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Review Article

Nanoparticles in therapeutic applications and role of albumin and casein nanoparticles in cancer therapy

Amrit Rai, Josphine Jenifer, Ravi Theaj Prakash Upputuri
PG Department of Biosciences, CMR Institute of Management Studies, Bangalore-560043, Karnataka, India

Background: Nanoparticles are widely used for various therapeutic treatments. Proteins have the advantage of being naturally occurring, with relative lack of toxicity and antigenicity, but easy biodegradability and potent ability to bind other compounds. Proteins have been produced in nanoformulations, which have been made by various methods including desolvation and precipitation. Biopolymeric nanoparticles including proteins are used in delivery of genes and drugs, and in tissue engineering. Protein nanoparticulate drug delivery systems are used to treat several diseases because of their high drug-binding capacity, biocompatibility, nutritional value, stability, and potent site-specific action. Among the various proteins, casein and albumin nanoparticles are used widely as nanocarriers for various therapeutic drugs.
Objectives: To review the types of nanoparticles and their role in therapeutic applications, and the important role of casein and albumin nanoparticles in cancer therapy.
Methods: A literature search based on nanoparticles, their types, and their roles in medical applications was conducted using websites including Google Scholar, SpringerLink, ResearchGate, and the U.S. National Institutes of Health's National Library of Medicine's National Center for Biotechnology Information PubMed database including PubMed Central covering from 2000 to 2016.
Results: Nanoparticles, especially albumin and casein had been found effective drug delivery systems.
Conclusion: Protein based nanoparticles such as albumin and casein nanoparticles are widely used for targeting cancers and can be used as effective vehicles for the delivery of various anticancer drugs.

Keywords: Albumin nanoparticles, casein nanoparticles, coacervation, nanoprecipitation, protein nanoparticles

The word "nano" is derived from the Greek word "nânos" meaning dwarf [1]. The prefix "nano-" has had ever-increasing applications to the spectrum of knowledge [2]. Nanoscale particles are composed of natural, synthetic or semisynthetic materials such as proteins, metals, and plant secondary metabolites. Nanospheres are nanometric solid-core spherical particulates often containing a drug embedded within the sphere or absorbed onto its surface [3]. Engineered nanoparticles are intentionally designed and have been created with physical properties tailored to meet the needs of specific applications such as drug delivery to various parts of the body [4]. Particles with a size ranging below 10 nm are of extreme interest because the physical and chemical properties of the particles arising from the quantum size effect confers on them great potential for use in applications in the electronic, chemical, and mechanical industries; technologies related to using catalysts, drugs, sensors, pigments; and in magnetic and electronic materials [5]. Pharmaceutical nanoparticles are defined as solid, submicron-sized materials that act as a drug carrier, which may be biodegradable, or not [2]. One of the vital applications for nanoparticles is in the field of cancer treatment for which several new nanoparticles have been synthesized and marketed [6]. The major aims of designing nanoparticles as a delivery system are to control particle size, surface properties, and ability to release pharmacologically active agents to achieve the site-specific actions of drugs at a therapeutically optimal rates and dosage regimens [5, 6]. To understand the enhanced interactions between nanoparticles and biological fluids, detailed studies have been performed in vivo, and in vitro on tissues, cells, and proteins [7]. Many biological processes take place at a nanoscale level. A combined application of biology and nanotechnology can perhaps meet challenges such as compatibility with the body metabolism [7, 8]. In a biological medium, nanoparticles may interact with biomolecules such as proteins,

Correspondence to: Ravi Theaj Prakash Upputuri, PG Department of Biosciences, CMR Institute of Management Studies, Bangalore 560043, Karnataka, India. E-mail: ravi.theaj@cmr.ac.in

nucleic acids, lipids, and metabolites because of their nano size and large surface-to-mass ratio [9]. Low concentrations of drug-loaded nanoparticles are found to be nontoxic to the human cells, and considered safe as antimicrobial agents [8, 9].

This narrative review covers nanoparticles that have been widely employed as carriers to enhance the therapeutic efficacy of various anticancer drugs, and their role in targeting cancers.

Type of nanoparticles

Liposomes

Liposomes are tiny synthetic spherical vesicles that can be constructed as lipid bilayers by incorporating cholesterol and phosphatidyl choline, which reduces the leakage of contents from liposome nanoparticles. Because of their size and amphiphilic nature, liposomes are well suited for drug delivery. Liposomes can be used as carriers for numerous types of molecules, for example to encapsulate antioxidants and biotic elements in drug delivery systems [9]. Liposome nanoparticles made from lipids or polymers could be constructed to improve the pharmacological and therapeutic potential of drugs [9, 10]. Liposomes, can also be used as imaging agents [11]. The imaging moiety together with a drug-delivery function creates a theranostic agent (with combined diagnostic and therapeutic capabilities) [9, 11]. An early observation was that it is difficult to retain some types of molecules entrapped in the liposome interior [12].

Cyclodextrins

Cyclodextrin nanoparticles are a new approach to macromolecular drug delivery systems comprising chitosan and carboxymethyl-β-cyclodextrin. This drug-delivery system is fundamental to oral pharmaceutical formulations incorporating the formation of drug complexes, with advantages for the drugs including enhanced solubility, dissolution, stability, bioavailability, and reducing or avoiding gastrointestinal damage, and an unpleasant smell or taste [13]. Nanoparticles in the range of 231–383 nm showed a positive ζ potential ranging from +20.6 to +39.7 mV, and were stable in simulated intestinal fluid of pH 6.8 at 37°C for at least 4 h, which demonstrates excellent biocompatibility and a unique ability and ultimate capacity to control drug delivery [14]. Cyclodextrins, with lipophilic interior cavities and hydrophilic exterior surfaces, are able to interact with various types of guest molecules to form noncovalent inclusion complexes. Chemically they are cyclic oligosaccharides have 6 D-(+) glucopyranose units linked by α-(1, 4) glucosidic bonds [13, 15]. The utility of cyclodextrins based on cyclodextrin complexation and nanotechnology would help to camouflage the unwanted characteristics of the encapsulated drug and lead to synergistic or additive effects, and would produce better therapeutic outcomes by prolonging the life of healthy cells [14, 15, 16]. A recent outcome in processing amphiphilic cyclodextrins includes biodegradable methoxy poly-(ethylene glycol)-poly-(lactide) nanoparticles for controlled delivery of dacarbazine [16].

