



# USE OF CHITOSON NANO-PARTICLE IN CANCER TREATMENT: AN REVIEW

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## Abstract

Chitosan is a versatile polysaccharide of biological origin. Due to the biocompatible and biodegradable nature of chitosan, it is intensively utilized in biomedical applications in scaffold engineering as an absorption enhancer, and for bioactive and controlled drug release. In cancer therapy, chitosan has multifaceted applications, such as assisting in gene delivery and chemotherapeutic delivery, and as an immunoadjuvant for vaccines. The present review highlights the recent applications of chitosan and chitosan derivatives in cancer therapy.

**Keywords** chitosan, source, structure, physicochemical properties, newly modification of chitosan nanoparticle.

## Introduction

It is well established that cancer has become one of the most serious threat to human health. It is estimated that there will be 12 million cancer deaths worldwide in 2030.<sup>1</sup> Especially in China, as a developing country with a large population, cancer incidence and death rates keep rising year by year due to environmental pollution. According to an approximation, six persons are diagnosed with cancer every passing minute. This serious situation has brought a heavy burden to the society and patients. Among existing cancer treatments, chemotherapy is by far the most employed form of intervention, however, patients face many severe problems caused by chemotherapeutic agents, such as detrimental side effects, drug resistance and high cost. In the process of exploring novel cancer therapy, thanks to the progress in material science and process technology, Nano drug development has emerged as a promising approach to overcome the shortcomings of conventional chemotherapeutic agents.

The antitumor effect is achieved by carriers delivering drug selectively to the tumor cells. Therefore, the role of carriers in DDS is vital. The carriers for loading anticancer drugs are commonly composed of amphiphilic polymers or hydrophilic biopolymer.

Based on the source of the composition matrix, drug carriers can be divided into two classes: chemosynthetic and natural. Among chemically synthesized polymers, polyesters, poly (ether-esters), polyurethanes, and poly- carbonates, have received considerable attention. Specifically, poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid)(PLA), poly (-caprolactone)(PCL) or their polyethylene glycol (PEG)-copolymers are well studied and widely used as drug carriers.<sup>3</sup> Natural biopolymers such as chitosan, collagen, cellulose, and fibrin, are also investigated intensively in pharmaceutical field owing to their unique characteristics.

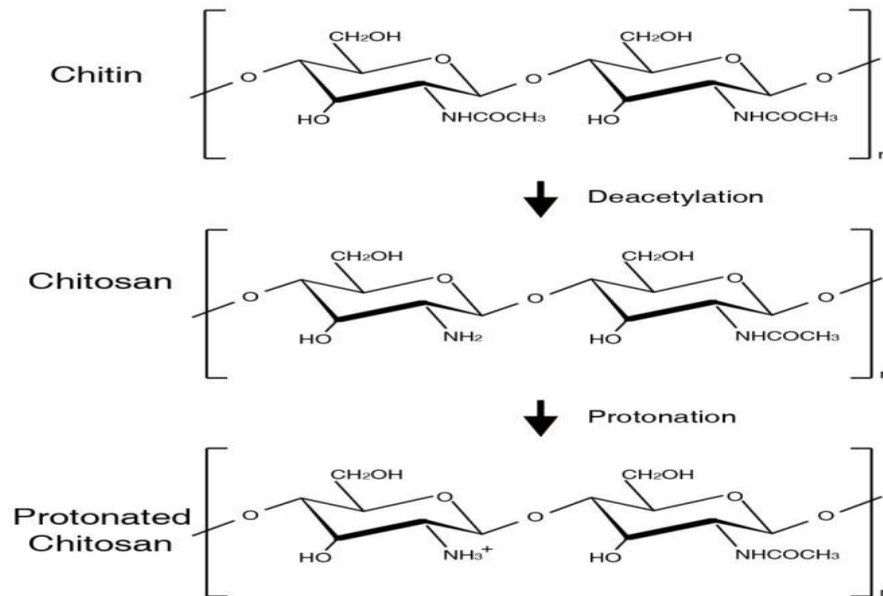
In particular, chitosan nanoparticles (CSNPs) have drawn considerable attention as anticancer drug delivery carriers because of their easy accessibility, excellent stability, low toxicity, and easy modification.<sup>6</sup> Herein; we aim to assess the various aspects of chitosan-based nanoparticles for drug delivery in cancer treatment.

## SOURCE, STRUCTURE, AND PHYSICOCHEMICAL PROPERTIES OF CHITOSAN

**Source and Structure** as a natural polysaccharide, chitosan is manufactured on a large scale by alkaline N-deacetylation of chitin in commercial production. Chitin is an abundant biopolymer isolated from the exoskeleton of crustaceans, such as crabs and shrimps.<sup>7</sup> Deacetylation of chitin and protonation of chitosan is shown in the (Figure 1). The proportion of the two repeating units (glucosamine and N-acetylglucosamine units) determines the degree of deacetylation of the polymer.

