Send Orders for Reprints to reprints@benthamscience.ae

Current Pharmaceutical Design, 2015, *21*, 000-000

Recent Advances on the Development of Pharmacotherapeutic Agents on the Basis of Human Serum Albumin

Fang Liu*, Jing Mu, Bengang Xing*

Fang Liu

Division of Chemistry and Biological Chemistry, Nanyang Technological University, Singapore 637371, Singapore

Bengang Xing

Abstract: Human serum albumin (HSA), a major transport protein component in blood plasma, has been reported recently to play many important roles in pharmacotherapeutics development. Owing to its promising intrinsic binding capability of drug molecules, HSA offers favorable characteristics and can be directly used as its monomeric formula or can be fabricated into protein based nanoparticle platforms to realize the effective delivery of therapeutic molecules into targeted diseases areas. In addition, HSA can also serve as a protein stabilizer or environment-responsive moieties to hybridize with the functional materials including polymers or inorganic nanoparticles through the covalent reactions or electrostatic interactions, and can thus greatly alter the relevant biological distribution and pharmacokinetic behavior to improve their therapeutic efficacy. By right, extensive studies have been conducted to develop HSA-conjugated pharmacotherapeutic agents toward effective *in vitro* and *in vivo* diseases diagnosis and treatment. The current review gives an in-detail account of the latest progresses of HSA-based carriers as functional protein materials, mainly with respect to its conjugation types, formulation aspects, and importantly their promising applications towards enhanced drug delivery and medical diagnosis.

Keywords: Human serum albumin, Drug delivery, Nanoparticle, Self-assembly, Pharmacotherapeutic agents, Covalent conjugation, Electrostatic interactions.

INTRODUCTION

Controlled release of therapeutic agents at location of disease areas with desired dosage will be of clinical significance to guarantee the determinative therapeutic and diagnostic consequence while minimizing the drug resistance and other side effects in the process of the treatment. So far, various types of delivery vehicles including liposomes, polymeric nanoparticles and nanoscale protein materials have been developed to achieve the precise and safe drug release by means of the enhanced permeability and retention (EPR) effect [1-3]. Amongst the available materials in drug delivery system, the nano/micro structures formed by proteins, a kind of natural biopolymers, have gained increasing attention due to their promising features of high bioavailability and favorable drug loading capacity [4, 5]. Moreover, unlike the synthetic polymers and other kinds of biopolymers such as polysaccharides, the delivery platforms based on natural proteins are easily amenable to residue specific modifications, which allow the precise control of their structures and functions in living systems. Such natural protein based delivery vehicles with site directed attachment of drugs and functional groups could therefore indicate profound effects towards the enhanced *in vivo* efficacy and therapeutic index over traditional treatment process [6].

Human serum albumin (HSA) is a major soluble protein (35–50 g/L human serum) in our blood and involved in many important bio-functions such as the maintenance of osmotic pressure and delivery of nutrients from circulating system to cells. HSA has been found highly soluble in aqueous solutions (up to 40% w/v) at pH 7.4 and extreme stable under a wide pH range from 4 to 9. In addition, as a natural transport protein, HSA has also been found to be able to bind with various endogenous and exogenous drug molecules in physiological environment. These intrinsic properties make HSA an attractive protein material for delivery of a wide variety of drugs or diagnostic agents toward the biomedical usage [7, 8]. Moreover, due to its favorable stability, biodegradability, promising size distribution and easily controlled surface chemistry, HSA has become the most commonly used biopolymers-based nanocarriers for the purpose of drug/gene delivery and medical diagnosis [9, 10].

Herein, we give an overview on the latest progress of HSA-based protein carriers for the improved drug delivery. Since some HSA-based protein transport systems have been well summarized in recent years [11, 12], in this review, we will mainly focus on the recent developments regarding the systematic strategies for the design and fabrication of HSA-based drug carriers and their relevant *in vitro* and *in vivo* applications based on literatures reported within the latest few years. Upon a general introduction to the structural and chemical properties of human serum albumin, the selected cases will be discussed in detail with respect to the conjugation types and formulation aspects between drug molecules and HSA moiety. The recent advances on improved drug delivery system through monomeric HSA-drug complexes, HSA-based protein particles and HSA-polymer/inorganic nanoparticle hybrids will also be extensively described. The final section summarizes future prospective for HSA carrier protein as promising scaffold toward the biomedical applications in targeted therapeutic delivery and medical diagnosis.

1. INTRINSIC STRUCTURAL AND CHEMICAL PROPERTIES OF HUMAN SERUM ALBUMIN

As the most abundant protein component in blood plasma, human serum albumin (HSA) accounts for about 60% of total plasma proteins [13]. This protein is encoded by the *ALB* gene on chromosome 4 and produced by the liver. Native HSA is synthesized as a non-glycosylated single chain at a rate of 0.7 mg/hour per gram of liver, which equates to roughly 10-15 grams of HSA synthesized daily. HSA has a molecular weight of 66.5 kDa with the primary sequence consisting of a single polypeptide chain with 585 residues and 17 pairs of disulfide bridges [13]. The amino acid composition of HSA is unique because it only has one tryptophan (Trp214), and only one free cysteine (cys 34), while the ionic amino acid residues such as glutamic acid, arginine and lysine are abundant. Such native

*Address correspndence to this author at the Division of Chemistry and Biological Chemistry, Nanyang Technological University, Singapore 637371, Singapore; E-mail: Liuf0019@e.ntu.edu.sg; Bengang@ntu.edu.sg

1381-6128/15 $58.00+.00

residue sequences endow albumins a high total charge property (with the net charge of -15 at physiological pH), and thus promoting its solubility [14, 15]. The X-ray structure analysis reveals that HSA contains three homologous domains (I, II and III). These domains form a heart shape confirmation, and each of them contains 10 alpha helixes that can be further classified into two sub-domains (A and B) (as showed in Fig. **1**). Basically, native HSA has excellent stability in physiological conditions with an average half-life of 15-20 days, which makes this protein an important transporter for a number of biomolecules. Such favorable stability is partly contributed from its 17 intramolecular disulphide bridges [16], which thus holds the great advantages as a protein material in the fabrication of biopharmaceutical products.

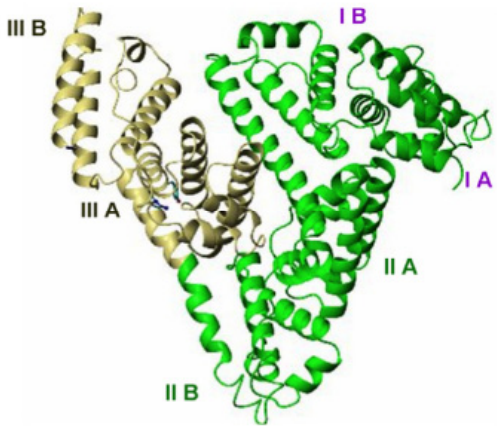

Fig. (1). The crystal structure of HSA and the locations of sub-domains from X-ray diffraction studies. The principal drug-binding sites on albumin are IIA and IIIA. The structure was obtained from the Protein Data Bank.

Numerous biophysical studies also revealed that HSA has several amphiphilic regions consisting of a number of alpha-helixes. Contributed from the amphipathicity and flexibility of alpha-helix reach regions, HSA represents the great potentials for binding of various ligands with promising affinity (10^{-4} to10^{-6} M), particularly for those with amphiphilic property. Although some ligands (e.g. fatty acid etc) were found binding to albumin at multiple sites, the IIA and IIIA subdomains have been proved to be major binding sites for the different types of ligands. In detail, the main binding forces for ligands affinity to subdomain IIA are derived from enthalpy, indicating the ionic interactions would be major factors for this subdomain. In contrast, the major binding forces for subdomain IIIA were entropy-driven, implying the hydrophobic interactions are the main contributing factors for ligands binding to the pocket of IIIA [17, 18]. Such interactions with HSA protein will influence the distribution, metabolism and excretion of small molecules in living systems [12, 19].

Furthermore, owing to its favorable flexibility, HSA also represents reversible conformation changes in the solutions with different pH values. Those conformation changes are thought to provide the structural basis for controllable encapsulation and release of drug molecules [20]. It is proposed that after binding with ligands, HSA–ligand complex could circulate through the body. When it reaches the target cells, the local pH changes at the cell membrane region may induce the conformation change of HSA, thereby leading the loose of caged molecules from HSA [21, 22]. Although the signal pathway for native HSA trafficking has not been fully recovered yet, generally, the protein was thought to bind to the albumin receptor proteins (e.g. gp60) for translocation across the endothelial cells [23]. Moreover, in some cases, the ligand binding may even influence biodegradation of HSA. For example, the binding fatty acid was found able to increase the half-life time of HSA in circulation system [24, 25].

2. RECENT ADVANCES IN HSA BASED PROTEIN MATERIALS FOR PHARMACEUTICAL USAGE.

Generally, tumor vascular abnormality allows enhanced accumulation of nano-sized drug cargos in tumor tissues rather than plasma or other organs. Such so-called enhanced permeability and retention (EPR) effect may decrease the renal clearance of nano-sized drug moieties, and thus increasing their circulation time in living system [26]. Usually, macromolecules with molecular weight of 40 to 800 kD employed as carriers for the development of tunable nano-sized prodrugs typically have hydrodynamic radii between 2 nm and 10 nm, and their biological behaviors will be mainly dominated by EPR effect [11, 27-29]. It should be noted that the monomeric human serum albumin protein has an effective hydrodynamic diameter of 7.2 nm, could thus work as carriers and enable the efficient delivery of therapeutic molecules on the basis of such EPR effect. Moreover, due to its promising bioavailability and drug loading capacity, HSA has often been used to form protein nanoparticles which can be employed for the purpose of drug or gene delivery [5, 6, 30]. In this section, the recent progress in the preparation and application of HSA based protein materials will be summarized according to their conjugation types and formulation aspects.

2.1. HSA-based Protein Conjugates for Therapeutic Design

2.1.1. HSA-Drug Conjugates Derived from Non-covalent Interactions

In terms of its unique biophysical properties, native HSA represents a number of binding sites for various types of drug molecules. Curry etc. summarized the ligand binding capacity of HSA based on the crystallographic studies up to 2005. For example, some acidic lipophilic endogenous compounds such as fatty acids, bilirubin and hemin etc are likely binding to HSA in multiple recognition sites. Some commonly used drug molecules including those with negative charges in physiological conditions are found priority bound to the binding sites mainly exist in subdomain IIA and IIB (Fig. **2**) [16]. More recently, Hazai etc [31] also indicated a systematic docking analysis to investigate the relationships between the chemical structures of a drug and the binding features to HSA and they found that apolar moieties (e.g. the hydrophobic tail of a fatty acid) of the ligand molecules were likely to be buried in the hydrophobic cleft in binding site.

Due to the promising binding of hydrophobic ligands to HSA, the strategy to enhance the binding affinity of a drug molecule toward HSA by introducing an additional hydrophobic part to the drug molecule structure has therefore become a well recognized method in the rational design of pharmaceutical prodrugs. For instance, metal-based platinum drugs such as cisplatin, carboplatin and oxaliplatin, have been widely used in clinics to treat a variety of solid tumors, which have been proposed to effectively suppress tumor progression by their interactions with DNA in tumor cells [32]. Unfortunately, the lack of tumor specificity, severe toxic side-effects, and acquired or intrinsic tumor resistance of platinum drugs remain the serious problems in clinical practice. Therefore, systematic studies aimed at designing and preparing new strategies to further improve the therapeutic index and minimize side effects associated with cancer treatments are currently the topics of consideration. To this end, Lippard's group recently proposed a new drug design by incorporation of platinum (IV) prodrugs with native HSA proteins [33]. In this typical study, a series of platinum (IV) molecules have been modified with fatty acid chain at axial position. Among them, the platinum (IV) complex with length of C16 which could form a non-covalent adduct with ratio of Pt and HSA: 1: 1 (Fig. **3**). The *in vitro* stability studies showed this well optimized Pt-HSA complex has significant longer life time (6.8 h)

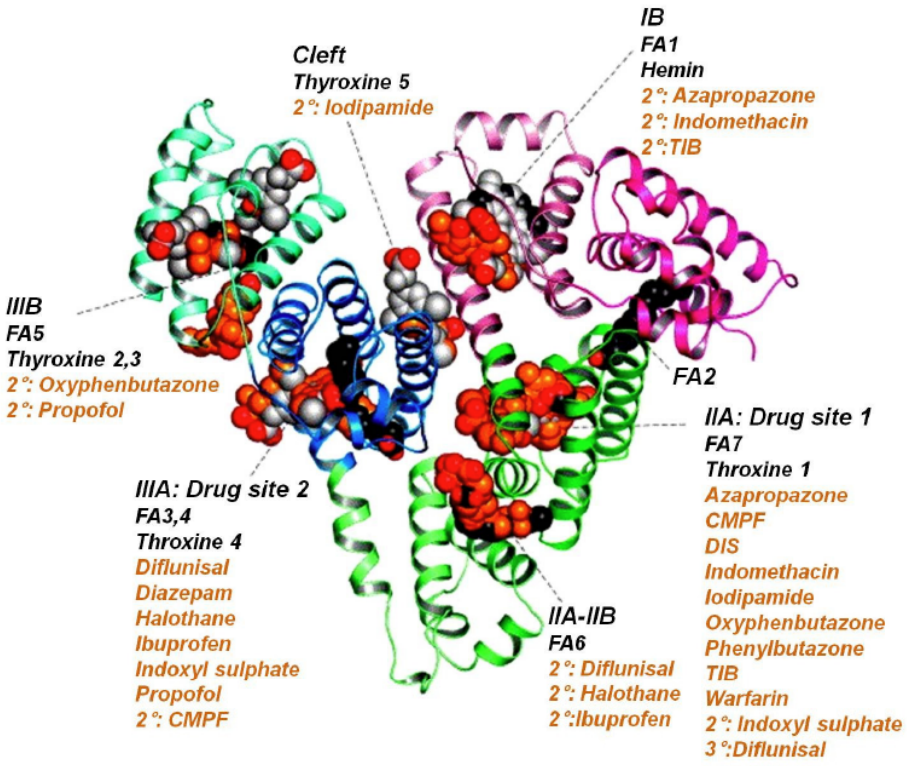

Fig. (2). Summary of the ligand binding capacity of HSA based on crystallographic studies. Ligands are depicted in space-filling representation; oxygen atoms are marked as red; all other atoms are coloured dark-grey, light grey and orange, respectively [16]. [Reprinted by copyright permission of Elsevier]

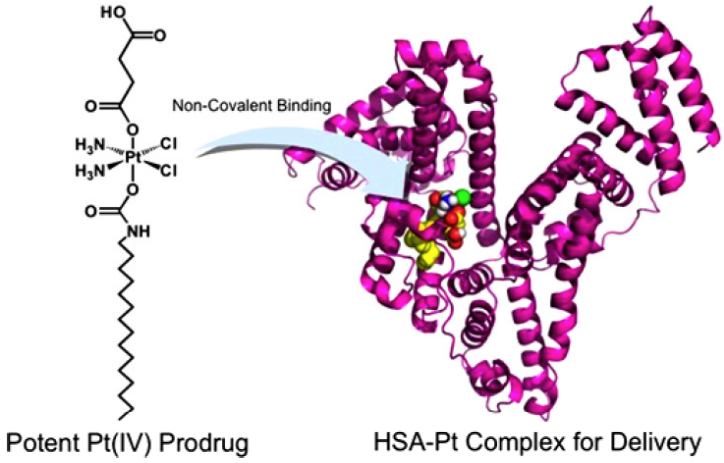

Fig. (3). Schematic illustration of the incorporation of a platinum (IV) prodrug with HSA [33]. [Reprinted by copyright permission of American Chemical Society]

in whole human blood than that of cisplatin (20 min) or satraplatin (6 min). Moreover, the non-covalent metal drug and HSA protein complex also indicated enhanced *in vitro* anticancer activity when compared with cisplatin.

Inspired by the hypothesis that the increased hydrophobic interactions with human serum albumin could potentially enhance the bioavailability of platinum antitumor prodrugs, similar design was also applied in other non-platinum metal complexes [34-37]. For example, recently, Keppler and Walsby etc demonstrated systematic studies through a series of pyridine-based Ru III derivatives (as shown in Fig. **4**) for antitumor applications [34, 38]. They confirmed that the pretreatment of those Ru III prodrugs with HSA could greatly improve anticancer activities *in vitro*. The detailed mechanism was further analyzed by crystallographic studies and EPR measurements, which clearly indicated that the enhanced non-coordinate interactions might increase the solubility of these metal complexes, and such unique interactions could effectively inhibit unexpected protein-metal coordination, thus improving their antitumor activities [34].

Together with metal based antitumor prodrugs, some amphiphilic dye molecules were also reported to bind with HSA structure through electrostatic and hydrophobic interactions [39, 40]. For example, a near-infrared dye IR825 was found to form HSA-IR825 complex with 1: 1 ratio [40, 41]. Such HSA-IR825 complex indi-

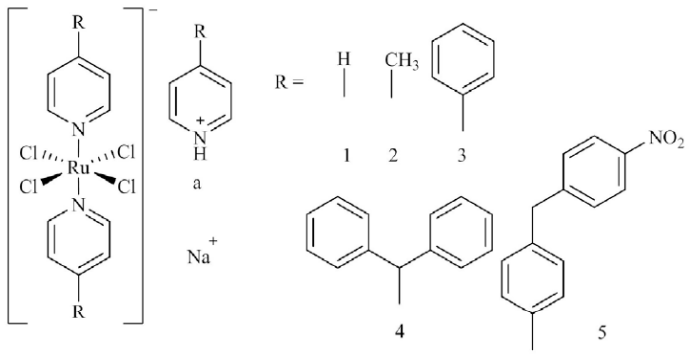

Fig. (4). The chemical structures of typical Keppler-type Ru III agents and their modified analogues synthesized by Walsby's group. Picture adapted from reference [34].

cated greatly enhanced (e.g. 100-fold) fluorescence emission under 600 nm compared to that of free IR825. Meanwhile, a rather high absorbance but low fluorescence QY at 808 nm was observed. By taking those unique properties, this photoactive HSA-IR825 complex indicated a robust imaging guided photothermal effect for *in vivo* tumor treatment when upon the light irradiation at 808nm [40]. Typically, the *in vivo* animal imaging and cancer phototherapy showed that HSA-IR825 effectively accumulated in tumor sites with highest fluorescence signals than other organs, and therefore exhibited strong photothermal effects. Tumors of mice in the treatment group were completely ablated by photothermal therapy within 16 days after treatment (Fig. **5**). Similarly, another MRI imaging guided photodynamic cancer therapy system based on Gd (III)-tetraphenylporphyrin complex was also reported recently when upon its non-covalent conjugation with native HSA protein [42]. Such HSA conjugation would lead to the enhanced uptake of the porphyrin-Gd complexes by tumor cells, and thus demonstrated specific cell toxicity to the targeted tumor cells.