Dendrimers

Dendrimers are typically globular macromolecules with a tree-like architecture ranging from 1.5 to 10 nm that are synthesized using a template approach with dendrimers that later initiate and direct the extension of a nanometric crystals within the framework of an encapsulating host. Gold–dendrimer nanocomposites have been synthesized using a simple colloidal approach based on utility of polyamidoamine dendrimers with succinamic $[(CH_2)_2(CO_2H)]$ acid terminal groups and dodecanediamine core. Nanoparticles with a spherical and highly crystalline structure, dimensions ranging from 3 nm to 60 nm, and polydispersity depending on the synthesis conditions, have been produced to enhance the dendrimer drug-delivery system [17]. The ability of such nanocomposite particles to induce calcification when coating a polymer substrate has also been investigated [17, 18]. Dendrimers have multiple types of functionalized groups at their periphery that have been proven to be effective against HIV as nonspecific microbicides [18]. A few dendrimers have presented a dual nature mechanism, such as four-generation polyamidoamine (PAMAM) branched naphthalene disulfonic surface groups ending in SPL2923 or polysulfonate dendrimer BRI2923, which prevent not only the entry of HIV-1, but also later steps in the reverse transcriptase/integrase phases of the viral replicative cycle [17, 18]. The delivery of drugs using such nanoparticles helps to enhance the therapeutic effectiveness of the drugs and reduce adverse side effects associated with high dosage by improving their pharmacokinetics [19].

Metal nanoparticles

Metal nanoparticles have been used extensively

in various biomedical applications because of their small size-to-volume ratio and excellent thermal stability [20]. Drug delivery based on such nanoparticles have shown to be a promising strategy for treatment of malignant brain tumors [20, 21]. Functional derivation of gold nanoparticles with carboxyl and alcohol groups can allow their conjugation with antibodies, for example against *E. coli* O157:H7 [20]. Gold nanoparticles have been incorporated into immobilization of enzymes to offer an inert and biocompatible system [20-24]. Gold nanoparticles have been synthesized and functionalized because of their low toxicity and ease of detection facilitating their potential applications in the field of medicine and biology [22, 23].

Carbon nanotubes

Carbon nanotubes are an allotrope of carbon in the form of a cylindrical graphene nanostructure and have unique properties that are useful in a wide variety of applications such drug delivery. Based on their property and structure, they can be classified into a single walled (0.4–3 nm), double walled (1–3 nm), and multiwalled (2–100 nm) carbon nanotubes [24]. Carbon nanotubes are sufficiently small enough to penetrate through the holes in tumors or to transport DNA, and their large surface-to-volume ratio imparts a good platform for efficient translocation of drugs and for ultrasensitive glucose detection [24]. Carbon nanotubes can be functionalized with bioactive peptides, proteins, nucleic acids, and drugs, and used to deliver their cargos to cells and organs [24-26]. Initially, carbon nanotubes were capped for drug loading and essentially two approaches were included in filling carbon nanotubes, i.e. during synthesis or after synthesis [24, 25]. An application of carbon nanotubes is to create new conjugates with highly promising and enhanced pharmacological profiles [25] and their intrinsic physicochemical properties enable covalent and noncovalent binding of several therapeutic drugs, and provide potential candidate nanoscale structures for drug development [24-26].

Quantum dots

A quantum dot is a nanoparticle composed of any semiconductor material such as silicon, cadmium selenide, cadmium sulfide, or indium arsenide [27, 28]. One of the most remarkable advantages of using quantum dots in drug delivery is that they can be traceable and provide the ability to elucidate pharmacokinetics and pharmacodynamics in drug candidates [27]. Quantum dots have the ability to enhance the efficiency of solar cells because of their ability to convert light photons to electrical energy [27-29]. Quantum dots are also known as semiconductor nanocrystals and have become an indispensable tool in biomedical research, especially for multiplexed, quantitative, and long-term fluorescence imaging and detection [27, 29]. Quantum dots loaded with therapeutic drugs can be decorated with protein, peptide, or other biomolecule pendants to allow loaded drugs to interact functionally within living systems. They have a tremendous impact on the enhancement of a wide range of fields including catalysis, computing, photonics, energy, and medicine. Quantum dots have found a variety of uses in vitro and in vivo for the delivery of the drugs [27]. They are considered to have great potential as novel molecular probes for both carcinoma imaging and targeted delivery, and may ultimately play a role in cancer diagnosis and therapy [27, 29].

Polymeric nanoparticles

Polymeric nanoparticles are solid colloidal particles with a diameter ranging between 1 and 1000 nm, having a major application in drug delivery and drug targeting. They offer a few specific advantages over liposome nanoparticles [30, 31]. Biodegradable or polymeric nanoparticles have been used as drug delivery carriers because of their enhanced bioavailability, encapsulation, controlled release, and less toxic properties enhancing the therapeutic value of encapsulated drugs [30]. Polymeric nanoparticles are used in multiple performance materials such as high impact resistant polymers and for encapsulation [31]. Highly advanced analytical techniques and computer simulations allow us to measure structure and develop control strategies to produce structured particles [30, 31]. Polymers in so-called “smart” drug delivery systems are rapidly developing various new solutions for therapeutic interventions using nano products [32]. Drugs encapsulated in polymeric nano systems could provide sustained controlled release of both hydrophilic and hydrophobic drugs minimizing unwanted side effects by avoiding peaks of drug concentrations, while providing adequate dose [31, 32]. The main function of biodegradable polymeric nanoparticles for controlled drug delivery has important therapeutic potential [31, 33]. Targeted polymeric nanoparticles could be applied to deliver chemotherapies to tumor cells with maximum efficacy and minimizing cytotoxicity on healthy tissues [31-33].

Polymeric protein nanoparticles

Albumin

Albumin can be used as a nanocarrier, and nanospheres and nanocapsules of this protein are biodegradable [6]. Many drugs and endogenous molecules bind to albumin [9, 10], which acts as a depot and transporter protein [8]. The high solubility of albumin (up to 40% w/v) at pH 7.4 makes it an attractive macromolecular carrier, which has the ability to accommodate a wide variety of drugs. Albumin is stable at a pH range of 4 to 9 and has thermal stability of up to 60°C when heated for 10 h without any deleterious effects [8, 10]. Albumin is mostly used to prepare nanospheres and nanocapsules [6]. Aspirin (acting as an inflammatory and antiplatelet agent) and curcumin (with presumed anticancer properties) are a couple of drugs that have been encapsulated within albumin nanoparticles, which with their properties of bioacceptability and biodegradability, serve as an attractive vehicle for drug delivery [34].

Gelatin

Gelatin is a proteinaceous material used for the preparation of nanoparticles [6-8]. The most widely used gelatins are animal proteins obtained by the controlled hydrolysis of collagen, the major component of the skin, bones, and connective tissues [6, 9]. The two main types of gelatin are Gelatin-A and Gelatin-B, which are produced by either acid or base hydrolysis, resulting in proteins with a different isoelectric point (pI), molecular weight, amino acid composition, and viscosity [7, 9, 10]. Microspheres and nanoparticles of gelatin could be used as drug-delivery vehicles with the advantages of being inexpensive and having low antigenicity. Gelatin can be modified using crosslinking agents such as glutaraldehyde, which provides gelatin with stability and an appropriate shape for drug delivery [6]. Two different molecular weight types (4 kDa and 20 kDa) of fluorescein isothiocyanate dextran have been studied to determine the drug loading efficiency and in vitro release behavior of gelatin nanoparticles [35].