In the process of exploring novel cancer therapy, thanks to the progress in material science and process technology, Nano drug development has emerged as a promising approach to overcome the shortcomings of conventional chemotherapeutic agents.

Fig.1:- chemical structure of chitin, chitosan and protonated chitosan Nano particle.



### Physicochemical Properties:-

#### a. Water Solubility :

The aqueous solubility of chitosan has a great effect on its processing conditions and application range. Chitosan is insoluble in aqueous medium at neutral pH but is soluble in slightly acidic environment owing to the amine groups on its backbone. However, the solubility of chitosan in neutral and basic pH media can be improved by quaternization to form trimethyl ammonium chitosan derivatives. Moreover, the molecular weight of chitosan also greatly influences its solubility and degradability. Chitosan and its derivatives having lower molecular weights and lower degrees of deacetylation exhibit greater solubility and faster degradation.<sup>8</sup> Owing to the presence of protonatable amine groups, chitosan is a positively charged polymer at acidic pH.

#### b. Biodegradability:-

Biodegradability is a key property of polymers which are used as carriers in DDS and as scaffolds in tissue engineering. It not only determines the application potential and range of biomaterials used in clinic, but also controls the metabolic fate of these polymers in the body. Chitosan, is one of the best widely used biodegradable biopolymers, not only contains abundant amino groups, but also possesses hydrolysable glycosidic bonds in its backbone. Consequently, chitosan can be degraded to nontoxic oligosaccharides of

variable length by proteases, largely lysozyme, in vivo. Subsequently, the produced oligosaccharides can be incorporated in metabolic pathways or excreted out.<sup>10</sup> Its degradation rate is related to its molecular weight, the degree of deacetylation (DD), material shape, size, and administration route. Generally, higher molecular weight chitosan has a lower degradation rate. Similar trend is observed with DD, the degradation rate decreases with an increase in the DD.

Chitosan is often used as a scaffold or carrier of active biomolecules in tissue engineering. For this purpose, it is necessary for chitosan to possess enough persistence time to allow the growth and extension of newborn tissue.

For a variety of applications as a relevant candidate for absent or damaged tissue and organ, chitosan scaffolds can be easily processed into different shapes by methodologies, such as hydrogels, foams or sponges, and fibrous membranes. Based on the adopted processing methods and inherent properties of chitosan matrix, these biomaterials with various morphologies may undergo in vivo degradation in days to weeks. As a chitosan derivative (N, O-carboxymethyl chitosan, with molecular weight of 200 kDa and 92% of DD) hydrogel persisted for ~5 days when it was used as a wound healing dressing.<sup>11</sup>

### c. Toxicity:-

Among natural polymers, chitosan is widely regarded as non-toxic, biocompatible and biodegradable polysaccharide. Several products based on chitosan have been proved by the FDA for use in wound dressing.

However, when chitosan and its derivatives are used to deliver drugs and genes, the toxicity of Nanosized chitosan particles should not be ignored because the nanoparticles may enter systemic circulation from the gastrointestinal tract, nasal cavity, or alveolar sacs, thereby causing various levels of toxicity to the human body.

The toxicity level of CSNPs may be related to several factors including the properties of the parent material which was used to prepare the nanoparticles, particle-size, and the interacting cell type. Detoxifying organ in the human body, hepatic toxicity of CSNPs should be taken into consideration. CSNPs are known to show certain negative effects on hepatic cells. CSNPs.<sup>15</sup>

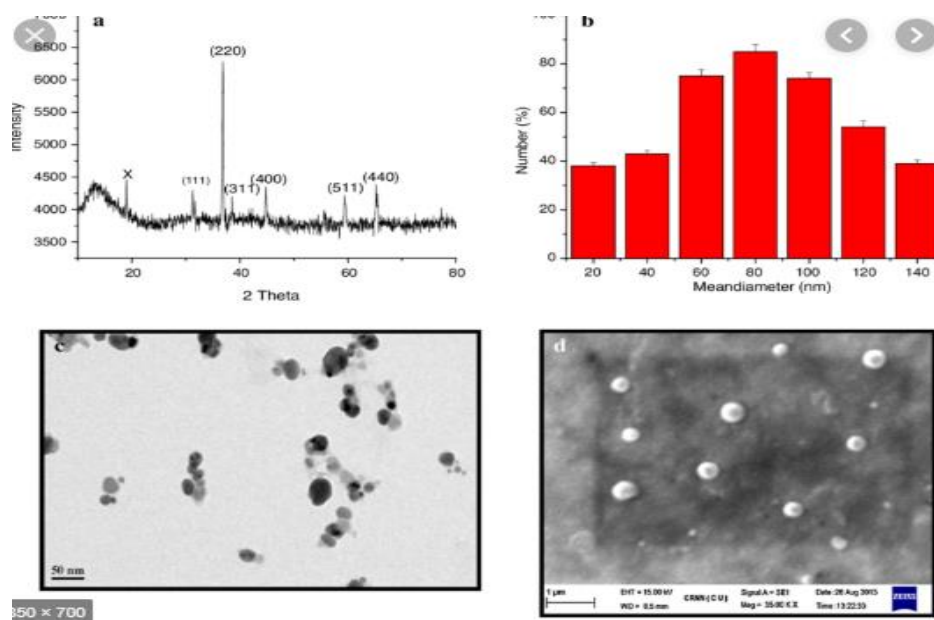


Fig: 2 Mechanism of toxicity caused by chitosan Nano- particle.

