CHAPTER 15

Self-assembled protein–drug nanoparticles for drug delivery

Riham R. Mohamed
Faculty of Science, University of Cairo, Department of Chemistry, Giza, Egypt

1. Introduction

Supramolecular self-assembly has recently caught the interest of academics all over the world due to its peculiar physical and chemical characteristics [1]. The arrangement of molecules in these nano-assemblies has made it feasible to improve novel devices that can communicate with living cells and provide an influence that can be employed in a variety of applications such as catalysis, diagnostics, biomaterials sensors, drug delivery, and nanocomposites [1].

Based on their excellent drug-loading capabilities, biocompatibility, and capacity to encapsulate hydrophobic active therapies, protein nanocarriers have been proposed as useful delivery stages for many medications [2].

2. Self-assembly

As demonstrated in Fig. 15.1, self-assembly is the process through which disordered molecules naturally form well-ordered structures as a result of unique local interactions between the individual components [3].

In the development of newer nanomaterials, the self-assembly process is critical. A cell, for example, contains a broad range of complex structures, including lipid bio membranes, folded proteins, protein combinations, structured nucleic acids, molecular machineries, and so forth, all of which have shown an inclination for self-assembly.

- It helps to generate common materials such molecular crystals, liquid crystals, semi-crystalline, and phase-separated polymers. It also occurs in large molecules, providing new opportunities for their employment in material sciences and delivery applications.
- It suggests the simplest and most straightforward solution.

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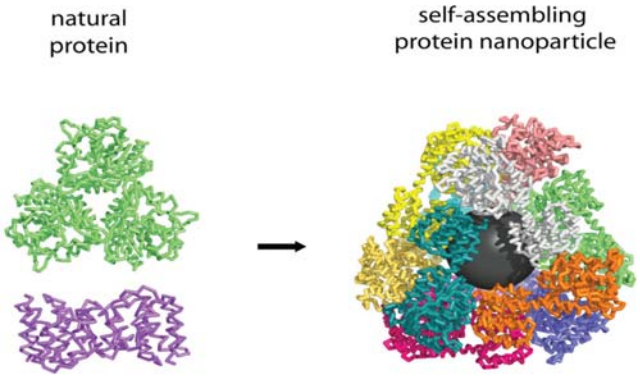

Figure 15.1
Schematic diagram of self-assembled protein nanoparticles.

Proteins derived from both animals and plants have been discovered to be effective medication and gene delivery vehicles. Protein nanoparticles are nontoxic and can be modified on the surface to adjust their residence time and target selectivity [4]. Furthermore, protein nanostructure encapsulation preserves both protein functioning and therapeutic agents [5]. Some of the most common protein-based nanoparticle systems discovered as nanocarriers are mentioned further.

3. Collagen nanoparticles

A fibrous, biocompatible protein known for its distinctive triple helix form is collagen [6]. Collagen is possible to cross-link itself to make hard solids. It was chosen for drug delivery applications and is made composed of sponges, fibers, sheets, nano, and microparticles for encapsulating medicinal substances [6], as shown in Fig. 15.2.

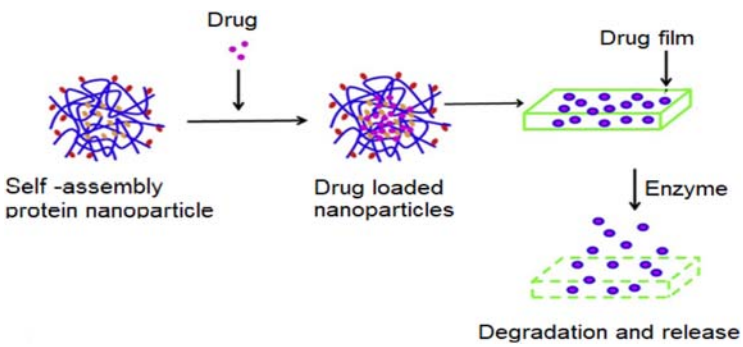

Figure 15.2
Protein film containing drug nanoparticles for localized drug delivery.

