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Albumin nanostructures as advanced drug delivery systems

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Abstract

Introduction—One of the biggest impacts that the nanotechnology has made on medicine and biology, has been in the area of drug delivery systems (DDSs). Many drugs suffer from serious problems concerning insolubility, instability in biological environments, poor uptake into cells and tissues, suboptimal selectivity for targets and unwanted side effects. Nanocarriers can be designed as DDSs to overcome many of these drawbacks. One of the most versatile building blocks to prepare these nanocarriers is the ubiquitous, readily available and inexpensive protein, serum albumin.

Areas covered—This review covers the use of different types of albumin (human, bovine, rat, and chicken egg) to prepare nanoparticle and microparticle-based structures to bind drugs. Various methods have been used to modify the albumin structure. A range of targeting ligands can be

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Declaration of interest

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attached to the albumin that can be recognized by specific cell receptors that are expressed on target cells or tissues.

Expert opinion—The particular advantages of albumin used in DDSs include ready availability, ease of chemical modification, good biocompatibility, and low immunogenicity. The regulatory approvals that have been received for several albumin-based therapeutic agents suggest that this approach will continue to be successfully explored.

Keywords

Human serum albumin; bovine serum albumin; ovalbumin; nanoparticles; drug delivery systems

1. Introduction

The discovery of innovative approaches for the selective delivery of therapeutic biomolecules (drugs, proteins, and genes) used for the treatment of disease has captured the interest of both experimental investigations and literature reviews [1–4]. In recent years, the field of targeted delivery systems has expanded to include a variety of nanoparticles (NPs) that have become a very important technology for drug carriers [5–8].

Among the widespread types of biomolecules used for targeted delivery, albumin protein has attracted attention of researchers for its selective delivery capabilities, nontoxicity, and non-immunogenicity [9,10]. Up to now, various types of albumin protein have been isolated including ovalbumin (OVA) (derived from egg white), human serum albumin (HSA), bovine serum albumin (BSA), and rat serum albumin (RSA) and have been used for different biomedical applications [11–13]. Among various albumin species, serum albumin has significantly captured the attraction of researchers. The key intrinsic features of albumin are the high stability and high solubility in water and diluted salt solution [9]. On the other hand, albumin has a striking potential of binding to lipophilic molecules. For example, several compounds such as hormones and fatty acids are transported by albumin protein in the body [14].

According to recent literature reviews, a wide variety of different molecules and substances including drugs, genes, peptides, vaccines, and antibodies can be effectively bound to the albumin matrix that can act as a versatile carrier [13,15–17] (Figure 1). So, in recent years, albumin has attracted a significant amount of interest among protein-based nanomaterials; it represents an important carrier with great potential in controlled delivery applications which can lead to suitably targeted delivery of various drugs and endogenous molecules [9,18,19]. Besides, albumin has been shown to have a high capacity of binding to a wide variety of drugs [9]. In this regard, in medicinal therapeutic approaches, drug delivery systems (DDSs) based on colloidal albumin NPs have attracted much attention due to their advantages including a high drug loading and entrapment capacity, good biocompatibility and biodegradability [20,21]. Furthermore, albumin nanocarriers are considered to be reliable and efficient carrier systems with features such as facile preparation, controllable well-defined size, and desirable surface modification characteristics [21].

Several surface modification methods are applied in order to improve binding of drugs to albumin particles in the solution followed by promoting drug-targeting abilities [20] (Figure 1). In this regard, the incorporation of therapeutic drugs in albumin-based carriers can be accomplished via several chemical or physical methods including covalent bonding to functional groups on albumin particles [22,23] or adsorption of drugs on the particle surface [20]. It is well known that functional groups facilitate the targeting ligands conjugation [15].

Furthermore, diverse efforts have been conducted for developing therapeutic anticancer DDSs [24,25]. Albumin nanocarriers have been shown to be effective particles as efficient carriers in improvement of the tumoricidal activity of anticancer drugs [26]. Therefore, the significant impact of albumin nanocarriers in cancer therapy is strongly confirmed [27].

In the present review, the above topics in addition to different albumin nanostructures and synthesis methods such as emulsion-based methods, coacervation methods, self-assembly, and nanoparticle albumin-bound technology (Nab-technology) processes will be discussed to aid the understanding and development of the albumin-based delivery systems. Also, the main applications of albumin-based DDSs for cancer therapy and targeting of other organs will be reviewed.

2. Albumin types, structures, and binding sites

2.1. HSA

Mammalian albumins are a family of globular and water-soluble proteins, produced in the liver, and have the highest abundance in human blood plasma (approximately 35–50 g/L). Albumin is a heart-shaped molecule with a molecular weight range of 65–70 kDa and a half-life in the body of 19 days. It is stable within the pH range of 4–9 and can withstand heat up to 60°C for 10 h [28,29]. HSA is a single polypeptide with 585 amino acids with a high cysteine and low tryptophan content [28]. The secondary structure of albumin consists of 67% α-helix and possesses 17 disulphide bridges along with 6 turns [30,31]. The three-dimensional (3D) shape of HSA is an alpha-helical ellipsoid shape with three homologous domains I, II, III (that can be thought of as flexible spheres in a row) that are cross-linked by disulfide bridges (schematically depicted in Figure 2) [9,32]. Each one of these three domains has a pair of subdomains called ‘A’ and ‘B’ [29]. Due to the presence of different drug-binding sites in the molecular domains, albumin has a high binding capacity and multifunctional ability to transport many drugs, fatty acids, bilirubin, ions (Na^+, K^+, Ca^{2+}), and a variety of hormones. In the blood stream, an important function of albumin is the maintenance of osmotic pressure [33,34]. As albumin is a protein with low immunogenicity and no toxicity, good general availability, and has been reported to have preferential uptake in tumor tissues, coupled with good biocompatibility and biodegradability, these properties make it an ideal carrier for drug and gene delivery [33,35].

Numerous studies have been used HSA-based DDSs for delivery of different anticancer drugs such as doxorubicin (DOX) [37,38] and paclitaxel [39].

2.2. BSA

BSA is a serum albumin protein with 69-kDa molecular weight. Similar to the human one, BSA is widely used for drug delivery since its abundance, biocompatibility, biodegradability, nontoxicity, and non-immunogenicity. Furthermore, BSA is accepted in the DDSs because of water-solubility improvement of prodrugs, low cost, and proper delivering properties [33,40]. For example, a study is used from these advantages for BSA NP-based delivery of tacrolimus in purpose of reduction of its kidney uptake/distribution and functional nephrotoxicity [41]. The results showed the initial burst release followed by sustained release as well as reduced functional nephrotoxicity. Similar initial phase of rapid decrease in tacrolimus concentration for Prograf (as control) and drug–BSA NPs and slower decline and delayed blood clearance with increased circulation time for drug–BSA NPs were also reported. The possible immunologic response developed against BSA *in vivo* could be a limitation for its clinical applications and encourage substitution with other types of albumins (particularly HSA) [33].

2.3. RSA

RSA prepared from rat serum has structural properties similar to other albumin species with a molecular weight of 64.3 kDa and an isoelectric point at pH 8.6. There are only a few reports for the use of rat albumin protein compared to the other albumin species [42].

2.4. OVA

OVA is the main storage protein in egg white and is a monomeric phosphoglycoprotein with a molecular weight between 42 and 47 kDa and an isoelectric point around pH 4–5. It has a 3D structure with a helical reactive loop arrangement and 365 amino acids in the polypeptide [43]. OVA is very economical compared with other proteins and, considering its ability to form emulsions, gels, foams, combined with its sensitivity to heat and pH, is a good choice for drug delivery applications [33,44,45]. In contrast to mammalian serum albumins, OVA is strongly recognized by the immune system and can be used in antigen delivery systems. The creation of a conjugate between poly(propyl-acrylic acid) and OVA increased MHC-1 presentation and T-cell activation, so it was suggested that it could be vaccine delivery system that targets CD8-expressing T cells [46].