## NEWLY MODIFICATION OF CHITOSAN NANO PARTICLE

Molecular backbone of chitosan contains reactive amino and hydroxyl groups that can be readily decorated with various ligands, functional groups and moieties. After the appropriate modification, beneficial properties can be obtained including enhanced solubility, biocompatibility, active targeting, etc.

**1. Chitosan Nanoparticles Modified by Carbon-Based Materials:-** Carbon nanotubes (CNT) are carbon cylinders composed of benzene rings. Although their insolubility in water and organic solvents may cause toxicity, they are being applied in biomedical fields as carriers to deliver protein, drugs, and sensors for DNA detection, inter alia. For the purpose of improving water-solubility and multi- functionalization, they can be chemically modified by conjugation with entities such as proteins, peptides, and biopolymers. On the other hand, the use of organic solvents may limit polymer functionalization.

To knowing the problem, Li et al. developed multi-walled carbon nanotube chitosan nanoparticle (MWCNT CSNP) hybrids by an ionotropic gelation process. The in situ synthesis technique was conducted at extremely mild room temperature condition without the use of any toxic solvents. The prepared MWCNT-CSNP hybrids improved the model protein immobilization efficiency 0.8 times and simultaneously decreased

the cellular toxicity by ~50% compared with carboxylate MWCNT. 20 MWCNTs with varying length were also conjugated with chitosan-folic acid nanoparticles (CS-FA NPs) by ionotropic gelation process. The surface functionalization improves the transfection efficiency and decreases the cytotoxicity of MWCNTs.<sup>2</sup>

**2. Chitosan Nanoparticles Modified by Natural Polysaccharide:-** Heparin is a biodegradable, biocompatible, and negatively charged polysaccharide with many carboxylic groups in its molecular structure. It is moderately stable in vitro but it may be corrupted by hydrolysis and in vivo enzymolysis. Thus, it can be used to stabilize functional nanoparticles via the ionic interaction between cationic chitosan and anionic heparin. A study reported by Yuk et al. explain that gold-deposited iron oxide NPs immobilized into the glycol chitosan/heparin network showed enhanced tumor-specific targeting (Fig. 3). Lai et al. used heparin- processed chitosan NPs to load cytolethal distending toxin (CdtB) for the treatment of gastric cancer. Like CdtB itself, the CdtB-encapsulated nanoparticles could enhance cell-cycle arrest at G2/M leading to apoptosis. The mechanisms for CdtB-encapsulated nanoparticles-induced cell death was mediated by ATM-dependent DNA damage checkpoint responses. HA modified DDS can specifically improve drug accumulation in cancer cells over-expressing CD-44. For example, polyelectrolyte complex nanoparticles are completely depends upon hyaluronic acid/chitosan (HA/CS) showed potent cytotoxicity and higher uptake efficiency in C6 cells after loading a water-insoluble curcuminoid. Deng et al. evidenced simultaneous co-delivery of chemotherapeutic agent doxorubicin (DOX) and tumor suppressive miRNA-34a into triple negative breast cancer cells by HA-CSNPs. It not only improved the antitumor activity of DOX, but it also suppressed tumor cells migration by targeting Notch-1 signaling. Apart from the above mentioned polysaccharides, other polysaccharides such as alginate, starch, pectin, and carboxyl-methyl cellulose are also used to fabricate polyelectrolyte complexes with chitosan for drug delivery.

**3. Chitosan Nanoparticles Modified by Chemically Synthesized Copolymer:-** Many chemically synthesized copolymers like polyesters and polyamides have been used directly as drug carriers owing to their excellent biocompatibility and controllable degradability. These are also be used to pick up the biological and chemical properties of chitosan by chemical conjugation. Poly(lactic-co-glycolic acid) (PLGA) is a biocompatible and degradable polymer that has been widely used to encapsulate and deliver various chemotherapeutic drugs, siRNAs, DNAs, peptides, and proteins. Chitosan-processed PLGA nanoparticles can also be protect biologic agents from degradation during systemic circulation, thereby enhancing therapeutic efficacy. Martin et al. reported that PLGA-chitosan (chitosan with a low molecular weight of 2.5 kDa) nanoparticles have the capacity to transport large amounts of surviving siRNA across the urothelium and/or to the tumor site, in that way rising the therapeutic response. Poly(N-isopropylacrylamide) (PNIPAA) is a widely studied polymer with excellent thermo sensitivity. The PNIPAA-processed CSNPs has the potential to display unique thermo-responsibility. Zhang et al. arranged self- assembled chitosan-graft-