For example, gold nanoparticles and collagen nanoparticles were joined via electrostatic interactions and then mineralized to generate a collagen–gold network gel that might be used for cytotoxicity against cancer cells [7]. Additionally, the combination of collagen and alginate revealed a significant and practical method for ophthalmic applications [8]. Additionally, 17-estradiol hemihydrate was coated with isolated collagen extracted from the aquatic sponge *Chondrosia reniformis*, which was then spread out in hydrogel and given to postmenopausal patients [9]. When delivered locally, cisplatin-loaded nanoparticles made of a mixture of collagen and hydroxyapatite with a structure resembling that found in bone were utilized to stop the spread of osteosarcoma cells [10].

Another study described the creation of reservoir and matrix nanoparticles type for the transport of the dense extract and oil of *Calendula officinalis* utilizing a cross-linked mixture of collagen and gelatin. In contrast to the limited cytotoxicity they exhibited against healthy keratinocyte cells, both nanoparticles types demonstrated steady discharge and substantial poisonousness against liver and breast tumor cells [11].

Despite the fact that collagen-based medication delivery methods are frequently utilized, either by itself or in combination with other substances, one of their drawbacks is that they are difficult to handle. This has prompted researchers to look for further protein-based delivery systems and collagen-derived proteins for related uses.

3.1 Gelatin nanoparticles

It is a partially hydrolyzed version of collagen that is more water soluble. When cooled, it solidifies into a gel-like structure after dissolving in hot water [12].

Gelatin is an intriguing choice for the administration of medicinal drugs since it is easily modified due to the amine and hydroxyl groups presence. Due to variations in swelling and cross-linking intensity, the kind and origin of the gelatin utilized for drug delivery may have a significant impact on the drug release [13].

The antiglaucoma medication latanoprost was combined with curcumin-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles and encapsulated, as illustrated in Fig. 15.3. Gelatin's temperature sensitivity allowed the formulation to travel to the delivery location while sustained on the ocular surface in an injectable form. The 7-day continuous administration successfully reduced oxidative stress and intraocular pressure, outperforming standard medication administration [14].

Additionally, gelatin has encapsulated inorganic nanoparticles to provide a distinctive structure for therapeutic uses. Gelatin hydrogels were loaded with nanoparticles of cerium oxide whose surface is covered with interleukin-17 (IL-17) aptamer to reduce mouse model brain inflammation. Gelatin helped to shield the aptamers from structural deterioration and alteration, which may be used to treat inflammation-related brain injury [15].

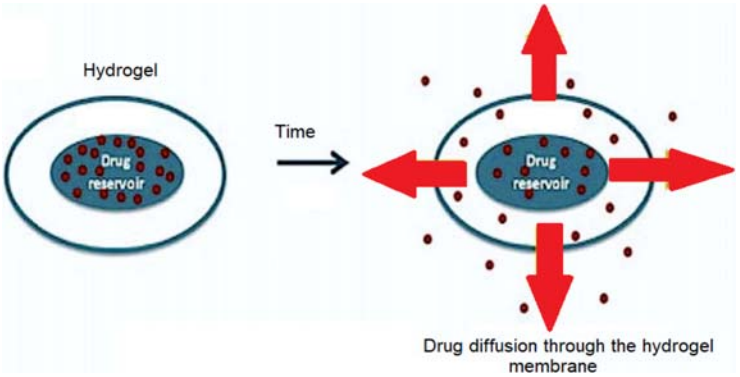

Figure 15.3
Diffusion of the drug throughout the hydrogel structure.

Gelatin and silver nanoparticles were used as an in-situ crosslinking system for the eye's antiangiogenic and antibacterial action. Due to gelatin's thermal reactivity, biocompatibility, and bioadhesion to the ocular surroundings when delivered intrastromally [16], these properties were exploited when administered intrastromally [16].

Erythrocyte cell membranes were used to modify the surface of gelatin nanoparticles to give them a nonimmunogenic nature, which enabled continuous release of the anticancer compound berberine [17].

3.2 Elastin nanoparticles

Elastin is a crucial protein that makes up the extracellular matrix. It allows connective tissues flexibility so they can regain their original shape once a modifying stimulus has been removed [18]. In order to effectively transport cancer drugs to prostate cancer cells, elastin-like polypeptides (ELPs) were combined with docetaxel-uploaded liposomal transporters and surface-adapted with gastric-discharging peptide [19].

