ISSN: 0976-4607
Review Article

International Journal of Innovative Pharmaceutical Research

Journal homepage: www.ijipr.com

Role of Chitosan Nanoparticles in Cancer Therapy

Aruna U*[1], Rajalakshmi R[1], Indira Muzib Y[2], Vinesha V[1], Sushma M[1], Vandana KR[1] and Vijay Kumar N[1]

[1]Dept of Pharmaceutics, Sree Vidyanikethan College of Pharmacy,
A. Rangampet, Tirupati - 517102, India.

[2]Institute of Pharmaceutical Technology, Sri Padmavathi Mahila University, Tirupati - 517502, India.

ABSTRACT

Nanotechnology is considered as a promising area to develop targeted drug delivery system using particulate systems as carriers for small and large molecules. Chitosan nanoparticles are good drug carriers because of their good biocompatibility and biodegradability, and can be readily modified. As a new drug delivery system, they have attracted increasing attention for their wide applications in loading protein drugs, gene drugs, and anticancer chemical drugs, and also provide versatile routes of administration including oral, nasal, intravenous, and ocular. The first part of the review is concerned with the cancer treatment with nanoparticles. The subsequent section covers with characteristics of chitosan, methods of targeting the cancer and applications.

Keywords: Nanoparticles, Chitosan, Cancer therapy.

INTRODUCTION

Cancer remains to be one of the leading causes of death worldwide. Over the past several decades' significant advancements have been made in fundamental understanding of cancer biology, which has in turn lead to better diagnostic and treatment methods. A major reason for this is our inability to administer therapeutic agents selectively to the targeted sites without adverse effects on healthy tissue. Current therapeutic strategies for most cancers involve a combination of surgical resection, radiation therapy, and chemotherapy (Erkki Ruoslahti, 2010).

Conventional chemotherapy can wreak havoc on healthy tissue, causing painful side effects, and it's not always effective. At the same time, there is the huge risk that the drugs entering the body could also possibly kill the healthy cells near the affected region. This is mainly because the drugs can not differentiate cancer cells from the others and also have some disadvatages for the delivery of conventional drug (Jiao, 2011).

Disadvantages of conventional drug to treat cancer

- Within the stroma of tumour cells, the interstitial space separates tumour cells and blood vessels. This distance cannot be crossed by the conventional anticancer drugs.
- Because of poor blood supply to tumour cells, the concentration of drug reaching tumour cells is decreased.
- Due to the absence of lymphatic network the development of high pressure gradient in the interstitial space which obstructs the convective flow of drug to tumour cells (Vivek Kumar *et al*., 2010).

In order to overcome this, nanotechnology became an emerging trend in the delivery of drugs to target the tumour cells and avoid harm to the normal cells. Moreover, it can dramatically reduce the side effects.

Nanoparticles are colloidal carriers with dimensions on the nano scale i.e., 1 to 1000 nm (Jun Jie Wang, 2011). They are particularly attractive for cancer treatment due to their small size, varied composition, surface functionalization, and stability which provide unique opportunities to interact and target the tumor microenvironment. These interactions of nanoparticles with the tumor include aiding in small molecule transport to the intracellular organelles to induce the greatest cytotoxic effect.

Biological nanoparticles are mainly developed for drug delivery systems as an alternative to liposomal technology, in order to overcome the problems related to the stability of these vesicles in biological fluids and during the storage. Polymer nanoparticles from biodegradable and biocompatible polymers are good