Generally, the presence of hydrophobic moieties in a drug molecule structure could enhance its affinity to HSA indeed, thus stabilize the drug-HSA protein conjugates for the purpose of improving drug stability and circulation retention. Although, this strategy has been extensively used in the design of anticancer macromolecular drugs, in some cases, HSA binding, if too strong, could also potentially prevent the recognition of drug molecules from the targeted enzymes or proteins, and therefore greatly compromised the treatment efficacy [43, 44]. For example, diflunisal, a salicylic acid derivative, has been widely used in the clinics for its anti-inflammatory activity. Because of its strong binding ability to HSA, the major diflunisal molecules have been found unavailable for reaction with the target enzyme during the treatment [45]. To ensure therapeutic efficacy, extreme high dosing levels of diflunisal drug molecules (> 250 mg twice daily) have to be often applied in hospitals. However, such high dose may easily cause undesirable side effects (e.g. gastrointestinal irritation etc), especially during chronic therapy [46]. Therefore, in order to optimize the reasonable binding affinity of this drug molecule to albumin protein, rather than to use drug molecule itself, Liang etc proposed a new diflunisal-HSA complex by incorporation of diflunisal prodrug into HSA. This prodrug-HSA complex displayed a relative weak intramolecular interaction than the complex formed by diflunisal and HSA [44]. The reduced binding affinity of prodrug to HSA holds great advantages in effective release of prodrugs from albumin carrier into the circulation, which thus significantly improved the drug bioavailability.

2.1.2. HSA-based Protein Drug Conjugates Derived from Covalent Interaction

Although there is an increasing interest in generation of drug-protein complex on the basis of electrostatic interactions, covalent conjugation remains the most commonly used approach in the field since it offers more stable HSA-drug complexes. Additionally, HSA represents numerous accessible functional groups due to the presence of abundant highly reactive amino acid residues including aspartic acid (36), glutamic aicd (61), lysine (59) and cysteine (35) etc [7]. Therefore, various types of HSA-drug complexes can be easily achieved by well-established coupling reactions between drug molecules and these reactive functional groups observed in HSA protein structure.

2.1.2.1. Target Cysteine Residues on HSA for Covalent Interaction with Drug Molecules

In the end of 1990s, Kratz etc. firstly developed a novel prodrug concept by introducing drugs onto HSA through the specific reaction between maleimide tagged drug molecules and the thiol group in cysteine (cys34) in protein structure. As a proof of concept, a doxorubicin-HSA conjugate was developed by conjugation of maleimide bearing doxorubicin (DOX) prodrug DOXO-EMCH to the cysteine-34 position on native HSA [12, 47, 48] (Fig. **6**). Typically, this prodrug contained an acidic environment sensitive hydrazone linker, which allowed free doxorubicin release either in intracellular acidic organelles (e.g. late endosome and lysosome etc) or in the slightly acidic environment often presented in tumor tissue. The preclinical study showed that this HSA bounded DOX prodrug system produced very promising antitumor effects. When compared with free doxorubicin, this DOX-HSA conjugate exhibited significantly reduced cardiotoxicity [49, 50]. Moreover, the phase I study also demonstrated that such drug-HSA covalent conjugate was able to induce tumor regressions in breast cancer, small cell lung cancer and sarcoma. Currently, the further Phase Ib (studies based on the combination treatment with other anti-cancer drugs such as ifosfamide and gemcitabine are still under processing now [51].

Similar design was also applied in other metal based drug molecules. For example, very recently, Christian and coworkers developed a new functional platinum (IV) prodrug for enhanced antitumor application by in situ targeting to HSA proteins in blood [52]. Typically, Pt(IV) complexes with –OH groups at both axial positions were functionalized with maleimide moieties (As showed in Fig. **7**). Such maleimide modified Pt(IV) complexes could specifically react with thiol groups in free Cysteine residues (e.g. cys34) in human serum albumin structure. The *in vivo* results clearly demonstrated that this new Pt(IV) prodrug had highly potent anticancer activity against CT-26 tumors, which therefore provided a feasible strategy to enhance tumor-targeting of platinum (IV) drugs *in vivo*.

Thereafter, targeting the free cysteine residue (cys34) on HSA has successfully become one of the mostly used strategies in the design of drug-HSA conjugates. Actually, apart from small molecule drugs, therapeutic reagents based on polypeptide structures

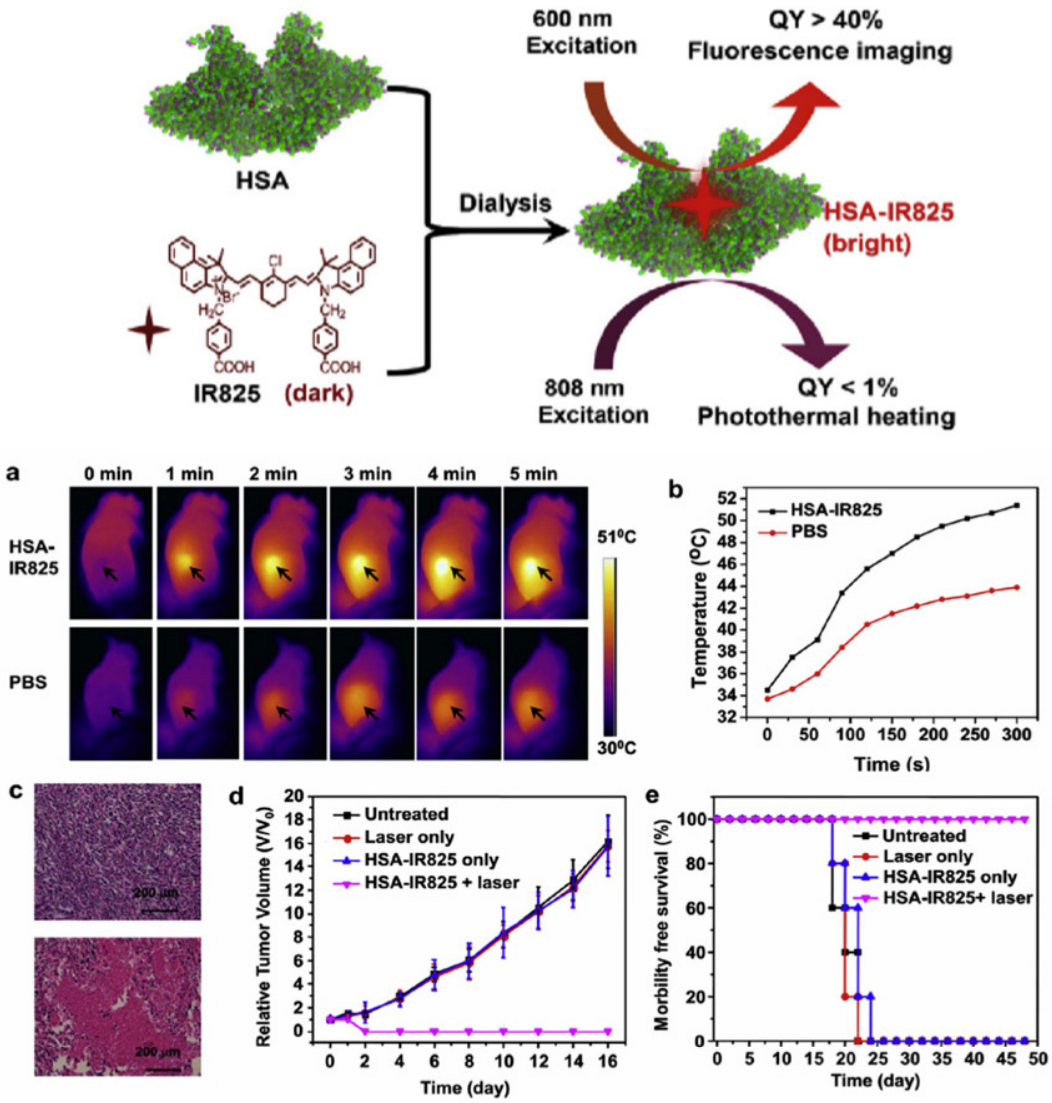

Fig. (5). Schematic illustration of the formation of HSA-IR825 complex and photothermal therapy on tumor-bearing mice. (a) IR thermal images of 4T1 tumor-bearing mice with (upper row) or without (lower row) i.v. injection of HSA-IR825 under the 808-nm laser irradiation. (b) The tumor temperature changes based on IR thermal imaging data in (a). (c) H&E stained slices of an untreated tumor and a tumor after HSA-IR825 induced photothermal ablation. (d) The tumor growth curves of mice after various treatments indicated (5 mice per group). (e) Morbidity free survival from different treatments. Picture modified from reference [40].[Reprinted by copyright with the permission of Elsevier]

were also reported to covalently conjugate with HSA carrier. For instance, polypeptide C34, one peptide sequence derived from the HR2 region of glycoprotein gp41 that helps HIV enters T-cells, has been well-known as a potent inhibitor against HIV fusion/entry. Recently, Stoddart etc reported a peptide-HSA macromolecular therapeutic conjugate (PC-1505) based on the reaction between maleimide-C34 derivative and the free cysteine residue of HAS [53] (Fig. **8**). The inhibition effect against HIV fusion of this macromolecular inhibitor was further evaluated in comparison with free polypeptide C34, and also T-20, another synthetic peptide as control that targets the conformational rearrangements of gp41. PC-1505 was approximately three times more potent than unbounded peptide in reducing the levels of HIV-1 RNA in the implants. And the authors have also found that PC-1505 has more sustained activity than T-20 against T-20-sensitive clone NL4-3G in the *in vivo* model [53]. Similar design was also reported by Xie etc., in which a modified anti-HIV peptide MPA was conjugated to HSA through specific maleimide-thiol addition. The resulted albumin-peptide FB006M displayed potent inhibitory activity against a number of laboratory and clinical isolates of HIV-1 *in vitro* and *in vivo* [54].

Furthermore, the biological conjugation of therapeutic reagents to HSA can be performed not only on the unique free cysteine, but also the other cysteines in unfolded HSA. For example, Ming etc. reported a HSA based macromolecular conjugates for targeted delivery of therapeutic oligonucleotides. In their system, native HSA was properly treated with reducing reagent DTT to disrupt the intracellular disulfide bridges. A tumor targeting peptide RGD conjugated siRNA was conjugated to HSA through disulfide linkage between the thiols from siRNA and that from cysteines in HSA. The resulted HSA conjugate has a hydro-dynamic diameter of 13 nm, and has demonstrated great gene delivery effect in *in vitro* study [10].

2.1.2.2. Target Lysine Residues on HSA for Covalent Interaction with Drug Molecules

The unique cysteine residues indeed provide promising reactive sites for preparation of drug-HSA (1: 1) conjugates based on the

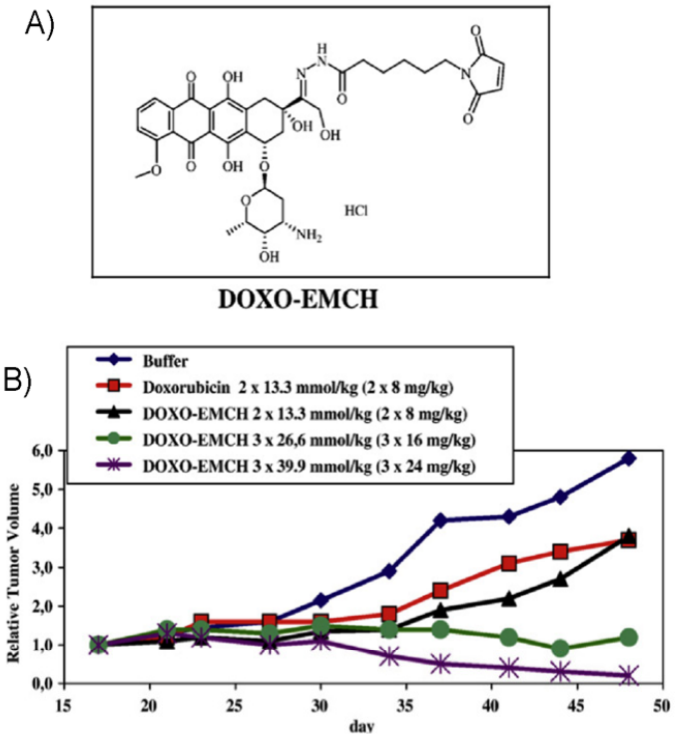

Fig. (6). (A) The chemical structure of DOXO-EMCH.(B) Curves depicting tumour growth inhibition of subcutaneously implanted MDA-MB-435 xenografts under therapy with doxorubicin and DOXO-EMCH [48]. [Reprinted by copyright with the permission of Copyright Clearance Center]

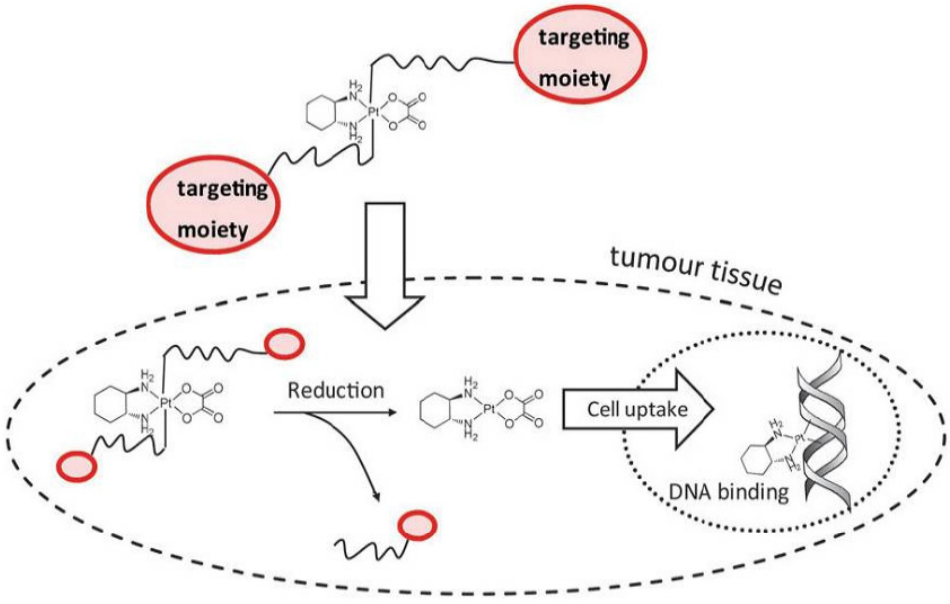

Fig. (7). (A) Illustration of maleimide-functionalized platinum(IV) prodrugs [52]. [Reprinted by copyright with the permission of RSC]

highly selective reaction between thiol and maleimide. However such strategy may have obvious limitation in the fabrication of multivalent drug-HSA conjugates because only one free cysteine on HSA is available. Although reducing reagent denatured HSA offers the possibility for multivalent conjugation by targeting cysteines, HSA transport protein may easily lose its native formula during such reductive treatment. Compared to the limited free cysteine residue in HSA structure, there are 59 lysine residues in HSA that represent numerous reactive amino groups, and several of them are also chemically accessible [55].

The first drug-HSA conjugate currently evaluated in phase I/II clinical studies was a methotrexate-HSA complex (MTX-HSA), which was prepared by directly conjugating MTX to lysine residues of native HSA [56, 57]. Although HSA offers numerous reactive lysine residues for MTX conjugation that allows high drug loading capacity, preclinical studies has revealed that low drug loading rates

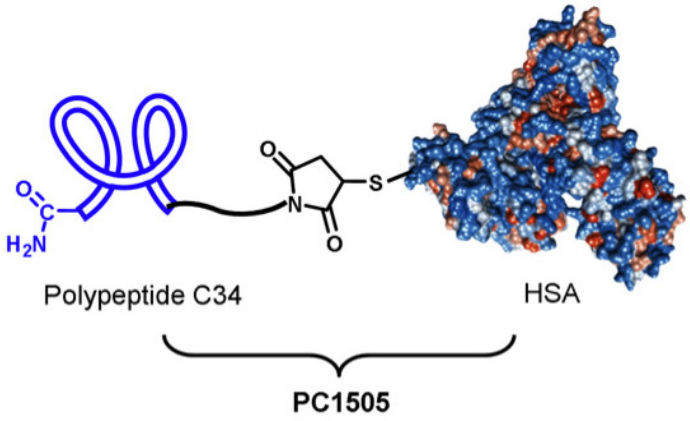

Fig. (8). Illustration of the chemical structure of PC-1505. [Picture adapted from Ref [53]]

(< 3: 1) would be very crucial for optimal tumor targeting. Phase I study has implied an excellent toxicological profile, which allows safe administration for cancer therapy [56].

As one kind of commonly used photosensitizers, porphyrins have been extensively applied in fluorescence imaging and in photodynamic treatment (PDT) of tumors, mainly attributed to their unique photochemical and photophysical properties [59]. Several natural porphyrins have intrinsic carboxyl groups such protoporphyrin IX (PpIX) and Chlorin e6 (Ce6), thus can serve as promising materials for the preparation of therapeutic photosensitizer-protein conjugates by direct conjugation to lysine residues in carrier proteins [58, 60]. Recently, a tumor-targeting HSA based photodynamic therapy (PDT) system has been developed by conjugating an attractive hydrophobic PDT reagent, Ce6, to native HSA through the coupling reaction between carboxyl groups on Ce6 and the ε-amide group of lysine side chain in HSA with the assistance of coupling regents of EDC/HOSu [58]. The authors found that the amount of Ce6 on a single HSA did not increase more than approximately 14 molecules when continually increasing the ratio of Ce6 to HSA during the coupling reaction. Under aqueous conditions, HSA-Ce6 conjugate formed stable and soluble self-assembled nanoparticles through hydrophobic interactions between Ce6 molecules with size distribution of 80–100 nm in diameter. The *in vivo* distribution and therapeutic efficacy of Ce6-HSA-NPs was then evaluated in HT-29 tumor-bearing mice. Compared with free Ce6, Ce6-HSA conjugates exhibited promising tumor target effect and prolonged retention time in tumor site. As indicated in Fig. **9**, at the same irradiation conditions, Ce6-HSA treatment leads to a significantly greater tumor volume reduction than control group with the treatment of free Ce6 [58].