Adenovirus entrapment into protein nanoparticles limited the adenovirus in the tumor in contrast to free adenoviral vectors, which demonstrated significant targeting distant organs like the liver as an off-target according to in vivo experiments in mice having neck and head tumor [20]. From the numerous research, it is clear that ELP-based nanocarriers work well as continuous drug discharge storage that might be further discovered for usage in medical fields.

From the numerous research, it is clear that ELP-based nanocarriers work well as continuous drug discharge storage that may be further discovered for usage in medical fields, as shown in Fig. 15.4.

3.3 Fibroin nanoparticles

Fibroin is one of the important proteins in silk. Two types of silkworms, *Bombyx mori* and *Antheraea mylitta*, are normally kept apart from it. The silk fibroin from *Bombyx mori* has a disulphide bond that covalently connects the light chain and heavy chain. The structural integrity of fibroin is preserved by a third glycoprotein chain, P25, which forms strong noncovalent linkages with the light and heavy polypeptide chains [21]. Because fibroin has a high glycine ratio, the polypeptide chains can pack closely together to form a dense, beta-sheet-based structure with a high tensile strength [22]. Fibroin has a high level of biocompatibility and less biodegradation and the capacity for chemical modification, which qualifies it as a potential drug delivery system candidate [23].

Fibroin nanoparticles can now be produced using a variety of techniques. These include supercritical fluid procedures, desolvation, capillary microdot technologies, salting out, and microemulsion [23]. To facilitate target-specific delivery, fibroin nanoparticles, magnetic iron oxide (Fe_3O_4), and the chemotherapy medication doxorubicin were proposed for directed administration to drug-sensitive and doxorubicin-resistant MCF-7 breast cancer cells. The magnetic field effect and the clathrin-mediated method of nanoparticle adoption into the cells were used to validate drug transport to both types of cells [24], as illustrated in Fig. 15.5.

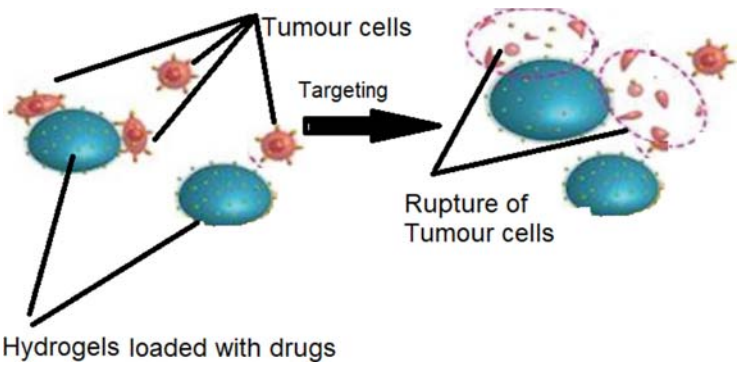

Figure 15.4
Schematic diagram of the tumor cell connection to stimuli-responsive hydrogel. External stimulus such as temperature or pH can cause hydrogels expansion and tumor cells rupture.

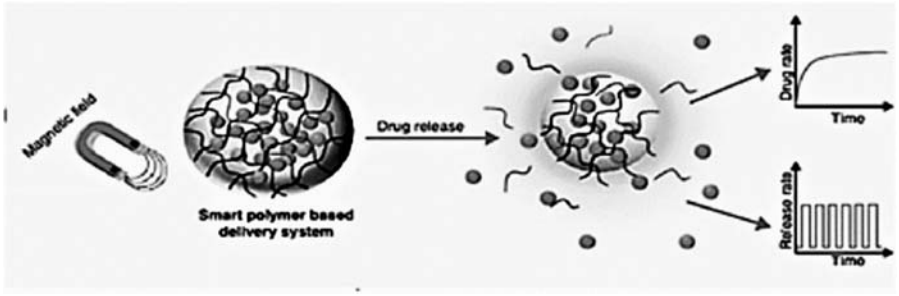

Figure 15.5
Magnetic field stimuli controlling drug delivery.