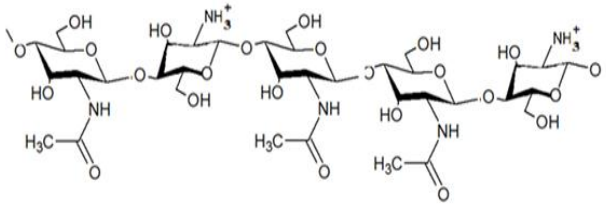
***Corrosponding author**

Aruna U
Email id: arunapharma21@gmail.com

candidates for drug carrier to deliver the drugs, because they are expected to be adsorbed in an intact form in the gastrointestinal tract after oral administration. With the development of material processing technology, nanomaterial is being widely used. Chitosan, the deacetylated derivative of chitin, is one of the abundant, renewable, nontoxic and biodegradable carbohydrate polymers, and available largely in the exoskeletons of shellfish and insects. (Shi XY and Fan XG, 2002; Jin MX and Hu QH, 2008). Chitosan has been widely applied as a functional biopolymer in food and pharmaceutics. Chitosan is known to have various biological activities including immune enhancing effects, antitumoral, antifungal, and antimicrobial activities. The unique characteristics of chitosan nanoparticles could provide a higher affinity for negatively charged biological membranes and site-specific targeting *in vivo*. Chitosan nanoparticles could elicit dose-dependent inhibitory effects on the proliferation of various tumour cell lines, while low toxicity against normal human liver cells.

Many drugs have problems of poor stability, water insolubility, low selectivity, high toxicity, side effects and so the drug carriers play a significant role in resolving these problems. Chitosan nanoparticles are the drug carriers with wide development potential and have the advantage of slow/controlled drug release, which improves drug solubility and stability, enhances efficacy, and reduces toxicity. Because of their small size, they are capable of passing through biological barriers in vivo (such as the blood–brain barrier) and delivering drugs to the lesion site to enhance efficacy. Modified nanoparticles also have other properties such as improved drug targeting. Under the action of enzymes in vivo, biodegradable nanoparticles can produce water and carbon dioxide without adverse effects, and have thus become the focus of increasing research.

Fig.1 Structure of Chitosan



Biological properties of chitosan

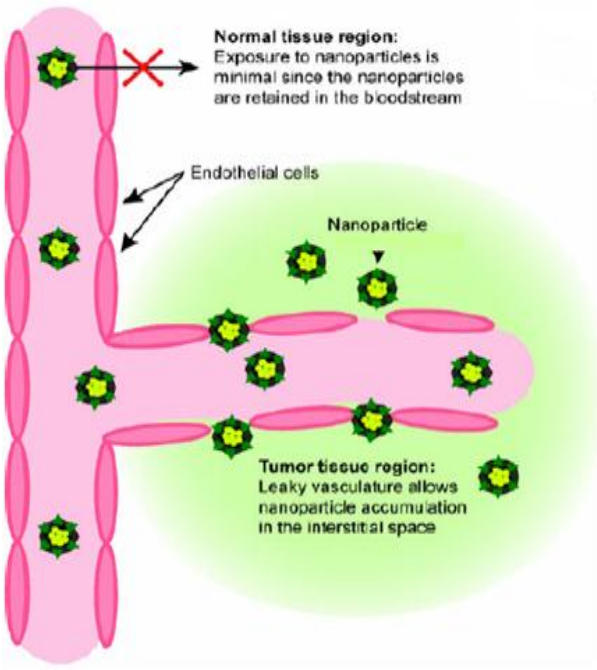
Bio adhesiveness

The amino and carboxyl groups in the chitosan molecule can be combined with glycoprotein in mucus to form a hydrogen bond, leading to an adhesive effect. As mucoprotein in mucus is positively charged, chitosan and mucus are attracted to each other to prolong the retention time of drugs and continuous drug release in vivo as well as improve drug bioavailability (Dudhani AR and Kosaraju SL, 2010).

Biodegradability and safety of chitosan

The biodegradability of chitosan is important for its use in drug delivery systems. Chitosan of suitable molecular weight can be cleared by the kidney *in vivo*, while that of excessive molecular weight can be degraded into fragments suitable for renal clearance (Kean T, Thanou M, 2010).

Fig.2 Anti tumour activity of chitosan nanoparticles



Chitosan can act on tumor cells directly to interfere with cell metabolism, inhibit cell growth, or induce cell apoptosis. It also has an antitumor role through improving the body's immune function (Cao J, Zhou NJ, 2005). It was showed that low-molecular-weight chitosan and chito-oligosaccharide could inhibit tumor growth in S180-bearing mice (Maeda Y, Kimura Y, 2004). It was found that a diet containing chitosan could reduce the generation of precancerous lesions in colon cancer induced by azomethane compounds (Torzsas T *et al*., 1996). In vitro antitumor testing of chitosan nanoparticles indicated that inhibition rate of 500 mg/L chitosan nanoparticles was 27% on Hela cells of cervical cancer, 23% on liver SMMC-7721 cells, 29% on gastric cancer BGC-823 cells, and as high as 55% on breast cancer MCF-7 cell (Zhou SH, 2007). These studies suggested that chitosan had antitumor effects in vitro and in vivo, leading to good prospects for their application as a supplementary antitumor drug and drug carrier. Studies have also indicated significant differences in antitumor activity of nanoparticles prepared by chitosan from different producers, and chitosan nanoparticles also had a selectivity for tumour cells (Fang GJ, 2007).