2.5. Binding sites on HSA

According to Sudlow's classification of the different binding modes between drug ligands and HSA, two major binding sites are located in subdomain IIA and IIIA, respectively [29,47,48]. The site IIA that has been denoted as the ‘warfarin binding site’ is a preformed, large, flexible multichamber cavity located within the core of subdomain IIA, and this site binds bulky heterocyclic, negatively charged compounds such as amantadine, azapropazone, and azidothymidin [29,49]. Hydrophobic interactions are predominant for drug binding at this site [36,50]. Site IIIA is called the ‘indole and benzodiazepine binding site’ and is topologically similar to the IIA site, but this site binds to compounds with more pronounced negative charges such as diazepam, halothane, ibuprofen, propofol, and non steroid anti-inflammatory drugs [29,49]. In this site, hydrophobic and electrostatic interactions and hydrogen bonding are crucial to understand the drug binding [36,50]. A

binding pocket in the subdomain IB has recently been recognized as a secondary binding site for some other compounds with different properties such as indomethacin, iophenoxic acid, naproxen, myristic acid, warfarin, lidocaine, and bilirubin [29]. Because of this finding, it has been proposed that subdomain IB could be a third drug-binding region of HSA [51,52] and therefore the 'Three-Site Model' for drug-binding regions of HSA was proposed [29]. In a recent study, Wang et al. investigated the albumin-binding interactions with anticancer cytotoxic drugs such as camptothecin, etoposide, teniposide, bicalutamide, and idarubicin at the scale of atomic details [32]. They suggested that subdomains IIA and IIIA were more favored for small hydrophobic and anionic molecules, while subdomain IB showed a high affinity for complex heterocyclic drugs and endogenous ligands [32].

The structural flexibility of albumin, and of the resulting albumin NPs, allows reversible binding of different compounds to the carrier and allows transfer to their target tissue [32]. The binding sites of different albumin species are very similar, particularly in terms of size, number of amino acid constituents, and biological function [53]. But there is about 25–30% variability in arrangement of amino acid sequence between HSA, RSA, and BSA [54]. Structure and frequency of albumin-binding sites will have a great effect on the pharmacokinetics, drug delivery/design, and therapeutic efficacy [55].

3. Albumin nanostructures

3.1. Synthesis and preparation methods

There are several different techniques that can be used to prepare albumin NPs, including desolvation, emulsification, 'Nab-technology,' thermal gelation, green chemistry, and nano-spray drying.

3.1.1. Desolvation—In the desolvation or coacervation process for preparing albumin NPs, ethanol is added drop by drop to an aqueous solution of albumin in order to achieve the desolvation of the albumin solution. The stirring of the solution must be continued until the whole solution becomes turbid. Because of the diminishing water solubility, the albumin phase separates and aggregates. In addition, through gradual addition of glutaraldehyde solution, particle cross-linking occurs and discrete albumin NPs form [17,33,56]. Incubation of the particles at different temperatures under constant stirring can be used in order to avoid the addition of the cross-linker [56]. Figure 3 shows the stepwise preparation of albumin NP through desolvation method followed by formation of a core–shell structure.

A study reported that the lowest concentration of glutaraldehyde which is required for manufacture of albumin NPs with good stability is about 40% [56]. Sadeghi et al. explained that compared to the use of pure acetone, ethanol, and mixtures of ethanol with acetone resulted in more spherical NPs [57]. Another study used a desolvation method with a membrane contactor for the large-scale production of BSA NPs, with comparable characteristics to particles that were produced by small-scale methods [58]. Moreover, it was suggested that the desolvation technique, which was used to prepare folate-coated BSA NPs, was promising and effective for delivering poorly water-soluble drugs [59]. Fisetin-loaded HSA NPs with an 84% encapsulation efficiency and a diameter of 220 ± 8 nm were reported using this method. Initial burst release and then sustained and slow release was obtained *in*

vitro [60]. Particle properties including size may be affected by desolvating agent, cross-linker, pH of HSA solution, centrifugation, and other characteristics of preparation method resulting in the desired particle size [33].

3.1.2. Emulsification—In this approach, albumin NPs are formed by the homogenization of aqueous albumin droplets contained in an oil phase. According to this method, drug and cholesterol are dissolved in a mixture of chloroform and ethanol, and then, this solution is added to aqueous albumin solution. A crude emulsion is prepared by shearing the resulting mixture, and then, this preparation is homogenized using a high-pressure homogenizer. After these steps, evaporation under vacuum is used to produce an organic dispersion of the drug–albumin NPs [41]. In the emulsification method, two different approaches are used for stabilization of the albumin NP: thermal heating or chemical treatment using a cross-linking agent [33].

3.1.3. Nab-technology—Nab-technology is a specific method used to prepare albumin NPs and can also be used for the encapsulation of lipophilic drugs into the NPs. The drug and HSA are mixed in an aqueous solvent. Drug–albumin NPs are then synthesized by passing the solution through a jet nozzle under high pressure. The size range of the NPs produced is about 100–200 nm [33]. A recent study formed a lapatinib-loaded HSA NPs (LHNP) preparation by adding a lapatinib–chloroform–ethanol mixture to the HSA solution and then the solution was subjected to high shear forces to form a coarse emulsion. This emulsion was passed through a micro-fluidizer and followed by evaporation and filtering of the NPs suspension to achieve the final emulsification. Thereafter, the NPs were frozen, lyophilized, and stored. The drug can be added to the chloroform and ethanol before the process of emulsification. In this study, the LHNP showed high cytotoxicity resulting in apoptosis of tumor cells and serious damage to tumor spheroids was demonstrated (Figure 4(a)) [61].

Nab-paclitaxel (the first FDA-approved nanotechnology-based drug) was manufactured by this method for the treatment of metastatic breast cancer and recently for other types of cancers [64,65]. This product has particles of about 130 nm diameter and shows increased drug tumor accumulation and superior antitumor efficacy compared to conventional drugs as found using this formulation in preclinical and clinical experiments [9].

3.1.4. Self-assembly—Another popular process for preparation of albumin NPs is self-assembly. Adding a lipophilic drug and decreasing the number of amine groups on the surface of the protein increases the hydrophobicity of albumin which causes the HSA to undergo self-assembly and results in formation of micelles [33]. In this regard, a study added succinylated cholesterol to BSA followed by stirring under argon. Dissolution of the cholesteryl (Chol)–BSA and then gradual addition of paclitaxel was used to produce a albumin–drug composite (paclitaxel–Chol–BSA) under stirring conditions. The paclitaxel–Chol–BSA structure was prepared via self-assembly with high colloidal stability in comparison to structures without Chol. The NPs had a 147.6 ± 1.6-nm hydrodynamic diameter and a high drug-loading capacity [62]. Paclitaxel–Chol–BSA NPs acted as an efficient agent showing sustained drug release (Figure 4(b)). Administration of paclitaxel–Chol–BSA NPs resulted in enhanced cellular uptake compared to paclitaxel–BSA and

paclitaxel dissolved in Cre/EtOH (i.e. Cremophor® El:ethanol [1:1]) (Figure 4(c)). The cytotoxicity evaluation of paclitaxel–Chol–BSA NPs against cancerous cells using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay showed lower viability and higher cytotoxicity of paclitaxel induced by these NPs, compared to paclitaxel–BSA and paclitaxel–Cre/EtOH as controls (Figure 4(d)).