3.4 Keratin nanoparticles

Nails, hair, horn, feathers, and vertebrate skin all include the fibrous, water-insoluble protein known as keratin [25]. The nonpolar amino acids alanine, leucine, isoleucine, valine, and phenylalanine are abundant in this sequence. It is distinguished by its mechanical strength. Methionine and cysteine, two amino acids that contain sulfur, are also present in significant amounts. Keratin was made more stable by disulfide connections that connected the cysteine residues and extensive hydrogen bond networks [25].

The sequence's nonpolar side chains interact hydrophobically, stabilizing the alpha helical domains that contain more disulfide bonds. Because of its acid tolerance, keratin does not degrade [25].

Keratin has attracted interest for its usage as a medication transport carrier for its low immunogenicity, pH sensitivity, and biocompatibility. Because integrins are overexpressed in tumor tissue. Integrin-bonding motifs in keratin make it easier for it for accumulation, as well as improved permeability and retention [26]. As a result, keratin nanoparticles are an intelligent choice for cancer cell passive targeting.

Keratin nanoparticles created by crosslinking and accumulating were used to contain the anticancer medication doxorubicin. The triple-negative MDA-MB-231 cells were high susceptible to drug-encapsulated protein nanoparticles than other breast cancer cell lines [27].

It has been proposed to use a mixture of keratin and carboxymethyl cellulose to create a sponge that contains the antibacterial compound clindamycin found in halloysite nanotubes. The keratin component controlled the water concentration, stabilized the carboxymethyl cellulose hydrogel, and made sure that the medication was released consistently. The keratin-containing sponge efficiently stopped *Staphylococcus aureus* and encouraged the growth and multiplication of fibroblasts on its surface [28].

These results imply that this sponge has the potential to be used as a wound-healing material. Since its primary benefit in medicine distribution is yet completely untapped for therapy, more study is necessary to fully comprehend the advantages of this protein for therapy.

3.5 Zein nanoparticles

Zein fits to the group of proteins known as alpha prolamines that were first identified from maize [29]. These hydrophobic amino acid remains, which include leucine, proline, glutamine and alanine, are abundant in this group of proteins and help to build alpha helices [29]. The Food and Drug Administration, a governing authority in the United States, has classified it as a generally-regarded-as-safe item [29].

Zein has long been used to cover confectionery ingredients in the food business. Its versatility to create nanoparticles using a variety of processes, including nanoprecipitation, phase separation, and liquid–liquid dispersion, together with its biodegradability and biocompatibility have made it useful as a drug transport system. Researchers have found that zein nanoparticles have an isoelectric point of 6.2, have low colloidal stability, and are more likely to combine at pH levels higher than five and when salt is present [29].

The anticancer medication 5-fluorouracil was enclosed in zein nanoparticles created by phase separation, with an encapsulation efficiency of roughly 60%. When administered to mice using a mouse model, the nanoparticles displayed a strong eruption discharge of more than 50% in phosphate buffer solution at both pH 6.8 and 7.4 and inactively collected in the liver, making it the ideal model for the treatment of hepatic malignancies [30]. For enhancing bioavailability and drug stability, zein nanoparticles have been successfully used to encapsulate a number of phytochemicals, including peppermint oil lutein, gallic acid, quercetin, naringenin, resveratrol, thymol, rutin, mint oil, and luteolin [31].

Zein nanoparticles were used to encapsulate a phytochemical called terpinen-4-ol with a 91% encapsulation rate. Those nanoparticles were evaluated as a probable melanoma therapy strategy [32]. Citronella oil's active components, geraniol and R-citronellal, were encapsulated in zein nanoparticles. These formulations had been suggested as Tetranychus urticae Koch mite pest repellents [33].

Zein nanoparticles can now be used in new ways in the field of agriculture, thanks to the nanoparticles' ability to entrap more than 90% of the active components and improve their durability. When 5-fluorouracil, a chemotherapeutic drug, and ZnS:Mn quantum dots were co-entrapped into zein nanoparticles for theranostic uses, another emerging use of zein was established. The nanoparticles' quantum dots allowed researchers to visualize the

breast tumor cells MCF-7, and 5-fluoruracil showed cytotoxicity depending on concentration [34].

