USE OF BIODEGRADABLE MICRO AND NANO-PARTICLES IN VACCINE DELIVERY

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SUMMARY

Effective immunizations in recent years become most important in terms of new antigens, adjuvants and routes of vaccination. Vaccination by oral route remains the preferred route for both patients and practitioners. To improve the bioavailability of peptides, proteins and antigens, they are associated with colloidal carrier e.g. polymeric nanoparticles. Synthetic polymers are choosen for longer duration of drug release, but they are limited by the use of organic solvents and relatively harsher formulation conditions. Amongst the different classes of biodegradable polymers, the thermoplastic aliphatic poly (esters) like poly (lactide) (PLA), poly (glycolide) (PGA), and especially the copolymer of lactide and glycolide, poly (lactide-co-glycolide) (PLGA) have generated immense interest due to their favorable properties such as good biocompatibility, biodegradability, and mechanical strength. Also, they are easy to formulate into different devices for carrying a variety of drug classes such as vaccines, peptides, proteins, and micromolecules. Also, they have been approved by the Food and Drug Administration (FDA) for drug delivery. In this mini-review, various formulation aspects of these biodegradable polymers, in the micro and nano range, were discussed.

KEY WORDS: Nanoparticles; oral delivery; Vaccine carriers; Biodegradable polymer; PLGA; Adjuvant.

1. INTRODUCTION

Vaccination against infectious disease, which has focused on the induction of systemic humoral and cellular immunity, is an effective and useful intervention that has been generated by centuries of medical research. Immunization efforts against infectious diseases such as polio, smallpox and measles have saved innumerable lives by eradicating or decreasing the occurrence of the disease and have contributed to today's increased life expectancy.

Several factors have been largely responsible for the inability of vaccines to protect against infectious diseases. One of the most significant factors includes the unavailability of vaccines against intracellular pathogens, or infected or altered cells, such as malaria and HIV, which rely on cell-mediated immunity[1-3]. Second, progress in the field of adjuvants for use with human vaccines has been inadequate, and the range of adjuvant

activities represented by existing adjuvants, mainly aluminium compounds, is limited, often prohibiting optimization of the magnitude and direction of the immune response. Third, high compliance with booster vaccine doses have left significant fractions of people in developing countries not fully immunized due to lack of access and education of these vaccination[4]. Finally, incompletely immunized women (mostly in developing countries) cannot pass immunity to neonates, leaving newborns susceptible to infections, e.g., umbilical cord infections[5].

To cross these limitations biodegradable polymers can be used which are natural or synthetic in origin and are degraded in vivo, either enzymatically or non-enzymatically or both to produce biocompatible, toxicolocally safe by products which are further eliminated by the normal metabolic pathways.

2. Types of micro and nano particles used in the delivery

The polymers selected for the parental administration must meet several requirements like biocompatibility, drug compatibility, suitable biodegradation kinetics and mechanical properties, and ease of processing[6,7]. Natural biodegradable polymers like bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatin, and hemoglobin have been studied for drug delivery. The use of these natural polymers is limited due to their higher costs and questionable purity[8]. Polyamides, polyaminoacids, poly alkyl cyano acylates, polyesters, polyortoesters, polyurethanes, and poly acrylamide have been used to prepare various drug loaded devices[6-13]. Amongst them, the thermoplastic aliphatic poly esters like PLA, PGA and especially PLGA have generated tremendous interest due to their excellent biocompatibility and biodegradability. These polymers are used in the vaccine delivery in micro or nano range. Moreover, PLGA microspheres have several additional advantages, such as the ability to elicit CTL responses, and the potential for mucosal immunization and DNA delivery. In addition to the depot effect, smaller PLGA microparticles (e.g., <10µm) were demonstrated to have adjuvant activity via their uptake by microphages and dendritic cells (DCs), and their localization in lymph nodes[14], and to induce CTL responses[15-19]. Despite the excellent biocompatibility of PLGA, the mild inflammatory response produced by PLGA microspheres has also been hypothesized as being involved in their adjuvant characteristics[20]. Most significant were reports of long-lasting antibody responses, many neutralizing above protective levels, in numerous animal models following a single dose of PLGA microparticles encapsulated antigens[21].