poly (N-isopropylacrylamide)/ carboxymethyl cellulose nanoparticles to load 5-fluorouracil (5-FU). The obtained polyelectrolyte complex nanoparticles were thermosensitive and had an average diameter of about 200 nm. Poly amido amine- dendrimers are a new type of synthetic polymer characterized by a branched spherical shape and a high density surface charge. Zhang et al. grafted carboxyl group-poly (amidoamine) onto carboxymethyl chitosan for preparing core-shell nanoparticles. The self-assembled dendrimer nanoparticles did not display significant cytotoxicity in the range of concentrations below 3.16 mg/ml and showed excellent properties as highly potent and non-toxic intracellular carriers for protein delivery. In totaling to these, other polymers, like polyethylene glycol, methoxy poly(ethylene glycol)-poly(lactic acid) (mPEG-PLA), poly(ethylene glycol)-poly(- caprolactone) (PEG-PCL), have also been grafted onto chitosan for fabricating various carrier nanoparticles.<sup>32-34</sup> For example, used paclitaxel and rutin as model drugs for the estimate of the controlled release capability of the chitosan-PEG-PCL nanoparticles.

**4. Chitosan Nanoparticles Modified by Low Molecular Weight Compounds:-** The compounds with low molecular weight offer unique characteristics in modification of nanoparticles. The processed NPs may show targeting efficacy, lower toxicity, longer flow time, etc. Folate, a low molecular weight compound, is often used as a modifier owing to high-expression of folate receptor in many cancer cells. Folic acid also be covalently conjugated to chitosan molecules via its gamma-carboxyl moiety. The folic acid-tagged hydrophobic-modified chitosan. NPs has been shown to load curcumin and successfully deliver the drug to folate receptor over-expressed cancer cells. Oleic acid is often used to prepare magnetic iron oxide nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs), and the oleic acid-decorated Fe<sub>3</sub>O<sub>4</sub> NPs are usually encapsulated in various carriers for use as imaging probes to detect tumors. However, the interface compatibility between Fe<sub>3</sub>O<sub>4</sub> NPs and carrier can greatly influence the loading efficacy and imaging quality. Oleic acid is used to modify the polymeric carriers to improve the interface compatibility.

After grafting with oleic acid, oleoyl-chitosan self-assembles into core-shell structures in aqueous solution and provides the effective core compartment for loading, A high-performance nanoparticle is set by in fact complexing succinate-processed chitosan with folic acid-modified chitosan. The resulting nanoparticles have an average diameter and zeta potential of 110.0 nm and 18.6 mV, respectively. These are very stable in aqueous suspension and are readily engulfed by oral cancer cells via folate-receptor-mediated endocytosis. Therefore, this kind of chitosan nanoparticle is an excellent vector for oral-specific delivery of 5-aminolevulinic acid for fluorescent endoscopic detection, and it can be used for photodynamic detection of oral cancer. Jin et al. prepared N-octyl-O-sulfated chitosan (NOSC) with a viscosity average molecular weight of 65-70 kDa for the preparation of PTX-loaded micelles.<sup>38</sup> The PTX micelles showed superior blood persistence, tumor accumulation, and therapeutic efficacy after intravenous injection into the tumor-bearing mice. D, L-Lactic acid has also been used as a modifier to decorate chitosan by grafting lactic acid onto amino groups in chitosan. After inducing the lactyl segment into the chitosan backbone, the resulting

nanoparticle showed a high protein encapsulation (96%) and a prolonged drug release rate (15%) over 4 weeks. Glycyrrhetic acid (GA) is one of the most important bioactive compounds of licorice and is used broadly as medicine for the treatment of many diseases. It is second-hand as a ligand to target liver because of the abundance of receptors for GA on the hepatocyte membrane. Used GA-processed sulfated chitosan (GA-SCS) to prepare doxorubicin-loaded micelles (DOX/SA-SCS micelles). The IC<sub>50</sub> of the prepared micelles against liver cancer cells (HepG2 cells) was 54.7 ng/mL, remarkably lower than that of the no-GA-modified micelles. Moreover, the DOX/SA-SCS micelles showed higher affinity for the liver cancer cells (HepG2 cells) than for the normal liver cells (Chang liver cells). Another study reported that 5-FU-conjugated GA-modified chitosan nanoparticles can target the liver, and have significantly inhibited tumor growth in an orthotropic liver cancer mouse model.