Gliadin and legumin

Gliadin is a gluten protein found in wheat that has bioadhesive properties, and has been explored for oral and topical drug delivery applications [8]. Nanoparticles of gliadin exhibit a tropism for upper gastrointestinal regions [6]. Gliadin has been identified as a polymer for the preparation of nanoparticles with the potential to adhere to mucus membranes, and is attractive because of its biodegradability, biocompatibility, and natural origin [8-10]. Its hydrophobicity and solubility allow the design of nanoparticles capable of protecting loaded drugs and controlling their release [12]. Proteins of this sort have neutral and lipophilic amino acid residues that promote hydrogen bonding with the mucosa, and lipophilic residues that can interact with biological tissues through hydrophobic interactions [6, 8, 12]. Cyclophosphamide, an anti-breast cancer drug has been loaded onto gliadin nanoparticles. Breast cancer cells cultured with the cyclophosphamide bearing nanoparticles for 24 h became apoptotic, as confirmed by western blotting [36]. Legumin is an albuminous storage protein in pea seeds (*Pisum sativum* L.), which has the ability to form self assembled nanoparticles after clustering or chemical crosslinking with glutaraldehyde [6]. Methylene blue has been used as a model for hydrophilic drugs and could be loaded to about 6.2% within legumin to study the potential release of drugs from the legumin nanoparticles [37].

Zein

Zein is a prolamine-rich protein that is comprised of a high proportion of hydrophobic amino acids, proline, and glutamine [6]. This protein can be extracted from proteinaceous bodies of the endosperm of the corn kernel [6, 8]. The main applications of such proteins are for films and coatings. Zein is a GRAS (generally recognized as safe) polymer approved by the U.S. Food and Drug Administration for human applications such as for drug delivery systems [6, 9]. Nanoparticles of zein proteins have been prepared to encapsulate various drugs, such as ibuprofen and bioactive compounds such as 5-flurouracil, which serves as a drug for esophageal, stomach, pancreatic, breast, and cervical cancers [6, 38].

Milk proteins

These involve two major proteins, beta lactoglobulin (BLG) and casein. BLG has a gelling property, and is resistant to peptic digestion, so it could be used in drug delivery systems. Casein micelles are used as nanovehicle carriers to deliver calcium and amino acids from mother to offspring. These carriers are unstable at unphysiological temperature, pH, and water activity. The micelles contain 10 to 100 casein molecules and are held by hydrophilic and hydrophobic

domains, which favors the changes in conformation. These proteins are used in drug delivery systems based on hydrogels and stabilizers. [6]. Casein nanoparticles when loaded with putative anti-cancer drugs such as curcumin serve as a potential drug delivery system for cancer treatment [39].

Albumin and casein nanoparticles

Compared with other nanoparticles, protein nanoparticles have been widely used in drug delivery applications because of their biocompatibility, low antigenicity, and enhanced bioavailability. The major advantages of using casein and albumin nanoparticles is that various types of drugs can be encapsulated by simple modifications, achieving their controlled and sustained release. Therefore, this present review mostly considers casein and albumin nanoparticles as promising tools for delivery of various anticancer drugs.

Preparation

Several methods have been employed for the preparation of casein and albumin nanoparticles. Some of the methods most frequently used are discussed following.

Coacervation

Coacervation or electrostatically-driven liquid–liquid phase separation is used to prepare nanoparticles of casein, which is soluble in alkaline solution, and albumin, which is soluble in water. These proteins are treated with solvents such as ethanol and acetone to form nanoparticles that are cross-linked by adding glutaraldehyde or glyoxal. Several factors affect the size of the particles, including the amount of solvents used during their synthesis, higher pH, which produces smaller particles, while higher salt concentrations neutralize the charges on particles causing clumping [6]. For albumin nanoparticles, acetone and ethanol are used as solvents to produce crosslinked particles, which form a separate phase then stabilized by coating the surface of particles with polylysine. Hydrochloric acid and γ-rays are also used to crosslink and generate particles [6, 40]. The mechanism for crosslinking is depicted in **Figure 1**, and the mechanism for coacervation in **Figure 2**.

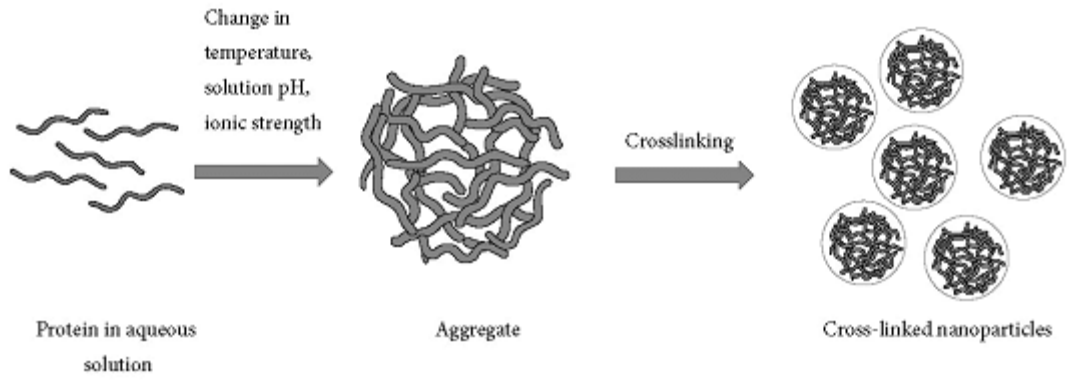

Figure 1. Protein nanoparticles formed by a crosslinking procedure. Reproduced from Warangkana Lohcharoenkal, Liying Wang, Yi Charlie Chen, and Yon Rojanasakul. Protein nanoparticles as drug delivery carriers for cancer therapy. BioMed Research International. 2014, Article ID 180549, 12 pages. http://dx.doi.org/10.1155/2014/180549 (reference [6]) under a Creative Commons Attribution 3.0 Unported (CC BY 3.0) license.

