chitosan-poly (γ-glutamic acid) nanoparticles were recorded ranging from 5.1 to 36.8 nm. Finally, insulin loaded these nanoparticles were applied on rat (Caco-2 cell monolayers). *In vivo* results clearly indicated that the insulin loaded Chitosan-poly (γ-glutamic acid) nanoparticles could effectively reduce the blood glucose level in a diabetic rat model. [9]

In an another study, Low molecular weight chitosan/hyaluronic acid (LMWCHA) nanoparticles were made by using commercially available chitosan (MW of 5kDa) and hyaluronic acid (MW of 64 kDa) with a ratio of 4:1 (w/w). Average diameter and zetapotenital were obtained 146 ±1 nm with a polydispersity index of 0.073 and +32 mV, respectively. Finally, these low molecular weight chitosan/hyaluronic acid based nanoparticles were used to transfect plasmid DNA (pEGFP) with approximately 25 % higher transfection efficiency in HEK 293T cells in an optimized condition (pH 6.4-6.8, 0.25 µg plasmid/well, and 4 h incubation time). [10]

As innovative attempt, low molecular weight chitosan LMWC (MW 6 kDa) conjugated gold nanoparticles was prepared and used as a potent plasmid DNA delivery system both *in vivo* and *in vitro* in BALB/c mice. LMWC conjugated gold nanoparticles had an average particle diameter of 4.5 nm. On the other hand, atomic force microscopy (AFM) showed that LMWC conjugated gold complex have a size of approximately 250-450 nm. Immunological analyses showed that LMWC conjugated gold nanoparticles are capable of inducing the enhanced humoral (10-fold) and cellular responses (100-1000 fold) after immunization with hepatitisBs antigen specific (HBsAg) plasmid. [11]

LMWC based nanoparticle was also been used as bovine serum albumin (BSA) protein carrier. This LMWC nanoparticle (degree of deacetylation of 86.6 %), a protein carrier, was prepared by using incorporation method and incubation method with effective sonication in presence of cross linker polyanion tripolyphosphate (TPP). The zetapotential and particles size of unloaded LMWC-TPP nanoparticles were ranging +46.3 to +55.7 mV and 182.7 to 222.6 nm, respectively. In contrary, size analysis under TEM indicates that the size range of BSA loaded LMWC-TPP nanoparticle is around 250-350 nm with zetapotential of +43.2 to +54.1 mV. [12]

Potentially amphiphilically modified paclitaxel loaded LMWC nanoparticle was synthesized to develop comparatively better drug delivery system. Molecular weights of chitosan used as precursor were 5, 10, and 20 kDa. Paclitaxel unloaded- LMWC nanoparticles had particle sizes ranging from 198 to 233 nm and zetapotentials ranging from -44.1 to -50.5 mV. Whereas, paclitaxel encapsulated LMWC nanoparticles had a particle size ranges from 134 to 147 nm and zetapotentials ranging from -24.2 to -43.5 mV. In this way this paclitaxel loaded LMWC nanoparticles increased the efficiency of hydrophobic anticancer drug paclitaxel delivery by reducing the side effects of nephrotoxicity, neurotoxicity, and cardiotoxicity. [13]

Retinol-encapsulated LMWC (M_W of 18 kDa) nanoparticles were synthesized using ultrasonication to reduce the toxicity of retinol for modern therapy of dermatological treatment of wrinkled skin. Average particle size retinol- encapsulated LMWC nanoparticles were around 100.6 to 254.5 nm according to the drug contents. Zetapotentials were recorded ranging from +51.9 to + 74.84 mV. [14]

Therefore, these new approaches showed that the LMWC nanoparticles preparations are feasible and prepared LMWC nanoparticles can be applied as potent nano vehicles for delivery of nucleic acids (plasmid DNA as vaccine), proteins

(BSA) and drugs (insulin, paclitaxel, and retinol). Recently, it was proven that depolymerisation approach is another very effective approach for LMWC based nanoparticle preparation. Depolymerisations [15-19] were mainly followed by mainly chemical oxidation, acid hydrolysis, and enzymatic degradation (Table 1). This approach showed much successful amelioration in delivery of drugs, proteins and nucleic acids especially for tumour targeting.