Another study prepared a BSA NP-based DDSs using self-assembly between negatively charged cyclic peptide (cRGD)-modified BSA loaded with DOX and a positively charged cell-penetrating peptide (KALA) through electrostatic interactions (Figure 4(e)) [63]. Han et al. used this method for the fabrication of cationic BSA NPs for gene delivery and treating meta-static lung cancer [12].

3.1.5. Other methods—Thermal gelation is another approach for preparation of albumin NPs and is a sequential process including protein–protein interactions and heat-induced unfolding. For example, in one approach, solutions of albumin and lysozyme are mixed. The pH of this mixed solution was regulated at 10.3 and afterward, it was stirred and heated. NPs prepared in this way had a spherical shape and a core–shell structure [33].

NP production using natural materials, called ‘green chemistry’ or green synthesis, can be used to provide safer NPs with reduced adverse side effects. For example, Lam et al. used glucose instead of glutaraldehyde as a cross-linking agent for modification of BSA to prepared NPs for delivery of berberine with reduced potential biological toxicity [66]. Using glucose and BSA, both of which have good biocompatibility and biodegradability, can lead to more rapid degradation of NPs in biological environments.

The last process, nano-spray drying, is commonly utilized for manufacturing a dry powder. To generate droplets, nano-spray dryers use a vibrating mesh technology. At the first stage, a piezoelectric crystal drives the spray head, then the mixture passes through a small spray cap which has a thin perforated membrane named ‘spray mesh.’ The piezoelectric actuator moves at an ultrasonic frequency and its movement causes the spray mesh to vibrate up and down. The vibration causes production of numerous droplets with a precise size [33]. This method can be used for preparation of dry powder with uniform-sized particles from a liquid phase.

Because of the possibly undesirable use of organic solvents and cross-linking agents in emulsification, the desolvation method can be an alternative due to its robustness and reproducibility. On the other hand, Nab-technology particles can be used for intravenous applications without using any surfactants and denaturing of the HSA [33]. In addition, thermal gelation can be used for fabrication of nanoscale hydrogels, and self-assembly can be used for high loading of poorly water-soluble drugs, and lastly, nano-spray drying provides a single-step continuous and scalable process for preparation of NPs [33].

3.2. Different albumin nanostructures

Albumin nanostructures can have different geometrical shapes and structures including albumin NPs, albumin microspheres, albumin-coated liposomes, albumin microbubbles, and albumin nanocapsules.

3.2.1. Albumin NPs—Albumin NPs have some particular benefits such as easy preparation and being reproducible. Because various drugs and also charged molecules are able to bind to albumin protein, they can effectively be incorporated into the matrix of the albumin NPs [22]. The presence of functional groups, such as amino and carboxylic groups on the surface of the NPs, allows covalent bonds to be formed with targeting ligands [67]. Albumin NPs are appropriate vehicles for gene therapy and drug delivery because they do not show undesired interactions with serum. They have also been utilized as efficient carriers to increase the tumoricidal activities of anticancer drugs [23].

3.2.2. Albumin microspheres (microparticles)—The usage of albumin microspheres as drug delivery vehicles was first suggested by Kramer in 1974 [68]. The reasons for using albumin microspheres are (1) making drugs more bio-compatible, (2) protecting cargos from oxidation and degradation, (3) controlled and targeted release of the active payload [69]. To produce albumin microspheres, albumin is chemically cross-linked using glutaraldehyde. They can be also prepared by adding an organic solvent and then stabilizing the micro-spheres at high temperature. The size range of these micro-spheres is 1–100 μm [70]. Smaller particles (1–3 μm diameter) accumulate in solid tumors as well as liver and kidney; and particles with size greater than 15 μm can be used for lung targeting [9]. A study utilized egg albumin microspheres to release microencapsulated vitamin A [71]. In another study, Lee et al. used arginine-conjugated albumin microspheres to inhibit division and migration of lung cancer cells [72].

3.2.3. Albumin microbubbles—Another type of albumin carrier is the form called microbubbles. These are spherical and contain a composition of one or more gases. The size of albumin microbubbles is approximately the same size as red blood cells (6–8 μm diameter). A single gas or a mixture of multiple gases can be used to prepare microbubbles that have applications in both imaging and therapy [73–75]. The properties of microbubbles can be divided into functional properties and structural properties. The ability to be injected, a good efficiency to scatter ultra-sound forming a contrast agent, and favorable biocompatibility are their functional properties. Their small size (1–10 μm), a density different from their biological environment providing compressibility within the surrounding body tissue, a surface that allows the attachment of various ligands, and the uniformity of their shell thickness are their structural properties [76,77]. Microbubbles can be used in *in vitro* and *in vivo* drug and gene delivery applications. For example, a study found that albumin microbubbles coated with polyethyleni-mine (PEI) are an appropriate nonviral gene carrier with high transgene efficiency and low cytotoxicity [74].

3.2.4. Other albumin types—Core–shell structure of albumin nanocapsules is developed as carriers for delivery of anti-inflammatory drugs [78]. Since the fundamental role of activated synovial macrophages in rheumatoid arthritis, an inflammatory disease, in a study, Rollett et al. designed a nanodevice using folic acid (FA)-functionalized HSA nanocapsules to reduce macrophage populations without side effects on normal tissues [79].

Albumin-coated liposomes are prepared by using electro-static interaction between anionic-charged albumin molecules and cationic-charged liposomes [80]. A study performed by Weecharangsan et al. suggested that albumin-coated liposomes were effective vehicles to

deliver antisense oligodeoxyribonucleotides (ODN) [81]. ODN reduces bcl-2 mRNA and protein expression level so it was used to treat human KB epidermoid carcinoma.

The variability of albumin structures in terms of size, shape, and composition offers new opportunities for delivery of different therapeutic materials in a controlled and precise manner. The stability issues of microparticles suggest that the fabrication of nanosized particles will show better stability, more flexibility for surface modification, and increased preferential uptake in pathological sites.

4. Modification and targeting of albumin NPs

As mentioned earlier, due to albumin chemical structure and presence of different functional group, albumin NPs provide more opportunities for modifying the surface of the particle through non-covalent and covalent bonding, allowing them to be used for different drug targets by introducing new components to the albumin NPs surface [10,82]. In order to improve the efficiency of nanostructures in targeting and delivery, different linkers and spacers can be used. These materials improve stability of the drug in the circulation while facilitating the drug release at the targeted location. Researchers can optimize carriers for specific applications via altering linker/spacer characteristics such as material type, structural length, and bond type. The following sections discuss the most investigated albumin NPs modification methods as well as their targeting.

4.1. Surfactant

To investigate the effect of surfactant-modified HSA, Zucchi et al. employed DOX-conjugated HSA NPs, coated with the surfactant, polysorbate 80, in order to study the cellular uptake and explore the toxicity in an animal model at two different exposure times (15 and 30 days) [83]. Surface-coated HSA NP bound to DOX and favorably altered the drug pharmacokinetics and reduced cardiac and testicular toxicities. Similarly, it was observed that the formulation favorably altered the toxicological profile of DOX [84]. The mechanism of toxicity was not clear, but the most probable explanation was that surface-coated NPs had lower uptake by cells and consequently caused less damage to the cells, whereas free NPs might lead to more cytotoxicity due to degradation of the uncoated NPs and release of the drug within the cells.

4.2. Polyethylene glycol

Polyethylene glycol (PEG)ylation is the chemical surface modification of proteins or particles with PEG and has been applied in numerous studies using DDSs. As a modifier attached to the surface of NPs, PEG possesses the following advantages: PEG reduces the NPs immunogenicity and enhances NPs accumulation in tumors via extending the circulation half-life more than 50-fold thus promoting the enhanced permeability and retention effect. Kouchakzadeh et al. used methoxy PEG (mPEG)–succinimidyl propionate to PEGylate the amino groups of BSA NPs. They demonstrated that PEGylated NPs released the drug (5-flurouracil) much slower than non-PEGylated NPs. It was suggested that the presence of the PEG layer acted as a barrier to drug diffusion [85].