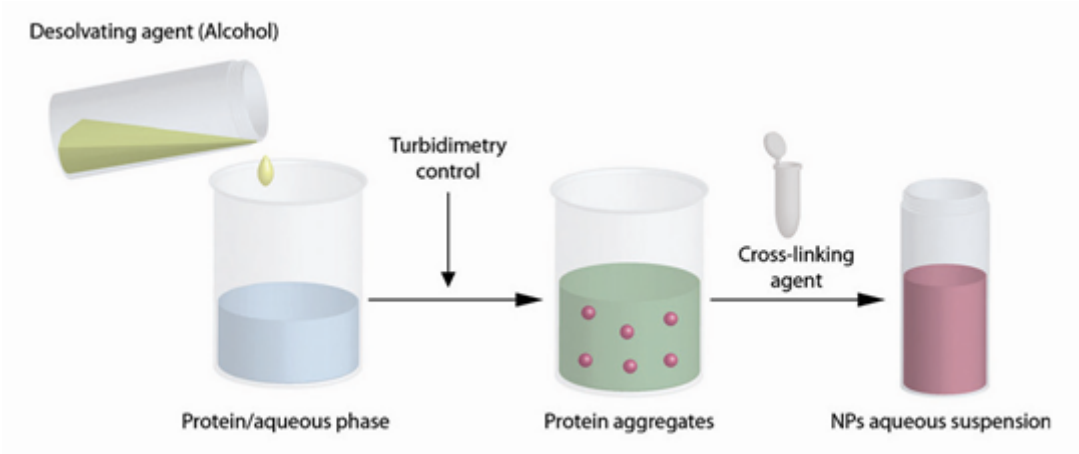

Figure 2. Process involved in coacervation for the preparation of nanoparticles and crosslinking the particles using glutaraldehyde. Reproduced from Julien Nicolas, Simona Mura, Davide Brambilla, Nicolas Mackiewicz and Patrick Couvreur. Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. Chem. Soc. Rev. 2013; 42(3):1147-1235 (reference [40] http://dx.doi.org/10.1039/C2CS35265F) with permission of The Royal Society of Chemistry. © The Royal Society of Chemistry 2013.

Emulsion

Protein nanoparticles are prepared in the form of a nanosphere by suspending them in oil by using homogenizer at a high speed or ultrasonic shear, forming the particles at the water–oil interface of the solution. Surfactants such as Span 80, phosphatidylcholine, or sodium dodecyl sulfate are added to stabilize the particles [6]. Protein nanoparticles formed using an emulsion method are crosslinked by adding glutaraldehyde (chemical crosslinking) or particles could be crosslinked by thermal-based crosslinking at 60°C for 20 min and then the solution centrifuged, and pellets washed with ethanol in the case of certain proteins like albumin or whey proteins [41]. The particle size was found to be larger than that for particles obtained in the coacervation method [42]. The emulsion process involves two steps, emulsion diffusion is the first, in which polymer (protein) was completely dissolved in solvent (oil), at high shear stress, into which drug was added, then a stabilizer solution (sodium dodecyl sulphate) is added, and mixed well using a magnetic stirrer. The particles are obtained from the aqueous phase by nanoprecipitation. The process involved in emulsion diffusion method is shown in the **Figure 3.** The second step is emulsion solvent evaporation, the protein (casein and albumin) is dissolved in appropriate solvents (e.g., chloroform or methanol), then the protein solution is mixed with the surfactant under high-shear force (on a magnetic stirrer) to form stable emulsified droplets. The organic solvent present in the droplets is removed under vacuum. Nanoprecipitation then occurs, forming the nanoparticles [42]. The scheme for evaporation method is shown in the **Figure 4.**

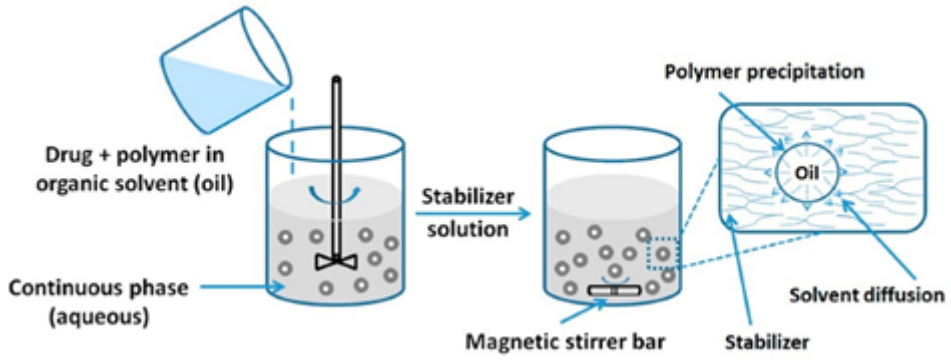

Figure 3. Emulsion diffusion method (protein encapsulated into the oil diffuses out and found in the outer water phase, to form harder particles). Reproduced from Yichao Wang, Puwang Li, Thao Truong-Dinh Tran, Juan Zhang, and Lingxue Kong. Manufacturing techniques and surface engineering of polymer based nanoparticles for targeted drug delivery to cancer. Nanomaterials 2016; 6(2):26 (reference [42] http://dx.doi.org/10.3390/nano6020026) under a Creative Commons by Attribution 4.0 (CC-BY 4.0) license.

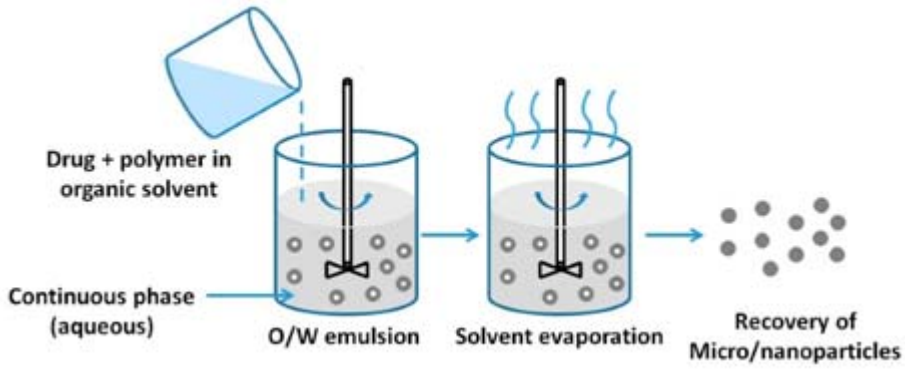

Figure 4. Emulsion evaporation method (emulsification of protein solution into water (O/W emulsion is oil-in-water) and removal of solvent from the polymeric solution). Reproduced from Yichao Wang, Puwang Li, Thao Truong-Dinh Tran, Juan Zhang, and Lingxue Kong. Manufacturing techniques and surface engineering of polymer based nanoparticles for targeted drug delivery to cancer. Nanomaterials 2016, 6(2), 26 (reference [42] http://dx.doi.org/10.3390/nano6020026) under a Creative Commons by Attribution 4.0 (CC-BY 4.0) license.