The likelihood of its use in medicine is constrained by the fact that the regular dimension of these particles was around 800 nm, and the nanoparticles were similarly cytotoxic to L929 fibroblasts. For clinical translation, additional surface feature variation is needed. Extremely hydrophobic curcumin was captured using hollow zein nanoparticles. In comparison to their solid equivalents, the method demonstrated improved curcumin encapsulation and preservation. The improved hydrophobic interface between the hollow zein shell and curcumin was to blame for this. The hollow zein nanoparticles demonstrated consistent curcumin discharge in virtual gastrointestinal fluid, indicating that they are promising candidates for both nutraceutical and medication delivery [35].

Numerous drug-loaded nanoparticles have been covered with zein for usage in oral formulations as zein-coated nanoparticles have demonstrated better oral bioavailability. Zein was applied to chitosan nanoparticles that had been loaded with selenoaminoacids in order to increase bioavailability [36]. For oral administration in the *C. elegans* model, zein nanoparticles containing the antidiabetic medication glibenclamide have been reported [37].

3.6 Albumin nanoparticles

A spherical protein called albumin is suitably water soluble. There are numerous sources of albumin, including serum, soybeans, cereals, and milk. Bovine serum albumin (BSA), ovalbumin, and human serum have all frequently been used to isolate it (human serum albumin, HSA). Depending on where it comes from, albumin can range in molecular weight from 47 to 69 kDa [38].

Albumin was used as a drug carrier because of the substantial amount of charged amino acid residues that allowed it to encapsulate polar drug molecules. For the existence of nonpolar amino acid remains in albumin's amino acid sequence, it can also lodge hydrophobic medicines. The albumin network's high number of drug-active sites confirms excellent encapsulation efficiencies [39].

Additionally, albumin may be easily used in a variety of procedures, including emulsion, desolvation, and coacervation techniques, to create nanoparticles. BSA and ovalbumin, which perform gels and respond to changes in pH and temperature, are readily available and hence affordable choices for drug administration [39].

HSA is extensively used as a drug transport agent as it is secure, exhibits great pH and thermal stability, can permeate tissues, and is biodegradable [39]. Medical studies have demonstrated that the very hydrophobic anticancer medication paclitaxel, when encapsulated in human serum albumin, outperforms the free drug in terms of prolonging life time.

This product, known as Abraxane, has been proved to lessen the side effects of free paclitaxel, including sensory neuropathy and neutropenia. It has been discovered that Gp60 and the acidic secreted protein, which are rich in cysteine (SPARC), are typically shown in many types of epithelial malignancies and interfere with the internalization of albumin nanoparticles [40].

Bovine serum albumin was used to more effectively bind the medications doxorubicin and curcumin to B16F10 lung cancer cells, which led to a noticeable decline in the cancer spheroids' vitality and metastatic potential. Studies done in vivo showed that the double drug-loaded albumin nanoparticles had a larger buildup in the lung tumor flesh. This might be as a result of albumin recognizing Gp60 in the cancer cells along with passive targeting brought on by the increased distribution and retention, a typical feature of dense tumors with permeable vasculature and a poorly formed lymphatic linkage [41].

By attaching nanoparticles surface to folic acid and entrapping a curcumin difluorinated derivative, we were able to achieve internalization of the albumin carrier by cancer cells. When compared to the untargeted carrier and the free medicine, the modified nanoparticles demonstrated improved cytotoxicity against SKOV3 cancer cells [42].

3.7 Gliadin nanoparticles

One of the main components of gluten is made up of glutenin and gliadin. Gliadin is a prolamine that contains the amino acids glutamine and proline as its major residues [43]. The shapes of gliadins include α, β, γ, and ω. However, being abundant in rye, barley, and wheat, gliadin had not yet been widely used for medication transport. Its bias against sensitive persons with enteropathy is a significant factor restricting its widespread biological applications [44].

Rare trials to alter gliadin nanoparticles for medication transport purposes had been documented recently. Mostly, gliadin nanoparticles had been created using precipitation in a nonsolvent (antisolvent method) and during their synthesis; both temperature and pH have an effect [45].

Successful candidates for oral delivery, gliadin nanoparticles had been shown to show good mucoadhesiveness in stomach mucosa and had enhanced the encapsulated carbazole bioavailability and consistent discharge [46]. There have also been some gliadin-based oral preparations for the administration of clarithromycin [47] and α-tocopherol [48].