Another study designed surface-modified albumin NPs in the form of two different PEG–HSA conjugates: poly (thioetheramido acid)–PEG copolymer-grafted HSA and mPEG-grafted HSA (HSA–mPEG) [86]. Rose bengal (RB) was used as a model drug and encapsulated into the NPs with different polymer coatings. The drug loading in HSA–mPEG NPs was much lower than unmodified HSA due to fewer drug–protein-binding sites being available in the modified HSA–mPEG. It was suggested that the existence of the steric hydro-philic barrier provided by PEG on the surface of the NPs prevented enzymatic digestion of NPs in the presence of enzymes and consequently led to very slow release of drug (RB) from modified HSA [86]. Zhang et al. reported that PEG-coated BSA could be utilized to stimulate bone formation after implantation in animal models. The BSA NPs were decorated with PEG-modified PEI and loaded with bone morphogenetic protein-2, using human C2C12 cells. The result of PEGylated PEI-coated BSA NPs showed a reduction in the cytotoxicity of native PEI compared with non-coated NPs [87].

4.3. Folate

Folate acts as a targeting ligand which shows high affinity for folate receptors. Folate receptors are overexpressed in human cancer cells, in particular epithelial ovarian cancer. Conjugation of folate to proteins permits receptor-mediated endocytosis by selective internalization of protein into the cytoplasm [88]. The carboxylic group of FA can be covalently conjugated to the amino groups on the surface of proteins or NPs. Nonconjugated folate (known as FA) has a small molecular weight and is non-immunogenic, but upon binding to proteins retains its affinity for the folate receptor. The large number of applications of the folate-conjugated albumin NPs in DDSs suggests the specificity of this conjugated system for cells expressing the folate receptor. Moreover, folate-conjugated albumin NPs increase the targetability of drugs to the tumor cells. Due to the encapsulation of anticancer drugs, they promote uptake and protect the drugs from degradation.

For example, Zhao et al. studied the folate-decorated BSA NPs where encapsulated paclitaxel were introduced to human prostate cancer cell line. The results showed that conjugated NPs had better drug delivery compared to unconjugated NPs. More importantly, folate-modified BSA NPs promote natural property of NPs, enhance cellular uptake, and significantly increase water solubility of paclitaxel [89].

Shen et al. prepared FA-conjugated albumin nanospheres (FA–AN) using desolvation and cross-linking with glutaralde-hyde, and L-lysine was used as a capping agent. The result of this process was successful fabrication of FA–AN with a particle size of 150–200 nm as a delivery platform for DOX into HeLa cancer cells and aortic smooth-muscle cells (AoSMC). It was found that FA–AN were taken up into HeLa cells after 2 h incubation, whereas nontargeted AN were not. The AoSMC cells (noncancer) treated with FA–DOX–AN remained more viable compared to HeLa tumor cells. The selectivity for HeLa cells over AoSMC was attributed to overexpression of the FR-alpha on HeLa [90].

4.4. Apolipoproteins

Apolipoproteins (Apos) have been successfully used to transport drugs across the blood–brain barrier (BBB) and into the brain. The rationale is that lipoproteins are naturally

transported into the brain, and Apo recognition by endothelial cells could mediate this. Apos can adsorb or covalently bound to the surface of albumin NPs. It has been reported that covalent coupling of ApoE to HSA NPs strongly enhanced drug transport and uptake into the brain to reach neurons [91–94]. These surface-modified albumin NPs are biodegradable and carry reactive groups such as thiol, amino, and carboxylic acid on their surface for binding to ligands [93]. Second, these Apo-modified albumin NPs resemble endogenously circulating lipoproteins, and can bind to lipoprotein receptors at the BBB, undergo transcytosis into the endothelium, leading to endocytosis into the brain [91,94]. Third, they protect active drugs and agents from degradation and decrease their side effects [91]. Zensi et al. studied ApoE-modified albumin NPs in an animal model to examine the cellular uptake into the brain. Conjugation was carried out by introducing sulfhydryl groups into the Apos, which were then stirred with HSA NPs for 12 h at room temperature. ApoE-modified NPs (but not plain NPs) were detected in brain endothelial cells and neurons after intravenous injection into mice [91]. Similarly, Wagner et al. co-incubated bEnd3 cells with ApoE–HSA and low-density lipoprotein (LDL) and showed an increase in binding affinity of ApoE to the LDL receptor and increased cellular uptake. It was suggested that LDL induced a conformational change in the ApoE structure, which could lead to an increase in the binding affinity [93]. In another study, Apos E3, A–I, and B-100 were covalently attached to HSA NPs loaded with loperamide [92,94]. Loperamide is a potential antinociceptive drug that normally does not cross the BBB. It was found that ApoE-modified HSA NPs had a significant antinociceptive effect.

4.5. Monoclonal antibodies

Many monoclonal antibodies (mAb) have been designed to selectively bind to overexpressed tumor markers. The human epidermal growth factor receptor-2 (HER2) has been used as a tumor marker for detection and treatment of metastatic breast cancer. The humanized anti-HER2-specific antibody (trastuzumab) combined with DOX could offer an effective therapy to woman with HER2 overexpressing breast cancer [22,95]. Anhorn et al. showed that trastuzumab-modified HSA NPs loaded with DOX were taken up by breast cancer cells (SKBr3) and effectively released the drug [95]. The same conjugated system showed receptor-mediated endocytosis into different cell lines (BT474, MCF7, and SK-BR- 3) in a time- and dose-dependent fashion [22].

Attachment of a novel mAb (1F2, directed against the extracellular domain of HER2) to the surface of PEGylated HSA NPs showed good binding to HER2 receptors on the surface of BT-474 cells [96]. Direct antibody coupling to the conjugated system led to higher binding affinity and significant cytotoxicity in BT-474 cells.

The overexpression of the epidermal growth factor receptor (EGFR) has been observed in many malignancies such as color-ectal, head, neck, non-small-cell lung, ovarian, breast, and prostate cancers. mAbs such as DI17E6 and cetuximab can recognize the EGFR [97,98]. The thiolated antibody (DI17E6) was covalently bound to the surface of HSA NPs and loaded with DOX. The targeted vehicle showed specific targeting and cytotoxicity to EGFR-positive melanoma cells [98]. Löw et al. prepared cetuximab-modified HSA NPs and tested

them against EGFR-overex-pressing colon carcinoma cells [97]. The results showed specific anticancer effects, with no nonspecific uptake into normal tissue.

5. Albumin conjugates for drug delivery

There are various approaches which can be used to load the albumin NPs with the desired drug, including covalent bonding, surface coating, and electrostatic adsorption which is possible due to the high number of functional groups available in the primary structure of albumin. As previously explained, site I and site II of HSA are responsible for most drug binding, but other binding sites may be loaded with different concentrations of individual drugs [33].

5.1. Albumin–drug conjugates

Several methods have been proposed to classify albumin–drug conjugates such as those based on fabrication methods [21], the structure of NPs [9,55] or the structure of the NP–drug conjugate [9,21,99], and the type of interaction [21], receptors [100], or target tissue [9,16] and improvement of drug half-life [100]. In the present work, we divide these conjugates based on the water solubility of the drugs that have been encapsulated.