In order to improve liver-targeted drug delivery, galactosylated Nano-carrier is another ideal choice. Asialo-glycoprotein receptors are expressed predominantly on hepatocytes for clearance of galactose-terminated glycoprotein's, and have been identified on a continuous human hematoma cell line, HepG2. Therefore, the therapeutic activity can be improved by the interactions between the ligand and liver cancer cell receptors. Cheng et al. second-hand galactosylated chitosan (GC) to prepare 5-FU loaded nanoparticles (GC/5-FU). Analysis of apoptosis pathways indicated that GC/5-FU up regulates p53 expression at both protein and mRNA levels. In vivo antitumor assessment showed that the sustained release of GC/5-FU nanoparticles was more capable at targeting hepatic cancer cells than 5-FU monotherapy in the mouse orthotropic liver cancer mouse model. Owing to its excellent active targeting ability, galactosylated chitosan has been widely used as Nano carriers for delivering various therapeutic drugs and genes in cancer treatment.

**5. Chitosan Nanoparticles Modified by Peptide, Protein:** - The bioactive molecules including peptides and proteins are also used to prepare functionalized chitosan NPs with unique functions. In order to improve selective delivery of anti-tumor drugs to tumor sites, Herceptin was conjugated with gemcitabine-loaded CSNPs (HER2-Gem-CSNPs). The targeted NPs displayed noticeable cytotoxicity along with an improved S-phase arrest, important to apoptosis in contrast with free gemcitabine and unconjugated gemcitabine-loaded nanoparticles due to higher cellular binding with eventual uptake and prolonged intracellular retention. Thus, HER2-Gem-CSNPs are able to offer an efficient and targeted release of gemcitabine for pancreatic cancer treatment. Silk fibroin also insoluble protein with low immune or inflammatory response and favorable biological response characteristics. So, silk fibroin-modified CSNPs are very appropriate for chemotherapeutic delivery in cancer treatment because of their better stability, low toxicity, simple and mild preparation methods. **Zhou et al.** developed a kind of up conversion nanoparticle (UCNP-Ppa-RGD) by using a photosensitizer.



## FUNCTIONS OF CHITOSAN NANOPARTICLES

Even though chitosan Nano- formulations are seldom used in cancer treatment up till now, multi-functional CSNPs are showing promise in personalized therapy. The Nano particle may show improved anti-tumor efficacy and specific targeting ability due to modification of chitosan Nano carriers or encapsulation of multiple therapeutic agents.

- **Enhancing Anti-Tumor Efficacy:-** In clinical treatment, many anticancer drugs with traditional formulation are being despised due to certain disadvantages such as poor water solubility, short circulation time in vivo, poor targeting and high side effects. Nano medicine holds a great potential in resolving these problems. Nanoparticles have been considered to be effective carriers because they can stay unrecognized during blood circulation, reduce the adverse reactions and increase the therapeutic efficacy.<sup>3</sup> As one of drug carriers, CSNPs may show enhanced antitumor efficacy in cancer treatment owing to many unique characteristics. Water soluble chitosan nanoparticle's can improve water solubility of hydrophobic drugs. The biodegradability of chitosan matrix may ensure that encapsulated drugs are released in a controlled fashion. The small-sized CSNPs can pass through biological barriers in vivo and deliver drugs to the tumor site owing to the enhanced permeation and retention or active targeting ability. The specific targeting of modified CSNPs can pick up bioavailability of therapeutic agents and decrease systemic toxicity. Therefore, antitumor efficacy can be enhanced by multi-functional CSNPs or multiple drug loading strategy. For example, co-delivery of paclitaxel and surviving sRNA-expressing plasmid (iSur-pDNA) by folate-modified amphiphilic linoleic acid and poly(-malic acid) double grafted CSNPs exhibited enhanced antitumor efficacy and extended endurance period as compared with single delivery of PTX or iSur-pDNA.
- **Improving individual Targeting Tumor:** - According to the targeting model, therapeutic Nano particle are generally separated into passive and active targeting nanoparticles. As nominated above, passive targeting nanoparticles accretion in tumor tissues mainly depends on the enhanced permeation and retention. Active targeting based on ligand-receptor interactions is an important way of increasing specific targeting ability of drug delivery system. It has been intensively studied in various drug delivery systems. The nanoparticles based on galactosylated chitosan (GC) and 5-FU can be more effective at targeting hepatic cancer cells than 5-FU monotherapy in the orthotropic liver cancer mouse model because Assail-glycoprotein receptor (ASGPR) found on membranes of the phagocytes shows specificity for glycoprotein's. Glycyrrhetic acid-prepared sulfated chitosan (GA-SCTS) micelles specific target the liver cancer cells (HepG2 cells) and

exhibit quick and significant ability to target the liver in vivo. An a palmer conjugated hyaluronan /CSNPs were prepared and used as carriers for targeted delivery of 5-FU by Ghasemi. The prepared NPs showed significantly higher targeting ability in (MUC1+human colorectal adenocarcinoma as compared with the free drug.