Although the drug-HSA conjugate can be easily prepared by the reactions between the carboxyl groups on drug molecules and lysine residues on HSA, in some cases, additional pretreatment of these therapeutic molecules or HSA structure are also necessary when the therapeutic molecules do not have a carboxyl group. For example, the commonly used antitumor drug doxorubicin (DOX) does not have free carboxyl groups in the structure. In order to prepare relevant DOX-HSA conjugation through its targeting to lysine residues of HSA, in a recent report, Yang etc introduced an extra linker group such as 4-pentynoic acid to firstly conjugate with native HSA through its NHS ester derivative to form the alkynyl group functionalized HSA (Fig. **10**). Meanwhile, an azide tagged pH sensitive motif was conjugated to DOX molecule to form a DOX prodrug. This prodrug was then reacted with HSA through "click reaction" to afford a pH sensitive DOX-HSA protein conjugate. The authors found that the as-prepared DOX-HSA conjugate

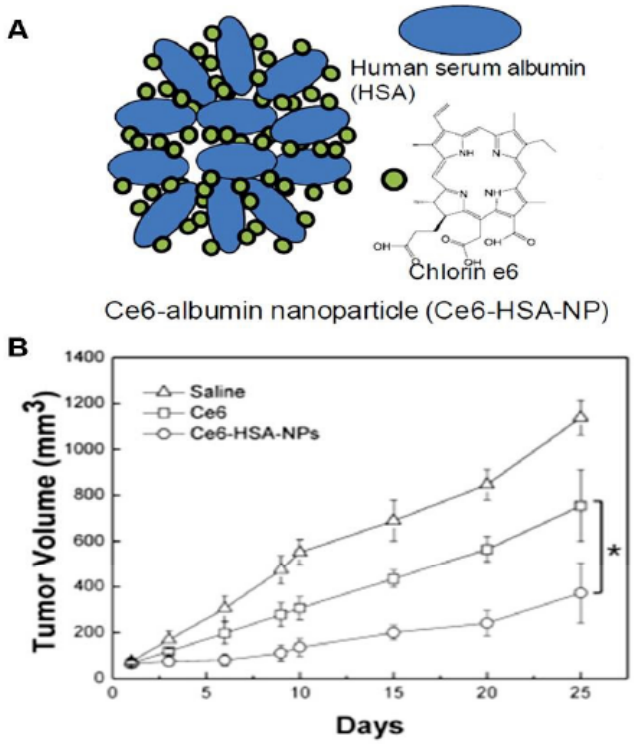

Fig. (9). A) Illustration of self-assembly of Ce6-HSA conjugates, B) Tumor growth with treatments. Data represent mean ± s.e. ($P < 0.05$) [58]. [Reprinted by copyright with the permission of Ivyspring]

can deliver DOX drug molecules into lysosomes of tumor cells, which was confirmed by co-staining with lysotracker blue. Moreover, the greatly improved cellular uptake and cytotoxicity was observed on the DOX-HSA conjugate when compared with free DOX prodrug alone or the control DOX-HSA conjugate without an acid-labile group [61].

In terms of numerous lysine residues in one protein structure, it is difficult to achieve specific conjugation of a therapeutic reagent to the expected site in HSA carrier. Non-specific reaction may limit the prospect toward obtaining a chemically homogeneous protein-drug conjugate [62]. Therefore, reliable methods for site-specific labeling on HSA for the purpose of obtaining new homogeneous protein therapeutics are highly desirable [63]. Besides the conjuga-

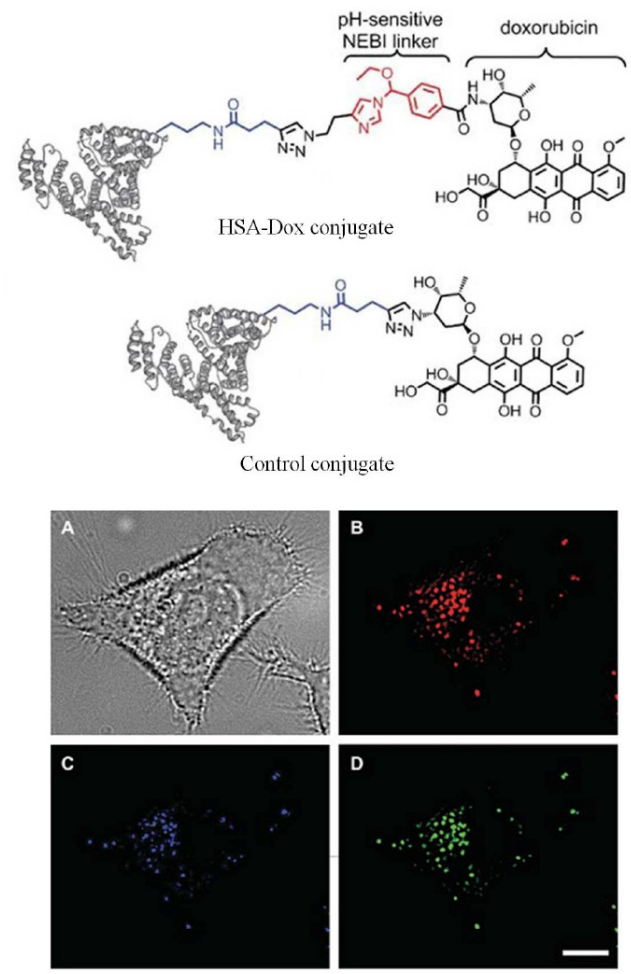

Fig. (10). Structure of a HSA-doxorubicin conjugate comprising a pH-sensitive linker and structure of a doxorubicin-HSA conjugate comprising acid-stable linkers. Fluorescence imaging studies for the internalization and localization of HSA-doxorubicin conjugate in human ovarian carcinoma 2008 cells: (A) transparent contrast image; (B) intrinsic red fluorescence of doxorubicin; (C) Lyostracker blue and (D) merged image [61]. [Reprinted by copyright with the permission of RSC Publishing]

tion of lysine residues in HSA structure through N-hydroxy-succinimide ester based chemistry, some other reactions have also been found to react with HSA lysine residues in a highly specific manner, which could serve as new targets to achieve site-specific labeling of drugs with HSA protein carrier [64]. For example, Carlos and co-workers developed a series of labeling reagents based on the structure of TAK-242 (As shown in Fig. **11**), a potent inhibitor to block protein–protein interactions between TLR4 and its adapter proteins by covalent binding to cysteine or lysine in targeted protein structure [65]. The analogues based on such inhibitor structure may probably demonstrate high reactivity and specificity for Lys64 in HSA. To test the *in vivo* stability, *D*-biotin was conjugated to HSA through this new developed method and the commonly used maleimide/thiol reaction respectively. The biotinylated HSA conjugates were administered to BALB/c mice. Blood was collected at different time intervals and analyzed by Western blot. As indicated in Fig. **11**, the biotinylated HSA conjugate formed by their present strategy was more stable than that prepared by maleimide-thiol chemistry [64]. This new conjugation method may find great utility for the generation of drug-HSA therapeutics with well defined components and improved stability.

2.1.3. New Strategy in Preparation of Drug-HSA Conjugate

Besides the lysine and cysteine based chemistry in the preparation of HSA-drug conjugates, other abundant amino acid residues potentially applied for HSA conjugation will be aspartic acid and glutamic acid, which contain carboxyl groups and are also accessible to process coupling reactions between therapeutic reagents and HSA carrier protein. Usually, these reactions based on aspartic acid or glutamic acid are not suitable for synthesis of monomeric HSA-drug conjugate. Because directly activating carboxyl groups on HSA may cause unexpected cross-linking between Asp/Glu residues and intrinsic lysine residues on HSA, which may lead to uncontrollable protein aggregates. Up to date, there are various new chemicals have been developed for the labeling on different kinds of amino acids residues on proteins [66, 67]. But not many of them have been applied in therapeutic reagent preparation because of their limited selectivity, relative poor conjugation yield, extreme reaction conditions and poor bioavailability. New strategies for protein conjugation with minimized protein manipulation and without the assistance of hazardous reagents are still attracting great interests. Among the various possibility, enzyme catalyzed conjugation would be such an ideal option, which has been increasingly proven for the replacement of chemical catalyzed reactions [68-70].

For example, tyrosinases and related phenol oxidases are the most actively studied enzymes. These enzymes can oxidize a broad range of phenols into o-quinones, which are highly reactive toward various nucleophiles [69]. (As shown in Fig. **12**) In line with this direction, it would easily introduce phenols tagged small dug molecules or macromolecular therapeutics (e.g. antibodies etc) to carrier proteins (e.g. HSA etc). More recently, by using tyrosinases from *Agaricus bisporus (*AbT*)*, an antibody (e.g. mouse monoclonal antibody) functionalized drug delivery vehicle (mAb-HSA) has been developed. To prove of concept, a mAb that can bind to MHC class II molecule (Major Histocompatibility Complex class II molecules are normally found only on antigen-presenting cells) in immune cells was selected to conjugate with native HSA. The reaction was performed by addition of tyrosinase enzyme into the mixture of mAb, HSA and excessive amount of phenolic linker molecule. The further studies confirmed that mAb-HSA conjugate could still recognize its antigens after the structural conjugation of mAb. Such one-step enzymatic strategy may offer a great alternative for preparation of functional protein based drug delivery vehicles [71].

2.3. Genetically Modified HSA Protein for Therapeutic Design

Proteins or polypeptides are now well recognized as important classes of therapeutics of clinical and commercial interests. Among them, antibody-based drugs are the largest and fastest growing class of protein therapeutics in the USA. However, the majority of peptides and especially protein drugs are often suffered from their poor tissue penetration and low stability of at physiological conditions [72]. Fortunately, many obvious evidences also indicated that the pharmacokinetic properties of protein drugs can probably be improved by covalent coupling to a suitable carrier. Human serum albumin has been widely used as a protein carrier for small dugs and proteins drugs due to its unique features of long life of circulation, high accumulation in the target tissue, as well as low toxicity [73]. However, chemically conjugation of protein drugs to HSA may potentially disrupt the three-dimensional shape, and thus subsequently block the targeted functions. Moreover, recent advances on current DNA recombinant and synthetic technologies enable polymeric albumins or albumin fragments to be easily produced. By right, gene manipulation techniques already light on the possibility of making HSA-carrier drug fusion proteins with desirable therapeutic properties [74]. Up to now, genetic fusion of a protein therapeutic reagent (e.g. antibodies etc) to HSA carrier has been proved to be an effective way to facilitate the delivery of protein therapeutics into targeted diseases areas. There are numerous other

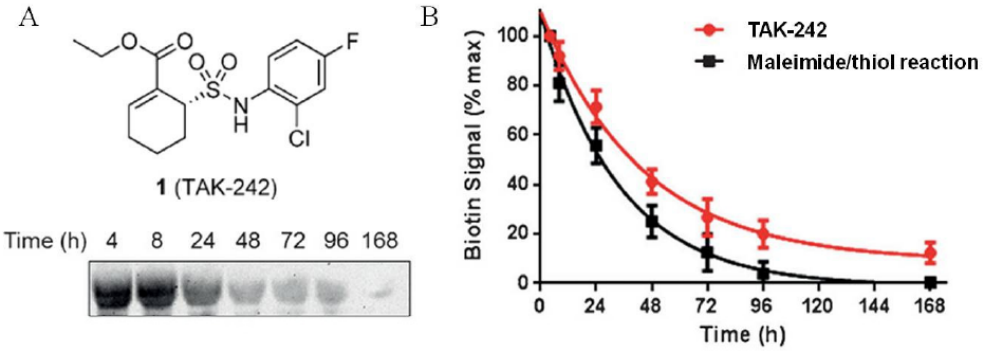

Fig. (11). Structures of TAK-242 derivative used for HSA conjugation and stability data of HSA conjugates in mice. (A) Representative Western blot for biotinylated HSA conjugates through the newly presented labeling method and commonly used maleimide/thiol reaction. (B) Averaged remaining biotin conjugate values. Picture modified from reference [64].

reviews that have already explained the importance of such genetic fusion with protein carriers [12, 75]. In this section, we will only cover some very latest progresses in the fields regarding the conjugation of protein therapeutics with HSA.

Recently, Yang etc. described a new recombinant protein system and its potential application prospect for rheumatoid arthritis (RA) therapy [76]. It is known that that Interleukin-1 (IL-1) plays critical roles in the development of several chronic inflammatory diseases, including RA, and blocking the binding of an excess of IL-1 to receptor by its receptor antagonist has been proved to be therapeutic potential in several IL-1 associated autoimmunity diseases [77]. Basically, the receptor antagonist analogue Interleukin-1 (IL-1) based IL-1ra was genetically fused to HSA to form a IL-1ra-HSA fusion protein. *In vitro* inhibition effect of the fusion protein against cytolytic activity of IL-1β to A375.S2 cells was then studied in comparison with free IL-1ra and carrier protein HSA. Only slight reduction of inhibition ability was observed compared with IL-1ra, indicating similar function to that of IL-1ra.

Uptake and biodistribution of IL-1ra-HSA was then evaluated by intravenous injection of radiolabeled fusion protein (^{131}I-IL-1ra-HSA) into mice with collagen-induced arthritis (CIA) and healthy control mice (As shown in Fig. **13**). The maximal accumulation of fusion protein (4.2 ± 0.3% of the initial injected amount) in arthritic paws was achieved at13 h post injection that is 7 fold higher than that in healthy hind paw. As contrast, radiolabeled IL-1ra itself just exhibited background level accumulation in both inflamed and healthy hind paws. Moreover, the uptake of the fusion protein was significantly lower than those of IL-1ra in liver and lung in both arthritic and healthy mice, which fusion with HSA can significantly increased the tissue specificity and reduce nonspecific accumulation [76].

Similarly, the strategy by fusing an antagonist protein of a specific factor receptor to HSA has also been applied by Kontermann etc. in the design of therapeutics against the signal pathways of tumor necrosis factor (TNF) [78]. As a pleiotropic cytokine, tumor necrosis factor (TNF) plays important roles in mediation of immune responses. Two kinds of TNF receptors TNFR1 and TNFR2 were involved in TNF based signaling pathways. The activation of TNFR1 by membrane TNF (mTNF) or soluble TNF (sTNF) could induce pro-inflammatory and pro-apoptotic signals [79]. During the systematic studies, a humanized anti-TNFR1 single-chain fragment variable (scFv IZI-06.1) was selected as the druggable protein, which selectively inhibited binding of TNF and lymphotoxin alpha to TNFR1.

The fusion protein scFv–HSA was produced by Chinese hamster ovary (CHO-K1) after gene transfection with CEMAX shuttle vector pCV072 containing the relative coding sequence. Actually, prior to this study, the same group has developed a scFv fusion protein by converting it to a fully human IgG1 antibody (ATROSAB), which showed great potential in treatment of inflammatory diseases [80]. In current study, binding of scFv-HSA to human TNFR1 was examined by ELISA in comparison with ATROSAB and scFv IZI-06.1 (Fig. **14**). Compared to ATROSAB with bivalent interaction, the authors assumed that relatively lower inhibitory activity of the scFv-HSA could be exclusively attributed to the reduced affinity due to monovalent interaction with TNFR1. The authors claimed that further studies will be required to compare

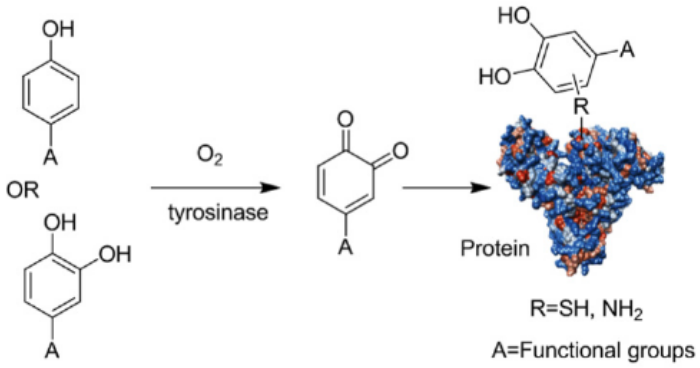

Fig. (12). Illustration of tyrosinase induced cross-linking of protein and other functional ligands [69].

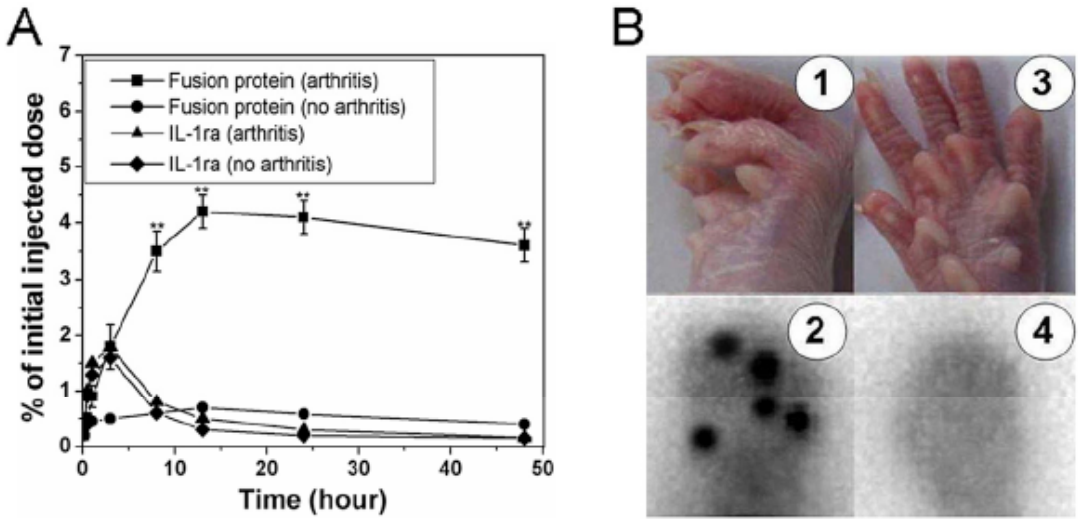

Fig. (13). (A) Uptake kinetics of ^{131}I-labeled fusion protein and ^{131}I-labeled IL-1ra in hind paws of mice with and without CIA. (B) Autoradiogram for the selectively accumulation of the fusion protein in the inflamed joint of mice. (1) and (2), inflamed paw; (3) and (4), normal paw [76]. [Reprinted by copyright with the permission of BioMed Central Publishing]

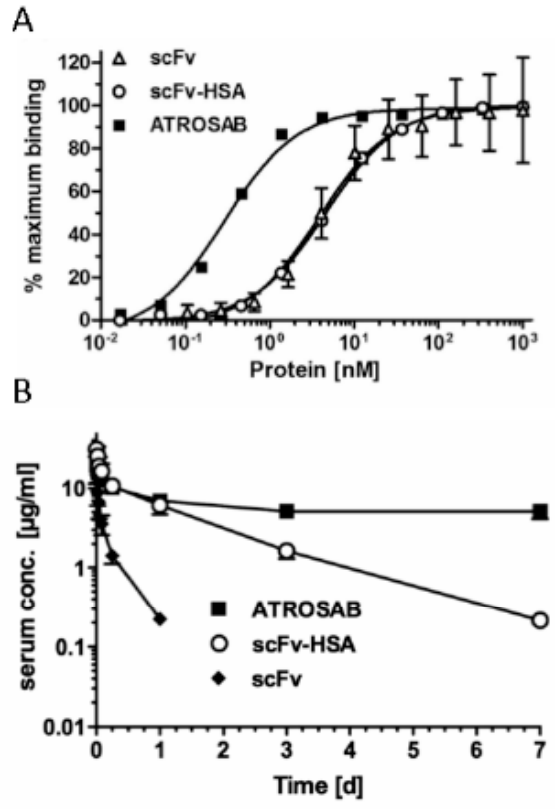

Fig. (14). A) Binding of scFv-HSA, ATROSAB and scFv IZI-06.1 to human TNFR1-Fc in ELISA; B) Pharmacokinetics of scFv-HSA and ATROSAB in female CD1 mice. Protein levels were determined by ELISA. ScFv IZI-06.1 was included for control [78]. [Reprinted by copyright with the permission of Oxford University Press]

scFv-HSA with ATROSAB including the studies in experimental models of inflammatory and neurodegenerative diseases.

