BIOMEDICAL RESEARCH JOURNAL

Volume 35, 2020

Synthesis of protein nanoparticles for drug delivery

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Abstract: In the past decades, nanoparticles have attracted increasing attention in biomedicine field due to their high drug-loading effectiveness, well selectivity for target tissues, and long-acting time in circulation. Among them, drug delivery system based on protein nanoparticles has been widely studied aiming to overco-me deficiency of drug-loaded materials, such as low biocompatibility, inefficient biodegradability and high toxicity. In this review, we briefly introduced the drug delivery system based on protein nanoparticles. The methods for synthesizing protein nanoparticles as well as its therapeutic efficacy as drug delivery system were considered. And we also discussed the application and prospects of protein nanoparticles in drug delivery.

Key words: Protein nanoparticles, drug delivery, cancer, UV illumination.

Introduction

Recently, much attention has been attracted on explo-ring nanoparticles as drug delivery materials, which aims to overcome the following challenges: 1) Large doses of organic solvents should be used for loading some anti-can-cer drugs, such as doxorubicin, paclitaxel and cisplatin, due to its poor solubility in aqueous solution, which may leading to the toxicity for the drug delivery system; 2) high side-effect of many drugs due to their low efficien-cy in targeting to specific tissue and cells; 3) very short acting time in circulation for many drugs (1-6). Thus, many biomaterials, such as organic polymers, liposomes, proteins and silica, have been developed as drug delive-ry carries (Table 1) (7-10). Furthermore, the methods of preparing nanoparticles for drug delivery were also being widely explored. These synthesized nanoparticles as drug delivery systems (DDS) have many advantages of high bioactivity efficacy, less side-effect for health tissues, long-acting time for its controllable release (10-12). And many characteristics of these biomaterials, such as the sizes, surface charge, morphology, biocompatibility and biodegradability, were generally considered for synthesis of these nanoparticles (10-12).

Although many mentioned advantages have been stu-died for the drug delivery of nano-materials, there are still

Table 1. The comparisons of advantages or disadvantages of each material.

Materials	Organic polymers	Liposomes	Proteins	Silica
Advantages	reduce the toxicity of the drug molecules with higher dose applied and increase the rate for improved therapeutics	non-invasiveness for tissues and individual cells; relatively nontoxic	biocompatibility, biodegradability, low toxicity; abundant renewable sources; high drug binding capacity	with controlled particle size and morphology; with high chemical stability
Disadvantages	carry a limited number of drug molecules; non-biodegradable properties	thermodynamically unstable and tend to fuse; can undergo lipid exchange with lipoproteins resulting in vesicle instability; poor drug encapsulation	hard to control particle size and exhibit batch-to batch variation	the fabrication often involves harsh chemicals and energy intensive laborious methods

some challenges in preparing these drug-loaded nanopar-ticles and increasing the drug-loading efficiency. And the therapeutic efficiency also limited by the toxicity of drugs for health tissues and cells due to the low tumor-targeting efficiency (13, 14). Liposomes have been used as potential drug delivery carriers due to its advantages in protecting drugs from degradation, targeting to special tissue and decreasing the toxicity or side effects (15). However, the application of liposome is limited due to its inherent pro-blems, such as low encapsulation efficiency, rapid release rate of hydrophilic drugs in serum and poor stability for its storage (15). Besides, many other biomaterials, such as or-ganic polymers, proteins and silica, have been developed as drug carries (16-18). Each biomaterial was found to be available due to some of its advantage characters, while it usually has some disadvantages in other fields.

Among these available biomaterials as potential drug carrier systems, protein-based nanoparticles that synthe-sized by variety of natural proteins and engineered syn-thetic polypeptides, has very promising potential for drug delivery (19). Having the brilliant characters of biocom-patibility, biodegradability and low toxicity, the protein-based NPs are naturally self-assembled protein subunits of the same protein or a combination of proteins making up a complete system (20, 21). And proteins can offer a lot of moieties which are accessible to modify little mole-cules for drug-binding, imaging or targeting entities (22, 23). Due to the great quickly development of nanotech-nology recently, the preparation of protein-based NPs has been focused on many proteins, such as albumin, gelatin, gliadin and legumin. Based on the previous studies, we proposed that the protein-based nanoparticles have more potential applications in drug delivery in the future. Thus, in this review, we aim to discuss the fabrication of protein-based NPs and its applications in drug delivery.

Synthesis of protein-based nanoparticles

Up to now, people have paid attentions on the synthe-sis of nanoparticles and many methods including sono-chemistry, colloidal method, thermal decomposition, hy-drothermal method, and microemulsion method (24-26), have been developed for synthesizing nanoparticles as drug delivery carriers for earlier diagnosis and treatment of some diseases. However, many of these methods were always limited by the toxicity of drugs for normal cells and tissue due to the difficulty of loading hydrophobic agents for administration (27-29). There are two major developed pathways for synthesis of protein nanoparticles: through crosslinking with functional groups of native protein mo-lecules or the derivative groups modified on the surface of protein molecules (30, 31).