Microparticles of size less than 125µm are suitable for vaccine delivery purpose. However, size of particles have importance in delivery and revealed the advantages of nanoparticles over the microspheres. More specially, the number of nanoparticles which cross the intestinal epithelium is greater than the number of microparticles which cross the intestinal epithelium is greater than the number of microspheres, and that not only the M cells but also the normal enterocytes are involved in the transport. Polymer nanoparticles are particles less than 1 µm diameter that are prepared from polymer. In recent years, significant research has been done using nanoparticles as oral drug delivery vehicles. In this application, the major interest is in lymphatic uptake

of the nanoparticles by the Peyer's patches in the GALT (gut associated lymphoid tissue). Peyer's patches are characterized by M cells that overlie the lymphoid tissue and are specialized for endocytosis and transport into intraepithelial spaces and adjacent lymphoid tissue. Nanoparticles bind the apical membrane of the M cells, followed by a rapid internalization and a shuttling to the lymphocytes[22,23]. The size and surface charges of the nanoparticles are crucial for their uptake. It has shown that microparticles remain in the Peyer's patches while nanoparticles are disseminated systemically[24].

Nanoparticles have a further advantage over larger microparticles, because they are better suited for intravenous (i.v.) delivery. The smallest capillaries in the body are 5-6 µm in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 µm, without forming aggregates, to ensure that the particles do not form an embolism.

Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, biological macromolecules, etc. and can be formulated for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver, spleen, or made for long-term systemic circulation. Four of the most important characteristics of nanoparticles are their size, encapsulation efficiency, zeta potential (surface charge), and release characteristics[25].

Diverse strategies have been developed to improve the bioavailability of peptide and protein drugs and vaccines, encapsulated in polymeric nanoparticles. Two main approaches prevailed to significantly improve transport: (i) by modifying surface physiochemical properties of nanoparticles, or (ii) by coupling a targeting molecule at the nanoparticle surface.

3. Physico-chemical and biological properties of PLGA

The understanding of the physical, chemical, and biological properties of the polymer is helpful, before formulating a controlled drug delivery device because it directly influence other factors like the selection of the microencapsulation process, drug release from the polymer device, etc[26].

The polymer PLA can exist in an optically active stereoregular form (L-PLA) which is semi-crystalline in nature and in an optically inactive racemic form (D, L-PLA) which is amorphous. The use of D,L-PLA is preferred over L-PLA as it enables more homogeneous dispersion of the drug in the polymer matrix[27,28]. Lactic acid is more hydrophobic than glycolic acid and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water, and subsequently degrade more slowly[29-31]. The physical properties such as the molecular weight and the polydispersity index affect the mechanical strength of the polymer and its ability to be formulated as a drug delivery device[32-34]. Also these properties may control the polymer biodegradation rate and hydrolysis. The commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to their molecular weights. The T_g (glass transition temperature) of the PLGA copolymers are above the physiological temperature of 373°C and hence they are glassy in nature. The biodegradation rate of the PLGA copolymers are dependent on the molar ratio of the lactic and

glycolic acids in the polymer chain, molecular weight of the polymer, the degree of crystallinity and the T_g of the polymer. PLGA degrades into lactic acid and glycolic acid. Lactic acid enters the tri-carboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water. Glycolic acid is either excreted unchanged in the kidney or it enters the tri-carboxylic acid cycle and eventually eliminated as carbon dioxide and water[27].

4. Manufacturing techniques of various biodegradable PLGA devices

4.1 Microparticles

From a number of micro-encapsulation techniques, the choice of technique selected on the basis of nature of the polymer, the drug and the duration of the therapy. There are following methods used for the manufacturing of the biodegradable polymer.

4.1.1Solvent evaporation and solvent extraction process

4.1.1.1 Single emulsion process[35]

This process involves oil-in-water (o/w) emulsification. One of the disadvantages of the o/w emulsification method is poor encapsulation efficiencies of moderately water-soluble and water-soluble drugs.

4.1.1.2 Double (multiple) emulsion process

This is a water-in-oil-in-water (w/o/w) method and is best suited to encapsulate water-soluble drugs like peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs like steroids[36].

4.1.2 Phase separation (coacervation)[37]

This process consists of decreasing the solubility of the encapsulating polymer by addition of a third component to the polymer solution in an organic solution. The coacervation process is mainly used to encapsulate water-soluble drugs like peptides, proteins, and vaccines.