5.1.1. Water-soluble drugs loaded onto albumin—For water-soluble hydrophilic drugs, there are three main methods to load the drug into albumin NP [33,101]: (a) incubation of drug with the preformed albumin NPs, (b) mixing of drug and albumin solution followed by cross-linking of the NPs (incorporation), and (c) addition of the drug to glutaraldehyde solution and then cross-linking formation of NPs. In all of these categories, the drug can either be adsorbed on the surface of the NP or incorporated into the main matrix of the NPs [33,102]. Using the incubation method, high drug loading is obtained in the first hour of incubation and then the amount of loaded drug becomes constant. The incorporation method can be used for loading the highest amount of drug, while the last method (c) is similar to the first approach (a) but taking a longer period of time.

Tacrine was the first drug to receive FDA approval for Alzheimer's disease (AD), but suffers from low bioavailability, a short elimination half-life, and occurrence of side effects including hepatotoxicity. Luppi et al. prepared BSA NPs carrying cyclodextrins to deliver tacrine via the intranasal route. They prepared NPs using a coacervation method and then used the incubation method to load the drug. They investigated the physicochemical properties of the NPs including the mucoadhesive property and the drug-permeation behavior and found an interesting *ex-vivo* drug-permeation profile [103]. Bae et al. prepared DOX-loaded HSA NPs that also had surface modification with transferrin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in order to target different tumor types *in vitro* [38].They showed that these NPs had cytotoxic and proapoptotic activities, showed synergistic cytotoxic effects against cancer cells, and also localized to tumor mouse model of cancer. Similarly, in a very recent study, TRAIL–DOX–HSA NPs prepared by the incorporation method were used to treat drug-resistant lung cancer *in vivo* [37].

Some other water-soluble drugs have been delivered by various types and formulations of albumin NPs, for example ganciclovir and phosphodiester oligonucleotide analog (anti-

cytomegaloviral compounds) [104], antisense oligonucleotides against polo-like kinase 1 [67], 5-fluorouracil (anticancer drug) [105], sodium ferulate (anti-hepatic fibrosis compound) [106], gabapentin (treatment of epilepsy) [107] with high stability and reduced side effects.

5.1.2. Insoluble/poorly water-soluble drugs loaded onto albumin—Several different methods have been used to load water insoluble or poorly soluble drugs into albumin NPs. The two major methods are entrapment and encapsulation. Drugs may bind to NPs by electrostatic adsorption of charged molecules or by covalent linkage between the drug and the albumin matrix of the NPs [33]. To accomplish these purposes, several fabrication methods have been used such as Nab-technology and self-assembly.

Paclitaxel has low water solubility and when delivered in a formulation with high concentration can induce hypersensitivity reactions. Zhao et al. reported the *in vitro*-targeted delivery of BSA NPs loaded with paclitaxel and further decorated with folate to target folate receptors on a human prostate cancer cell line [108]. They reported a drug-entrapment efficiency of 95.3% and a drug-loading efficiency of 27.2%. They demonstrated a significant increase in paclitaxel water solubility, the NPs had desired surface properties, high stability, and showed specific targeting to cancer cells. Another study investigated vinblastine sulfate-loaded BSA NPs conjugated with folate for tumor-targeted drug delivery [109]. They investigated the physiochemical properties of these NPs and demonstrated a drug-entrapment efficiency of 84.83% and a drug-loading efficiency of 42.37% and proposed that this structure could be effective in tumor targeting.

Delivery of some other drugs such as HI 6 dichloride monohydrate and HI 6 dimethane sulfonate (treatment of intoxication by organophosphorus [OP]) [110], aspirin [111], methotrexate (anticancer drug) [112], platinum (IV) prodrugs such as cisplatin (treatment of cancer) [35], ruthenium-based anticancer drugs [113], diclofenac (an anti-inflammatory drug), and tacrolimus (as a immunosuppressant) [41] have also been investigated to improve their stability and solubility.

5.1.3. Other compounds—Conjugation of BSA to a water-soluble phthalocyanine (ZnPcC4) was investigated in a new approach to cancer therapy called sonodynamic therapy (SDT). SDT is the synergistic effect of sonosensitizers excited by ultrasound [114]. They found efficient sonodynamic activity of ZnPcC4–BSA against HepG2 human hepatocarcinoma cells caused by production of intracellular reactive oxygen species. To improve drug delivery of albumin-based nanostructures, other biomolecules can be attached such as alginate (to improve the swelling behavior of NPs) [115] and Apos A–I and B-100 that have been proposed to increase delivery across the BBB by interacting with the scavenger receptor SR-BI [92,94].

6. Albumin NPs for delivery to specific organ systems

As mentioned above, albumin is an excellent material for construction of nanostructured-based DDSs, especially considering its good controlled-release properties, its ability to tune the hydrophilicity/hydrophobicity, defined primary structure, and the existence of charged

amino acids [33,56]. Its excellent interaction profile is one of the important factors in its pharmacokinetic and pharmacodynamic properties [116]. Table 1 lists recent investigations of albumin DDSs for different malignancies and tissue targeting.

6.1. Albumin-based nanostructures for brain targeting

In recent years, the targeted delivery of albumin-based NPs has been investigated for different neurological and neurode-generative diseases. As one example, treatment of AD has been investigated using albumin NPs with the capability to overcome the BBB. AD is a neurodegenerative disease that represents the most common form of dementia among old people (over the age 65 years) and is expected (without efficient diagnosis or therapeutic intervention) to double every 20 years [124]. Due to the growth of the incidence of this disease over time, finding efficient strategies for its detection and treatment in early stages is urgent [103]. DDSs for AD treatment should be designed and targeted to provide controlled release and complete absorption of drugs at objective site [103]. Brain-targeted drugs must cross the BBB which acts as a barrier against drug delivery in the treatment of AD and other brain disorders [125]. Due to the accumulation of Aβ in the brain after AD, one effective strategy may be the elimination of the Aβ peptide from the brain, but it would need to cross the BBB in the reverse direction [126] to be cleared via the blood stream. HSA can clear Aβ from the CNS and act as effective inhibitor of Aβ fibrillization [127] indicating potential application of albumin for optimized NPs design.

Tacrine is a licensed drug for the treatment of AD but suffers from low bioavailability, short half-life, and hepatotoxicity. A study prepared BSA NPs attached to beta cyclodextrin and its hydrophilic derivatives for their potential advantages on drug solubility, absorption, and nasal delivery to reach specific sites of the brain. The results showed favorable nasal drug permeation, improved nasal drug bioavailability, decreased lag time, and potential application for AD treatment particularly using most selective types of cyclodextrin derivatives [103].

Furthermore, dual-functionalized albumin-based NPs were prepared using cRGD, KALA, and DOX loading. Through this structure, improved results were shown including brain tumor targeting via integrin targeting of cRGD, enhanced cell penetrating of KALA, and pH responsibility via disassembly of nanostructure. Furthermore, higher intracellular drug accumulation and rapid DOX release were reported [63].

6.2. Liver targeting

Albumin has often been exploited in selective delivery systems targeting the cells of the liver [22,66]. Coupling biomolecules to albumin has paved the way for the robust advances in development of novel drugs against liver diseases by improving short-term administration and by reducing extrahepatic side effects [128]. Numerous studies have been focused on albumin NP-based liver delivery systems for treatment of different hepatic diseases such as viral hepatitis, hepatocellular carcinoma (HCC), liver micrometastases, and hepatic fibrosis.

DOX is considered as a therapeutic drug in treatment of HCCs. For example, (6-maleimidocaproyl) hydrazone derivative of DOX conjugates containing galactose residues are used as a delivery system in liver tumor targeting. Favorable results are obtained with

this formulation such as enhanced antitumor efficacy and a better toxicity profile. Selective and rapid binding of this structure to albumin and the high plasma stability of the albumin-bound form made it a clinical candidate for intravenous applications [129]. DOX can be either extracellularly or intracellularly released due to its acid-sensitive hydra-zone linkage contained in the prodrug. Because of conjugate binding to the asialoglycoprotein receptor, they can selectively be taken up by HCC cells.