Targeting of chitosan nanoparticles

Positive charges of chitosan have selective adsorption and neutralizing effects on the tumor cell surface. As a drug carrier, it has a targeting function to liver, spleen, lung, and colon (Park K *et al*., 2007). Doxorubicin–chitosan polymeric micelles had excellent drug-loading properties, were suitable for targeting the liver and spleen, and significantly reduced drug toxicity to the heart and kidney (Xu XY, 2008).

Metabolism of chitosan nanoparticles

Nanoparticles are recognized as foreign matter in vivo and are absorbed by antibodies generated in the human body. Plasma protein, lipoprotein, immune protein, and complement C protein in plasma are also adsorbed on nanoparticles, accelerating the reorganization of the reticuloendothelial system. Nanoparticles are engulfed by macrophage and cleared from the body's circulation (Mei ZN, 2002). The bridge between nanoparticles and macrophage is formed because of plasma protein adsorbed on the nanoparticle surface. The ability of nanoparticles to adsorb plasma protein is determined by surface charge of nanoparticles, thereby influencing the transfer intensity of nanoparticles by macrophage (Redhead H *et al*., 2001). Nanoparticles with polarity and high surface potential as well as amphipathic or hydrophilic nanoparticles are engulfed less and have a longer circulating time *in vivo* (Nam *et al*., 2009).

Cancer-targeted drug delivery using chitosan and its derivatives

The critical bottleneck of conventional cancer chemotherapeutics includes high toxicity of most anticancer drugs, due to indiscriminate distribution of drugs towards disease and healthy cells. In addition, anticancer drugs often suffer from poor solubility in water and thus need to use organic solvents or detergents for clinical applications, resulting in undesirable side effects such as venous irritation and respiratory distress. Therefore, designing a distinct carrier system that encapsulates a large quantity of drugs and specifically targeting tumor cells is indispensable for successful cancer therapy.

Passive targeting

The origin of the EPR (enhanced permeability and retention) concept dates back to the late 1970s, when Maeda et al. discovered the selective accumulation of macromolecular drugs in tumor tissues. The specific passive accumulation of macromolecules was attributed to defective tumor vasculature with disorganized endothelium at the tumor site and a poor lymphatic drainage system. Since then, researchers have capitalized this concept for the delivery of various drugs by conjugating them with polymers or encapsulating within nanoparticles. Nowadays, it was evident that the long circulating macromolecules (polymer–drug conjugates) and nano-sized particulates (such as micelles and liposomes) accumulate passively at the tumors due to the EPR effect (R Duncan, 2003).