4.1.3 Spray drying[38]

Contrary to these methods, the spray drying method is very rapid, convenient, easy to scale-up, involves mild conditions, and is less dependent on the solubility parameter of the drug and the polymer.

4.2 Nanoparticles

Nanoparticles (nanospheres and nanocapsules) could be prepared by the same methods as those described for microparticles, except that manufacturing parameters are adjusted to obtain nanometer-size droplets. Nanoparticles constituted of synthetic polymers are usually prepared by dispersion of preformed polymers. Several techniques can be used, mainly chosen in function of the hydrophobicity of drugs to be encapsulated. The nanoprecipitation method is employed to encapsulate lipophilic drugs, forming nanospheres. Recently, this technique has been adapted to encapsulate hydrophilic compounds into PLGA and PLA nanoparticles. Small polydispersity indices are easily and rapidly obtained by nanoprecipitation[39]. The solvent evaporation method is used to encapsulate either hydrophobic or hydrophilic drugs.

4.3 Evaluation parameters influencing nanoparticle properties

It was found that key parameters modulating nanoparticle size during the formulation process like the number of

homogenization cycles, the addition of excipient to the inner water phase, the drug concentration and the oil–outer water phase ratio, affect the size of PLGA nanoparticles. The concentration and nature of the surfactant influence nanoparticle size e.g., a high concentration of surfactant reduces the size of complexes. At last, polymer molecular weight also influences the size of particles; the higher the polymer weight, the smaller and less poly-dispersed the nanoparticles[37]. Hydrophobicity and surface charges are greatly influenced by polymer composition.

Mucosal absorption of nonparticles can be improved either by modifying their surface properties or by coupling a targeting molecule at their surface. Modification of nanoparticle surface properties can be achieved either by coating nanoparticle surface with hydrophilic stabilizing, bioadhesive polymers or surfactants or by incorporating biodegradable copolymers containing an hydrophilic moiety in the formulation. Poly (ethylene glycol) (PEG) has been employed as nanoparticle coating in drug delivery applications for its stabilizing properties. Due to mucoadhesive properties, chitosan has been one of the most employed polymers to coat nanoparticle surface. Such modifications ultimately enhance the mucosal permeability. Different types of targeting molecules are also tried e.g. the lectin family. Lectins conjugate to polymeric nanoparticles and significantly increase their transport across the intestinal mucosa by efficiently increasing interactions with mucus. Thus, surface properties of nanoparticles can be modified either by improving non-specific interactions with the cell apical surface or by grafting a specific ligand targeting epithelial intestinal cells.

5. Concluding remarks:

Proteins are typically unstable when encapsulated in PLGA microspheres unless careful measures are taken to prevent instability pathways. Protein unfolding and aggregation in the polymer as well as adsorption of the protein to vessels used to monitor release kinetics has complicated in vitro characterization of PLGA microparticles. Also, attempts to distinguish immune responses generated by stable and unstable PLGA-antigen preparations have been complicated by the lack of positive control. Several deleterious stresses (e.g., organic solvents, pH, and moisture) cause or accelerate the antigen instability during microencapsulation, storage and release of the antigen from PLGA microparticles. Few understood features of in vitro quality control and in vivo immune response need to be resolved in order to advance the development of PLGA microspheres. Additionally, difficulties to reach very large scale manufacturing because of the physical–chemical complexity of some of the microencapsulation methods may have hampered the ability to reduce manufacturing costs.

Several exciting initiatives have been pursued to overcome these impediments. Various formulation strategies have been evaluated to improve the stability of PLGA-encapsulated antigens, including trial-and-error, empirical methods and mechanistic approaches.

MPs of PLGA are in the market, while NPs are being successfully used to deliver peptides and proteins in addition to a host of therapeutics over times extending upto days, weeks or even months in controlled manner, such products need to be developed and marketed.

Nowadays micro and nanoparticles are preferable choice for vaccine delivery due to their easy intracellular uptake and enhanced therapeutic efficacy. Due to their sub-cellular size they can penetrate deep into tissues through fine capillaries and are generally taken up efficiently by the cells. These systems can be used to provide targeted drug delivery, and to improve oral bioavailability, to improve stability of therapeutic agents against enzymatic degradation. Biodegradable polymers are preferable for the preparation of the micro- nanoparticles because they do not need to remove from the body. Moreover, for shorter duration of action they are chosen over synthetic polymers having all above mentioned advantages.

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