➤ **Prolonging Blood Circulation Time:** - During the development of effective drug delivery nanoparticles, one major obstacle is the rapid clearance from blood. The ability of drug-loaded nanoparticles to circulate in the bloodstream for a prolonged period of time is often a prerequisite for successful targeted delivery. They should evade the phagocytic uptake by reducing opsonization by blood proteins, hence increasing the bioavailability of the drug. The in vivo fate of nanoparticle is mainly dependent on the chemical and physical properties of the NPs, including size, surface charge and surface chemistry. In order to overcome these obstacles, some shielding groups including PEG, polyvinyl alcohol, and polysaccharides are adsorbed or grafted on the surface of NPs for masking the NPs because these groups or polymers can hinder the hydrophobic and electrostatic interactions that help plasma proteins bind to particles. For the purpose of decorating NPs, PEG seems to be an ideal candidate and has been extensively used to coat the surface of NPs, as it successfully weakens the uptake by cells of mononuclear phagocytic system (MPS) and leads to extended blood circulation time. Some CSNPs also exhibit extended blood circulation time after PEG modification. Showed that PLGA-CS-PEG nanoparticles showed spectacular continuation in blood circulation, as well as reduced macrophage uptake, with only a small amount of the nanoparticles sequestered in the liver. Long blood circulation of drug also has great advantage in the treatment of blood malignices.

➤ **Reversal of Multi-Drug Resistance Multi-drug resistance (MDR):-**is a major barrier for imitating the therapeutic effects of chemotherapeutic agents in cancer treatment. Once a patient suffers from MDR, the therapy efficiency is reduced and leads to the failure of the treatment. P-glycoprotein-mediated resistance is the most extensively studied MDR pathway. It has been confirmed that nanoparticles may be able to reverse the drug resistance because it may avoid recognition by the P-glycoprotein (P-gp) efflux pump by means of being enveloped in an endosome when entering the cell, leading to high intracellular drug concentrations. To overcome MDR, used amphiphilic N-octyl-O-sulfate chitosan (NOSC) copolymer to pre- pare paclitaxel-encapsulated micelles (PTX-M). Owing to the combination of the inhibiting pre- effect of NOSC and the bypassing P-gap action of the intact PTX-M, the micelles had superior blood persistence,

tumor accumulation, and therapeutic efficacy after intravenous injection into the tumor-bearing mice. Besides loading of the one chemotherapeutic agents, CSNPs are also used to deliver genes for the purpose of MDR reversal. used magnetic-Fe<sub>3</sub>O<sub>4</sub> chitosan nanoparticle- encapsulated MDR1 siRNA to investigate the effects of MDR1 gene on the reversal of MDR in the glioblastoma cell line. Their results revealed that the expression of MDR1 at both the mRNA and P-gp protein level decreased, which increased sensitivity to chemotherapy in vitro also confirmed that the thiolated glycol chitosan could deliver Pre-targeted poly-siRNA and form stable nanoparticles. The resultant nanoparticles are not only defend siRNA molecules from enzymatic degradation, but also accumulate in adriamycin-resistant.

- **Crossing Blood-Brain Barrier:-** The biological boundary between the brain and the blood, the blood-brain barrier (BBB) takes liability for transporting necessary nutrients and protecting the brain. The BBB constitutes an efficient organization of tight junctions between endothelial cells in the brain tissue.

The barrier can prevent harmful substances including chemotherapeutic agents entering into the brain interstitial and protect the cells of nervous system However; the existence of BBB brings tremendous negative impact on therapeutic effects of brain tumors when chemotherapeutic drugs are used. Therefore, various nanoparticles are being the optional strategies for overcoming the problem. Especially, it has been demonstrated that cationic nanoparticles showed prominent efficacy in permeating the BBB due to their cationic charge. As a cationic polymer, CSNPs receive much attention in preparation of Nano formulations or Nano probes for the treatment and diagnosis of brain cancers. used chitosan as a linker and stabilizer to develop a targeting Nano probe (NPCP-CTX-Cy5.5) for the purpose of selective accumulation in brain tumors across the BBB. The Nano probe is comprised of an iron oxide nanoparticle coated with a PEGylated chitosan-branched copolymer, a targeting ligand, chlorotoxin (CTX), and a near-IR flu-orphore.