Oridonin is an effective drug against primary liver cancer; however, its poor solubility in water as well as its short biological half-life limits its clinical use. On the other hand, galactosylated BSA (GBSA) can act as an effective drug carrier to target liver tissue [130]. In a study by Li et al. [23], oridonin-loaded GBSA NPs were developed for liver targeting. The amount of galactose on the NPs surface was correlated with hepatocyte targeting as well as reducing the toxic effects of the free aldehyde moieties. The oridonin GBSA NPs showed suitable stability and controlled release features.

Furthermore, a study used BSA NPs for delivery of berberine as an antiproliferative agent against activated hepatic stellate cells in liver fibrosis [66]. It was shown that this nano-particulate formulation led to a therapeutic effect at a lower dose in comparison to the free drug and could be used as antidote for liver damages of fibrosis.

7. Cancer treatment

The characteristics of albumin NPs make them appropriate candidates for tumor-targeted drug delivery to different stages of cancer. In this regard, multifunctional nanostructures based on albumin have provided a new paradigm for advanced drug delivery and theranostic applications. For example, a recent study introduced a composite NP consisting of Au nanoclusters embedded in BSA NPs for DOX delivery to cervical cancer HeLa cells and for cell imaging through photonic excitation (Figure 5(a)) [131]. The nanostructures showed good stability and retention of their luminescence and were well taken up by cells. Furthermore, additional properties of NPs included favorable biocompatibility, suitable quantum yield, and ability to track drug release. Figure 5(b) illustrates the cytotoxicity assessment of HeLa cells treated with Au nanoclusters embedded in BSA NPs loaded with DOX via an MTT-based cell viability assay. The nontoxicity of the empty composite NPs and the toxicity of the DOX-loaded NPs were shown. Another study developed HSA NPs loaded with fisetin showing selective toxicity toward breast cancer cells (MCF-7) [60].

In addition, application of albumin NPs for direct delivery into tissue is another efficient approach. A study constructed conjugates from apoptotic TRAIL protein–HSA–DOX–octyl aldehyde HSA NPs (TRAIL/Dox HSA-NPs) with synergistic cytotoxic and apoptotic effects on H226 lung cancer cells [37]. In an animal model, gradual drug release with good decrease in tumor size was reported through application of TRAIL/Dox HSA-NPs in comparison to TRAIL or Dox HSA-NPs alone (Figure 6).

8. Conclusions

In recent years, albumin-based DDSs have captured considerable attention from researchers, and a range of technologies based on albumin nano/microparticles have been developed.

These technologies are being exploited in therapeutic biomedical applications including anticancer DDSs, gene delivery, and also theranostic applications. Albumin nanostructures provide many favorable properties for drug delivery applications including biocompatibility, biodegradability, easy surface modification, and improvement in the water solubility of poorly water-soluble drugs. Various preparation methods, surface modification mechanisms, and different structures of albumin NPs have been exploited to deliver cargos and targeting to specific sites.

Investigations are still ongoing into the exact nature of the albumin protein–drug interactions; identifying and characterizing binding sites; decreasing immunologic responses; targeting of different tumor cell types; and delivery to a wider range of tissues, organs, and complex tumors. Application of albumin structures and their exceptional properties for theranostic applications and other synergistic approaches can also be used for preparation of novel nanocarriers. Furthermore, optimization of albumin-based nanostructures and their specific targeting is very important factors for improvement of their therapeutic efficacy and more future clinical applications.

9. Expert opinion

In recent years, albumin-based DDS have captured a great deal of attention by researchers in the pharmaceutical sciences, and diverse techniques based on preparation of albumin-based micro/nanoparticles have been developed for targeted therapy of cancer, infections, and other conditions. Albumin DDSs containing optical and ultrasound-based imaging agents or other reporter molecules can also be used for diagnostic and imaging applications or theranostics (combination of diagnostic and treatment in a single structure). Albumin-based delivery systems can be used for gene therapy applications where nucleic acids need to be protected from degradation in the bloodstream and delivered into target cells. The entirely natural origin, good biocompatibility, and low immunogenicity of albumin protein make it the protein of choice for preparing DDSs. Several approaches have been developed for fabrication of nanomaterials, surface modification, and drug/gene loading. Furthermore, various micro/ nanoparticles with different physicochemical characteristics have been proposed for tissue-specific targeting. Despite these advances, there are still challenges in the field of albumin-based DDSs. For example, nanoparticulate delivery systems including albumin NPs require a simple and one-step synthesis method, especially for scale-up in the pharmaceutical industry. Additionally, less investigated issues in nanomedicine may create new challenging discussions in the future, for example the long-term impacts of NPs (including albumin NPs) on the human body, or their biological toxicity may harm the environment.

On the other hand, biological challenges of drug delivery must be resolved. Despite many studies on targeted delivery systems, there is still no approved clinical product in the field of active targeting. Furthermore, the biological properties of tumors and malignancies must be considered. For example, stimulus-responsive DDSs and gene carriers that respond to different physiological cues in diseased or cancerous tissue (e.g. the acidic microenvironment of tumors) have been extensively investigated [8,132]. The addition of stimulus-responsive moieties into the structure of albumin NPs would be a potential future

research field. On the other hand, due to the important impact of albumin protein on the formation of the protein corona, short- and long-term effect of biological media on the albumin NPs with different shape/size/charge should be investigated.

Nab-paclitaxel was approved and marketed for metastatic breast cancer treatment in 2005 and for first-line treatment of metastatic non-small-cell lung cancer in 2012 and metastatic pancreatic cancer in 2013. In addition, several clinical trials are under development for treatment of metastatic melanoma, pancreatic cancer and for delivery of other hydrophilic/hydro-phobic drugs [16,21]. The regulatory approvals that have already been obtained for several albumin-based therapeutic agents suggest this approach will continue to be a profitable route for future endeavors. Optimization of the different nanostructures and their specific targeting abilities, together with further toxicological studies, will be very important for future clinical applications.

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Article highlights

- Albumin (from serum or chicken eggs) is the most investigated protein for preparation of protein based drug delivery nanostructures.
- Advantages include ready availability, natural origin, ease of chemical modification, good biocompatibility, biodegradability, and low immunogenicity.
- Albumin has a range of natural molecular binding sites that can be used for drug delivery.
- Albumin nanostructures can solubilize insoluble drugs, reduce toxicity to surrounding normal cells, and protect nucleic acids from degradation.
- Molecular-recognition ligands can be attached to albumin DDSs to allow targeting to specific cells and tissues including cancer.
- Albumin-based DDSs have already received several regulatory approvals and more can be expected.

This box summarizes key points contained in the article.

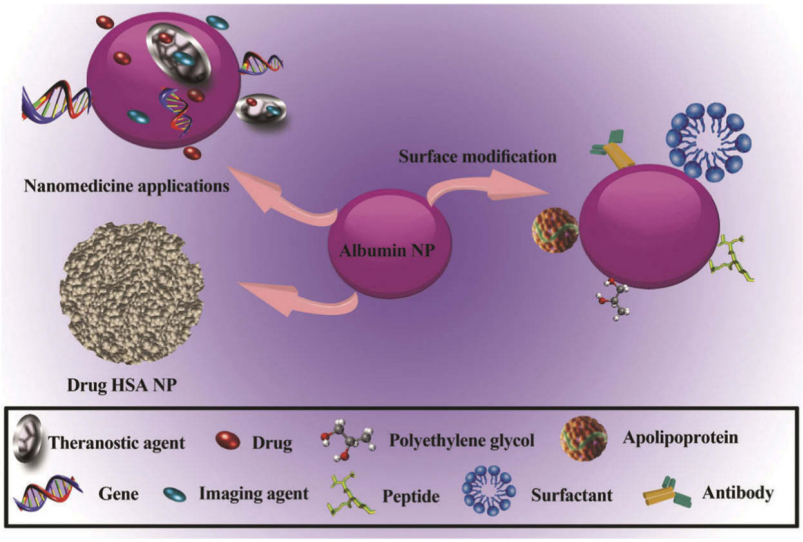

Figure 1.

