Review

Protein-based nanocarriers as promising drug and gene delivery systems

Ahmed O. Elzoghby [a], Wael M. Samy [b], Nazik A. Elgindy [a],*

[a] Department of Industrial Pharmacy, Faculty of Pharmacy, Alexandria University, El-Khartoum Square, Azarita, Alexandria 21521, Egypt
[b] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon

A R T I C L E I N F O

A B S T R A C T

Among the available potential colloidal drug carrier systems, protein-based nanocarriers are particularly interesting. Meeting requirements such as low cytotoxicity, abundant renewable sources, high drug binding capacity and significant uptake into the targeted cells, protein-based nanocarriers represent promising candidates for efficient drug and gene delivery. Moreover, the unique protein structure offers the possibility of site-specific drug conjugation and targeting using various ligands modifying the surface of protein nanocarriers. The current review highlights the main advances achieved in utilizing protein nanocarriers as natural vehicles for drug and gene delivery tasks with respect to types, advantages, limitations, formulation aspects as well as the major outcomes of the in vitro and in vivo investigations. The recently emerged technologies in the formulation of protein nanocarriers including using recombinant proteins as alternatives to native ones and new non-toxic crosslinkers as alternatives to the toxic chemical crosslinkers are also discussed.

Contents

1. Introduction

In recent years, there has been a considerable interest in the development of novel drug delivery systems using nanotechnology [1,2]. Polymeric materials used for preparing nanoparticles for drug delivery must be at least biocompatible and best biodegradable. The

* Corresponding author. Tel.: +20 3 4878248; fax: +20 3 4873 273.
E-mail address: d_nazik4@yahoo.com (N.A. Elgindy).

use of natural polymers has been very well described in the literature for fabrication of nanoparticles [3,4]. Obviously, there is growing interest in developing protein nanocarriers as GRAS (generally regarded as safe) drug delivery devices due to their exceptional characteristics, namely biodegradability, nonantigenicity, high nutritional value, abundant renewable sources and extraordinary binding capacity of various drugs. Proteins have the possibility of less opsonization by the reticuloendothelial system (RES) through an aqueous steric barrier in addition to their excellent functional properties including emulsification, gelation, foaming and water binding capacity [5–7]. Moreover, protein nanoparticles can be easily prepared and scaled up during manufacture [6,7].

Owing to multiple functional groups in the primary sequences of polypeptides, protein nanoparticles can be exploited to create different interactions with therapeutic compounds and subsequently form three-dimensional networks offering a variety of possibilities for reversible binding of active molecules, protecting them in a matrix as well as specific targeting to the site of action [6,7]. Furthermore, protein nanoparticles possess acceptability as metabolizable naturally occurring components. Hydrolysis of proteins by digestive enzymes generates bioactive peptides that may exert a number of physiological effects in vivo [5]. This review embodies an in-depth discussion of the nanoparticulate drug delivery systems that make use of proteins as drug carriers.

2. Animal proteins

Animal proteins represent good raw materials since they have the advantages of synthetic polymers and the advantages of absorbability and low toxicity of the degradation end products [8].

2.1. Gelatin

Gelatin is a denatured protein that is obtained from collagen by acid and alkaline hydrolysis. It has a long history of safe use in pharmaceuticals, cosmetics, as well as food products and it is considered as GRAS material by the FDA [9]. It has a relatively low antigenicity because of being denatured. Its functional groups are accessible for various chemical modifications, which may be especially useful in developing targeted drug delivery vehicles [10]. Gelatin is a polyampholyte having both cationic and anionic groups along with hydrophobic groups. The repeating sequences of glycine, proline and alanine amino acid triplets are responsible for its triple helical structure [11]. Gelatin nanoparticles have been reported for successful delivery of various drugs including anticancer [8,12], anti-HIV [13], antimalarial [14], antimicrobial [15,16], skeletal muscle relaxant [16], analgesic [17], anti-inflammatory [18], anti-diabetic [19,20], topical ophthalmic [21] drugs, protein synthesis inhibitors [22], tissue-type plasminogen activator [23] as well as gene delivery [10,24]. Various methods including desolvation [25], coacervation-phase separation [12], emulsification-solvent evaporation [14], reverse phase evaporation [26] and nanoprecipitation [16] have been used to prepare gelatin nanoparticles.