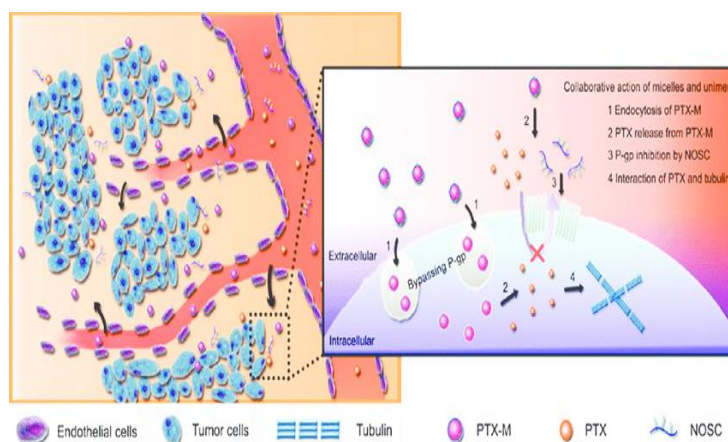


Fig .3 The mechanism of enhanced accumulation of PTX inside tumor cells by PTX-M via a combination mechanism of the inhibiting P-gp effect of NOSC and the bypassing P-gp action of the intact PTX-M.

**6. Diagnosis, Detection and Imaging:-** With the process of Nano medicine and molecular imaging, a variety of tumor-targeting nanoparticles have been designed and extensively studied for cancer theranostics. The functional NPs can chemically interact with biomarkers to alter the signals for imaging and provide biological information on pathological lesions. Therefore, they are expected to achieve early diagnosis and personalized therapy in cancer treatment in the near future. Owing to excellent biocompatibility and available functional groups on chitosan, it is often used to encapsulate or decorate other nanoparticles for preparing various multifunctional nanoparticles. For the reason of disease diagnosis and detection, Yang et al. prepared a high- concert nanoparticle by using alginate to in fact complex with folic acid-modified chitosan for fluorescent endoscopic detection of colorectal cancer. Developed a probe using nanoparticles of miR-155 MB self-assembled with chitosan (CS-miR-155 MB) to image the expression of miR-155 in lung cancer cells. The CSNPs shows the superior fluorescence potency and transfection efficiency, thus can be used for detecting miRNA expression in living cells.

**7. Photodynamic Therapy and Imaging: -** Photodynamic therapy (PDT) is becoming a shows potential and non-invasive process for cancer treatment owing to its unique therapeutic characteristics. Under light irradiation at certain wavelength, photosensitizer (PS) produces cytotoxic singlet oxygen ( $^1O_2$ ) to kill tumor cells through apoptosis or necrosis. Moreover, the selective accumulation of photosensitizers in tumor tissues also can be employed in photodynamic imaging (PDI) because the photosensitizers can provide an intense fluorescence signal. However, the photosensitizers are greatly limited in clinical use owing to their poor water solubility, non-specific skin photo toxicity, inadequate tumor-targeting selectivity, etc. One way for overcoming these shortcomings is the use of Nano carriers to deliver photosensitizers for enhancing their tumor specificity by the so-called EPR effect. Therefore, chitosan is often used as coating polymer to modify photosensitizers because of its nontoxicity, excellent biocompatibility, good water-solubility and availability for further modification of functional groups.

**8. Thermotherapy: -** Because of the thermo sensitivity of tumor cells, thermotherapy has been an important support to other forms of cancer treatments in clinics. Taking the advantage of passive/active targeting ability of nanoparticles, chemotherapeutic agents and functional nanoparticles can be encapsulated simultaneously into CSNPs for combination therapy. As a kind of thermotherapy, magnetic fluid hyperthermia (MFH) is a new approach based on Nano technology to deposit heat power in deep tissues by over- coming limitations of conventional heat treatments. After infiltration into the target tissues with Nano sized magnetic particles, the power of an alternating magnetic field is transformed into heat. Developed by reverse micro emulsion method using the Nanocages as magnetic cores and chitosan as the matrix. In vivo experiments showed that

tumor temperature  $42.6 \pm 0.0^\circ\text{C}$  within 10 min in the alternating. Photodegradation of the photodynamic dye ICG. Meso-porous silica provided the second photo protection for ICG by facilitating the formation of ICG aggregates. Magnetic field after injection; and the temperatures in the right hepatic lobes and the rectum were significantly lower than in the tumor and the constant temperature could last up to 30 min. The CSNPs can specifically target liver cancer tissue by static magnetic field and with the application of alternating magnetic field, effectively raise tumor tissue temperature and facilitate tumor apoptosis. Therefore, the combination of chemotherapy and MFH is likely to be a new safer and efficacious therapy model in cancer treatment. Photo thermal therapy is an attractive technique for treating solid tumors in a minimally invasive manner by using NIR laser light-generated heat to destroy tumor cells, Light-absorbing materials e.g., photo thermal conducting agents, as the source of photo thermal effect, play a key role in photo activated cancer therapy. Among reported NIR absorbents, gold Nano- crystals, including gold Nano rods, silica-cored Nano shells, and gold Nano-cages, were most extensively investigated due to their excellent biocompatibility. Used DOX-conjugated chitosan derivatives to cover gold Nano rods (GNRs) for preparation of functional Nano carriers (DOX-CS-GNR). Because of the combination of chemical and photo thermal effects, the prepared Nano carriers showed good optical properties.