Reactive oxygen species (ROS) are the primary pathogenic molecules produced in microbial infections including viral lung infections [81]. It has been found that oxidative stress plays important roles in the pathogenesis and development of influenza induced acute lung injuries (ALI) [82]. Thioredoxin-1(Trx), a small redox-active protein (12k Da) with ubiquitous expression in human body, has been proposed to have the potentials in the treatment of influenza virus-induced ALI. However, the therapeutic impact of Trx is greatly limited because of its extreme short half-life in plasma of mice (1 h) and rats (2 h) [83]. In order to develop long-acting form of Trx, Maruyama etc. proposed a recombinant Trx-HSA fusion protein.[84] The results indicated that Trx-HSA had good redox regulation properties and could be used as a novel and effective approach for preventing contrast-induced nephropathy (CIN).

More recently, the therapeutic impact of Trx-HSA in the treatment of influenza-induced ALI has also been evaluated by the same group.[85] Typically, the influenza-induced ALI model mice were produced by intratracheal injection of influenza virus A/PuertoRico/8/34(H1N1) suspension. Since influenza infection may cause induction in the infiltration of inflammatory cells, especially neutrophils, the protection efficacy of all tested reagents were examined by counting the number of total cells and neutrophils in the bronchoalveolar lavage fluid (BALF). As shown in Fig. **15**, the Trx-HSA treated mice demonstrated a significant small number of total cells and neutrophils in BALF than those treated with saline or Trx. In addition, Trx-HSA could significantly suppress the elevation of protein levels in BALF, which was often associated with the acute lung damage. The authors thus offered a potential therapeutic protein agent for the treatment of various acute inflammatory disorders.

2.3. HSA-based Protein Nanoparticle Systems for Therapeutic Design

Among various kinds of nanostructures, protein nanocarriers such as HSA based nanostructures serve as an ideal platform for controlled delivery of bioactive agents due to their unique features of low toxic and biodegradable properties. Moreover, owing to the presence of functional groups such as aminos, thiols and carboxylic groups etc, HSA nanoparticles make themselves available for covalent conjugation to drug molecules or some specific targeting ligands. Up to date, HSA-based nanoplatforms have been success-

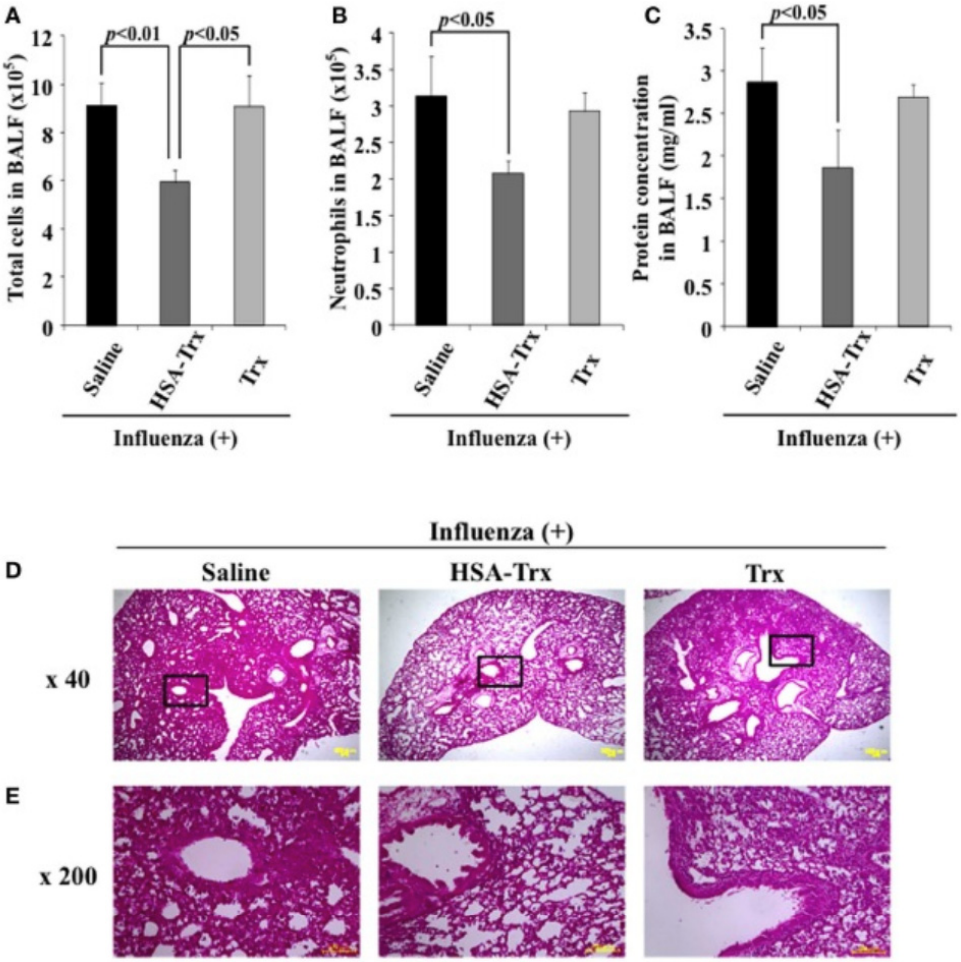

Fig. (15). Relative inhibitory effect of HSA–Trx or Trx on influenza-infected mice**.** The same injected dose (3.5 nmol/mouse) HSA–Trx or HSA was administered intravenously at 4 and 6 days after the virus infection. The numbers of **(A)** total cells and **(B)** neutrophils, or **(C)** protein concentration in BALF were determined at 8 days after virus infection. **(D)** Sections of pulmonary tissue were prepared at 8 days after virus infection, and subjected to histopathological examination (HE staining) [85]. [Reprinted by copyright permission of the Nature Publishing Group]

fully applied in delivery of many kinds of small drug molecules such as doxorubicin (DOX), paclitaxel and some druggable metal based complexes. Generally, like many other protein nanoparticles, HSA-based nanocarriers could be fabricated either through non-covalent binding strategies such as self-assembly or covalent conjugation strategies such as cross-linking reagents guided intermolecular conjugation. In this section, we will mainly focus on the latest progress on the preparation and application of HSA based protein nanoparticles.

2.3.1 Preparation of HSA-based Nanoparticles

By right, three typically different technologies have been described for fabricating albumin nanoparticles, mainly based on emulsion formation, desolvation or coacervation.[9, 86] With respect to the emulsion technique, basically, the preparation of HSA nanoparticle has been extensively studied under different conditions determined by concentrations, emulsification time and power, stirring rate, heat stabilization temperature and the types of the non-aqueous phase etc [87]. Despite successful in principle, however, involvement of organic solvents and surfactants during the preparation process would be the potential disadvantages, which would make emulsion and purification more complicated. To solve this issue, the desolvation process without organic solvents has therefore been proposed subsequently which has been extensively applied for preparation of albumin nanoparticles so far [86, 88].

As showed in Fig. **16**, in a typical desolvation process, nanoparticles are generated by a dropwise addition of a desolving agent (e.g. ethanol etc) to an aqueous solution of albumin (e.g. pH 7.0 - 10.0) under stirring until the solution become cloudy. During the addition of ethanol into the albumin solution, the self-assembled nanoparticle formed due to diminished water-solubility. To stabilize the albumin nanoparticles, a cross-linking reagent such as glutaraldehyde etc is then added into the solution, resulting condensation reactions between basic amino acid residues such as lysines or arginines in albumin structure. Based on this procedure, the albumin nanoparticles with well-defined mean particle sizes (approximately 100 nm) can be easily obtained under well controlled pump-injection system.

Moreover, in order to further reproduce HSA nanostructures with well-defined morphology and surface chemistry, some other desolvation techniques based on nano-spray drying [89], self-assembly [58, 90, 91] and disulfide stabilizing techniques [92, 93], have been also reported. So far, these new strategies have been extensively utilized in the synthesis of human serum albumin based drug nanocarriers for enhanced pharmacotherapeutic applications [94]. Among them, the method of spray drying takes great advantages in preparation of albumin-based nanocarriers with reproducible morphology and surface chemistry.

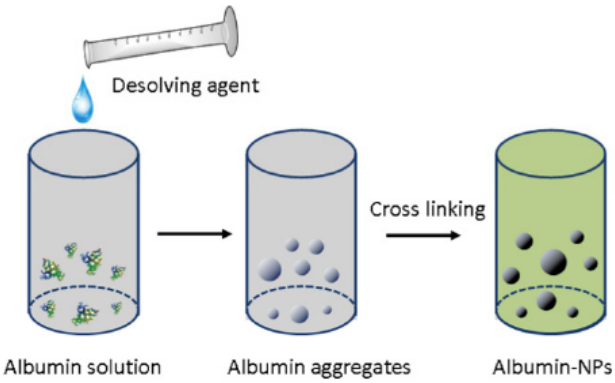

Fig. (16). Schematic illustration of albumin-nanoparticle synthesis by desolvation/coacervation method [88].

More recently, another new fabrication method in preparation of self-cross-linked HSA-NPs was reported by Cheng etc. As shown in Fig. **17**, the authors broke up the intramolecular disulfide bonds of HSA by glutathione (GSH), and then used a modified desolvation method to form the HSA-NPs. The obtained nanoparticles are quite stable in reducing free buffers, but highly sensitive to physiologically relevant reducing environment such as a solution containing GSH. Therefore, this new strategy may hold great potentials in preparation of multifunctional HSA-NPs for therapeutic applications [92].

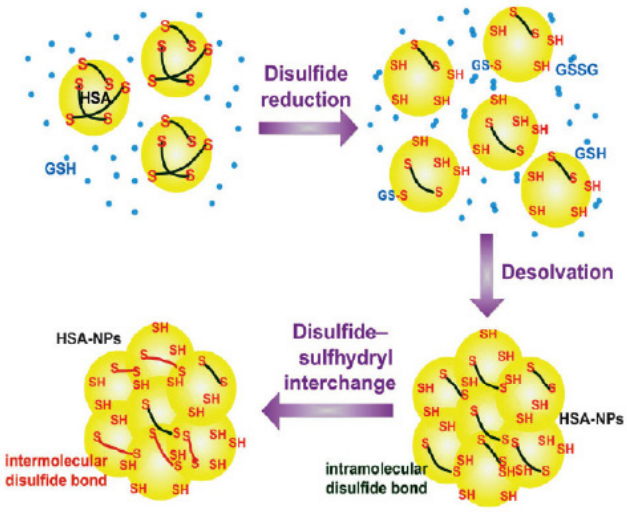

Fig. (17). Schematic of the preparation of the HSA-NPs by a reduction and desolvation method. [Reprinted by copyright permission of RSC]

2.3.2. Recent Pharmaceutical Design on the basis of HSA Nanoparticles

As nanocarriers to targeted delivery of therapeutic reagents, protein nanoparticles made of albumins offer unique properties of promising biodegradability, reproducible preparation, and more importantly, favorable surface chemistry. Furthermore, inspired by natural characteristics of protein transporters, HSA-based nanocarriers indicate great potentials in enhancing the circular retention and accumulation of drug molecules in diseases areas. So far, there are an increasing number of HSA-based nanodrugs are under clinical trials including radioactive ^{99m}Tc albumin aggregates for nuclear medicine diagnosis, albumin-paclitaxel (ABI-007) and albumin-docetaxel nanoparticles (ABI-008) for effective antitumor treatment [95]. Among them, the FDA approved albumin-paclitaxel nanoparticle (ABI-007 or Abraxane®), an injectable formulation of paclitaxel and HSA-based carrier with an average particle size of 130 nm developed by American Bioscience, has become the most advanced HSA-drug product for the treatment of various types of cancers such as metastatic breast cancer (in 2005), non-small-cell lung cancer (in 2012) and pancreatic cancer (in 2013) [96].

As showed in Fig. **18**, it has been proposed that albumin-based Abraxane® drug carrier has been transported by targeting to the glycoprotein receptor gp60 on the endothelial cells, which subsequently activated caveolin-1 dependent endocytosis of HSA nanoparticle across the cell membrane [97]. After that, endothelial cells could release HSA bound paclitaxel into the subendothelial space. Upon exposure to tumor interstitium, the accumulation process of albumin-drug in tumor cells could be possibly facilitated by SPARC (Secreted Protein, Acidic and Rich in Cysteine), a matricellular glycoprotein with high binding affinity to albumin, which has been found over-expressed in many cancers. Encouraged by the excellent clinical performance for such well-developed albumin-paclitaxel nanocarrier, there is a continuous interest in exploring new therapeutic candidates based on HSA-based nanoparticles [98-100]. For example, a widely used antitumor reagent, doxorubicin (DOX), has also been incorporated into HSA nanoparticles by a modified desolvation method and the *in vivo* anticancer study on H22 tumor-bearing mice revealed that such DOX-HSA nanoparticle exhibits significant therapeutic effect in inhibition of tumor growth. Moreover, compared with free DOX, a significant reduced cardiac toxicity was observed during the treatment with DOX-HSA-NPs [101]. Very recently, another novel self-assembled paclitaxel-HSA nanoparticle with an enhanced antitumor efficacy was also described [90]. Unlike most commonly used chemical strategies, where hazardous cross-linking agents are often used, a different self-assembling strategy to avoid toxic conjugating agents and organic solvents has been applied. In this approach, HSA carrier was firstly unfolded to expose the relative hydrophobic domains and followed by co-assembly with paclitaxel drug (PTX) into nanoparticles. The new PTX-HSA nanoparticle displayed a high drug loading capacity, and even nearly all drugs were encapsulated into the HSA nanocarriers [90]. As indicated in Fig. **19**, the imaging tracking analysis indicated that such HSA-PTX nanoparticle could greatly enhance the accumulation of dye molecules or drug molecules in tumors of H22 tumor-bearing mice. In order to evaluate of the *in vivo* anti-tumor efficacy, the aforementioned FDA approved anti-breast cancer HSA-bounded PTX drug, Abraxane®, was used as a control. The result indicated that such newly developed PTX-HSA nanoparticle is more efficient than Abraxane® in both suppression of tumor growth and elongation of the mouse survival time [90].

As described in section 2.2.1, a new strategy in preparation of self-cross-linked HSA-NPs without need of any toxic chemicals has been developed [92]. In a latest study, a novel paclitaxel (PTX) loaded HSA nanoparticle has been prepared based on the intermolecular refolding strategy. To prepare the PTX loaded HSA nanoparticle, a de-natural HSA/water solution was mixed with PTX/tertiary butyl alcohol solution in a microchannel reactor. The obtained NPs with size ranged from 140–180 nm was water soluble and remained stable for at least 48 hours in aqueous medium with no change in morphology. When exposed to reducing environment such as the GSH containing cell culture medium, cross-linked PTX-HSA-NPs exhibits rapid response with a quick PTX release, thus holds great potentials for controlled therapeutic applications [93].

Besides the small molecule drugs, the macromolecular therapeutic proteins can also be incorporated into HSA nanoparticle to achieve enhanced pharmacotherapeutic effect in HSA-based nanocarrier design. For instance, a mouse monoclonal antibodies (mAb) recognizing MHC class II molecule has been developed to covalently bind to HSA nanocapsules.[103] The HSA nanoparticle

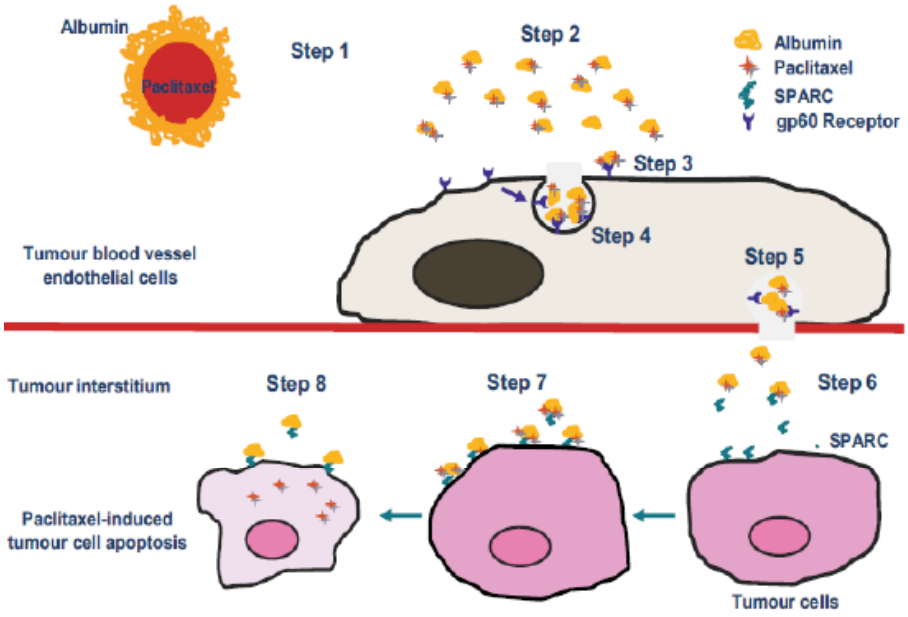

Fig. (18). Illustration of tumor accumulation mechanism of the nab-technology platform. Abraxane® targets to tumor cells through the translocation mediated by gp60 on the endothelial cells and SPARC at tumour cell membranes.[97] [Reprinted by copyright permission of Elsevier]

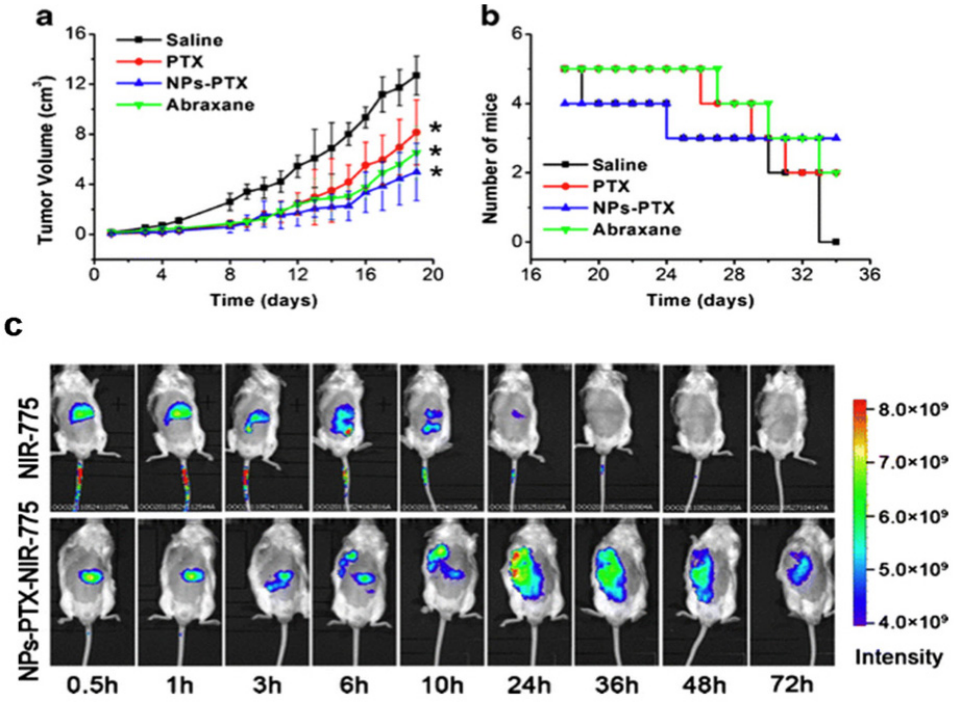

Fig. (19). Antitumor efficiency in H22 tumor-bearing mice. a) Tumor volumes during treatment. b) Survival of tumor-bearing mice in various groups; c) The NIRF images of H22 tumor-bearing mice after i.v. injection of free NIR-775 and the dye molecule labeled nanoparticle NPs-PTX-NIR-775[90]. [Reprinted by copyright permission of Springer International Publishing]

bound antibody maintained the binding ability to its antigen-presenting cells without any unspecific binding to MHC class II-negative cells [103]. Similarly, in addition to antibodies, therapeutic active enzymes can also be delivered by using HSA nanoparticles. For example, β-galactosidase, an essential exoglycosidase enzyme in the human body, can hydrolyze the β-glycosidic bond between a galactose and its organic moiety. Recently, this enzyme was co-assembled with 30Kc19-HSA nanoparticles to form an enzyme delivery nanosystem with enhanced cell penetration [103]. Basically, 30Kc19 protein and HSA were firstly fabricated into nanoparticles by using the desolvation method. The cargo protein β-galactosidase was then conjugated to the 30Kc19-HSA nanoparticles by glutaraldehyde guided cross-linking method. The results demonstrated that the 30Kc19 protein-HSA-β-galactosidase hybrid nanoparticles exhibited enhanced cellular uptake and beta-galactosidase activity when compared with the HSA-β-galactosidase nanoparticles (As indicated in Fig. **20**).