Electrospray

This process consists of a setup in which a protein solution is taken up in a syringe that is connected to an electrode from a high-voltage power supply. An oppositely charged metal foil electrode collector is placed opposite the syringe nozzle. When the solution enters the electric field from the nozzle of the syringe, it forms a tailored cone because of the surface tension. The cone breaks into droplets in the high electric field, and at selective condition the droplets reach the micro or nano level. These particles can be used for drug delivery systems [43]. The process involved in electrospray method for nanoparticle preparation is shown in **Figure 5.**

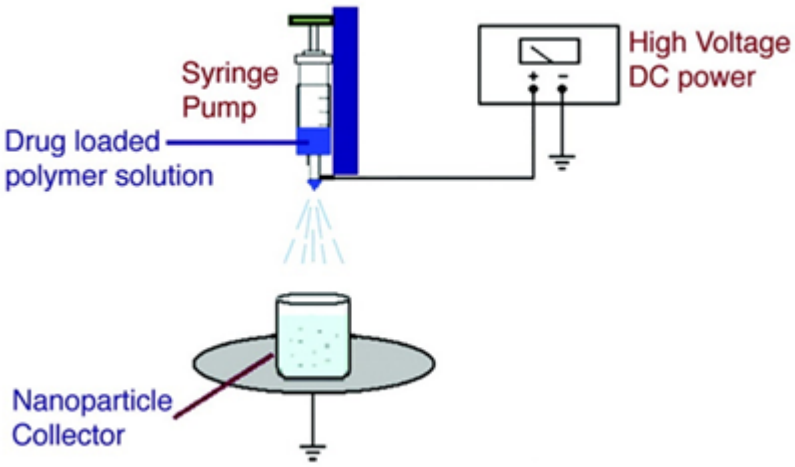

Figure 5. Electrospray method produces solid particles by solvent evaporation. Reproduced from Radhakrishnan Sridhar and Seeram Ramakrishna. Electrosprayed nanoparticles for drug delivery and pharmaceutical applications. Biomatter. 2013; 3(3): article e24281, (reference [43] http:// dx.doi.org/10.4161/biomatter.24281) under a under a Creative Commons Attribution-Non Commercial 3.0 Unported License.

Nanoprecipitation

The materials required for the nanosphere production are polymer (natural proteins like casein, albumin and enzymes), solvents for the polymers such as ethanol, acetone, hexane, and methylene chloride; and detergents [45]. Doxorubicin-loaded albumin nanoparticles were synthesized by nanoprecipitation [46]. The nanoparticles were synthesized by a rapid diffusion of the protein solution with the solvents, which results in surface tension at the interface between two liquids causing an increase in surface area, and leading to precipitation of protein nanoparticles. The process can take place in both the presence and absence of surfactants. The process is simple, rapid, and reproducible [47, 48]. The steps involved in nanosphere formation are shown in the **Figure 6.**

Complex coacervation

Proteins are amphoteric in nature, so their nanoparticles are prepared by first adjusting the pH to anionic or cationic (above or below the pI) charge. The charged protein binds with the polyelectrodes by electrostatic attraction, which are used to facilitate the DNA/oligonucleotide entrapment into nanoparticles. Albumin-polyethyleneimine–DNA nanoparticles are synthesized by combining albumin solution with polyethyleneimine and coacervation process is conducted by adding sodium sulphate solution containing DNA, the nanoparticles formed were stabilized by cross-linking with 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) to form particles in the range 300 to 700 nm [6, 50]. The mechanism involved in the complex coacervation process for DNA encapsulation into the particles is shown in **Figure 7**.

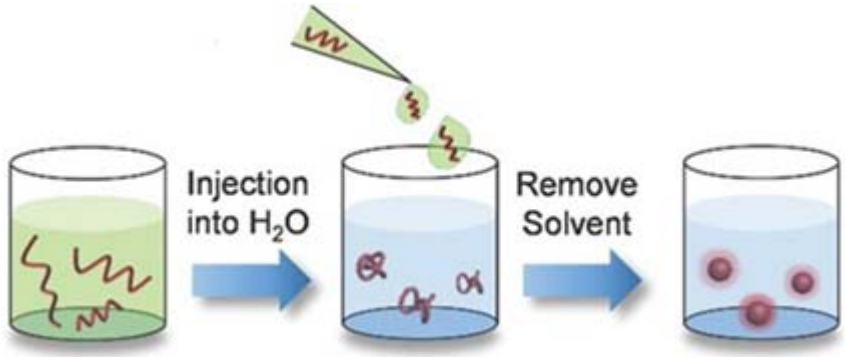

Figure 6. Nanoprecipitation method. The polymer solution is dissolved and injected into water under sonication, the nanoparticles are separated from solvents by evaporation (90°C). Reproduced from Yang-Hsiang Chan and Pei-Jing Wu. Semiconducting polymer nanoparticles as fluorescent probes for biological imaging and sensing. Part. Part. Syst. Charact. 2015; 32:11-28 (reference [49] http://dx.doi.org/10.1002/ppsc.201400123) with permission from John Wiley and Sons © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

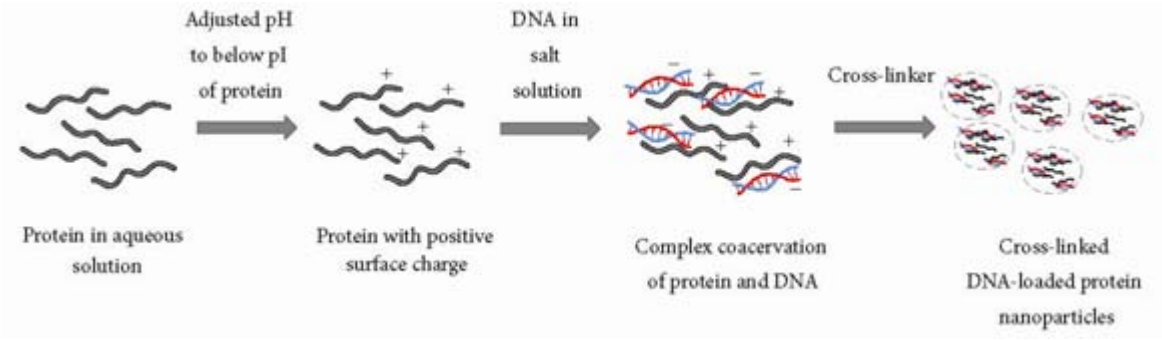

Figure 7. Complex coacervation method procedure and complex binding to DNA strand to form crosslinked nanoparticles. Reproduced from Warangkana Lohcharoenkal, Liying Wang, Yi Charlie Chen, and Yon Rojanasakul. Protein nanoparticles as drug delivery carriers for cancer therapy. BioMed Research International. 2014: article ID 180549, 12 pages. http://dx.doi.org/10.1155/2014/180549 (reference [6]) under a Creative Commons Attribution 3.0 Unported (CC BY 3.0) license.

Dialysis

This method is similar to nanoprecipitation, but a dialysis membrane with a molecular cutoff was used for nanoparticle preparation. The process that occurs during dialysis is that the nonsolvent portion of the polymers are mixed with solvents to form a homogenous mixture, which is then transferred into a membrane to enhance the gradual loss of solubility of proteins leading to the formation of nanoparticles (in suspension). The solvents used for the protein nanoparticle synthesis by dialysis affect the size and structure of the nanoparticles. This process is mostly used in encapsulation of protein into the polymer [44, 47]. The different methods involved in protein nanoparticle synthesis are illustrated in **Table 1**.