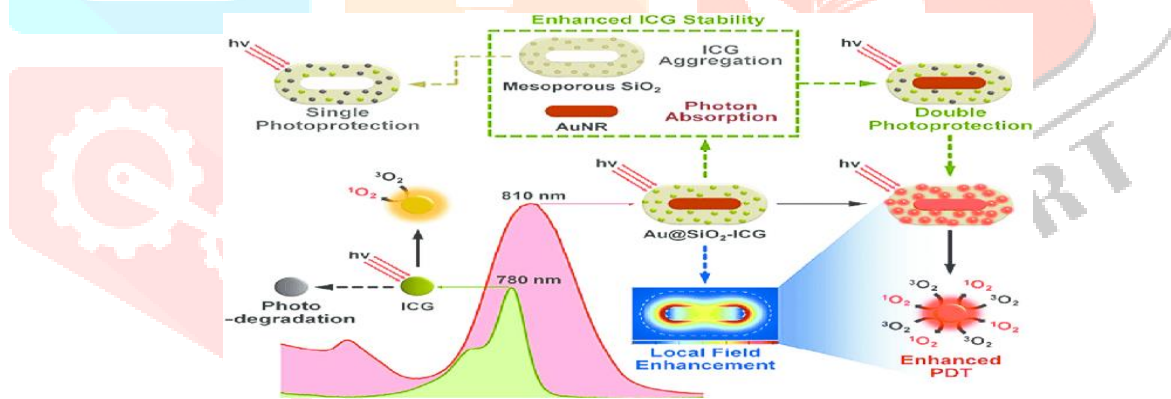


Fig.4-Schematic diagram of enhanced PDT by utilizing the surface plasmonic effect of the AuNR to simultaneously increase the absorption coefficient and reduce photodegradation of the photodynamic dye ICG.

**9. Gene Therapy:-**Gene therapy holds a promising alternative for cancer treatment. However, the major shortcoming of gene therapy is the gene transfection rate. Currently, there are two main types of vectors that are used in gene therapy, viral or non-viral gene delivery systems. Although the viral gene delivery system shows a high transfection yield, it still has many disadvantages, such as onco-genic effects and immunogenicity. Therefore, as the alternate, nonviral gene delivery system gains importance. Especially, numerous synthetic chemicals, natural polymers, or lipids have been used as nonviral vectors for enhancing the efficiency of gene delivery.<sup>111</sup> among them, as a natural nontoxic polysaccharide, chitosan has the

potential for gene delivery applications. Its biodegradability, biocompatibility, and the ability to protect DNA against DNase degradation are added advantages. RNAi-based therapy is a highly specific method for gene silencing which holds a dominant position in cancer gene therapy. However, the delivery efficiency of free short interfering RNA (siRNA) is quite low and most of the free siRNA is rapidly degraded following i.v. injection.

**10. Encapsulation of Metal Compounds:** - Several metals and their compounds find special applications in cancer diagnosis and treatment owing to their unique properties. For example, platinum compounds have significant antitumor efficacy and broad spectrum anti-cancer activity attributable to their remarkable cytotoxicity against tumor cells. Gold nanoparticles are often used in NIR photo thermal therapy due to their tunable surface Plasmon resonance property to convert NIR light into local heat. Magnetic iron oxide nanoparticles are also useful as a magnetic resonance imaging agent and tumor-targeting drug carriers because they have outstanding magnetic response.

**11. Encapsulation of Protein:** - Therapeutic peptides/proteins and protein-based antigens are being extensively utilized for the management and cure of many diseases, particularly oncologic and metabolic diseases. Although they are frequently used in clinic and almost exclusively administered by parenteral injections the therapeutic efficacy and biocompatibility are weakened.

## Conclusion

Chitosan, the natural biodegradable and non-toxic polymer, holds promise as a suitable material for biomedical applications. There are multifaceted applications of chitosan in cancer therapy, including gene delivery, chemotherapeutic delivery, and immunotherapy. Although chitosan-based drug delivery systems and gene delivery vectors are not yet approved by the FDA (Food and Drug Administration), great progress in cancer therapy research is being made. Physico-chemical characteristics, such as its cationic nature, molecular weight, DDA, and pH of transfection medium are major factors that influence the gene delivery efficacy of chitosan nanoparticles. The genetic material, i.e., siRNA or DNA, and cell type also contribute to the efficiency of transfection using chitosan vectors.

However, chitosan's low water solubility is a major limitation for gene and drug delivery applications. To improve the water solubility, new functional groups or addition of neutral polymers like PEG have been commonly employed. PEG addition also has the advantages of prolonged in vivo circulation and reduced bio-clearance of chitosan nanoparticles. Alone, chitosan has difficulty encapsulating hydrophilic drugs; therefore, conjugation strategies are employed to achieve high drug loading. Derivatization of chitosan with hydrophobic molecules or polymers has enhanced the ability of chitosan to encapsulate hydrophobic drugs.

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