The coacervation or equally coined desolvation pro-cess under relatively mild conditions is one of the most frequent methods to prepare protein-based NPs. In gene-ral, the protein solvent was extracted into an anti-solvent phase to make a colloidal system (32, 33). And the fol-lowing phase separation leads to the solid colloid phase that dispersed in another phase with the initial protein dissolved solvent and an anti-solvent. And then the an-ti-solvent and the initial solvent should be miscible (34-36). The size and output of protein nanoparticles are deter-mined by the pH value of the solution prior to desolvation (37).

Recently, people developed electrospray as a new tech-nique for synthesis of protein nanoparticles. And this me-thod has been used for synthesis of gliadin and elastin-like protein nanoparticles (38, 39). In general, high voltage was used on the solution with an emitter. The sizes of protein nanoparticles which contained by the emitted aerosolized droplets can be well controlled. And the therapeutic drugs and nucleic acids can be well loaded by the protein nano-particles with high efficacy through this pathway.

Although the synthesis methods of protein-based na-noparticles has been widely improved, the costly, toxicity chemical reagents introduction, and the difficulty in size controllable are still significant challenges in synthesis of protein-based NPs, thus greatly limits its application in biomedicine field (Table 2). Reducing the disulfide bonds of native proteins by UV illumination and thus inducing the self-assembly into protein nanoparticles is a potential good pathway to solve these problems. Prompers et al. firstly reported that the disulfide bonds in protein mole-cules can be broken by UV illumination (40). The tryp-tophans (Trp) residues neighbing disulfide bonds were thought to be responsible to this photo-reduction. Petersen et al. found that the quantum of free thiols in cutinase was increased after being illuminated by UV light (41). And Hanssens et al. found the similar photo-reduction phe-nomenon in another protein of goat α-lactalbumin (42). Xie et al. systemly studied the unfolding mechanism of lysozyme under UV illumination and they found that these proteins can self-assemble into protein nanoparticles only through UV illumination. Besides, both the size and morphology of these protein nanoparticles can be well controlled with changing the illumination time or inten-sity of UV light.

The application of protein-based nanoparticles in drug delivery

Protein-based nanoparticles have been widely used as drug delivery system (12). Here, we introduce several examples about the application of protein nanoparticles in drug delivery. Silk protein was reported as carries for gene delivery in vitro (43, 44). Many kinds of cytostatic drugs, such as 5-fluorouracil, paclitaxel and doxorubicin (DOX) have been reported for binding to albumin and thus si-

Table 2. The comparisons of advantages or disadvantages of each method.

Methods	Coacervation	Solvent extraction or emulsion process	Polyelectrolyte complexation	Salt precipitation	Heat denaturation
Advantages	relatively mild conditions	high encapsulation rate	high encapsulation efficiencies	a simple method	equipped with targeting moieties
Disadvantages	the method can easily promote agglomeration	lack relatively large particle sizes	strong pH influence	retain the risk of changed bioactivity and lost conformation; higher heterogeneity	lack relatively large particle sizes

gnificantly enhanced the anti-tumor efficacy (45-47). And the toxicity of these drugs was greatly decreased by loa-ding into the protein-based NPs (48). Gelatin was used in increasing the cellular responsive to foreign antigen as a kind of immunological adjuvant (49).

Albumin, such as ovalbumin and serum albumin (BSA and HSA), is a major protein in the circulation system. Albumin has very important functions in controlling os-motic pressure and loading nutrients to tissues and cells. Albumin acts as a depot and transportation protein for de-livery many therapeutic drugs and endogenous molecules.

[13] Albumin is very stable under the pH from 4 to 9 and can bear temperature of 60oC for 10 hours without unfol-ding (14). And the albumin is widely used in synthesis of nanospheres and nanocapsules for drug delivery due to its biodegradable, easy synthesis, and well-controlled sizes and easy modification for its function groups on the sur-face of molecular. Besides, the loaded drug can be released from albumin nanoparticles easily by protease digestion

(15). Albumin-bound NPs (nab) has been used to carry hydrophobic molecules and it can come into bloodstream through the pathway of endogenous albumin (50), avoi-ding the solvent-based toxicities for bodies. Besides, the albumin-bound paclitaxel has been investigated in re-ducing the risk of hypersensivity reactions without pre-medication and special intravenous tubing (51, 52). Two

Human serum albumin (HAS) based drugs, Abraxane and Albunex, have been tested having great clinical benefits and has been used on the market for many years (53, 54).

Elastinlike polypeptide (ELP) has been used for tissue targeting due to its environment-responsive character. The cell-penetrating peptides (CPPs) have been widely used in drug delivery (55). That is one of cell-penetrating pep-tides and has been reported as a carries for many proteins crossing over cell membranes (56). And the study of in vivo delivery of Fab fragments conjugated with Tat as previously reported shown that the Tat peptide has well special tissue localization (57).

Based on the photo-reduction mechanism, Xie et al. developed a one-step method to synthesize anticancer drugs loaded protein nanoparticles upon UV illumination (shown in Figure 1). This synthesis method was performed without adding any toxicity organic solvents or chemical denaturants. Besides, it was reported that these protein-na-noparticles showed rapid rate for drug release under acidic and reducing conditions while slowly at physiological en-vironment, suggesting that these protein nanoparticles are suitable for anti-tumor drug delivery.