Casein and albumin nanoparticles as drug carriers

Biopolymeric nanoparticles are used in delivery of molecules such as genes, drugs, and also in tissue engineering. They are stable, have nutritional value, biocompatible, of low toxicity, have a natural mechanism of degradation, and they have a high binding capacity for therapeutic drugs [53-56]. Protein-based drug delivery systems are used therapeutically in medicine [57, 58]. These delivery systems not only protect drugs from proteases and other enzymatic activity in the gastrointestinal tract, but also enhance the bioavailability of drugs, which are encapsulated in protein nanoparticles [58]. Casein is inexpensive, nontoxic, nutritional, readily available, and stable. It is a safe biocompatible and biodegradable protein. The biocompatibility of casein is noteworthy for oral delivery applications. Casein nanoparticles are easily digestible, excellent in emulsification, easily bind with the molecules or ions, and act as a shield against radiation. Because of these properties, casein is an ideal protein for drug delivery systems [53, 59-61].

Casein, along with oleic acid is used as a drug-loaded film for the immediate release of drug within the system, but the drug release rate decreases when tensile strength is affected by thermal treatment. Therefore, casein nanoparticles could potential be used as a film for tablet outer coatings [62, 63]. The phenytoin release rate was less when it was present in a film containing sodium caseinate and microcrystalline cellulose, than in the intact powder state [64]. Casein micelles are used to deliver various nutrients such as calcium, phosphate, and vitamins. Casein micelles encapsulated with nutraceutical compounds (fat-soluble vitamin D_2) have been prepared by nanoprecipitation and were found to enhance calcium metabolism in the body, the micelles protected the vitamin from photochemical degradation. The vitamins concentration loaded into micelles was found to be 5.5 times more than the free form of vitamin in the body [61].

Casein nanoparticles were used in delivery of lipids like docosahexaenoic acid (DHA), which is an ω-3 polyunsaturated fatty acid found to provide protection against certain cardiovascular disorders. A study of the DHA loaded casein nanoparticle showed that the lipids had high affinity to casein nanoparticles. DHA-loaded casein nanoparticles were prepared by addition of dissolved DHA (ethanol was as a solvent) into casein. The addition of calcium and phosphate to the nanoparticles produced reformed DHA-loaded casein nanoparticles. The particle size (288.9 ± 9.6 nm) remained the same even after thermal treatment at 74°C. These nanoparticles had a potent protective activity against DHA oxidation and particles conserve the biological effect of DHA in the body [65]. Controlled release of phenytoin sodium from casein microspheres was enhanced, with a high encapsulation efficiency of 76.5%. Nanoparticles

Table 1. Various methods involved in synthesis of protein nanoparticles

Synthesis No.	Method	List of protein nanoparticles synthesized	References
1.	Desolvation/ Coacervation	Casein, albumin (HSA, BSA), beta-lactoglobulin and elastin derived particles	[6, 48]
2.	Emulsion	Albumin (HSA, BSA), whey protein	[6, 48, 44]
3.	Electrospray	Gliadin, elastin-like peptide, albumin and gelatin	[6, 43]
4.	Nanoprecipitation	Gliadin and some enzymes like chymotrypsin, lysozyme	[51, 52]
5.	Complex Coacervation	Gelatin and human serum albumin	[6]
6.	Dialysis	Lysozyme, therapeutic protein (TGF-β1)	[47]

were found to be stable in simulated gastric fluid with release of less drug and sustained release of drug in the brain [66]. Magnetic drug targeting was found to target drugs specifically to the specific sites in the body. Particle uptake increased when metallic particles were encapsulated into casein nanocarriers, preventing rapid clearance of drug from the body. Iron oxide impregnated casein nanoparticles have been synthesized by in situ precipitation in alkaline medium, causing nanoparticle swelling and were analyzed for the intake of water. Swelling of nanoparticles was found to increase and decrease alternatively at various concentrations, pHs, and temperature. The tumescence of the particle increased when an external magnetic field was applied [67].

Metformin hydrochloride (MET) is used to treat Type II diabetes. In its free form, it has a low bioavailability and often the drug has to be administrated at regular intervals. On encapsulation of MET into casein micelles, release of drug was controlled and in a sustained manner, and loss of drug was inhibited. A drug release study was conducted in vitro and showed that 50% of the drug was released within 3 h and 85% of drug was released within 15 h after administration, proving a novel method to administer MET [68].

Bovine serum albumin (BSA), a serum protein with many hydrophobic binding sites, acts as a natural transporter of various ligands such as fatty acids, steroids, and even drugs. They can bind covalently or noncovalently with ligands including therapeutic drugs [69]. BSA nanoparticles synthesized using desolvation methods were tested as drug carriers to analyze the loading efficiency and release behavior. Rhodamine-B-loaded albumin nanoparticles have been prepared and analyzed for controlled release of drugs in the ear. When rhodamine-B is administered into ear, it is deposited in the osseous spiral lamina, but on entrapment into albumin nanoparticles, it has a potent effect on inner ear disorders through a sustained release of drug [70].

Antiretroviral therapy can increase the lifespan of patients infected with human immunodeficiency virus (HIV). The replication of HIV virus is controlled by antiretroviral therapy. Efavirenz, an antiretroviral drug has been loaded into BSA nanoparticles using a desolvation method and the particles coated with polysorbate 80. A study of release in vitro showed that initially 27.7% was released at a pH of 7.4, and that there was a sustained release of drug with about 75% being released after 36 h. BSA nanoparticles resulted in a prolonged and sustained release of Efavirenz in patients with HIV infection [71].

5-Flurouracil in free form is released at a high rate. BSA nanoparticles have been used as a macromolecular carrier for 5-flurouracil to achieve a sustained and controlled release of the drug. The nanoparticles were synthesized by desolvation and studies of drug release in vitro have been conducted. The nanoparticles contained 1.1 to 1.2 mg of drug entrapped in 200 mg of BSA with an encapsulation efficiency of about 30%. The nanoparticle suspension favored a constant release of 5-flurouracil for about 20 h without any burst release [72].

Sodium ferulate was loaded into BSA using a desolvation process and crosslinking by glutaraldehyde, which was used to target the hepatic cells. The crosslinked BSA nanoparticles loaded with sodium ferulate were found to have 80% entrapment efficiency (EE) and 16% loading capacity when 1.0 mL of glutaraldehyde was added per mg of BSA. The entrapment efficiency decreased when more glutaraldehyde was added in the crosslinking process. BSA nanoparticles with entrapped sodium ferulate have been used in liver-targeted drug delivery showing a higher drug distribution and lower drug concentration compared with sodium ferulate solution, with a sustained drug release in the liver [73].