Poly lactic acid as LMWBP based nanoparticle

Poly lactic acid is another outstanding biodegradable polymer that can be used for LMWBP nanoparticle preparation and therapeutic application. Layer-by-layer technique using a filtration approach was used to prepare low molecular weight (M_W 2000 $g.mol^{-1}$) poly-L-lactic acid (LMW-PLA) nanoparticles. Core LMW-PLA nanoparticles were prepared using either chloroform (CHL) or dichloromethane (DCM) as a solvent for PLA. Mean sizes of the nanoparticles, determined by photo correlation spectroscopy (PCS), were approximately 350 nm and 400 nm for the LMW-PLA-CHL and LMW-PLA-DCM nanoparticles, respectively. Polydispersity of the both particles remained typically below 0.1 indicating small size deviation. It had also been shown that zetapotential values of LMW-PLA-CHL and LMW-PLA-DCM nanoparticles were -53 and -30 mV, respectively at pH 7.0. Further, poly electrolytes like poly-allylamine hydrochloride, poly-sodium-4-styrenesulfonate were also used to coat the LMW-PLA-CHL and LMW-PLA-DCM nanoparticles to increase the stability of those nanoparticles. [20]

LMW-PLA nanoparticle (2000 $g.mol^{-1}$) loaded with sulbutamol sulphate (SS) and beclomethasone dipropionate (BDP) were also synthesized by a modified nanoprecipitation method for pulmonary delivery. The size distribution of SS-LMW-PLA was settled mainly in the range of 500-900 nm, while the diameters of the empty particles and the BDP-LMW-PLA particles were smaller, 300-500 nm. [21]

Recently, Estradiol loaded poly lactic acid-glycolic acid (PLGA) based low molecular weight (14.5 kDa) nanoparticles were prepared following emulsion-diffusion-evaporation method employing didodecyldimethyl ammonium bromide (DMAB) as chemical stabilizer to improve the bioavailability and estradiol release efficiency on oral administration. Particle size and zetapotential of this unloaded nanoparticle was around 90.9 nm and + 72.5 mV, respectively. Particle size and zetapotential were slightly increased to 98.3 nm and +78.9 mV after subsequent drug loading. This estradiol loaded LMW-PLGA nanoparticle mediated delivery not only reduced the cytotoxicity of tissues but also reduced the inflammation in liver, spleen, and intestinal segments (duodenum, jejunum and ileum) as compared to HMW-PLGA based delivery of estradiol. [22]

Poly-n-butyl cyanoacrylate as LMWBP based nanoparticle

Poly-n-butyl cyanoacrylic acid (PBCA) is also a potential biodegradable polymer. LMW-PBCA nanoparticles were produced by a dispersion polymerization carried out in water at a pH 3 and employing a polymeric stabilizing agent, dextran. Finally these nanoparticles were hydrolysed using esterase at pH 7 and 37^{o}C to reduce the particle size and molecular weight. Average particle size was substantially decreased ranging from 240 to 200 nm by esterase enzymatic activity. But molecular weight distribution was broadened suggesting chain scission. Little alterations in the average molecular weight of the hydrolysed polymers were obtained. This might be due to the hydrophilicity of the polymer, which increased as more side groups were hydrolyzed. [23]

In another study, dispersion polymerization process was performed to prepare LMW-PBCA nanoparticles using dextran as stabilizing agent. The drug insulin was loaded during the final stages of the particle synthesis. Insulin loaded LMW-PBCA nanoparticles had a molecular of 2.05 kDa and 254 nm particle size. Insulin unloaded nanoparticle had a molecular weight of 2 kDa and particle size of 240 nm. Still there was no effective influence of this dispersion method for nanoparticle synthesis regarding particle size and molecular weight. To this end, esterase enzymatic treatment was also applied on insulin loaded LMW-PBCA nanoparticles to reduce the particle size. Despite the enzymatic hydrolysis and least reduction in particle size, there was no reduction in residual polymer molecular weight suggesting a progressive loss of entire chains from the active surface. [24]