In gliadin hydrophobic matrix, which showed high stability in physiological medium but quickly discharged its payload in the existence of trypsin, all transretinoic acid was successfully trapped, according to early studies on gliadin nanoparticles. Comparing with uncross-linked gliadin nanoparticles, cross-linked nanoparticles with glutaraldehyde

demonstrated improved stability in aqueous conditions [49]. Celecoxib and disomin were co-encapsulated in lactoferrin-conjugated gliadin nanoparticles, which were created for oral transport to target liver tumor cells [50]. Although gliadin nanoparticles have been used to transport drugs with some positive therapeutic results, its bias in some people severely restricts their usage as a nano-carrier.

3.8 Lactoferrin nanoparticles

Milk and mammalian exocrine fluids are the main sources of lactoferrin, a globular protein that binds iron. The transferrin proteins group includes lactoferrin. It is a glycoprotein with a 76–80 kDa molecular weight range. It has been discovered that a high concentration of glycosylated residues increases lactoferrin's stability [51]. It is around 300 times more able than transferrin to bind to iron.

In addition to its vital function in the body's iron transport and uptake, lactoferrin also performs ribonuclease-like functions, promotes bone formation, and slows down bone resorption. Excellent antibacterial qualities are also present in lactoferrin [51]. Through receptor-mediated endocytosis, lactoferrin has exceptional capacity to bypass the obstacle of blood-brain. To successfully transfer medications to the brain, numerous experiments involving the attachment of lactoferrin to drug-burdened nanoparticles have been conducted [52]. The antioxidant curcumin was successfully delivered to neuronal cells using this character of lactoferrin. It was effectively modeled to provide improved neuroprotection for neuronal cells against rotenone-mediated poisonousness using curcumin-loaded lactoferrin nanoparticles [53].

With an extremely high encapsulation efficiency of over 95%, the antioxidant cichoric acid was effectively trapped in lactoferrin nanoparticles and demonstrated high antioxidant action to its nonencapsulated complement [54]. To kill melanoma cells, the chemotherapy drug 5-fluorouracil was contained inside lactoferrin nanoparticles. The encapsulation led to improved internalization and retaining in melanoma cells as well as a potentially effective discharge of the medication at endosomal pH, which improved cytotoxicity [55].

3.9 Legume protein nanoparticles

Legumin is a storage protein found in leguminous plants, such as peas, soy beans, beans, hemp seeds, and lentils, with a molecular weight of between 300 and 400 kDa [56]. It is occasionally referred to as vegetable casein because of its resemblance to the milk protein [56]. Legumes mostly contain residues of cysteine, leucine, glutamic acid, aspartic acid, and tyrosine amino acids. Legumin contains a lot of nitrogen. It has been established that legumin nanoparticles created through precipitation and glutaraldehyde cross-linking are nonimmunogenic and do not cause the development of antibodies.

However, mice developed a strong immunogenic response to the legumin protein. The change in immunogenicity was caused by the loss of antigenic epitopes in the legumin nanoparticles as a result of cross-linking with glutaraldehyde [57]. The discovery that legumin nanoparticles had a cytostatic effect was another intriguing finding in this study. This trait may be valued given that these anticancer drugs are delivered via nanoparticles.

Pea proteins have recently been working to create a robust vitamin D emulsion that has superior penetration in $CaCo_2$ cells, proving the application of these systems for creating formulations that are vitamin- and nutrient-enriched [58].

The benefits and drawbacks of a few protein nanoparticles are listed in Table 15.1 [59].

Table 15.1: Merits and drawbacks of each protein nanoparticles.

Substance	Advantages	Disadvantages
Silk protein fibroin	High firmness malleability High mechanical strength, Matching for various machining conditions Low immunogenicity biodegradability Biocompatibility	Cause immunogenic reactions Slow deterioration
Gliadin	Biocompatibility biodegradability Nonimmunogenicity Nontoxic	Huge particle size Rapid deterioration
Legumin	High firmness Bioadhesive Large surface area Tiny particle size Low immunogenicity	Low yield
Ferritin	High firmness High firmness pH firmness Thermal firmness Biodegradability	High cost
Human serum albumin	High firmness High solubility in physiological fluids Biodegradability Nonimmunogenicity Nontoxic Availability	Costy
Gelatin	Biocompatibility Biodegradability Ease of bridge Safety	Poor mechanical firmness Rapid deterioration

3.10 Lipoprotein

Normal nanoparticles called lipoproteins transport lipids throughout the body [60]. Lipoproteins are an intriguing and practical delivery vehicle due to a number of characteristics. Apolipoproteins that are embedded in phospholipids protect the triglycerides and cholesterol esters in the center of the various types of lipoprotein nanoparticles that are present [61]. Chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), and high-density lipoprotein (HDL; 7−13 nm) are some of the different classes of lipoproteins that can be categorized based on size and density (80−1200 nm) [62].