Chitosan–drug conjugates

The polymer–drug conjugates are composed of a water-soluble polymer that is chemically conjugated to a drug via a biodegradable spacer. The spacer is usually stable in the bloodstream but cleaved at the target site by hydrolysis or enzymatic degradation. Such drug conjugates can be selectively accumulated at the tumor site by the EPR effects, followed by release of the drug by cleavage of the spacer. Based on this concept, several polymer–drug conjugates have recently entered into phase I/II clinical trials. The representative example is N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-based drug conjugates such as HPMA copolymer – doxorubicin conjugate (PK1) and HPMA copolymer–doxorubicin conjugate containing galactosamine as a targeting moiety (PK2), developed for the treatment of primary or secondary liver cancer (L.W. Seymour, 1991). In recent years, chitosan–anticancer drug conjugates have also been investigated. Low molecular weight chitosan conjugated with paclitaxel (LMWC-PTX) was also synthesized by chemical conjugation of LMWC and PTX through a succinate linker, which can be cleaved at physiological conditions (E. Lee, 2008). This conjugate was evaluated as a carrier for the oral delivery of paclitaxel. LMWC (MW<10 kDa) exhibited more favourable characteristics than high molecular weight chitosan, such as lower toxicity and higher water solubility. Moreover, LMWC could quickly and reversibly open the tight junctions between human epithelial colorectal adenocarcinoma cells (Caco-2). This is a highly useful characteristic for a carrier of drug molecules, especially for oral delivery. LMWC-PTX was absorbed in the small intestine after oral administration and remained in its intact conjugate form until it reached the bloodstream. An advantage of LMWC-PTX for oral delivery of PTX is that LMWC-PTX has the ability to bypass the Pgp mediated barrier (efflux pump) in the gastrointestinal tract and CYP450-dependent metabolism in the intestine and liver. N-succinyl chitosan derivatives were conjugated with mitomycin C (MMC) using carbodiimide chemistry. Owing to the hydrophilicity of N-succinylchitosan, the conjugate is water-soluble when the MMC content in the conjugate is less than 12%. The N-succinylchitosan conjugates exhibited good antitumor activities against various tumours such as murine leukaemias (L1210 and P388), B16 melanoma, Sarcoma 180 solid tumor, a murine liver metastatic tumor (M5076), and a murine hepatic cell carcinoma (MH134) (Y Kato, 2004). Chitosan and its derivatives can be covalently cross-linked to prepare nano-sized particles as the drug carriers (M. Prabaharan, 2005). The cross-linking process involves formation of the covalent bonds between the chitosan chains and functional cross-linking agents. The representative chemical cross-linkers that have been widely used for chitosan include bi-functional agents such as PEG dicarboxylic acid, glutaraldehyde, or monofunctional agents such as epichlorohydrin (M Goldberg, 2007; M Bodnar, 2005).

Chitosan-based polyelectrolyte complex (PEC) nanoparticles

PECs, prepared by electrostatic interactions between oppositely charged polyions, have received considerable attention as carrier systems for drug and gene delivery (W Sun, 2008). The complex formation and the physical properties of PECs are influenced by many factors such as degree of ionization of the chitosan and anionic counterparts, chain flexibility, charge distribution over the polymer chain, pH, temperature, time of interaction, ionic strength, and concentration of

Applications of chitosan nanoparticles

S. No	Drug	Method of preparation	Polymers used	Applications	References
1	Amphotericin B	Dialysis	Polylactic acid-grafted chitosan	Ocular	Wenjun Zhou, 2013
2	Flutamide	Poly Electrolyte complex	Chitosan and dextran sulphate	Prostate cancer	A. Anitha, 2013
3	5-Fluorouracil	Emulsion droplet coalescence	Chitosan & eudragit S100	Colon cancer	Anto shering, 2011
4	Gemcitabine	Ionotropic gelation	Chitosan	Haemotological malagnancies	Katayoun Derakhshandeha, 2011
5	Doxorubicin		Thiolated chitosan	Breast cancer	Fatemeh Talaei, 2011
6	Silver		Chitosan	Lung cancer	Sanda C, 2011
7	Didanosine		Chitosan	Brain cancer	Al-Ghananeem, 2010
8	Curcumin		Chitosan and pluronic 127	Skin cancer	Thi Minh Phuc Le, 2013
9	Lomustine		Chitosan	Lung cancer	Archana mehrotra, 2011
10	5-aminolaevulinic acid		Succinate modified chitosan	oral cancer	Shu-Jyuan Yang, 2013
11	Paclitaxel	Polymerization	Thiolated Chitosan-coated PMMA	Colon cancer	SeyedehParinaz Akhlaghi, 2010
12	Camphothecin	Micro precipitation	N-trimethyl chitosan	Melanoma	Xian-Ping, 2010
13	oxaliplatin	Solvent evaporation	Chitosan & eudragit S100	Colorectal cancer	Anekant Jain, 2010
14	Carboplatin	Poly electrolyte complex	Chitosan-alginate	Retinoblastoma	Parveen S, 2010
15	5-aminolaevulinic	Ionotropic gelation	Chitosan	Colorectal cancer	Qin Tiana, 2009
16	Cisplatin	Self assembly	Glycol chitosan	Liver cancer	Li FR, 2008
17	Methotrexate	Complexation process	Chitosan	Breast and neck cancer	Daniele Rubert Nogueira, 2004