Versatile carrier systems based on albumin NP. Different approaches for modification of albumin NPs and their application for delivery of different biomolecules are shown.

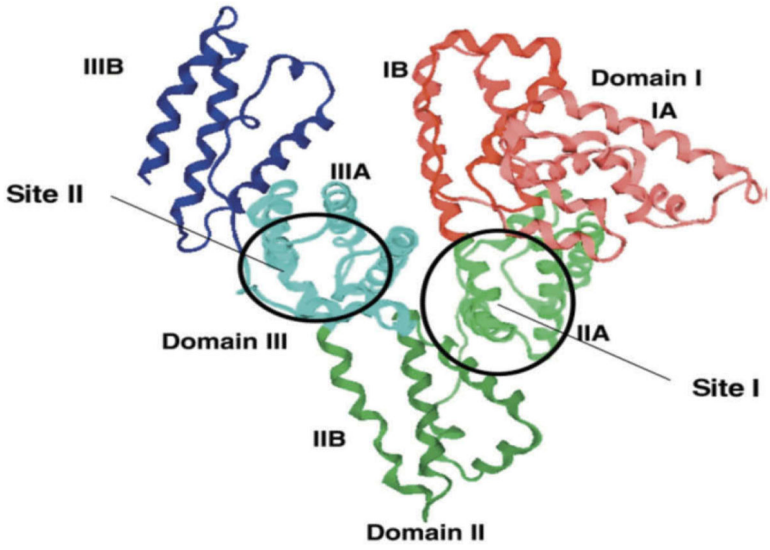

Figure 2.
Schematic of the crystal structure of HSA and its main binding sites. Reprinted from ref [36]. with permission from John Wiley & Sons.

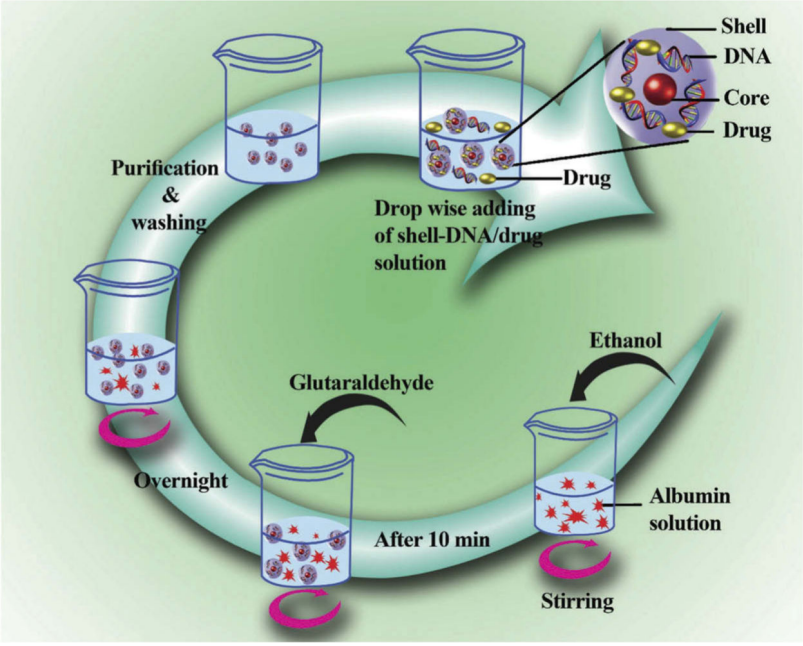

Figure 3.
Desolvation based preparation of albumin nanostructure for gene/drug delivery.

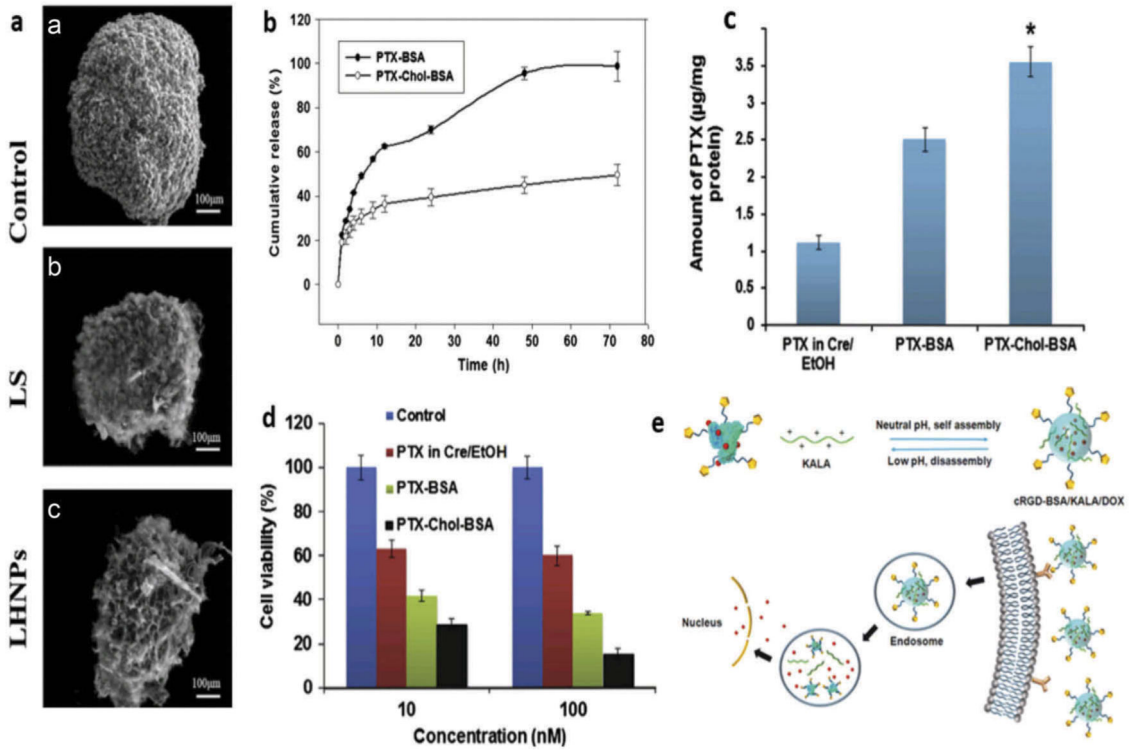

Figure 4.

(a) Scanning electron micrographs of 4T1 carcinoma spheroids, after incubation with lapatinib solution (LS) and LHNP in comparison to control group, (b) Cumulative release profile of paclitaxel from paclitaxel-BSA and paclitaxel-Chol-BSA, (c) Cellular uptake quantification of paclitaxel after incubation in MCF-7 cells for 1 h, (d) MCF-7 cell viability results after exposure to the formulations indicated, (e) Schematic illustration of the cRGD-BSA/KALA/DOX NP and their pH-triggered assembly and disassembly. (a Reproduced from ref [61]. Copyright 2015 with permission from ‘Elsevier’. b, c, d reproduced with from ref [62]. Copyright 2015 with permission from ‘Elsevier’; e reproduced from ref [63]. Copyright 2015 with permission from ‘American Chemical Society (ACS)’).