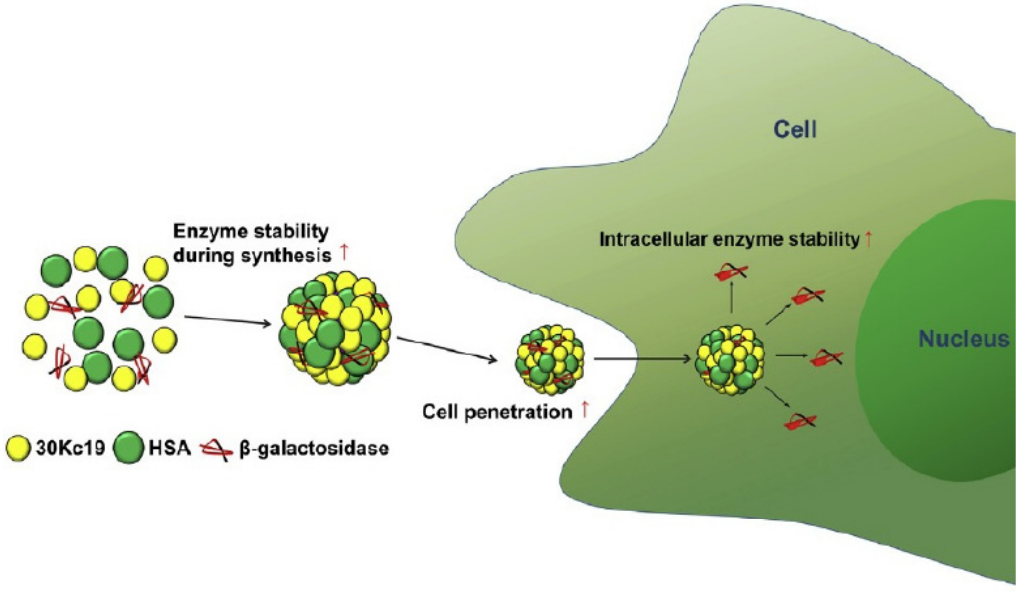

Fig. (20). Schematic illustration of fabrication and the intracellular delivery of enzyme cargo by the 30Kc19-HSA nanoparticles. Picture reedited from reference [103]. [Reprinted by copyright permission of Elsevier]

Inspired by the rational design of introducing an additional cell penetrating or specific targeting fragment to HSA-based nanocarriers for enhanced therapeutic behavior, another antitumor doxorubicin containing HSA nanoparticle with surface functionalization of TRAIL and transferrin (TF) was also developed by Youn etc very recently (Fig. **21**) [104]. Typically, TRAIL, a tumor necrosis factor (TNF)-related apoptosis-inducing ligand, was selected as the second bioactive molecule in addition to doxorubicin drug. Transferrin, a critical protein transporter, is known to guide the TF bound cargo molecules into cancer cells via the transferrin receptor (TFR)-mediated endocytosis pathway [105]. The *in vitro* study indicated that this HSA nanoparticle is able to inactivate various types of cancer cells including the DOX resistant MCF-7/ADR cell-line and a necrosis/apoptosis-less sensitive pancreas cell-line (CAPAN-1). In addition, the transferrin conjugated HSA-drug nanoparticles also exhibited enhanced tumor localization, suggesting excellent targeting roles of transferrin when bound to HSA carriers. Similarly, Trastuzumab (TMAB), an anti HER2 monoclonal antibody that is frequently used for breast cancer treatment, was also chosen to conjugate with antitumor drug Methotrexate (MTX) loaded HSA nanoparticle to form a cancer targetable HSA-MTX nanodrug [106]. The specific targeting roles of antibody functionalized HSA nanoparticles could increase the therapeutic efficacy of MTX on HER2 tumor cells, and meanwhile decrease the unexpected side effects on the healthy tissues or organs. Apart from the systematic conjugation of protein moieties to HSA-based nanocarriers, similar design by conjugation of a nucleolin-targeting DNA aptamer to HSA structure to achieve a tumor targetable protein-DNA nano-complex has been developed. Based on such novel aptamer-HSA-PTX nanoparticle, the anticancer drug paclitaxel (PTX) can be successfully delivered into MCF-7 cells with enhanced uptake compared with aptamer free HSA- PTX nanoparticle [107].

3. HSA–ORGANIC/INORGANIC HYBRID NANOSYSTEMS FOR THERAPEUTIC DESIGN

Although protein based nanomaterials (e.g. HSA-based nanocarriers) have been widely applied in therapeutics design, there still exist some potential drawbacks on pure protein-based materials such as lack of intrinsic targeting groups, limited proteolytic stability, and insufficient circulation times [108]. To minimize these technical shortcomings, further modification to optimize protein structures would be therefore highly required. Recent advances of organic micro/nanoparticles such as synthetic polymers and inorganic nanomaterials have individually or synergistically attracted increasing attentions in the last few decades attributed to their promising stability, controllable morphology and tunable surface chemistry. Therefore, fabrication of nanostructures by combination of the advantages from polymers or inorganic nanoparticles with unique properties of serum albumin protein carrier may generate novel biomaterials with more potential for potent biomedical applications.

3.1. HSA-Polymer Hybrids for Delivery of Therapeutic Reagents

The conjugation of synthetic polymers to protein materials may equip the hybrid materials with favorable properties such as tailored amphiphilicity, new types of self-assembly and good phase-separation activity [109]. Moreover, polymer functionalization can also enrich the surface chemistry of protein-polymer hybrids, and can thus facilitate the subsequent conjugation of other functional moieties including specific targeting ligands and environment-responsive linkage molecules. In general, there are two types of basic core-shell structures in all existing albumin-polymer hybrid systems (As illustrated in Fig. **22**). Similar to many other protein-polymer hybrids, the HSA-polymer nanoparticles can also be easily obtained via non-covalent and covalent conjugation strategies. Actually, HSA surface offers a pool of active groups such as amines of Lys residues, carboxylic acids from Asp and Glu residues, thiols from Cys residues, which can therefore facilitate both statistical and site specific chemical conjugations for the modification of protein structures [108]. Usually, covalent conjugation (e.g. through amide bonds etc) can make the HSA-polymer hybrids more stable under physiological conditions, and thus potentially minimize unexpected drug leaking. The driving forces toward non-covalent generation of protein polymer hybrids are derived primarily from the electrostatic or hydrophobic interactions. Although non-covalent conjugation strategies have been thought to be relatively easy for preparation of flexible materials for a wider application range, the relatively weaker interactions compared to robust covalent connections would

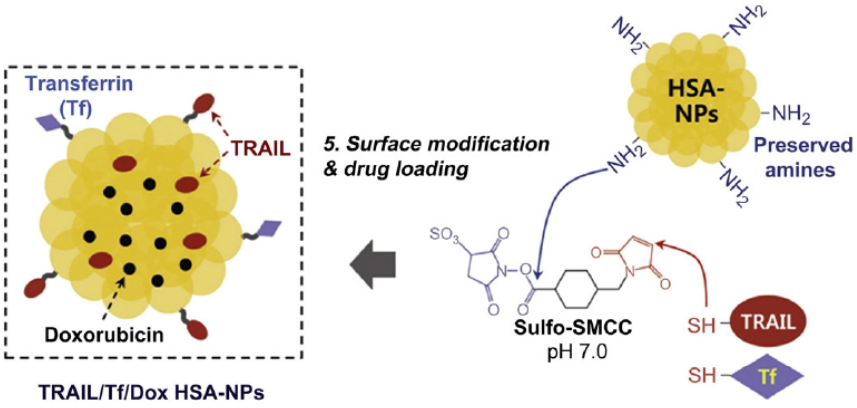

Fig. (21). Schematic illustration of the preparation of TRAIL/Tf/Dox HSA-NPs. Picture adapted from reference [104] [Reprinted by copyright permission of Elsevier]

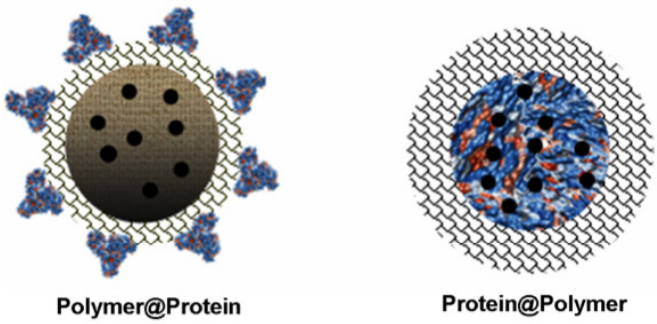

Fig. (22). Illustration of two fabrication strategies for protein-polymer hybrids preparation, polymer@protein/core-shell and protein@polymer/core-shell, the black dots stand for drug molecules [109].

be the potential concerns for the rational design of HSA-polymer hybrid carriers in their extensive applications in living system [109].

3.1.1. HSA-Polymer Hybrids through Covalent Interaction

Owing to the fact for their easy preparation and usually high production yield, the covalent conjugation has been widely used in the preparation of HSA hybrids. Normally, the reaction between EDC/NHS activated carboxyl acids and amines, and the reaction between maleimide and thiols are the most commonly used strategies to form HSA-polymer hybrids. Considering the biocompatible properties of polymeric materials, polyethylene glycol (PEG) has become the number one polymer option to improve the potentials of the therapeutic HSA-polymer hybrid carriers with minimum side effects and cytotoxicity to normal tissues [110]. In addition, PEGylation was also found to enhance the passive targeting ability of protein carriers through EPR effect. Since the first developed PEGylated albumin hybrids by Davies and Abuchowski in the 1970s, the strategy of HSA PEGylation has been continuously broadened in the relevant research areas [108, 109, 111-113].

Generally, HSA has been selectively PEGylated by a PEG-propionalde derivative (20 kDa) by utilizing the different pKa values of the α-amino group of the N-terminal residue and the ε-amino groups of the Lys residues [115]. The as-prepared HSA-PEG hybrids normally demonstrated prolonged half-life time and enhanced vascular retention, when compared with the native HSA alone. Recently, Lee etc reported a paclitaxel (PTX) delivery system using shell cross-linked PEG-HSA hybrid nanocapsules. Typically, PEG chain (5 kDa) was cross-linked onto HSA nanoparticles through NHS ester/amine and maleimide/thiol chemistry to form the core-shell structure. Due to the strong binding of PTX to HSA, the drug molecules were effectively encapsulated into the hydrophobic inner core of the cross-linked shell particle. Moreover, after loading with PTX, the HSA-PEG shell still represented reactive groups for the conjugation of a cell-penetrating peptide Hph1. Upon the assistance of Hph1, the PTX-loaded HSA/PEG nanocarries can preferentially accumulate in the targeted tumor site. Furthermore, the *in vivo* anticancer studies also indicated that the PTX-HSA/PEG nanoparticle can effectively suppress tumor growth when upon i.v. administration.

Besides numerous accessible functional amino and carboxyl groups in native HSA structure for the functionalization of PEG and other polymers, more reactive groups (such as the thiols on cysteines) could also become chemical accessible if HSA was denaturized by a proper reduction reagent. The enriched surface chemistry in HSA structure may make this protein-based carrier more available to hybridization with polymer materials, and to conjugation of drug molecules or other functional reagents including some specific targeting ligands. So far, a series of stable and highly well-defined albumin based co-polymers via novel denaturation and pegylation approaches have been developed [108, 116-118]. For example, a polymer albumin core–shell nano-transporter has been recently described for high capacity loading and controlled release of anti-tumor doxorubicin drug with enhanced anti-leukemia activity [119]. Typically, the native HSA has been firstly conjugated with ethylene diamine to form cationized protein surface followed by the conjugation with polyethylene (oxide)-2000 (PEO2000). The HSA-polymer conjugate was then denaturized to form an unfolded polypeptide with free cysteine residues for DOX-maleimide conjugation. The final DOX loaded protein-polymer conjugate is brought into water

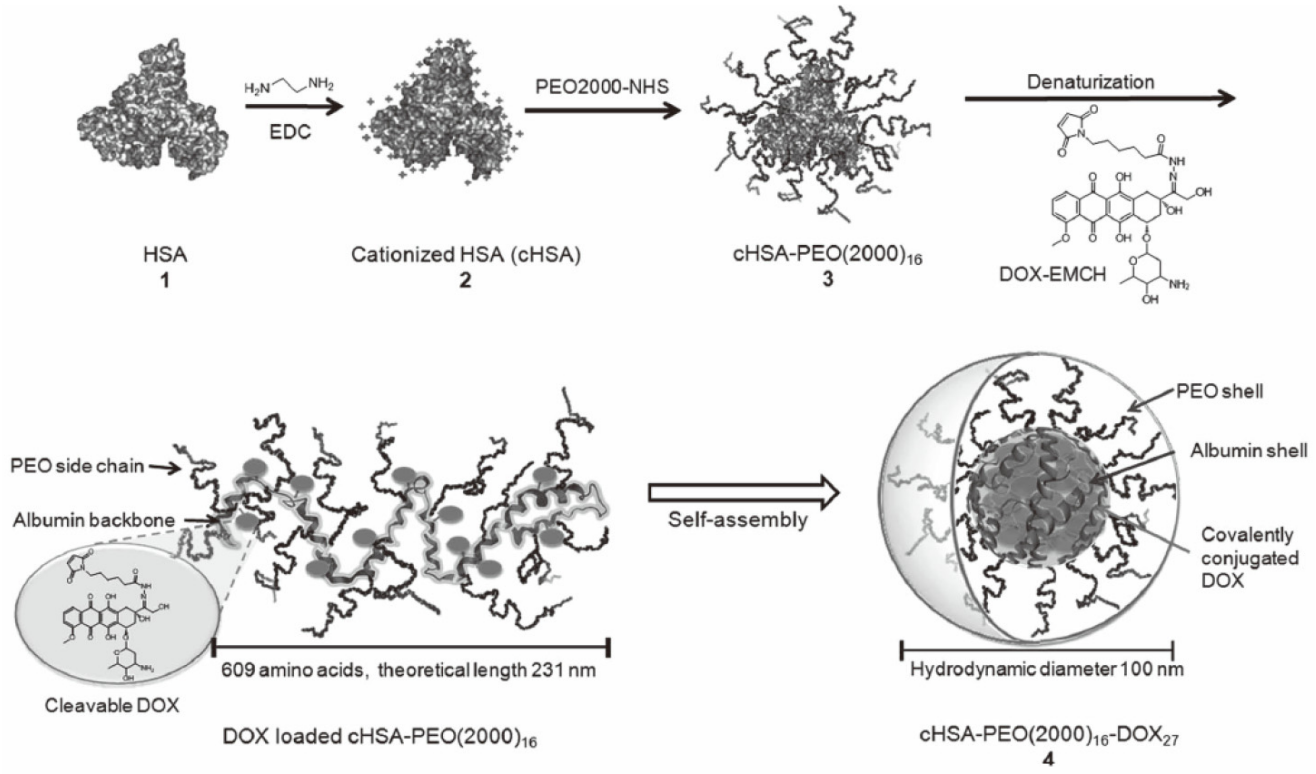

Fig. (23). Preparation of DOX loaded cHSA-PEO (2000)-DOX micelles [118] [Reprinted by copyright permission of Wiley]

to form the self-assembled core-shell structure (~ 100 nm in diameter) (Fig. **23**). The *in vitro* study and primary *in vivo* investigations indicated the great potential for the treatment of Leukemia, additional pharmacological studies will be required to completely understand the pharmacokinetics of this macromolecular HSA-based polymer drug carrier under *in vivo* conditions [118].

3.1.2. HSA-Polymer Hybrids through Non-covalent Interaction

The key driving forces toward the non-covalent conjugation of protein-polymer co-assembled nanostructures would be the electrostatic and hydrophobic interactions from the amino acid residues in protein structures and the functional groups in polymer materials. In terms of the isoelectric point of HSA (pI = 4.9) [120], human serum albumin usually displays a negative net charge under physiological pH. Therefore, the positively charged polymers have been often chosen to form the HSA-based polymer hybrids to improve the efficacy of therapeutic molecules or gene delivery, as the positively charged surface may facilitate the nanoparticle drug carries translocate into targeted cancer cells [121]. For instance, poly (ethylenimine)s (PEI) are such a kind of cationic polymers, which have been commonly utilized to non-covalently coat with HSA-based carriers to enhance the stability, and to improve the binding capacity of negatively charged therapeutic molecules including drug active proteins and siRNAs into living systems [122, 123]. Recently, upon the fabrication based on the well established ethanol desolvation technique, such hybridized PEI-HSA nanoparticles have also been used for the delivery of small molecular antitumor doxorubicin drugs [124].