the polymeric solutions (A Drogoz, 2007). The preparation of PEC nanoparticles is quite simple and can be easily performed under mild conditions without the use of toxic organic reagents. It has been demonstrated that chitosan can form PEC nanoparticles with various polyanions such as hyaluronic acid, chondroitin sulfates, alginate, carboxymethyl cellulose, carrageenan, heparin, and poly (acrylic acid) (A Denuziere, 1998).

Self-assembled chitosan nanoparticles

Polymeric amphiphiles can form self-assembled nanoparticles (SNPs) in an aqueous environment via hydrophobic interactions between the hydrophobic parts, primarily to minimize interfacial free energy. Since chitosan is a hydrophilic and cationic polysaccharide, chitosan-based SNPs can be readily obtained by chemically attaching the hydrophobic moiety to the backbone of chitosan and its derivatives. Enhanced accumulation at the tumor site can be achieved by conjugating the targeting moiety to the SNPs. By varying the degree of substitution of the hydrophobic moiety, it is easy to control the particle size and zeta potentials of the nanoparticles which are important parameters affecting biodistribution of nanoparticles in vivo. As described earlier, chitosan-based SNPs can encapsulate a quantity of hydrophobic drugs inside the nanoparticles. Studies using chitosan nanoparticles have been carried out for various anticancer drugs (JH Kim, 2006).

PEGylated chitosan nanoparticles

Engineering the surface of the chitosan nanoparticles with PEG has attracted increasing attention because of its great potential in the therapeutic applications. There are numerous publications that reviewed the importance and advantages of PEGylated nanoparticles for biological and pharmaceutical applications. PEGylation of chitosan nanoparticles can increase their physical stability and prolong their circulation time in blood by reducing the removal by the reticuloendothelial system (Z Zhao, 2009).

Active targeting — receptor-mediatedendocytosis (RME)

The accumulation of drugs in tumour tissue does not always guarantee successful therapy if the drug does not reach the target site of the tumor cell such as the cell membrane, cytosol, or nucleus. Therefore, a more effective mechanism should be employed such that the therapeutic agents are able to reach their molecular targets. Cancer cells often over-express some specific antigens or receptors on their surfaces, which can be utilized as targets in modern nanomedicine. Active targeting can be achieved by chemical alteration of

nanosized drug carriers with targeting components that precisely recognize and specifically interact with receptors on the targeted tissue (X Yang, 2008).

Physical targeting

Increasing efforts have been made to exploit physiological signals such as pH, temperature, ionic strength, and metabolites for targeted drug delivery applications (IF Tannock, 1989; T Yahara, 2003; HC Hurst, 2001). Of the various stimuli, pH and temperature have been widely investigated for the treatment of solid tumors. Numerous reports have demonstrated that neoplastic tissues could exhibit a lower pH value (acidosis) or a higher temperature (hyperthermia) than healthy tissue. Therefore, drug targeting to solid tumors can be achieved by designing stimuli sensitive drug carriers, which disintegrate and release the entrapped drugs in response to a lower pH or higher temperature specifically at the tumour site. The interstitial pH of the tumour plays a prominent role in cancer therapy. In a healthy human, the extracellular pH of the body tissue and blood is maintained around 7.4. In contrast, the tumor tissue exhibits substantially lower pH values varying from 5.7 to 7.8, depending on the tumor histology and volume (M Dellian, 1996). The decrease in extracellular pH values in the tumour tissue is primarily due to poor organization of the vasculature in the tumor, resulting in low blood pressure, local hypoxia, and accumulation of acidic metabolites. This difference in pH between tumors and normal tissue has stimulated many investigators to design novel pH-sensitive carriers (VA Sethuraman, 2008; ES Lee, 2008). In case of paclitaxel, whose primary site of action is the microtubule, its intracellular concentration is critical for its pharmacological effect. Therefore, efficient intracellular delivery of such drugs is essential to eradicate cancer cells. Recently, N-acetyl histidine conjugated glycol chitosan (NAcHis–GC), where histidine (with imidazole group, pKa value of 6.5) acts as pH-responsive fusogen, was developed for the efficient intracytoplasmic delivery of paclitaxel (JS Park, 2006).