Ofloxacin-coated BSA nanoparticles have been synthesized and their activity against *Pseudomonas aeruginosa* has been studied together with release of the antibiotic in vitro. The drug loading content and entrapment efficiency were found to be 65% and 85% respectively. The drug release was about 81%, with a controlled and steady drug release from the nanoparticle with a cumulative drug release of about 81% within 16 h. The nanoparticles used in delivery of antibacterial drugs, showed a sharp zone of inhibition of 21 mm in diameter, which demonstrates that the nanoparticles have a potent activity against *Pseudomonas aeruginosa* [74].

Casein and albumin as anticancer drug carriers

Casein as a drug delivery carrier

Casein and BSA nanoparticles have been used in the delivery of anticancer drugs. These carriers have attracted attention because of their low immunogenicity and have biodegradable, biocompatible, and versatile properties. They have the ability to evade the reticuloendothelial system, and this results in a

prolonged circulation time [64, 55]. The protein-based nanoparticles are used in anticancer drug delivery because they can reach the target tumor by penetrating barriers in body after administration with minimal loss of activity. They have a potent and specific role in killing the tumor cells, without affecting normal cells. These particles can release the drugs in a controlled manner increasing their therapeutic effects. These particles are easy to synthesize and their distribution can be monitored with feasibility for surface modification and metabolism [66].

The archetypical anticancer drug, cisplatin has been loaded into casein nanoparticles (which were crosslinked using transglutaminase). The antitumor activity of these nanoparticles was studied by administering them intravenously into hepatic H22 tumor-bearing mice. Zhen et al. [75] observed that these nanoparticles have a remarkable ability to penetrate barriers such as cell membranes to target the tumors and inhibit the growth and proliferation of the cancer. Cisplatin-loaded casein nanoparticles showed a 1.5-fold higher activity than free cisplatin [75].

Curcumin loaded onto the hydrophobic regions of β-casein micelles was assayed for its cytotoxicity on leukemia cells and its antioxidant activity was studied. The free form of β-casein does not have potent activity on tumor cells, even free curcumin shows low clinical efficacy and low bioavailability, but curcumin loaded β-casein micelles showed enhanced activity against human leukemia cell lines. Curcumin activity was increased about 2500-fold when it was encapsulated into the -casein micelles, there was also an apparent increase in the antioxidant activity [76].

β-Casein micelles have been used as a novel system to deliver chemotherapeutic paclitaxel and Tariquidar, a P-glycoprotein-specific transport inhibitor by oral administration. Multidrug resistance is a barrier to cancer treatment, so only P-glycoprotein-specific transport inhibitor was encapsulated separately into β-casein and administered along with paclitaxel (which was loaded into β-casein micelles) to treat gastric cancer. These nanoparticles showed a combined effect against gastric cancer cells [77].

β-Casein has been used to deliver the anticancer drugs like mitoxantrone to act against stomach cancer. Nanoparticles were administered orally and they effectively solubilized and stabilized the drugs [78]. The ionically cross-linked casein nanoparticles were used as a delivery matrix for the sustained release of less soluble flutamide, an anticancer drug. The nanoparticles with flutamide were produced by oil-in-water emulsification, and then cross-linked with sodium tripolyphosphate (a polyanionic cross-linker). These nanoparticles were administered intravenously to rats. After administration, the nanoparticles were observed to circulate in the blood for long time, with controlled release of the drug. A study of drug release in vitro was conducted and the drug loading content and efficiency of incorporation were found to be 8.7% and 64.55% respectively [79].

Casein nanoparticles loaded with magnetic iron oxide (CNIO) have been used to target and for imaging of pancreatic tumors. An amino terminal fragment (ATF) of urokinase plasminogen activator and cisplatin (CDDP) was prepared and entrapped into casein nanoparticles loaded with magnetic iron oxide (CNIO). This complex was administered into mice affected by pancreatic tumors and a drug release study found sustained release with high drug loading. The ATF of urokinase plasminogen activator and cisplatin-loaded casein nanoparticles loaded with magnetic iron oxide (ATF-CNIO-CDDP) were considered to have a potent theranostic role for targeting and imaging in cancer treatment, and these nanoparticles have shown enhanced therapeutic effects compared with the free form of cisplatin [80].

Hybrid nanocarriers such as poly-L-lactide-coglycolic acid (PLGA)–casein polymers have been used to entrap anticancer drugs such as paclitaxel and epigallocatechin gallate (EGCG), which inhibits specific signals. Emulsification followed by precipitation has been used to prepare nanoparticles with a uniform core and shell structure, with paclitaxel in the core and EGCG in the shell. EGCG was released first at the tumor site to enhance the activity and release of paclitaxel. These hybrid nanocarriers were helpful in achieving a sustained release of drug that would enhance the treatment even at low concentrations with low toxicity. The drug release was studied by administering the nanoparticles to rats, and these polymer–protein multilayered nanoparticles presented high potential for cancer therapy [81].

Paclitaxel together with EGCG encapsulated into PLGA-casein nanoparticles have been used in a model of breast cancer treatment. Paclitaxel activates the NF-κB pathway with many signal inhibitors. The activity of these nanoparticles showed sequential activity of EGCG followed by paclitaxel, which sensitized a paclitaxel-resistant breast cell line to

paclitaxel and promoted tumor metastasis and apoptosis. Paclitaxel-induced expression of P-glycoprotein was repressed by the nanocomplex both at the protein and gene levels. This nanocomplex produced a significant cytotoxic response on breast cancer primary cells, suggesting its translational value [82].

Platinum (Pt(II) complex) loaded β-casein nanoparticles have potential for the treatment of gastrointestinal cancers. The solubility of the Pt(II) complex was increased when it was entrapped into the casein nanoparticles. The cytotoxicity of both free Pt(II) complex and entrapped complexes were studied using colorectal carcinoma HCT116 cells, and there was an enhanced targeted delivery of drug on the tumor cells and Pt(II) complex was easily taken up by the tumor cells. The β-casein nanoparticles showed promise as an ideal nanocarrier for Pt(II) complex delivery [83].

The novel platinum drug (bipyridinemorpholine-dithiocarbamate Pt(II) nitrate) was entrapped into a stable nanovehicle system consisting of β-casein and chitosan nanoparticles. These nanoparticles were investigated and characterized. Their cytotoxicity against colorectal carcinoma HCT116 cells was studied and tumor cells were found to take up these platinum complexes easily. The uptake process was enhanced when the platinum drug was encapsulated into -casein and chitosan nanoparticles [84]. The disposition of polysaccharides and proteins were studied using tumor-bearing mice, in which BSA had prolonged circulation in the blood, was easily taken up by, and accumulated in the tumor [85].

Albumin as a drug delivery carrier

Metformin is predominantly used in the treatment of type II diabetes mellitus, but it was found that there is an association between pancreatic cancer and type II diabetes mellitus, so metformin loaded BSA nanoparticles were prepared by desolvation and their activity on MiaPaCa-2 cell lines studied. The toxicity of the nanoparticles towards the cancer cells was found to be higher than free BSA or free metformin [86, 87].