The result of in vivo experimental suggested that the

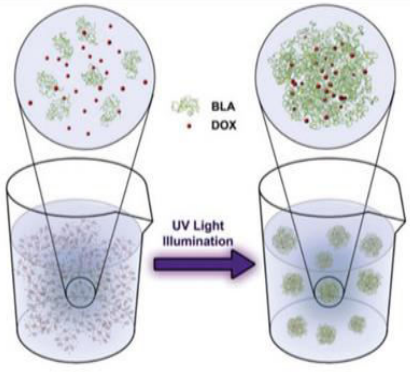

Figure 1. The Schematic of photo synthesis of BLA-DOX NPs (58).

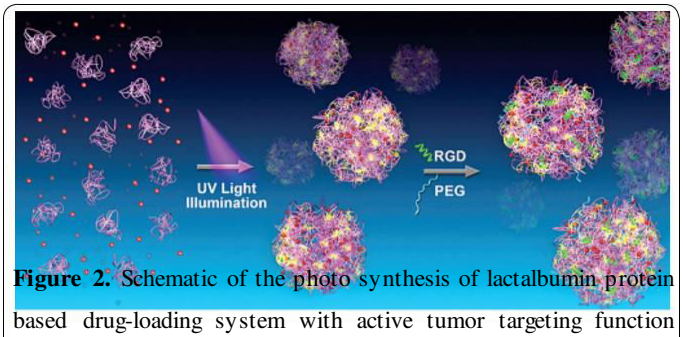

Figure 2. Schematic of the photo synthesis of lactalbumin protein based drug-loading system with active tumor targeting function (59).

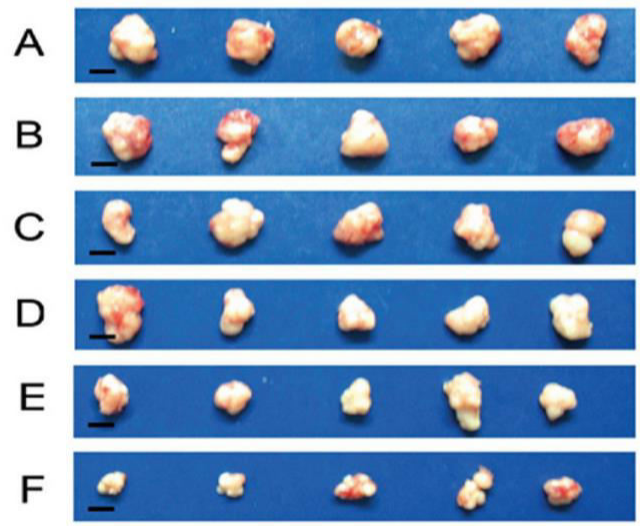

Figure 3. The photo of the with esophagus tumor ECA109 tumors dissected from male mice (6–8 weeks old, 18–22 g) pre-treated by vein injection every two days for a total ten times injection with PBS buffer A), RGD-PEG-BLA/NPs B) and free DOX C); and other drug-loaded protein nanoparticles: PEG-BLA-DOX/NPs D), RGD-BLA-DOX/NPs E) and RGD-PEG-BLA-DOX/NPs F), respectively. Each time, the dose of free DOX and DOX loaded in nanoparticles was 1.5 mg kg−1 (59).

RGD-conjugated protein nanoparticles have great affec-tion on tumor targeting and inhibition (figure 3). And this method of synthesis of protein nanoparticles based on photo-reduction can be extended to synthesize many other disulfide-containing protein-based nanoparticles for drug delivery, although some challenge for this photo-reduc-tion method, such as the potential decomposition for some drugs under high intensity of UV light illumination and the low drug-loading efficiently has to be overcome.

Conclutions

Protein, including variety of natural proteins and enginee-red synthetic polypeptides, based platforms of biomaterials has very promising potential for drug delivery. Many syn-thesis methods for protein nanoparticles were developed. And the protein characters of biodegradability, biocompa-tibility, and even the effect of particles sizes act significant roles on the application of protein nanoparticles in drug delivery. Protein nanocarriers have great potential appli-cation to the future development of anti-cancer therapeu-tics due to its high loading efficiency and targeting effect. And synthesizing methods will be further developed to further improve delivery of anti-cancer drugs, siRNA, and other therapeutic molecules by using protein nanocarriers.

And synthesis of multi-funtional protein nanoparticles for drug delivery will be another potential direction in bio-medicine. This review offers a whole summary about the synthesis, characters as well as the application of protein nanoparticles as drug delivery system and we hope that this will be a useful reference in the further development of protein nanotechnology.

Acknowledgments

This work was supported by the National Natural- Science Foundation of China (No. 31400810, 81200654), the Na-tural Science for the Youth Foundation (No. 51402153), the Natural Science Foundation of Jiangsu Province (No. BK20151016), the Shanghai City Health Bureau Project (20124104) and the Fundamental Research Funds for the Central Universities (No. KYZ 201321).

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