CONCLUSION

Chitosan is a multifunctional biopolymer with many interesting applications. The present review concluded from the above discussion that the chitosan with its versatile characteristics like adhesiveness, bio degradable nature and anti-tumour activity acts as a best carrier for the cancer therapy when compared to other natural polymers available.

REFERENCES

Al-Ghananeem AM, Saeed H, Florence R, Yokel RA, Malkawi AH. Intranasal drug delivery of didanosine-loaded chitosan nanoparticles for brain targeting; an attractive route against infections caused by AIDS viruses. *J Drug Target*. 2010;18(5):381-8.

Anekant Jain, Sanjay K Jain, N Ganesh, Jaya Barve, Aadil M Beg. Design and development of ligand-appended polysaccharidic nanoparticles for the delivery of oxaliplatin in colorectal cancer. *J.nanotechonology*. 2010;6(1):179-190.

Anitha, Saji Uthaman, Shantikumar Nair, Vinoth Kumar, Lakshmanan. Enhanced delivery system of flutamide loaded chitosan-dextran sulphate nanoparticles for prostate cancer. *Journal of Biomedical Nanotechnology*. 2013;9(3):335-40.

Anto Shering M, Kannan C, Sabari Kumar K, Sathish Kumar V, Suganeshwari M Formulation of 5-fluorouracil Loaded Chitosan Nanoparticles By Emulsion Droplet Coalescence Method For Cancer Therapy. *International Journal of Pharmaceutical & Biological Archives*. 2011;2(3):926-931.

Archana mehrotra, Ramesh Chand nagarwal, Jayanta Kumar. Lomustine Loaded Chitosan Nanoparticles: Characterization and in-Vitro Cytotoxicity on Human Lung Cancer Cell Line L132. *Chem. Pharm. Bull*. 2011;59(3):315-320.

Bodnar, Hartmann, Borbely. Preparation and characterization of chitosan-based nanoparticles. *Biomacromolecules*. 2005;6:2521–2527.

Daniele Rubert Nogueira, Lorena Tavano, Montserrat Mitjans, Lourdes Pérez, M. Rosa Infante. In vitro antitumor activity of methotrexate via ph-sensitive chitosan nanoparticles. *Eur J Pharm Biopharm*. 2004;57:123-131.

Dellian, Helmlinger, Yuan, Jain. Fluorescence ratio imaging of interstitial ph in solid tumours: effect of glucose on spatial and temporal gradients. *Br. J. Cancer*. 1996;74:1206-1215.

Denuziere, Ferrier, Damour, Domard. Chitosan–chondroitin sulfate and chitosan– hyaluronate polyelectrolyte complexes: biological properties. *Biomaterials*. 1998;19:1275-1285.

Drogoz, David, Rochas, Domard, Delair. Polyelectrolyte complexes from polysaccharides: formation and stoichiometry monitoring. *Langmuir*. 2007;23:10950-10958.

Dudhani AR, Kosaraju SL. Bioadhesive chitosan nanoparticles: preparation and characterization. *Carbohydr Polym*. 2010;81(2):243-251.

Duncan R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov*. 2003;2:347-360.

Erkki Ruoslahti, Sangeeta N Bhatia, Michael J Sailor. Targeting of drugs and nanoparticles to tumors. *Journal of cell biology*. 2010;188(6):759.

Fang GJ, Hong Y, Jiang YY. Comparison of antitumor effects of chitosan nanoparticles from different sources in vitro. *J Clin Rehab Tiss Engin Res*. 2007;11(48):9696–9699.

Fatemeh Talaei, Ebrahim Azizi, Rassoul Dinarvand. Thiolated chitosan nanoparticles as a delivery system for anti sense therapy: Evaluation against EGFR in T47D breast cancer cells. *Journal of controlled release*. 2011;3(2).

Fazil M, Md S, Haque S, Kumar M, Baboota S, Sahni JK, Ali J. Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting. *Eur J Pharm Sci*. 2012;47(1):6-15.