The size, density, lipid composition, main apolipoproteins, and function of these lipoproteins are characteristics. Typically, a density-based ultracentrifugation technique is used to extract lipoprotein nanoparticles from plasma [63].

4. Fabrication techniques of protein−drug nanoparticles for drug delivery

(1) Chemical approach, which usually employs sophisticated coacervation and emulsion techniques.
(2) A nano-spray drying approach and the electrospray technique.
(3) Desolvation-based self-assembly.

4.1 Chemical method

4.1.1 Emulsion/solvent extraction

Although it has been utilized to produce protein nanoparticles, emulsion/solvent extraction techniques are frequently used to extract polymer nanoparticles. A mixture of two or more incompatible liquids called an emulsion occurs when one or more of the liquids are distributed into the other liquid [64,65]. Using mechanical stirring or sonication, an emulsion (O/W or W/O) is formed by dispersing a polymer solution (in an organic solvent [O]) or protein solution (in an aqueous buffer [W]) and subsequently removing the solvent or nonsolvent to produce nanoparticles. To stabilize emulsion particles, a surfactant and a stabilizer are needed. In a physiological setting, surfactants are expected to have an impact on drug release rates and interactions with drug matrix. Protein nanoparticles require careful management of protein content and the relative volume fraction between the water and oil phases.

4.1.2 Polyelectrolyte complexation/complex coacervation method

By changing specific factors, such as the pH, proteins can be either cationic or anionic because they are amphoteric and have various charged functional groups. Other polymeric electrolytes and the charged protein may interact electrostatically. It is possible to create stable biocompatible nanoparticles using this contact between proteins and other polymers, which is dependent on pH to provide regulated delivery of bioactive therapies [66].

4.2 Physical method

4.2.1 Nanospray drying

The nanospray drying procedure is used to produce nanoparticles in liquid samples. When liquid samples are fired into chambers heated by nitrogen, carbon dioxide gas emerges from the injector in the path of the spray [67]. An electrode for collecting nanoparticles is situated at the bottom of the chamber. These electrodes cause the sprayed droplets to migrate electrostatically charged in the direction of the chamber bottom. Small-scale protein particles can be created using this quick and affordable approach.

Spray drying is employed in drug delivery systems because hydrophilic drugs can be enclosed in these spray-dried nanoparticles. Because solvent evaporation helps to keep the temperature of the nanoparticle droplets, this technique is utilized for materials that are sensitive to heat [68]. The benefit of this technique for nanoparticles creation is the allowance precise control of particle size by altering variables like nozzle size and particle spraying velocity. Surfactants are frequently used to stabilize polymer particles in the case of protein nanoparticles. To stabilize the nanoparticles, the surfactant addition changed the form of the particles into spheres.

4.2.2 Electrospray technique

Utilizing liquid atomization through electrospraying allows for the usage of materials at the submicron scale. In order to create an aerosolized droplet containing protein nanoparticles of colloidal size using this method, significant voltage application is required [69]. Using this technique, it is simple and highly effective to insert medicines and nucleic acids into nanoparticles. Additionally, solvent evaporation can be used to produce solid particles [70]. Depending on the type of medication delivery system, some factors, like the voltage applied, needle gauge diameter, the operating distance, and the flow rate, can be altered [71,72]. Electrospraying has several advantages, including repeatability, low cost, high encapsulation efficacy, and a quick, reliable way to make protein or carbohydrate polymer nanoparticles.