Various drugs loaded into casein and albumin nanocarriers and their role in targeting cancers are shown in **Table 2**.

Table 2. Drugs loaded into casein and albumin nanoparticles and their role in targeting cancers

Protein nanoparticle	Drugs loaded into the nanoparticles	Role of nanoparticles	References
Casein	Cisplatin	Antitumor activity, they have ability to the penetrate the cell membrane, targets and inhibit tumor growth	[75]
	Curcumin	Antioxidant activity, enhanced anticancer activity on leukemia cells	[76]
	Paclitaxel and tariquidar	To treat the multidrug resistance gastric cancer	[77]
	Paclitaxel and epigallocatechin gallate (EGCG)	EGCG enhances the paclitaxel at the tumor site, with low toxicity to cells	[81]
	Platinum (Pt(II) complex)	To treat the gastrointestinal cancer	[83]
Albumin	Metformin	To treat type II diabetes mellitus and pancreatic cancer	[86, 87]
	Albendazole	Strongly inhibits vascular endothelial growth factor. Highly toxic to ovarian cancer cells and less toxic to healthy cells,	[88]
	Gemcitabine	To treat pancreatic cancer, by inhibiting the growth of tumor cells, control drug release at tumor site	[89]
	Paclitaxel and docetaxel	Potent anticancer drug formulation, with less toxicity	[90]
	Rapamycin	Control and inhibit the cancer cell growth by controlling the signal pathway during abnormal cell growth	[91]

Albendazole, a broad-spectrum antihelminthic agent, has been used in the treatment of ovarian cancer. The action of albendazole involves weakening the formation of microtubules, and it strongly inhibits vascular endothelial growth factor in case of ovarian cancer with ascites. Albendazole has been formulated in a nanoform by entrapping it into BSA nanoparticles. Studies of drug release in vitro and in vivo have been conducted and found that about 93% of albendazole was released at 36 h, which was highly toxic to ovarian cancer cells and less toxic to healthy cells [88].

Because of the low solubility of paclitaxel, it is administrated at a higher concentration, which causes a hypersensitivity reaction. To overcome its low solubility, targeted delivery of drug was developed in form of nanoparticles by entrapping paclitaxel into BSA bound to folic acid. These nanoparticles were characterized and in vitro studies with a prostate cancer cell line and showed a cumulative release of about 46.6% at 36 h, which was sufficient to be cytotoxic to cancer cells leading to a decrease in their number. The release of drug was found to be in a sustained and controlled manner [48].

Gemcitabine, a drug used in treating pancreatic cancer, has been entrapped into BSA nanospheres to enhance its efficacy of action and prolong its retention in the circulation. Nanoparticles loaded with GEM have been synthesized by desolvation-crosslinking method. An 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT) assay demonstrated an antiproliferative effect of these nanoparticles on the human pancreatic cell line BXPC-3 and that the effect was more potent than that of free gemcitabine solution or BSA nanoparticles, with a high drug loading efficiency of about 83% and about 93% release of drug was in a controlled manner [89].

Folate and its conjugates have been used to target chemotherapeutic drugs to folate-receptor-positive tumor cells at the tumor site. The folate and folate receptors were found to be an ideal therapeutic in the case of many cancers. Gefitinib, an anticancer drug, has been loaded into the BSA nanoparticles decorated with folate bound to carboxymethyl-β-cyclodextrin. Gefitinib-loaded folate-decorated bovine serum albumin conjugated carboxymethyl-β-cyclodextrin nanoparticles (FA-BSA-CM-β-CD nanoparticles) were prepared. An in vitro study found that gefitinib release was higher after initial administration and followed by stable release demonstrating desired delivery system characteristics. An MTT assay demonstrated a cytotoxic effect FA-BSA-CM-β-CD nanoparticles on HeLa cells and enhanced activity on the tumor compared with free gefitinib and folate nanoparticles without drug loading [92].

Human serum albumin (HSA) nanoparticles have been used to target drug treatment of various cancer cell lines. Paclitaxel was entrapped into HSA nanoparticles using an emulsion solvent evaporation method and high pressure in a homogenizer. The growth of a MCF-7 human breast cell line was inhibited by the cytotoxicity of paclitaxel-HSA nanoparticles in a dose-dependent manner [93].

Paclitaxel and docetaxel, together known as Taxanes are used as a potent drug against breast cancer. The availability of Taxanes at the tumor site was found to be less and toxicity increased if more drug is administered [94]. This drawback was addressed by binding the drug to solvent-free nanoparticle albumin. Albumin loaded with paclitaxel was prepared and found to have a potent activity against the tumor by transcytosis (receptor-mediated transport), by binding to gp60 protein, which activates the tumor to produce secreted protein, acidic and rich in cysteine (SPARC), to inhibit cancer cells with less toxicity and enhanced antitumor activity [90].

Abraxane (nanoparticle albumin bound (*nab*)-paclitaxel) has been used to treat metastatic breast cancer, and was approved based on a clinical trial in 460 patients. The trail showed that for patients treated with Abraxane there was longer patient survival than in those patients treated with solvent-based paclitaxel in second line treatment [90]. Rapamycin has been used to control and inhibit cell growth and multiplication. The signal pathway activation of rapamycin kinase is likely to be abnormal in cancers. Therefore, rapamycin could be used as a treatment option to control the pathway. *Nab* technology has been used to bind rapamycin to albumin nanoparticles, administrated at various concentrations into 3 tumor-bearing rat models of cancer. Tumor growth was subsequently evaluated and the rapamycin was nontoxic even at higher concentrations with direct drug release at the tumor cells because of the nano formulation [95]. Cationic vectors together with serum protein allow removal of therapeutic agents from blood, and this strategy could be used as a delivery system in the treatment of lung cancer. Cationic BSA nanosized particles were prepared with entrapped siRNA and used to deliver the siRNA directly to target lung cancer cells. When the siRNA was delivered

to the tumor site, it accumulated the Bcl-2 specific siRNA through gene silencing, thus inhibiting tumor proliferation and causing apoptosis in a B16 cell model of lung metastasis; showing promise as a novel targeted drug-delivery system [96].

Conclusion

Protein-based nanocarriers are a widely recommended drug delivery systems because of their small size (ranging from 1 to 100 nm) that allows them to pass through multiple biological barriers providing an effective outcome at the target site. Protein nanoparticles have high encapsulation efficiency, allowing the incorporation of substantial amounts of drug within the nanoparticles, providing a sustained release of the drug to targeted sites. Nanoparticles allow rapid clearance of drugs after their action at tumor sites. Albumin and casein are biocompatible and can be easily metabolized by natural processes. Albumin and casein nanoparticles have excellent binding capacity for therapeutic drugs. Such protein-based nanoparticulate drug delivery systems have potential as efficient carriers of various anticancer drugs, and can be manipulated for targeted drug delivery.

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Conflicts of interest statement

The authors have no conflicts of interest to declare.

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