Goldberg, Langer Jia. Nanostructured materials for applications in drug delivery and tissue engineering. *J. Biomater. Sci. Polym. Ed*. 2007;18:241-268.

Hurst. Update on HER-2 as a target for cancer therapy: the ERBB2 promoter and its exploitation for cancer treatment. *Breast Cancer Res*. 2001;3:395-398.

Jiao PF, Zhou HY, Chen LX, Yan B. Cancer-targeting multifunctionalized gold nanoparticles in imaging and therapy. *Curr Med Chem*. 2011;18(14):2086-102.

Jin MX, Hu QH. Characterization and application in bioadhesive drug delivery system of chitosan. *Centr South Pharm*. 2008;6(003):324-327.

Jun Jie Wang, Zhao Wu Zeng, Ren Zhong xiaotian Xie, Guang Lin Zhou, Xiao Ri Zhan, Shu Ling Wang. Recent advances of chitosan nanoparticles as drug carriers. *Int J Nanomedicine*. 2011;6:765-774.

Katayoun Derakhshandeha, Sahar Fathi. Role of chitosan nanoparticles in the oral absorption of Gemcitabine. *International Journal of Pharmaceutics*. 2012;437:172-177.

Kato Y, Onishi H, Machida Y. N-succinylchitosan as a drug carrier: waterinsoluble and watersoluble conjugates. *Biomaterials*. 2004;25:907-915.

Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. *Adv Drug Deliv Rev*. 2010;62(1):3-11.

Kim Park, Kim Choi, Chung, Jeong, Park, IS Kim, Kwon. Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel. *J. Control Release*. 2006;111:228-234.

KS Snima, R Jayakumar, AG Unnikrishnan, Shantikumar, V Nair, Vinoth Kumar, Lakshmanan. O-Carboxymethyl chitosan nanoparticles for metformin delivery to Pancreatic cancer cells. *Carbohydrate Polymers*. 2012;6(3).

Lee, Gao, Bae. Recent progress in tumor ph targeting nanotechnology. *J. Control Release*. 2008;132:164-170.

Li FR, Yan WH, Guo YH, Qi H, Zhou HX. Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing miceself assembly. *Journal of Controlled Release*. 2008;127(1):41-49.

Lili Shi, Cui Tang, Chunhua Yin. Glycyrrhetinic acid-modified chitosan/poly (ethylene glycol) nanoparticles for liver-targeted delivery. *Biomaterials*. 2012;33(30):7594-7604.

Liu, Sun, Cao, Zhang, Yao, Lu, Luk. An investigation on the physicochemical properties of chitosan/DNA polyelectrolyte complexes. *Biomaterials*. 2005;26:2705-2711.

Maeda Y, Kimura Y. Antitumor effects of various low-molecular-weight chitosans are due to increased natural killer activity of intestinal intraepithelial lymphocytes in sarcoma 180-bearing mice. *J Nutr*. 2004;134(4):945-950.

Mei ZN, Yang XL, Xu HB. Biodegradable polymer long-circulating nanoparticle. *Chin J Hosp Pharm*. 2002;22(7):433-435.

Nam HY, Kwon SM, Chung H, et al. Cellular uptake mechanism and intracellular fate of hydrophobically modified glycol chitosan nanoparticles. *J Control Release*. 2009;135(3):259-267.

Park K, Kim J, Nam Y, et al. Effect of polymer molecular weight on the tumor targeting characteristics of self-assembled glycol chitosan nanoparticles. *J Control Release*. 2007;122(3):305-314.

Parveen S, Mitra M, Krishnakumar S, Sahoo SK. Enhanced antiproliferative activity of carboplatin-loaded chitosan-alginate nanoparticles in a retinoblastoma cell line. *Acta Biomater*. 2010;6(8):3120-31.

Prabaharan, Mano. Chitosan-based particles as controlled drug delivery systems. *Drug Deliv*. 2005;12:41-57.

Qin Tiana, Chuang-Nian Zhanga. Cancer cell detection by 5-aminolaevulinic acid-loaded chitosan nano-particles. *Cancer Letters*. 2009; 273:210-220.