Heparin, poly vinyl alcohol and gelatin as LMWBP based nanoparticles

Low molecular weight heparins (LMWHs) are negatively charged oligosaccharides used in the treatment of deep vein thrombosis and pulmonary embolism. Presence of carboxylic acid and sulphate groups in the glycosaminoglycan units of LMWH renders this highly negatively charge and limits the direct absorption via mucosa. To this end, polyehylenimines (PEIs) was used to enhance mucosal absorption of LMWHs by forming nano formulation LMWHs through electrostatic interactions with reduced negative surface charge. This nano formulation of PEI-LMWH was prepared by adding fixed concentration of LMWH (40$mg.ml^{-1}$) to aqueous solutions of varying concentration of PEIs. Experimental results showed that nanoparticle of 25 kDa PEI-LMWH formulation has a particle size of approximately 5 nm with a decreased negative zetapotential of -45 from -60 mV. [25]

LMW poly-vinyl alcohol (PVA) nanoparticle was synthesized by using NaOH modified method. Newly synthesized LMW-PVA nanoparticles have shown the average size ranging from 74 to 168 nm with the polydispersity index of <0.2 and zetapotential ranging from +13 to +19 mV. These LMW-PVA nanoparticles have been applied in Calu-3 human bronchial epithelial cell line (ATCC) to determine its uptake efficacy *in vitro* and these nanoparticles were shown effective translocation across the Calu-3 cell line. [26]

Table 1: Comparative analysis on depolymerisation techniques for LMWBP based nanoparticles preparation with therapeutic applications

LMWBP based NP [a]	CD [b]	~Size (nm)	NP-MW [c] (kDa)	Application
Chitosan [15]	Oxidation by $NaNO_2$	70.6	55	5-fluorouracil drug delivery
Chitosan-g-L-phenylalanine [16]	Complex coacervation with H_2O_2	80.0	4.4	Nucleic acid (DNA) delivery
Glycol- chitosan [17]	Acid degradation by HCl	230	20	Subcutaneous SCC7 tumor targeting
Chitosan [18] complexed with DNA	Acid degradation by HCl	220	21	HeLa cell and HepG2 cell line targeting
Chitosan [19]	Chitosanase enzyme action	385.2	20	Insulin delivery

[a] **LMWBP based NP:** Low molecular weight biodegradable polymer based nanoparticle. [b] **CD:** Chemical depolymerisation Process [c] **NP-MW:** Nanoparticle-molecular weight.

Cycloheximide (CHX)-loaded low molecular weight gelatin (LMWG) nanoparticles were made by a two-step desolvation method for intravascular delivery of a well known protein synthesis inhibitory drug CHX. LMWG were used in this study ranging from 22 to 87.5 kDa. Experimental results showed that formed CHX-LMWG particles are in the range 200-300 nm in presence of glutaraldehyde as an effective cross linker. [27]

OUTLOOK AND FUTURE PERSPECTIVES

Many say that 'LMWBP based nanoparticles are the best delivery systems of the nucleic acids, proteins and drugs in the near future' and some add 'and these always will be!' Indeed, instead of a large amount of research in the past and at present, major obstrucles remain to be overcome before a feasible practical process can be established for any approach to LMWBP based nanoparticles preparations for delivery of nucleic acids, proteins and drugs. Here we have concentrated on recent progresses on preparation techniques of LMWBP based nanoparticles with their therapeutic applications as nucleic acids, proteins and drugs delivery systems. As shown here, there have been substantial recent developments in LMWBP based nanoparticles preparations for delivery especially in terms of their physicochemical properties (like molecular weight, particles size), few *in vitro* applications and *in vitro* cytotoxicity tests. But the more important promising issues, such as the specific interactions of these LMWBP based nanoparticles with human organs, tissues, cells, microbial cells, biomolecules and cellular metabolism brought by these LMWBP based nanoparticles. Hence, the major upstanding question is "Can targeted drug, nucleic acid and protein delivery problems be resolved by application of LMWBP based nanoparticles without cytotoxic effects?" Attempting to address this challenge should be the focus of future research on LMWBP based nanoparticles as effective delivery systems.

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