Moreover, the HSA-based polymer hybrids can also be designed as polymer@HSA core-shell structure rather than HSA@polymer core-shell. For example, Dong etc proposed a type of new hybridized HSA-polymer core-shell nanoparticle for tumor environment-sensitive DOX drug delivery [124] (As shown in Fig. **24**). Basically, DOX drug molecules were firstly mixed with polyCG DNA fragments to afford DOX encapsulated DNAs, followed by the addition of the cationic gelatin (C-gel). The obtained core structure was then mixed with native HSA solutions to form the final core-shell complex, namely HDD, through electrostatic interaction between negatively charged HSA and positively charged the C-gel. Different from most delivery vehicles based on HSA where the covalent conjugation was required, in this design, HSA attached to the polymer nanoparticle as its native and active formula. The *in vivo* anticancer study indicated that such HSA coated nano-complexes (HDD) could quickly accumulate in the tumor site, leading to release of DOX upon the digestion of the gelatin and the DNA in the presence of protease and DNase. The results from both S180 tumor model and the orthotopic hepatic Heps tumor model suggested that HSA capsulated DOX containing nanoparticle exhibited a higher anticancer efficacy and a significantly lower cardiotoxicity than free DOX drug molecule.

Besides positively charged PEI and gelatin, cationic dendrimers such as Poly(amidoamine)/ (PAMAM) have also been used to conjugate with HSA through non-covalent electrostatic interactions. By right, PAMAM dendrimers have been widely investigated in the biomedical applications for effective therapy and diagnostics [126, 127]. Several studies have been conducted to investigate the binding between PAMAM and HSA protein, and the result indicated a complicated binding mechanism involved in electrostatic interaction, hydrophobic interaction and hydrogen bonds formation [128, 129]. By taking those great advantages, HSA has been recently introduced to PAMAM to form a self-assembled dendriplexes for plasmid DNA delivery [130]. The result showed that the incorporation of HSA to the dendrimer macro-systems can significantly enhance gene delivery effect and diminish the membrane damage by highly positively charged the PAMAM.

Moreover, non-covalent electrostatic interactions have also been applied to encapsulate HSA into self-assembled superamolecular systems such as liposomes etc. For instance, pirarubicin (THP) is an effective anthracycline analogue for the treatment

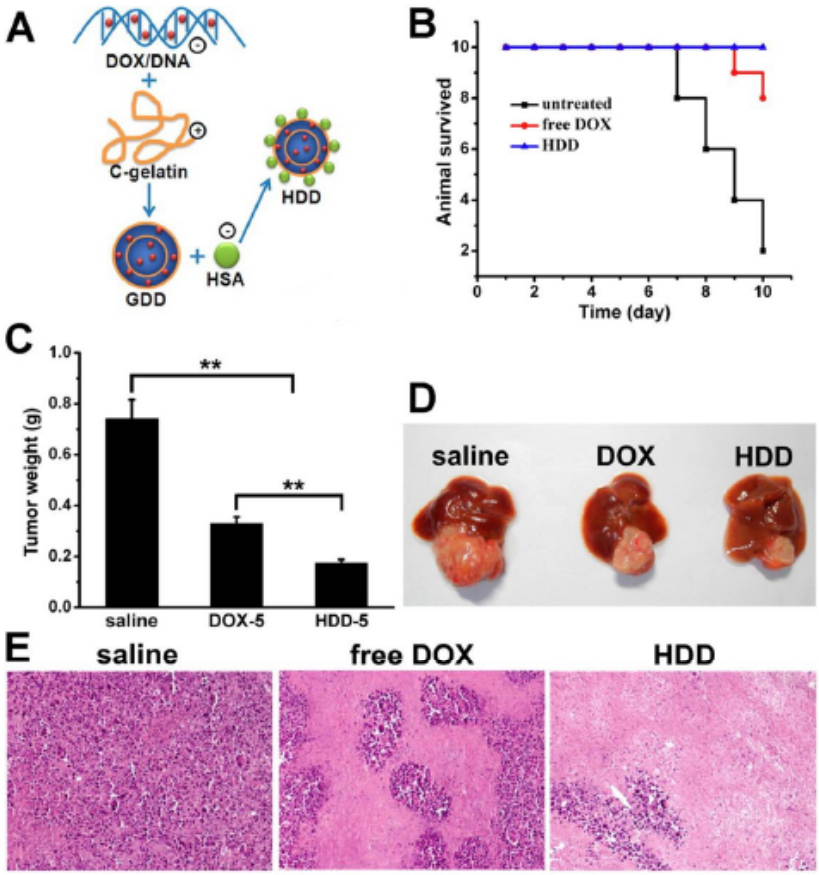

Fig. (24). Antitumor activity analysis in orthotopic Heps-bearing mice. (A) The schematic illustration of HSA coated nano-complexes (HDD). (B) Tumor sizes changes after iv injection of saline, free DOX, or HDD at a dose of 5 mg/kg body weight. (B) The survival curve of animals given free DOX or HDD. (C) The weights of tumors separated from animals that received different treatments. (D) Representative photographs of livers with tumors separated from animals that received different treatments. (E) H&E stained tumors taken from animals that received different treatments [124]. [Reprinted by copyright permission of American Chemical Society]

of various types of solid tumors. However, its promising utilities have been limited mostly due to the extensive side effects.

Very recently, a THP based oleic acid complex (THP-OA) through electrostatic interactions has been synthesized. Such THP-OA complex was subsequently incorporated into HSA containing liposome nanostructure, which was obtained by a simple high pressure homogenization method in the presence of egg yolk lecithin E80 and HSA proteins (Fig. **25a**). The *in vivo* study indicated that this novel pirarubicin-oleic acid complex albumin nanoparticle (THP−OA−AN) retained potent antitumor efficiency with significantly reduced side effects in comparison with free THP (Fig. **25b**) [131]. Such self-assembled protein drug liposome nanocomplex integrated the advantages of both liposome and HSA-based protein carrier, and thus enabled HSA easily compatible with small molecule pirarubicin drug molecules, thus providing a promising strategy to non-covalently assemble carrier proteins into superamolecular platforms for the enhanced pharmacotherapeutic applications *in vitro* and *in vivo*.

3.2. Fabrication of HSA-Inorganic Nanoparticle Hybrids

Besides HSA nano-hybrids on the basis of polymer materials, inorganic nanoparticles have also attracted significant academic and industrial interests in biomedical applications during the last few decades, due to their unique material- and size-dependent physicochemical properties, which usually are not achievable through traditional soft materials such as lipids or polymers based nanoparticles [132]. Generally, inorganic nanoparticles indicated unique optical, magnetic and other physical properties, which enabled them not only working as nano-sized delivery vesicles, but also serving as alternative probing materials to replace small molecules or organic fluorophores for their promising biomedical applications in molecular imaging and diseases therapy. Nano-sized inorganic delivery carriers can normally achieve their effective accumulation in the site of diseases interests, and can be transported or processed at the scales of tens of nanometers, mostly based on their endogenous transport mechanism in living cells or tissues. However, efficiency in relevant cellular trafficking and tissues targeting, and effective bioavailability for mostly existing inorganic nanoparticles remain the technical challenges in the fields which may potentially limit their biomedical applications *in vitro* and *in vivo*. Therefore, proper surface modification of particles with affinity ligands or biocompatible moieties is often necessary and required to retain the colloidal stability and to solve these technical obstacles [133].

As a highly functional transport protein in blood plasma, human serum albumin has been frequently utilized for their pharmacotherapeutic design. Extensive studies have confirmed that surface modification of inorganic particle with HSA designates several advantages such as improving the stability and solubility of nanomaterials. In addition, because of its excellent binding capacity to various therapeutic molecules, HSA on nanoparticle surface may also serve as an ideal vector for transport and release of drug molecules. Moreover, HSA also demonstrated promising plasma

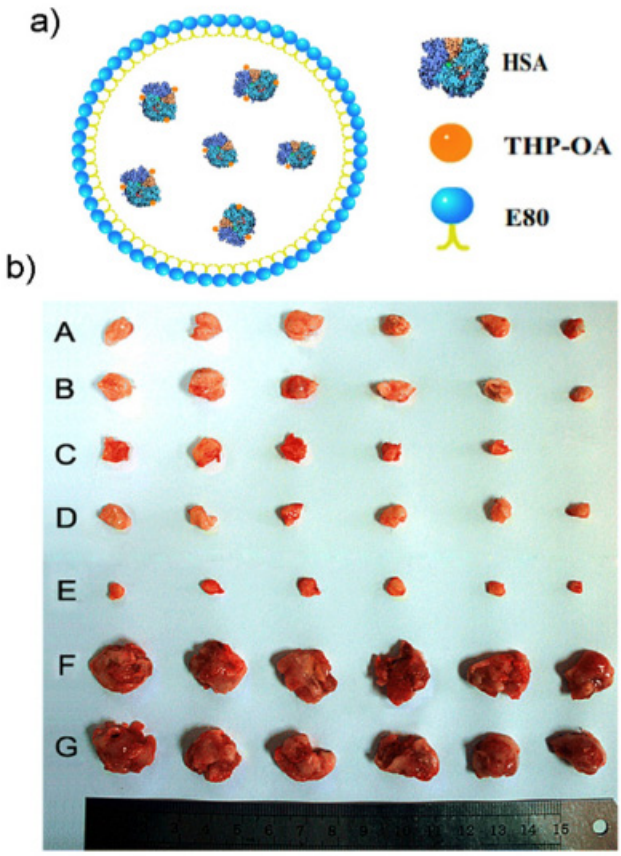

Fig. (25). a) Schematic illustration of THP−OA−AN. Tumor growth after application of different dose schemes of THP, THP−OA−AN, blank AN, and saline. b) (A) THP (5 mg/kg); (B) THP−OA−AN (5 mg/kg); (C) THP (10 mg/ kg); (D) THP−OA−AN (10 mg/kg); (E) THP−OA−AN (15 mg/ kg); (F) blank AN; (G) normal saline. Comparison between THP and THP−OA−AN in different dose schemes illustrates similar antitumor activity [130]. [Reprinted by copyright permission of American Chemical Society]

stability and long circular retention time, which makes it an excellent option for improving the accumulation of nanoparticles in targeted diseases sites through passive EPR effect [9, 134]. Similar to aforementioned strategies to functionalize HSA-based polymer hybrids, HSA can be also conjugated to inorganic nanoparticles through covalent linkage or non-covalent interactions depending on different purposes. In terms of the linkage through covalent conjugation, additional functional groups on various types of nanoparticle surface such as amines, carboxyl acids or thiols etc are usually indispensable. HSA can be therefore directly coupled to inorganic particle surface through the binding with free amino, carboxyl or thiol presenting residues in native HSA.

The biological behavior of inorganic nanoparticles may change when upon binding with HSA protein [134-140]. These studies have found that cellular uptake of nanoparticles is strongly affected by the physicochemical properties of a protein layer surrounding the nanoparticles. For instance, Krol etc. conjugated ^{98}Au radiolabeled gold nanoparticle with HSA and another blood proteins apolipoprotein individually. The biological kinetics of these gold nanoparticle protein conjugates (abl-AuNP and apoE-AuNP) have been systematically investigated in animal models when compared with a non-protein coated nanoparticle (citrate-stabilized AuNP) [142]. The authors concluded that protein conjugation can greatly increase the blood circulation time of AuNPs, and the HSA coated AuNPs could even move more deeper into the brain tissue close to the hippocampus after 19 hours post intravenous injection. Particularly, HSA coated AuNPs also indicated a higher retention in lung and brain in comparison with other particles with different surface modifications. These findings provided insightful information for the rational design of nanodrugs targeting to brains and lungs. Similar studies based on the binding of HSA protein to single-walled carbon nanotubes (SWCNT) also confirmed that albumin conjugation could enhance cellular internalization of SWCNT and could also further stimulate myeloperoxidase (MPO) based nanotubes biodegradation in human inflammatory cells [143].

Encouraged by their promising biological behaviors of HSA coated nanoparticles, great affords have been carried out to develop novel drug delivery nanoplatforms with enhanced therapeutic effects. For example, Chen etc reported a HSA-coated iron oxide nanoparticle formula for tumor targeted and magnetic resonance imaging (MRI) guided drug delivery [144]. The *in vitro* assays indicated that HSA capsulated nanoparticle (D-HINPs) can help antitumor drug DOX cross the cell membrane and accumulate into its cell nuclear targets. (Fig. **26**) The authors assumed that the polyamine layer may contribute positively charges to particles and thus partially enhance the cell internalization, and additionally, HSA coating may have played a positive role in tumor targeting. The further *in vivo* therapeutic studies indicated that this new designed nanoparticle exhibited comparable tumor suppression effect to Doxil, a FDA-approved nano-drug [145]. More importantly, the particle preparation can be easily scaled up, and the same technique can be readily applied to load other small-molecule-based therapeutics. Moreover, tumor targeted MR imaging is also available during the tumor treatment, which can not be achieved in the cases of free DOX or Doxil.

More recently, HSA hybridized graphene quantum dot nanoparticles with hyaluronic acid as cancer cell targeting ligand has also been developed. In this typical study, HSA worked mainly as protein carrier to achieve promising loading of gemcitabine drug molecules through electrostatic interactions. The *in vitro* studies displayed that such HSA hybridized nanoconjugate can effectively deliver gemcitabine into gemcitabine resistant pancreatic cancer cells [146]. Moreover, a HSA coated porous doxorubicin loaded gold shell nanoparticle has been reported for both tumor therapy and imaging, in this study, HSA serves as gold nano-shell stabilizer and a multi-branched polypeptide layer which enabled the conjugation of a NIR fluorescent dye and the tumor affinity ligand moiety of folic acid [147].

Besides extensive utilities in drug delivery, HSA-nanoparticle hybrids have also been well studied in biological sensing and imaging. By taking the advantages of good biocompability of HSA, Chen etc recently reported a type of dopamine-plus-HSA coated iron oxide nanoparticles for various cell labeling in a non-covalent conjugation manner. Compared to the conventional nano-sized labeling regents, these HSA-ion oxide nanoparticles demonstrated lower toxicity to cells, and showed better T2 contrast.[148] Similarly, a HSA stabilized quantum dot (QDs) with tumor target ligand folic acid (FA) has also been synthesized for *in vivo* tumor imaging with reduced non-specific toxicity [149]. As a nanoparticle stabilizer, HSA was deposited onto OD surface by modified emulsification/solvent evaporation methods. In this typical system, HSA has also offered abundant reactive carboxylic groups for the conjugation with a tumor targeting ligand Folic acid-PEG-NH_2. The FA conjugated HSA CIS/ZnS QDs is available for efficient tumor-targeted imaging. More recently, Margel etc have presented a new Bi_2O_3/HSA core-shell nanoparticle for X-ray imaging applications. Basically, Bi_2O_3 core particles were stabilized by coating with human serum albumin via a precipitation process. The *in vivo* trials confirmed the efficacy and safety of these Bi_2O_3/HSA core-shell nanoparticles for CT imaging [150]. Similarly, HSA-encapsulated gold nanoclusters (AuNCs) have also been developed very recently

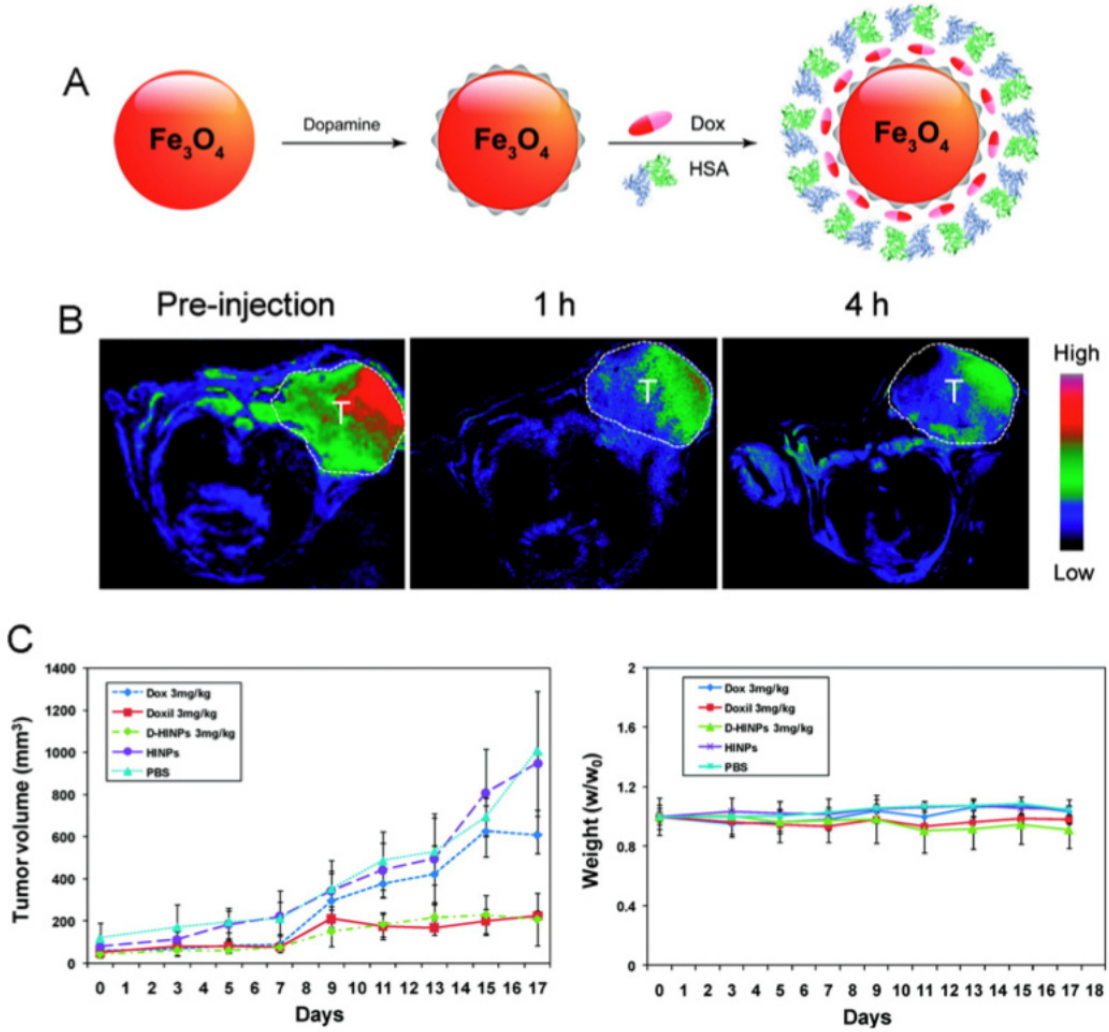

Fig. (26). A) Schematic illustration of the synthesis of D-HINPs; B) MR images taken before, and 1 and 4 h after the injection of D-HINPs (6 mg of Fe/mL). C) Left: tumor growth curves for treatment with D-HINPs, free DOX, Doxil all at 3 mg of Dox/kg and HINPs with same Fe concentration as in D-HINPs and PBS as control. Right: change of mouse body weight during treatment [143]. [Reprinted by copyright permission of American Chemical Society]

for selectively fluorescent labeling of *staphylococcus aureus* including those methicillin-resistant strains (MRSA) [134]. In addition to serving as nanoparticle stabilizer, HSA can also be used as responsive moieties for the detection. For instance, a new electrochemical biosensor based on the human serum albumin and graphene oxide modified indium tin oxide electrode has been developed for the discrimination of D-and L-tryptophan. The detection mechanism is based on the interaction between D- or L-tryptophan and HSA, which can generate analytical signals for further processing [151].