Redhead H, Davis S, Illum L. Drug delivery in poly (lactide-coglycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: in vitro characterisation and in vivo evaluation. *J Control Release*. 2001;70(3):353-363.

Sanda C Boca, Monica Potara, et al. Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible highly effective photothermal transducers for in vitro cancer. *Cell therapy*. 2011;311(2).

Sethuraman, Lee, Bae. A biodegradable ph-sensitive micelle system for targeting acidic solid tumors. *Pharm. Res*. 2008;25:657-666.

SeyedehParinaz Akhlaghi, Shahrooz Saremi, SeyedNasser Ostad, Rassoul Dinarvand, Fatemeh Atyabi. Discriminated effects of thiolated chitosan-coated pMMA paclitaxel-loaded nanoparticles on different normal and cancer cell lines. *Nanotechnology*. 2010;6(5):689-697.

Seymour LW, Ulbrich K, Wedge SR, Hume IC, Strohalm J, Duncan R. N-(2- hydroxypropyl)methacrylamide copolymers targeted to the hepatocyte galactosereceptor: pharmacokinetics in DBA2 mice. *Br. J. Cancer*.1991;63:859-866.

Shi XY, Fan XG. Advances in nanoparticle system for deliverying drugs across the biological barriers. *J China Pharm Univ*. 2002;33(3):169-172.

Shu-Jyuan Yang, Cha-Fu Lin, Min-Liang Kuo, Ching-Ting Tan. Photodynamic Detection of Oral Cancers with High-Performance Chitosan-Based Nanoparticles. *Biomacromolecules*. 2013;14(9):3183-3191.

Sun, Mao, Mei, Kissel. Self-assembled polyelectrolyte nanocomplexes between chitosan derivatives and enoxaparin. *Eur. J. Pharm. Biopharm*. 2008;69:417-425.

Tannock, Rotin. Acid pH in tumors and its potential for therapeutic exploitation. *Cancer Res*. 1989;49:4373-4384.

Thi Minh Phuc Le, Van Phuc Pham, Thi Minh Lua Dang, et al. Preparation of curcumin-loaded pluronic F127/chitosan nanoparticles for cancer therapy. *Adv. Nat. Sci: Nanosci. Nanotechnol*. 2013.

Torzsas T, Kendall C, Sugano M, Iwamoto Y, Rao A. The influence of high and low molecular weight chitosan on colonic cell proliferation and aberrant crypt foci development in CF1 mice. *Food Chem Toxicol*. 1996;34(1):73-77.

Vivek Kumar Gupta, et al. Nanoparticle Formulation for Hydrophilic & Hydrophobic Drugs. *Int. J. Res. Pharm. Sci*. 2010;1(2):163-169.

Wenjun Zhou, Yuanyuan Wang. Self aggregated nanoparticles based on amphiphilic polylactic acid- grafted chitosan copolymer for occular delivery of amphotericin B. 2013;8(1):3715-3728.

Xu XY, Zhou JP, Li L. Preparation of doxorubicin-loaded chitosan polymeric micelle and study on its tissue biodistribution in mice. *Acta Pharm Sin*. 2008;43(7):743-748.

Yahara, Koga, Yoshida, Nakagawa, Deguchi, Shirouzu. Relationship between microvessel density and thermographic hot areas in breast cancer. *Surg. Today*. 2003;33:243-248.

Yang, Zhang, Wang, Chen, Zhang, Gao, Liu. Self-aggregated nanoparticles from methoxy poly(ethylene glycol)-modi.ed chitosan: synthesis; characterization; aggregation and methotrexate release in vitro, Colloids Surf. B. *Biointerfaces*. 2008;61:125-131.

Zhao, He, Yin, Bao, Shi, Wang ,Tang, Yin. Biodegradable nanoparticles based on linoleic acid and poly(beta-malic acid) double grafted chitosan derivatives as carriers of anticancer drugs. *Biomacromolecules*. 2009;10:565-572.

Zhou SH, Hong Y, Fang GJ. Preparation, characterization and anticancer effect of chitosan nanoparticles. *J Clin Rehab Tiss Engin Res*. 2007;11(48):9688-9691.