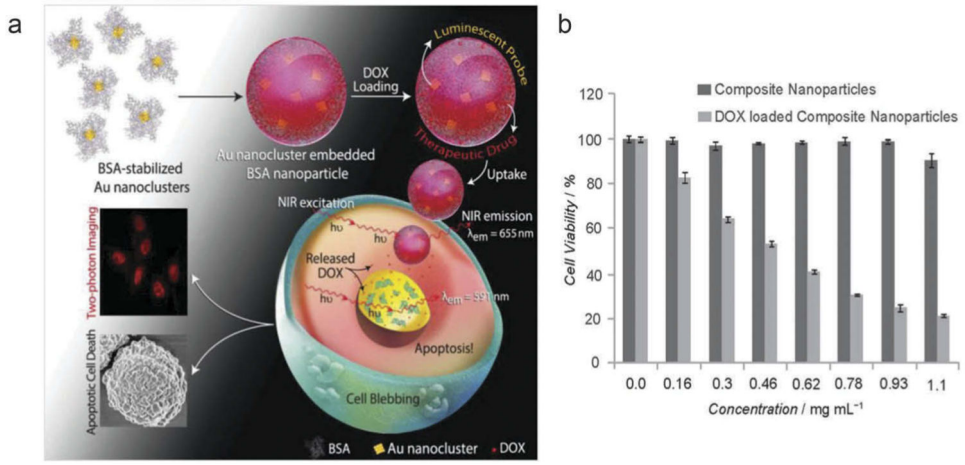

Figure 5.

a) Schematic illustration of the composite nanoparticles composed of Au nanoclusters embedded in BSA NPs and the consequent cancer cell uptake and DOX release inducing cell death. Cellular apoptosis and related two-photon imaging are also demonstrated, b) HeLa cell viability evaluation via MTT assay after treatment with various concentrations of Au nanocluster embedded BSA NPs and DOX loaded Au nanocluster embedded BSA NPs for 36 h. Reproduced from ref [131]. Copyright 2015 with permission from John Wiley & Sons.

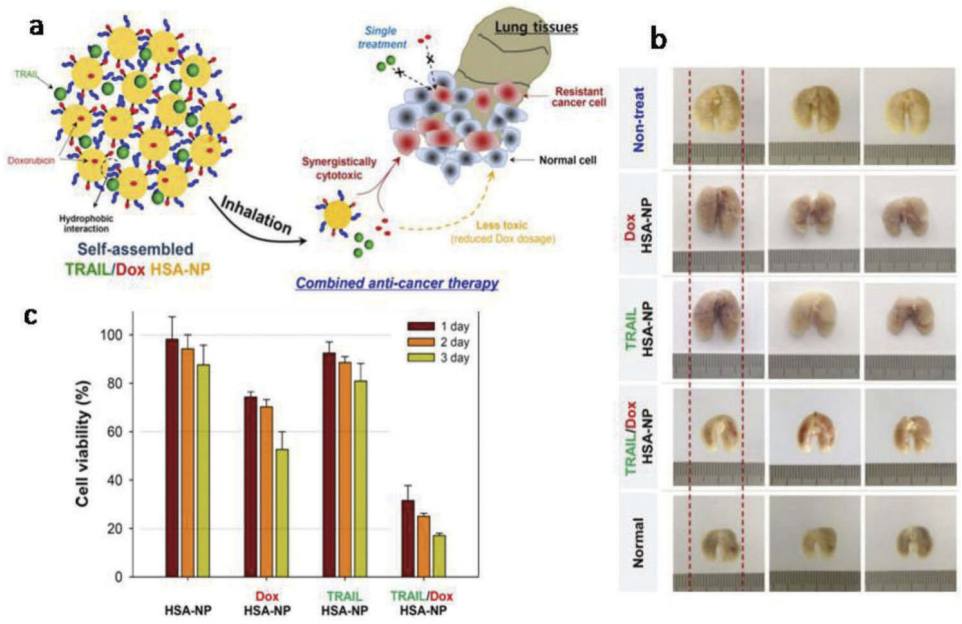

Figure 6.

a) Schematic illustration of TRAIL/Dox HSA-NPs inhalation based anticancer treatment, b) Photographs of the lungs of BALB/c nu/nu mice implanted with H226 cells with and without TRAIL/Dox HSA-NPs incubation, c) Cell viability of H226 cells via MTT assay after exposure to HSA-NPs, Dox HSA-NPs (6 μg/ml), TRAIL HSA-NPs (2 μg/ml), and TRAIL/Dox HSA-NPs (6 μg/ml of Dox + 2 μg/ml of TRAIL). Reproduced from ref [37]. Copyright 2015 with permission from ‘Elsevier’.

Table 1

Recent advances in tissue targeting with albumin-based DDSs.

Particle delivery system	Size (nm)	Aim of the research	Results	Reference
10-Hydroxycamptothecin-loaded HSA NPs	<200	New concept on loading strategy	Introduction of liquid compound synthesis method High entrapment efficiency Low-toxicity involvement *In vitro* significant antitumor activity with model drug	[117]
BSA–poly(L-lactic acid) NPs	118–156	Drug delivery in cancer	Long blood circulation half-time Good tumor inhibition efficacy and low side effects in a mouse tumor xenograft model Improved sustained release	[118]
Niclosamide encapsulated BSA NPs	199.9	Cancer drug delivery	Good *in vitro* therapeutic efficacy Good induction of apoptosis Excellent particle properties	[119]
HSA NPs	~340	Drug-resistant lung cancer	Antitumor efficacy with synergistic cytotoxicity and apoptotic activity Reduced tumor size Reduced drug doses and low side effects Enhanced bioavailability and sustained-release property of gemcitabine	[37]
Graphene quantum dot-labeled HSA NPs	56–250	Pancreatic cancer-specific drug delivery and bioimaging	Improved efficiency of drug on resistant pancreatic cancer cells Excellent bioimaging potential High biocompatibility	[120]
^{111}InBSA albumin NPs	~193	Biodistribution analysis of albumin NPs for pulmonary drug delivery	Greater retention time in lung tissue Slow clearance	[121]
BSA NPs	300	Anti-Alzheimer's drug delivery	Spherical-shaped and negatively charged NPs Increased drug loading Sustained drug release Enhanced tacrine delivery	[103]
Polysorbate 80-coated NPs	141.9	Epilepsy treatment	Delivery of gabapentin Increased drug concentration in the brain Reduced duration of all phases of convulsion Formation of a drug monolayer on the NPs surface	[107]
HSA NPs	~268	Treatment of organophosphorous intoxication	Rapid release of the antidote obidoxime drug	[122]
Galactosylated BSA spherical NPs	200	Liver targeting	Stable delivery and release of poorly soluble oridonin Entrapment efficiency: 58.2–72.4 Loading efficiency: 2.8–4.8 Inhibition of the LX-2 cell growth	[23]
BSA NPs	394	Liver fibrosis therapy	Exhibition from caspase 3 activation at low doses Inhibition from hepatotoxicity *in vivo* Sustained enzyme release	[66]

Particle delivery system	Size (nm)	Aim of the research	Results	Reference
β-Galactosidase-loaded HSA NPs	170–350	Enzyme delivery	Negligible toxicity to animal cells Enhanced cellular uptake and intracellular stability High drug entrapment	[13]
Chitosan–egg albumin NPs	352.9	Transdermal aceclofenac delivery	Sustained *ex-vivo* permeation High inhibition of swelling of rat paw edema	[123]

HSA: Human serum albumin; BSA: bovine serum albumin; NPs: nanoparticles; DDSs: drug delivery systems.