Desolvation of native gelatin by a desolvating agent (e.g., alcohol or acetone) produces large nanoparticles with a wide size range. Therefore, a two-step desolvation process was adopted by Coester et al. [25] to discard low MW gelatin molecules and then precipitate smaller nanoparticles with a narrow size distribution. Although the use of glutaraldehyde (GA) as a crosslinker leads to improvement of the mechanical properties and stability of nanoparticles, its high toxicity may limit the applications of the final product. Therefore, the use of non-toxic crosslinking agents was emerged as an alternative [17]. Genipin is a naturally occurring crosslinking agent with a negligible cytotoxicity (~10,000 times less than GA) [27]. Recombinant human gelatin (rHG) nanoparticles were prepared using genipin as a crosslinker and showed a great potential for protein drug delivery

in terms of sustained release, less initial burst and safety. About 50% of the model protein (FITC-BSA) was released from the nanoparticles over 10 days [28]. In another study, a mixture of a water soluble carbodiimide (CDI) and N-hydroxysuccinimide (NHS) was used by Qazvini and Zinatloo [17] as a non-toxic crosslinker of gelatin nanoparticles. Using paracetamol as a model drug, both drug entrapment and loading efficiency were higher in the CDI/NHS crosslinked nanoparticles (27 and 7.3%); than that of nanoparticles crosslinked with GA (10 and 4%), respectively [17]. The recombinant enzyme microbial transglutaminase, an acyltransferase that forms intra- and intermolecular isopeptide bonds in and between many proteins by crosslinking the ε-amino groups of the amino acid lysine to the side chain amide group of glutamine, was used by Fuchs et al. [29] to crosslink gelatin nanoparticles producing particles of defined size below 250 nm and narrow size distribution.

A coacervation-phase separation method was used to prepare paclitaxel-loaded gelatin nanoparticles where an aqueous solution of sodium sulfate was added slowly to gelatin solution containing Tween 20 followed by addition of isopropanol containing paclitaxel [12]. Compared to the conventional paclitaxel formulation which uses cremophor/ethanol as solubilizers, the nanoparticles showed longer retention and higher accumulation in organs and tissues (average of 3.2-fold). Gelatin nanoparticles were also prepared via a single water-in-oil emulsification/solvent evaporation method by mixing an aqueous phase of both gelatin and drug with an oil phase of polymethylmethacrylate then crosslinked with glutaraldehyde saturated toluene [14]. A reverse phase evaporation method was used by Gupta et al. [26] to prepare gelatin nanoparticles (~37 nm) inside the inner aqueous core of reverse micellar droplets formed by dissolving the surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT) in n-hexane [30]. In another study, Lee et al. [16] prepared drug-loaded gelatin nanoparticles by nanoprecipitation method using water as a solvent, ethanol as a nonsolvent and poloxamer as a stabilizer. About 80% of loaded tizanidine hydrochloride was released around 15 h after start-up of the release experiment. Recently, a novel water-in-water emulsion technique was successfully used by Zhao et al. [20] to prepare D,L-glyceraldehyde-crosslinked gelatin-poloxamer 188 nanoparticles as insulin pulmonary administration system. The nanoparticles promoted insulin pulmonary absorption effectively and showed good relative pharmacological bioavailability [20].
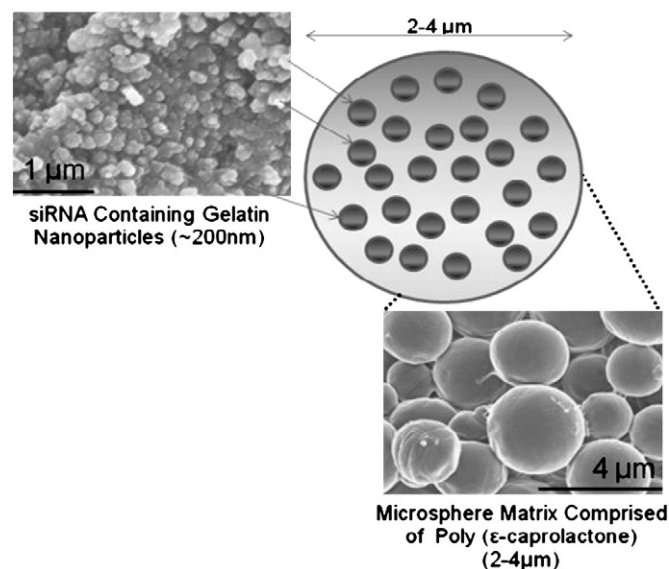

Fig. 1. Scanning electron micrographs of small interfering RNA (siRNA)-encapsulated type B gelatin nanoparticles and siRNA containing NiMOS [32].