The fact that HSA functionalization changed the biological behaviors of inorganic nanoparticles has been extensively applied in the design of functional nanomaterials for biomedical therapy and diagnosis. However, it must be noticed that the adsorption of HSA to the surface of nanomaterials may possibly influence HSA structure and function [152]. For example, after binding to silver nanoparticles, the reduced binding capacity to drug molecules (e.g. to warfarin and ibuprofen etc), loss of free thiols and antioxidant activity, and enhanced copper binding affinity have been observed in HSA [139]. For biomedical applications, loss of antioxidant activity could result in unexpected oxidative stress during the treatment process. The potential function alterations of HSA conjugated nanoparticle will lead to undesirable consequences, which should be taken into cautious consideration while using such nanoparticles for biomedical purposes.

4. CONCLUSIONS AND PERSPECTIVES

In summary, human serum album (HSA) with favorable stability, biodegradability and amphiphilicity has shown its superior advantages in the drug delivery and theranostic applications. HSA, encapsulating drugs or genes ether in its monomeric native formula or nanoparticle formula, has significantly improved the therapeutic efficacy of agents in a variety of human diseases including neutropenia, haemostasis, substance abuse, and oncology [7, 12]. So far, a number of HSA-based conjugates have been approved for human use and some are under clinical trials for future applications [108, 153-155].

Although the developed HSA-based materials have presented a lot of promising benefits, there are still many issues need to be carefully considered and addressed. The synthesis and bioconjugation steps in many cases require harsh conditions and even biohazardous chemical regents, which may potentially result in undesirable damage to HSA structure or functions. Sometimes even some mild reactions through non-covalent binding could also influence the natural performances of HSA. What's more, the question

of whether such alteration of HSA could affect the normal physiological behaviors has not been fully answered yet. This issue should be taken into consideration when using these strategies in fabrication of HSA-based therapeutics.

The successful and wide biological applications of nano-sized delivery platforms would greatly rely on the particle morphology, so as well in protein based nanomaterials. For instance, compared to HSA nanospheres, HSA nanotubes could encapsulate the target molecules more quickly without structural changes and also circulate in the bloodstream for longer time. Moreover, HSA nanotubes also have offered favorable biocompatible chambers for manipulation of virus and bacterial cells at single particle levels [156]. Despite recent progress on the fabrication of HSA protein-based nanomaterials, extensive efforts may still need to be further conducted to develop novel nanostructures to achieve the expected performance in the biomedical applications.

Moreover, there is no doubt that the well established albumin-based targeting strategy could facilitate to accumulate in malignant and inflammatory tissues due to the EPR effect, the specific affinity to the targeted disease sites remains a pressing and challenging concern. Incorporation of additional targeting ligands fused with HSA may compensate its shortcomings in improving the specific targeting [64]. However, the variety of active groups on HSA leads to failure of commonly used conjugating methods with site-selectivity, thus making it quite difficult to get a precisely defined product [10, 147, 149, 157]. Therefore, new site-specific labeling techniques are still highly desirable in the future research. Another interesting alterative to generate functional HSA would be the recombinant protein technologies [149]. Based on these techniques, not only specific targeting proteins or polypeptides could be fused to HSA, those biological molecules with other desirable functions such as protein therapeutics could be also used to adapt into native HSA structure.

Besides the promising activity towards delivery of various types of therapeutics into specific diseases area, protein based nanocarriers can also facilitate transport of molecular imaging agents for early stage of disease diagnosis and real-time monitoring of therapeutic response. It is believed that such imaging-guided diagnosis and treatment will direct us move toward the goals of personalized medicine, which, to a great extent, lies on the successful development of reliable platforms ensuing safe drug administration and reliable theranostics. By right, HSA based nanomaterials have been well investigated as such promising delivery platforms. Up to now, a number of clinic studies have proved the excellent therapeutic potential of HSA bound macro/nano-drugs. Furthermore, extensive efforts have also been greatly carried out recently in the development of HSA based imaging agents [144, 158-162]. Although HSA has been thought playing important roles in improving the targeting effects and reducing non-specific toxicity of imaging agents in living systems [149, 158], the advance of HSA-based theranostics with integration of both therapy and diagnosis is still under its infant stage. Far from adequate, the relevant studies should be the main focus of the next decades. Considering the attractive biochemical and biophysical properties, it is no doubt that HSA-based materials will continuously serve as one of the important options in this active research area.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENT

Declared none.

REFERENCES

[1] Sui M, Liu W, Shen Y. Nuclear drug delivery for cancer chemotherapy. J Control Release 2011; 155: 227-36.

[2] Kievit FM, Zhang M. Cancer nanotheranostics: improving imaging and therapy by targeted delivery across biological barriers. Adv Mater 2011; 23: H217-47.

[3] Peer D, Karp JM, Hong S, FaroKHzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nano 2007; 2: 751-60.

[4] Komatsu T. Protein-based nanotubes for biomedical applications. Nanoscale 2012; 4: 1910-18.

[5] Shimanovich U, Bernardes GJ, Knowles TP, Cavaco-Paulo A. Protein micro- and nano-capsules for biomedical applications. Chem Soc Rev 2014; 43: 1361-71.

[6] Lohcharoenkal W, Wang L, Chen YC, Rojanasakul Y. Protein nanoparticles as drug delivery carriers for cancer therapy. BioMed Res Int 2014; 2014: 180549.

[7] Sleep D. Albumin and its application in drug delivery. Expert Opin Drug Deliv 2014; 18: 1-20.

[8] Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside. Mol Aspects Med 2012; 33: 209-90..

[9] Elzoghby AO, Samy WM, Elgindy NA. Albumin-based nanoparticles as potential controlled release drug delivery systems. J Control Release 2012; 157: 168-82.

[10] Ming X, Carver K, Wu L. Albumin-based nanoconjugates for targeted delivery of therapeutic oligonucleotides. Biomaterials 2013; 34: 7939-49.

[11] Kratz F. Albumin as a drug carrier: Design of prodrugs, drug conjugates and nanoparticles. J Control Release 2008; 132: 171-83.

[12] Elsadek B, Kratz F. Impact of albumin on drug delivery-new applications on the horizon. J Control Release 2012; 157: 4-28.

[13] Sugio S, Kashima A, Mochizuki S, Noda M, Kobayashi K. Crystal structure of human serum albumin at 2.5 Å resolution. Protein Eng 1999; 12: 439-46.

[14] https: //kclpure.kcl.ac.uk/portal/files/12489435/Studentthesis-Beulah_Banfield_2014.pdf accessed on 9/12/2014.

[15] Peters Jr, T. In *All About Albumin*. Peters, T., Ed.; Academic Press: San Diego **1995;** 9-11.

[16] Ghuman J, Zunszain PA, Petitpas I, Bhattacharya AA, Otagiri M, Curry S. Structural basis of the drug-binding specificity of human serum albumin. J Mol Biol 2005; 353: 38-52.

[17] He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature 1992; 358: 209-15.

[18] http: //gradworks.umi.com/35/00/3500434.html accessed on 10/12/2014.

[19] Lexa KW, Dolghih E, Jacobson MP. A Structure-based model for predicting serum albumin binding. PLoS One 2014; 9: e93323

[20] Bos OJ, Labro JF, Fischer MJ, Wilting J, Janssen LH. The molecular mechanism of the neutral-to-base transition of human serum albumin Acid/base titration and proton nuclear magnetic resonance studies on a large peptic and a large tryptic fragment of albumin. J Biol Chem 1989; 264: 953-59.

[21] Le Gal JM, Manfait M. Conformational changes of human serum albumin *in vivo* induced by free fatty acids as studied by Fourier transform infrared spectroscopy. Biochim Biophy Acta 1990; 1041: 257-63.

[22] Wilting J, Van der Giesen WF, Janssen LH, Weideman MM, Otagiri M, Perrin JH. The effect of albumin conformation on the binding of warfarin to human serum albumin. The dependence of the binding of warfarin to human serum albumin on the hydrogen, calcium, and chloride ion concentrations as studied by circular dichroism, fluorescence, and equilibrium dialysis. J Biol Chem 1980; 255: 3032-7.

[23] Kim J, Bronson C, Hayton WL, Radmacher MD, Roopenian DC, Robinson JM, Anderson CL. Albumin turnover: FcRn-mediated recycling saves as much albumin from degradation as the liver produces. Am J Physiol-Gastr L 2006; 290: G352-60.

[24] Schnitzer JE, Oh P. Albondin-mediated capillary permeability to albumin. Differential role of receptors in endothelial transcytosis and endocytosis of native and modified albumins. J Biol Chem 1994; 269: 6072-82.

[25] Peters Jr T. In *All About Albumin*. Peters, T., Ed.; Academic Press: San Diego, 1995; 76-132

[26] Greish K. Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. Methods Mol Biol 2010; 624: 25-37.

[27] Greish K. Enhanced permeability and retention of macromolecular drugs in solid tumors: a royal gate for targeted anticancer nanomedicines. J Drug Target 2007; 15: 457-64.
[28] Maeda H, Fang J, Inutsuka T, KitamotoY. Vascular permeability enhancement in solid tumor: various factors, mechanisms involved and its implications. Int Immunopharmacol 2003; 3: 319-28.
[29] Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nat Rev Drug Discov 2010; 9: 615-27.
[30] Stollenwerk MM, Pashkunova-Martic I, Kremser C, *et al*. Albumin-based nanoparticles as magnetic resonance contrast agents: I. Concept, first syntheses and characterisation. Histochem Cell Biol 2010; 133: 375-404.
[31] Zsila F, Bikadi Z, Malik D, Hari P, Pechan I, Berces A, Hazai E. Evaluation of drug-human serum albumin binding interactions with support vector machine aided online automated docking Bioinformatics 2011; 27: 1806-13.
[32] Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003; 22: 7265-79.
[33] Zheng YR, Suntharalingam K, Johnstone TC, *et al*. Pt(IV) prodrugs designed to bind non-covalently to human serum albumin for drug delivery. J Am Chem Soc 2014; 136: 8790-8.
[34] Webb MI, Wu B, Jang T, Chard RA, *et al*. Increasing the bioavailability of Ru(III) anticancer complexes through hydrophobic albumin interactions. Chem Eur J 2013; 19: 17031-42.
[35] Enyedy ÉA, Dömötör O, Bali K, Hetényi A, Tuccinardi T, Keppler BK. Interaction of the anticancer gallium (III) complexes of 8-hydroxyquinoline and maltol with human serum proteins. J Bio Inorg Chem 2014; 1-12.
[36] Hummer AA, Heffeter P, Berger W, *et al*. X-ray absorption near edge structure spectroscopy to resolve the *in vivo* chemistry of the redox-active indazolium trans-[tetrachlorobis(1H-indazole) ruthenate(III)] (KP1019). J Med Chem 2013; 56: 1182-96.
[37] Dias DM, Rodrigues JP, Domingues NS, Bonvin AM, Castro, MM. Unveiling the interaction of vanadium compounds with human serum albumin by using 1H STD NMR and computational docking studies. Eur J Inorg Chem 2013: 4619-27.
[38] Lipponer KG, Vogel E, Keppler BK. Synthesis, characterization and solution chemistry of trans-indazoliumtetrachlorobis (indazole)ruthenate(III), a new anticancer ruthenium complex IR, UV, NMR, HPLC investigations and antitumor activity crystal structures of trans-1-methyl-indazoliumtetrachlorobis-(1-methylindazole)ruthenate(III) and its hydrolysis product trans-monoaquatrichlorobis-(1-methylindazole)-ruthenate(III). Metal-Based Drugs 1996; 3: 243-60.
[39] van der Vorst JR, Schaafsma BE, Verbeek, FP, *et al*. Near-infrared fluorescence sentinel lymph node mapping of the oral cavity in head and neck cancer patients. Oral Oncol 2013; 49: 15-9.
[40] Chen Q, Wang C, Zhan Z, *et al*. Near-infrared dye bound albumin with separated imaging and therapy wavelength channels for imaging-guided photothermal therapy. Biomaterials 2014; 35: 8206-14.
[41] Chen Q, Wang C, Cheng L, He W, Cheng Z, Liu, Z. Protein modified upconversion nanoparticles for imaging-guided combined photothermal and photodynamic therapy. Biomaterials 2014; 35: 2915-23.
[42] Luo J, Chen LF, Hu P, Chen ZN. Tetranuclear gadolinium(III) porphyrin complex as a theranostic agent for multimodal imaging and photodynamic therapy. Inorg Chem 2014; 53: 4184-91.
[43] Digilio G, Tuccinardi T, Casalini F, *et al*. Study of the binding interaction between fluorinated matrix metalloproteinase inhibitors and human serum albumin. Eur J Med Chem 2014; 79: 13-23.
[44] Yang F, Ma ZY, Zhang, Y, *et al*. Human serum albumin-based design of a diflunisal prodrug. Eur J Pharm Biopharm 2013; 84: 549-54.
[45] Sekijima Y, Dendle MA, Kelly JW. Orally administered diflunisal stabilizes transthyretin against dissociation required for amyloidogenesis. Amyloid 2006; 13: 236-49.
[46] http: //accessmedicine.mhmedical.com/book.aspx?bookID=374 accessed on 8/12/2014
[47] Kratz F, Müller-Driver R, Hofmann I, Drevs J, Unger C. A novel macromolecular prodrug concept exploiting endogenous serum albumin as a drug carrier for cancer chemotherapy. J Med Chem 2000; 43: 1253-6.
[48] Kratz F. DOXO-EMCH (INNO-206): the first albumin-binding prodrug of doxorubicin to enter clinical trials. Expert Opin Inv Drug 2007; 16: 855-66.
[49] Graeser R, Esser N, Unger H, Fichtner I, Zhu A, Unger C, Kratz F. INNO-206, the (6-maleimidocaproyl hydrazone derivative of doxorubicin), shows superior antitumor efficacy compared to doxorubicin in different tumor xenograft models and in an orthotopic pancreas carcinoma model. Invest New Drug 2010; 28: 14-9.
[50] Kratz F, Warnecke A, Scheuermann K, *et al*. Probing the cysteine-34 position of endogenous serum albumin with thiol-binding doxorubicin derivatives. Improved efficacy of an acid-sensitive doxorubicin derivative with specific albumin-binding properties compared to that of the parent compound. J Med Chem 2002; 45: 5523-33.
[51] http: //www cytrx com/pipeline accessed on 10/12/2014
[52] Pichler V, Mayr J, Heffeter, P, *et al*. Maleimide-functionalised platinum (IV) complexes as a synthetic platform for targeted drug delivery. Chem Commun 2013; 49: 2249-51.
[53] Stoddart CA, Nault G, Galkina SA, *et al*. Albumin-conjugated C34 peptide HIV-1 fusion inhibitor equipotent to C34 and T-20 *in vitro* with sustained activity in SCID-HU THY/LIV mice. J Bio Chem 2008; 283: 34045-52.
[54] Xie D, Yao C, Wang L, *et al*. An albumin-conjugated peptide exhibits potent anti-HIV activity and long *in vivo* half-life. Antimicrob Agents Ch 2010; 54: 191-6.
[55] Hettick JM, Siegel PD. Determination of the toluene diisocyanate binding sites on human serum albumin by tandem mass spectrometry. Anal Biochem 2011; 414: 232-38.
[56] Hartung G, Stehle G, Sinn H, *et al*. Phase I trial of methotrexate-albumin in a weekly intravenous bolus regimen in cancer patients. Clin Cancer Res 1999; 5: 753-9.
[57] Hartung G, Stehle G, Sinn H. Phase I-trial of a methotrexate-albumin conjugate (MTX-HSA) in cancer patients. Eur J Cancer 1997; 33: S249-50.
[58] Jeong H, Huh M, Lee S J, *et al*. Photosensitizer conjugated human serum albumin nanoparticles for effective photodynamic therapy. Theranostics 2011; 1: 230-9.
[59] Josefsen LB, Boyle RW. Unique diagnostic and therapeutic roles of porphyrins and phthalocyanines in photodynamic therapy, imaging and theranostics. Theranostics 2012; 2: 916-66.
[60] Wang RM, Komatsu T, Nakagawa A, Tsuchida E. Human serum albumin bearing covalently attached iron(II) porphyrins as O_2-coordination sites. Bioconjugate Chem 2004; 16: 23-6.
[61] Luong A, Issarapanichkit T, Kong SD, Fong R, Yang, J. pH-Sensitive, N-ethoxybenzylimidazole (NEBI) bifunctional crosslinkers enable triggered release of therapeutics from drug delivery carriers. Org Biomol Chem 2010; 8: 5105-9.
[62] Krantz A, Hanel AM, Strug I, *et al*. Site-specific labeling of a protein lysine residue by novel kinetic labeling combinatorial libraries. Comput Struct Biotechnol J 2014; 9: e201403001.
[63] Chen X, Muthoosamy K, Pfisterer A, Neumann B, Weil T. Site-selective lysine modification of native proteins and peptides via kinetically controlled labeling. Bioconjugate Chem 2012; 23: 500-8.
[64] Asano S, Patterson JT, Gaj T, Barbas CF. Site-selective labeling of a lysine residue in Human Serum Albumin. Angew Chem Int Ed 2014; 53: 11783-6.
[65] Jinno F, Yoneyama T, Morohashi A, Kondo T, Asahi S. Chemical reactivity of ethyl (6R)-6-[N-(2-chloro-4-fluorophenyl)sulfamoyl] cyclohex-1-ene-1-carboxylate (TAK-242) *in vitro*. Biopharm Drug Dispos 2011; 32: 408-25.
[66] Spicer CD, Davis BG. Selective chemical protein modification. Nat Commun 2014; 5: 4740.
[67] Baslé E, Joubert N, Pucheault M. Protein chemical modification on endogenous amino acids. Chem Biol 2010; 17: 213-27.
[68] Tanaka T, Kamiya N, Nagamune, T , N-terminal glycine-specific protein conjugation catalyzed by microbial transglutaminase. Febs Lett 2005; 579: 2092-6.
[69] Chen T, Embree HD, Wu LQ, Payne GF. *In vitro* protein-polysaccharide conjugation: Tyrosinase-catalyzed conjugation of gelatin and chitosan. Biopolymers 2002; 64: 292-302.
[70] Heck T, Faccio G, Richter M, Thöny-Meyer L. Enzyme-catalyzed protein crosslinking. Appl Microbiol Biot 2013; 97: 461-475.
[71] Rollett A, Thallinger B, Ohradanova-Repic A. Enzymatic synthesis of antibody-human serum albumin conjugate for targeted drug delivery using tyrosinase from Agaricus bisporus. Rsc Adc 2013; 3: 1460-7.
[72] Torchilin V. Intracellular delivery of protein and peptide therapeutics. Drug Discov Today: Technologies 2008; 5: e95-103.