4.3 Self-assembly

4.3.1 Self-assembly

When protein chains are dissolved in a solution with a concentration greater than the critical micelle concentration (CMC) at the critical solution temperature (CMT), protein micelles can be formed [73]. Hydrophobically altered proteins can self-assemble into micelle nanoparticles when introduced to aqueous solutions. Active molecules can also pass through hydrophobic cores, which can behave as a channel.

4.3.2 Desolvation

The most well-known method for creating protein-based nanoparticles is desolvation [22]. By adding desolvating chemicals, like acetone and ethanol, to protein mixtures with pharmaceuticals, the desolvation method creates nanoparticles.

Desolvating chemicals change the structure of proteins and make them less soluble, which causes protein nanoparticles to precipitate. The production of particles occurs as the particle size grows and is accomplished by gradually increasing the quantity of particles of the same size [22].

Crosslinking chemicals like glutaraldehyde GA are used to crosslink nanoparticles after they have produced. By using the desolvation approach, protein nanoparticles can regulate particle size in accordance with reaction conditions [74]. The main factors affecting particle size are the protein content, desolating agent, additive rate, reaction pH, and reaction temperature. Smaller nanoparticles can be created under conditions of high pH and low protein concentration.

4.4 Characterization of protein nanoparticles

4.4.1 Polydispersity and particle size

For nanoparticle systems, the two most important characteristics are particle size and size distribution [22]. Many researches have confirmed that when it comes to applications involving drug delivery, nanoparticles offer significant advantages over microparticles. Since they exhibit significantly more intracellular absorption due to their tiny sizes when matched with microparticles, and since they are comparatively moveable, nanoparticles are typically used in a wide variety of biological agents [74]. According to a recent study, the capillary size of the spleen causes nanoparticles larger than 230 nm to concentrate there. Other research revealed that the size of the nanoparticles also affects how well they penetrate cells.

According to studies, particle size has an impact on medication release. Drugs release quickly on the surface of or close to tiny particles because they have a bigger surface area.

Larger particles, however, can encase more medications and release them more gradually. Additionally, smaller particles suffer greatly from agglomeration when nanoparticle dispersion is stored or transported [75].

4.5 Particle morphology

Electronic and medicinal applications can be created by utilizing a substance's physical and chemical properties at the nanoscale [76]. Understanding whether nanoscale materials show adverse effects, such as toxicity, is crucial. To fully understand the outcomes from cell culture and animal models, nanomaterials' toxicity should be characterized along with their physical and chemical features.

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) are two techniques for observing the structure of nanoparticles [77–79]. An extremely high-resolution scanning probe microscope with a resolution that is around 1000 times better than the optical diffraction limit is an AFM or scan force microscope (SFM).

The surface of the sample is scanned by a high-intensity electron beam in an SEM, an electronic microscope, in order to examine surface photos of the sample. A signal containing sample data, including composition, electrical conductivity, and surface topography, is produced as a result of the electron's interaction with the sample atoms.

4.6 Surface charge

By assessing the surface charge, density, and hydrophilicity, it is possible to anticipate how effective the surface alteration will be. Zeta potential measurements of nanoparticles in aqueous solutions are the most typical method for determining the surface charge.

The polydispersity index is another tool for gauging nanoparticle distribution. The stability of colloidal solutions is significantly influenced by particle contact.

The stability of particle interactions is also predicted by the zeta potential measurement [78]. Zeta potential is a metric for particle interaction. Additionally, most colloidal systems that are water-soluble are stabilized via electrostatic repulsion; the stronger the repulsion, the less close the particles are to one another [80].

5. Conclusions

Anticancer medications, genetic materials, growth factors, peptide hormones, DNA, and RNA are just a few of the compounds that can be delivered using protein nanoparticles as carriers. In comparison to other carriers, protein nanoparticles have the merits of more stability and simple fabrication. Fibroin and albumin are the two proteins that are most

frequently used for medication delivery applications. On the other hand, research on the utilization of proteins and legumin has started to establish whether or not they are used in applications for drug delivery.

The nanoparticle synthesis procedures often employ processes like desolvation and complicated coacervation. While other physical and chemical techniques also have some drawbacks like low rates of flow or the requirement for surfactants removal, nanospray drying is still relatively new.

Therefore, more research should be done to get beyond these limitations. By adjusting characteristics including size, shape, and surface charge, the effectiveness of protein transfer can be developed by protein nanoparticles.

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