[73] Chaudhury C, Brooks CL, Carter DC, Robinson JM, Anderson CL. Albumin binding to FcRn: distinct from the FcRn-IgG interaction. Biochemistry 2006; 45: 4983-90.

[74] Tuan GCV, Kragh-Hansen U, Otagiri M. Pharmaceutical strategies utilizing recombinant human serum albumin. Pharmaceut Res 2002; 19: 569-77.

[75] Kratz F, Elsadek B. Clinical impact of serum proteins on drug delivery. J Control Release 2012; 161: 429-45.

[76] Liu M, Huang Y, Hu L, *et al*. Selective delivery of interleukine-1 receptor antagonist to inflamed joint by albumin fusion. BMC Biotechnology 2012; 12: 68.

[77] Goldbach-Mansky R. Blocking interleukin-1 in rheumatic diseases. Ann Ny Acad Sci 2009; 1182: 111-23.

[78] Berger V, Richter F, Zettlitz K, Unverdorben F, Scheurich P, Herrmann A, Pfizenmaier K, Kontermann R E. An anti-TNFR1 scFv-HSA fusion protein as selective antagonist of TNF action Protein. Eng Des Sel 2013; 26: 581-7.

[79] Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell Death & Differentiation 2003; 10: 45-65.

[80] Kontermann R E, Münkel S, Neumeyer J, *et al*. A humanized tumor necrosis factor receptor 1 (TNFR1)-specific antagonistic antibody for selective inhibition of tumor necrosis factor (TNF) action. J Immunother 2008; 31: 225-34.

[81] Spooner R, Yilmaz Ö. The Role of Reactive-oxygen-species in microbial persistence and inflammation. Int J Mol Sci 2011; 12: 334-52.

[82] Vlahos R, Stambas J, Selemidis S. Suppressing production of reactive oxygen species (ROS) for influenza A virus therapy. Trends Pharmacol Sci 2012; 33: 3-8.

[83] Nakamura H, Herzenberg LA, Bai J, *et al*. Circulating thioredoxin suppresses lipopolysaccharide-induced neutrophil chemotaxis. Proc Natl Acad Sci USA 2001; 98: 15143-8.

[84] Kodama A, Watanabe H, Tanaka R, *et al*. A human serum albumin-thioredoxin fusion protein prevents experimental contrast-induced nephropathy. Kidney Int 2013; 83: 446-54.

[85] Tanaka R, Ishima Y, Enoki Y, *et al*. Therapeutic impact of human serum albumin–thioredoxin fusion protein on influenza virus-induced lung injury mice. Front immunol 2014; 5: 561.

[86] Langer K, Balthasar S, Vogel V, Dinauer N, von Briesen H, Schubert D. Optimization of the preparation process for human serum albumin (HSA) nanoparticles. Int J Pharm 2003; 257: 169-80.

[87] Gallo JM, Hung CT, Perrier DG. Analysis of albumin microsphere preparation. Int J Pharm , 1984; 22: 63-74.

[88] Lin W, Coombes A, Davies M, Davis S, Illum L. Preparation of sub-100 nm human serum albumin nanospheres using a pH-coacervation method. J Drug Target 1993; 1: 237-43.

[89] Lee SH, Heng D, Ng WK, Chan HK, Tan RB. Nano spray drying: A novel method for preparing protein nanoparticles for protein therapy. Int J Pharm 2011; 403: 192-200.

[90] Ding D, Tang X, Cao X, Wu J, Yuan A, Qiao Q, Pan J, Hu, Y , Novel self-assembly endows human serum albumin nanoparticles with an enhanced antitumor efficacy. AAPS PharmSciTech, 2014; 15: 213-222

[91] Xu R, Fisher, M, Juliano RL, Targeted albumin-based nanoparticles for delivery of amphipathic drugs. Bioconjugate Chem 2011; 22: 870-8.

[92] Wang W, Huang Y, Zhao S, Shao T. Cheng Y. Human serum albumin (HSA) nanoparticles stabilized with intermolecular disulfide bonds. Chem Commun 2013; 49: 2234-6.

[93] Zhao S, Wang W, Huang Y, Fu Y, Cheng Y. Paclitaxel loaded human serum albumin nanoparticles stabilized with intermolecular disulfide bonds. MedChemComm 2014; 5: 1658-63.

[94] http: //digital.library.louisville.edu/utils/getfile/collection/etd/id/2727/filename/5524.pdf accessed on 15/12/2014

[95] Bououdina M. *Handbook of Research on Nanoscience, Nanotechnology, and Advanced Materials* IGI Global. 2014.

[96] http: //www cancer gov/cancertopics/druginfo/fda-nanoparticle-paclitaxel accessed on 18/11/2014

[97] Cortes J, Saura C. Nanoparticle albumin-bound (nab™)-paclitaxel: improving efficacy and tolerability by targeted drug delivery in metastatic breast cancer. EJC Supplements 2010; 8: 1-10.

[98] Kouchakzadeh H, Shojaosadati SA, Shokri F. Efficient loading and entrapment of tamoxifen in human serum albumin based nanoparticulate delivery system by a modified desolvation technique Chem Eng Res Des 2014; 92: 1681-92.

[99] Avachat A, Oswal Y, Gujar K, Shah R. Preparation and characterization of rivastigmine loaded human serum albumin (HSA) nanoparticles. Curr Drug Deliv 2014; 11: 359-70.

[100] Gaca S, Reichert S, Multhoff G, *et al*. Targeting by cmHsp70 1-antibody coated and survivin miRNA plasmid loaded nanoparticles to radiosensitize glioblastoma cells. J Control Release 2013; 172: 201-6.

[101] Yuan A, Wu J, Song C, *et al*. A novel self-assembly albumin nanocarrier for reducing doxorubicin-mediated cardiotoxicity. J Pharm Sci 2013; 102: 1626-35.

[102] Rollett A, Reiter T, Ohradanova-Repic A, *et al*. HSA nanocapsules functionalized with monoclonal antibodies for targeted drug delivery. Int J Pharm 2013; 458: 1-8.

[103] Lee HJ, Park HH, Kim J A, *et al*. Enzyme delivery using the 30Kc19 protein and human serum albumin nanoparticles. Biomaterials 2014; 35: 1696-704.

[104] Bae S, Ma K, Kim TH, *et al*. Doxorubicin-loaded human serum albumin nanoparticles surface-modified with TNF-related apoptosis-inducing ligand and transferrin for targeting multiple tumor types. Biomaterials 2012; 33: 1536-46.

[105] Li H, Qian ZM. Transferrin/transferrin receptor-mediated drug delivery. Med Res Rev 2002; 22: 225-50.

[106] Taheri A, Dinarvand R, Atyabi F, Ghahremani MH, Ostad SN, Trastuzumab decorated methotrexate-human serum albumin conjugated nanoparticles for targeted delivery to HER2 positive tumor cells. Eur J Pharm Sci, 2012; 47: 331-40.

[107] Wu J, Song C, Jiang, C, Shen X, Qiao Q, Hu Y. Nucleolin targeting AS1411 modified protein nanoparticle for antitumor drugs delivery. Mol Pharm 2013; 10: 3555-63.

[108] Wu Y, Ng DY, Kuan SL, Weil T. Protein-polymer therapeutics: a macromolecular perspective. Biomaterials Science 2015; DOI: 10 1039/C4BM00270A.

[109] Boyer C, Huang X, Whittaker MR, Bulmus V, Davis TP. An overview of protein-polymer particles. Soft Matter 2011; 7: 599-14.

[110] Banerjee SS, Aher N, Patil R, Khandare J. Poly(ethylene glycol)-Prodrug conjugates: concept, design, and applications. J Drug Deliv 2012; 17.

[111] Bhattacharya A, Prajapati R, Chatterjee S, Mukherjee TK. Concentration-dependent reversible self-oligomerization of serum albumins through intermolecular β-Sheet formation. Langmuir 2014; 30: 14894-904.

[112] Zhao T, Cheng Y, Tan H, *et al*. Site-specific chemical modification of human serum albumin with polyethylene glycol prolongs half-life and improves intravascular retention in mice. Bio Pharm Bulletin 2012; 35: 280-8.

[113] Byeon HJ, Min SY, Kim I, *et al*. Human serum albumin-TRAIL conjugate for the treatment of rheumatoid arthritis. Bioconjugate Chem 2014; 25: 2212-21.

[114] Zhao T, Yang Y, Zong A, *et al*. N-terminal PEGylation of human serum albumin and investigation of its pharmacokinetics and pulmonary microvascular retention. Bioscience trends 2012; 6: 81-8.

[115] Wu Y, Pramanik G, Eisele K, Weil T. Convenient approach to polypeptide copolymers derived from native proteins. Biomacromolecules 2012; 13: 1890-8.

[116] Wu Y, Chakrabortty S, Gropeanu, RA, *et al*. pH-responsive quantum dots via an albumin polymer surface coating. J Am Chem Soc 2010; 132: 5012-4.

[117] Kuan SL, Stöckle B, Reichenwallner J, *et al*. Dendronized albumin core-shell transporters with high drug loading capacity. Biomacromolecules 2013; 14: 367-76.

[118] Wu Y, Ihme S, Feuring-Buske M, *et al*. A core-shell albumin copolymer nanotransporter for high capacity loading and two-step release of doxorubicin with enhanced anti-leukemia activity. Adv Healthc Mater 2013; 2: 884-94.

[119] Ruso JM, Attwood D, García M, *et al*. Interaction of amphiphilic propranolol hydrochloride with haemoglobin and albumin in aqueous solution. Langmuir 2000; 16: 10449-55.

[120] Verma A, Stellacci F. Effect of surface properties on nanoparticle-cell interactions. Small 2010; 6: 12-21.

[121] Zhang S, Doschak MR. Uludağ H. Pharmacokinetics and bone formation by BMP-2 entrapped in polyethylenimine-coated albumin nanoparticles. Biomaterials 2009; 30: 5143-55.

[122] Abbasi S, Paul A, Prakash S. Investigation of siRNA-loaded polyethylenimine-coated human serum albumin nanoparticle complexes for the treatment of breast cancer. Cell Biochem Biophys 2011; 61: 277-87.

[123] Abbasi S, Paul A, Shao W, Prakash S. Cationic albumin nanoparticles for enhanced drug delivery to treat breast cancer: preparation and *in vitro* assessment. J Drug Deliv 2012; 2012: 686108.

[124] Zhu Q, Jia L, Gao Z, Wang C, Zhang J, Dong L. A tumor environment responsive doxorubicin-loaded nanoparticle for targeted cancer therapy. Mol Pharm 2014; 11: 3269-78.

[125] Kadlecova Z, Rajendra Y, Matasci M, *et al*. DNA delivery with hyperbranched polylysine: A comparative study with linear and dendritic polylysine. J Control Release 2013; 169: 276-88.

[126] Stiriba SE, Frey H, Haag R. Dendritic polymers in biomedical applications: from potential to clinical use in diagnostics and therapy. Angew Chem Int Ed 2002; 41: 1329-34.

[127] Giri J, Diallo MS, Simpson AJ. Interactions of poly(amidoamine) dendrimers with human serum albumin: binding constants and mechanisms. ACS Nano 2011; 5: 3456-68.

[128] Sisavath N, Le Saux T, Leclercq L, Cottet H. Effect of dendrimer generation on the interactions between human serum albumin and dendrigraft polylysines. Langmuir 2014; 30: 4450-7.

[129] Liu N, Hao Y, Yin Z, Ma M, Wang L, Zhang X. Self-assembled human serum albumin-coated complexes for gene delivery with improved transfection. Die Pharmazie-Int J Pharm Sci 2012; 67: 174-81.

[130] Zhou J, Zhang X, Li M, *et al*. Novel lipid hybrid albumin nanoparticle greatly lowered toxicity of pirarubicin. Mol pharm 2013; 10: 3832-41.

[131] Huang HC, Barua S, Sharma G, Dey SK, Rege K. Inorganic nanoparticles for cancer imaging and therapy. J Control Release 2011; 155: 344-57.

[132] Mahon E, Salvati A, Baldelli BF, Lynch I, Dawson KA. Designing the nanoparticle-biomolecule interface for "targeting and therapeutic delivery". J Control Release 2012; 161: 164-74.

[133] Chan PH, Chen YC. Human serum albumin stabilized gold nanoclusters as selective luminescent probes for staphylococcus aureus and methicillin-resistant staphylococcus aureus. Anal Chem 2012; 84: 8952-6.

[134] Cañaveras F, Madueño R, Sevilla JM, Blázquez M, Pineda T. Role of the functionalization of the gold nanoparticle surface on the formation of bioconjugates with human serum albumin. J Phys Chem C 2012; 116: 10430-7.

[135] Gebauer JS, Malissek M, Simon S, *et al*. Impact of the nanoparticle–protein corona on colloidal stability and protein structure. Langmuir 2012; 28: 9673-9.

[136] Ge C, Du J, Zhao L, *et al*. Binding of blood proteins to carbon nanotubes reduces cytotoxicity Proc Nat Acad Sci USA 2011; 108: 16968-73.

[137] Treuel L, Brandholt S, Maffre P, Wiegele S, Shang L, Nienhaus GU. Impact of protein modification on the protein corona on nanoparticles and nanoparticle-cell interactions. ACS Nano 2014; 8: 503-13.

[138] Mariam J, Sivakami S, Kothari D, Dongre P. Bioactivity of albumins bound to silver nanoparticles. Protein J 2014; 33: 258-66.

[139] De Paoli SH, Diduch LL, Tegegn, TZ, *et al*. The effect of protein corona composition on the interaction of carbon nanotubes with human blood platelets. Biomaterials 2014; 35: 6182-94.

[140] Dautova Y, Kozlova D, Skepper JN, Epple M, Bootman MD. Proudfoot D, Fetuin-A and albumin alter cytotoxic effects of calcium phosphate nanoparticles on human vascular smooth muscle cells. Plos One 2014; 9: e97565.

[141] Schäffler M, Sousa F, Wenk A, *et al*. Blood protein coating of gold nanoparticles as potential tool for organ targeting. Biomaterials 2014; 35: 3455-66.

[142] Lu N, Li J, Tian R, Peng YY. Binding of human serum albumin to single-walled carbon nanotubes activated neutrophils to increase production of hypochlorous acid, the oxidant capable of degrading nanotubes. Chem Res Toxicol 2014; 27: 1070-7.

[143] Quan Q, Xie J, Gao H, *et al*. HSA coated iron oxide nanoparticles as drug delivery vehicles for cancer therapy. Mol Pharm 2011; 8: 1669-76.

[144] Barenholz Y. Doxil®-The first FDA-approved nano-drug: lessons learned. J Control Release 2012; 160: 117-34.

[145] Nigam P, Waghmode S, Louis M, Wangnoo S, Chavan P, Sarkar D. Graphene quantum dots conjugated albumin nanoparticles for targeted drug delivery and imaging of pancreatic cancer. J Mater Chem B 2014; 2: 3190-5.

[146] Topete A, Alatorre-Meda M, Iglesias P, *et al*. Fluorescent drug-loaded, polymeric-based, branched gold nanoshells for localized multimodal therapy and imaging of tumoral cells. ACS Nano 2014; 8: 2725-38.

[147] Xie J, Wang J, Niu, G, *et al*. Human serum albumin coated iron oxide nanoparticles for efficient cell labeling. Chem Commun 2010; 46: 433-5.

[148] Lee JY, Nam DH, Oh MH, Serum-stable quantum dot--protein hybrid nanocapsules for optical bio-imaging. Nanotechnology 2014; 25: 175702.

[149] Aviv H, Bartling S, Grinberg I, Margel S. Synthesis and characterization of Bi2O3/HSA core-shell nanoparticles for X-ray imaging applications. J Biomed Mater Res B 2013; 101B: 131-8.

[150] Zor E, Hatay Patir I, Bingol H, Ersoz M. An electrochemical biosensor based on human serum albumin/graphene oxide/3-aminopropyltriethoxysilane modified ITO electrode for the enantioselective discrimination of d- and l-tryptophan. Biosens Bioelectron 2013; 42: 321-5.

[151] Mortimer GM, Butcher NJ, Musumeci AW, Deng ZJ, Martin DJ, Minchin RF. Cryptic epitopes of albumin determine mononuclear phagocyte system clearance of nanomaterials. ACS Nano 2014; 8: 3357-66.

[152] Zhang S, Kucharski C, Doschak MR, Sebald W, Uludağ H. Polyethylenimine-PEG coated albumin nanoparticles for BMP-2 delivery. Biomaterials 2010; 31: 952-63.

[153] Yuge S, Akiyama M, Komatsu T. An Escherichia coli trap in human serum albumin microtubes. Chem Commun 2014; 50: 9640-3.

[154] Yang M, Hoppmann S, Chen L, Cheng Z. Human serum albumin conjugated biomolecules for cancer molecular imaging. Curr Pharm Des 2012; 18: 1023-31.

[155] Xu Y, Luo J, Chen Z. Zn^{2+}-responsive bimodal magnetic resonance imaging and fluorescence imaging agents and their interaction with human serum albumin. Eur J Inorg Chem 2014; 20: 3208-15.

[156] Alexander VM, Sano K, Yu Z, *et al*. Galactosyl human serum albumin-NMP1 conjugate: a near infrared (NIR)-activatable fluorescence imaging agent to detect peritoneal ovarian cancer metastases. Bioconjugate Chem 2012; 23: 1671-9.

[157] Chen Q, Liang C, Wang X, He J, Li Y, Liu Z. An albumin-based theranostic nano-agent for dual-modal imaging guided photothermal therapy to inhibit lymphatic metastasis of cancer post surgery. Biomaterials 2014; 35: 9355-62.

[158] Bu L, Ma X, Tu Y, Shen B, Cheng Z. Optical image-guided cancer therapy. Curr Pharm Des 2013; 14: 723-32.

Received: January 15, 2015 **Accepted: February 24, 2015**