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Review Article

Chitosan-Based Hydrogel Nanoparticles for Cancer Therapy

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ABSTRACT
Cancer, an uncontrollable growth of cells, is among the leading causes of mortality and morbidity throughout the world. Malignant neoplasms are difficult to treat diseases because of their single in kind characteristics such as tissue invasion, metastasis, evading reticuloendothelial system (RES) and so forth. In recent decade polymeric nanoparticulate systems has gained special attention in drug delivery and targeting among all biocompatible nanoforms. Among these systems, chitosan-based hydrogel nanoparticles have been wildly utilized for drug delivery purposes. The usage of chitosan nanogels in cancer therapy significantly improved in recent years. The various cancers were the target of chitosan nanogels. Also, modification of other delivery systems with chitosan were much reported. The aim of this study is the review and update of the recent studies on chitosan nanogels applications in cancer therapy by focus on cancer based classification.

Keywords: Chitosan, Nanogels, Cancer, Chemotherapy.

INTRODUCTION

Cancer, an uncontrollable growth of cells, is among the leading causes of mortality and morbidity throughout the world. Malignant neoplasms are difficult to treat diseases because of their single in kind characteristics such as tissue invasion, metastasis, evading reticuloendothelial system (RES) and so forth[1]. Angiogenesis is a two edge blade like hallmark which along with giving special abilities to neoplasmic cells, can be used as a therapeutic agent delivery pathway[2]. Chemotherapy with special drugs is the most common treatment has been used to deal with neoplasms which has obtained remarkable successes in inhibiting proliferation of tumor cells and eliminating of cancerous ones[3]. But there are many obstacles against this form of treatment such as multi drug resistant cancer cells, non-specificity of drugs and the following adverse side effects on the non-target cells and healthy tissues (e.g. anorexia, hair loss or severe ones like renal failure, cardiac arrest or even death[4, 5]) low biodegradability and low biocompatibility of drugs and insolubility of hydrophobic drugs in hydrophilic environment of the body which limit their extended usage[6].

Polymeric nanoparticulate systems in drug delivery

In recent decade polymeric nanoparticulate systems such as micells, nanoparticles, dendrimers as a subcategory of pharmaceutical nanomaterials has gained special attention in drug delivery and targeting among all biocompatible nanoforms. These nanosized particles can easily escape from the reticuloendothelial system (RES) because of their small size (with a size range from 10 to 1000 nm in diameter-their size ranged from 10 to 1000nm). They are used as carriers to transfer drugs specifically to cancer sites with the lowest interaction with normal cells[7-12].

Hydrogel nanoparticles

Hydrogel-based nanoparticles (nanogels) as a member of the nanoparticles family can contain high water content (sometimes over 90% wt.) because of their high functional groups (e.g. –OH , -CONH, -CONH2 and SO3H) (13). This feature presents high compatibility to these colloidal systems[10,11,14-16].

Chitosan/chitin-based hydrogel nanoparticles

Chitin is a natural polymer exploited from some crustaceans. When the degree of chitin's alkaline deacetylation reaches to about 50%, it will turn to chitosan (α (1-4)-2-amino-2-deoxy β-D-glucan). Drug and gene delivery, mucosal vaccination and tissue engineering are some noticeable application of chitosan[17-19]. There are published some review articles about chitosan nanogels in the past years. The aim of this study is the review and update of the recent studies on chitosan nanogels applications in cancer therapy by focus on cancer based classification.

Chitosan/chitin-based hydrogel nanoparticles for breast Cancer therapy

Breast cancer, a heterogeneous disease is the first cause of cancer related death among women population leading to 23% of all cancer mortalities according to WHO (World Health Organization) reports in 2012. One third of women in Asia are in danger of this disease in their lifespan[20]. Breast cancer treatment is (mainly) based on markers like estrogen and progesterone receptor and human epidermal receptor 2 (HER2). The less receptors exist on the breast cancer cells surface, the more complexities will counter face the therapy procedure[21,22].

An ionically cross-linked chitosan nanoparticle (CH-NP), a translucent and flexible acetylpolyamidoamine (Ac-PAMAM)–thiolated chitosan (TCS) hydrogel nanoparticle films and a glycol chitosan nanoparticles with hydrotropic

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2-(4-(vinylbenzyloxy)-N,N-diethylnicotinamide) (VBODENA-COOH) oligomers (HO-CNPs) and a glycol chitosan-5β-cholanic acid (HGC) conjugate nanoparticles are some nanogels prepared to deliver docetaxel (DTX), letrozole (LTZ) and paclitaxel (PTX) and camptothecin (CPT) to breast cancerous cells, respectively[23-25]. The DTX-CH-NP had encapsulation efficiency ranged from 78-92% and DTX release profile was from 77.46 ± 1.17% to 73.79 ± 0.79% for different concentrations of DTX which reduced MDA-MB-231 cancer cells viability to 85%. Ac-PAMAM–TCS films could have 31% w/wspherical LTZ particles loading efficiency. 86.3%±4.0% and 89.2%±3.1% in vitro drug release were indicated after 5h when the hydrogel had 15% and 20% drug, respectively. PTX-HO-CNPs exhibited about 96.8% drug loading efficiency at PTX (10 wt.%)-HO-CNP which was more than that of Abraxane® and a considerable stability in aqueous media. The average tumor weight reduction of PTX-HO-CNPs was approximately 10.7 and 2.8 fold more than that of saline and Abraxane®, respectively. Furthermore PTX-HO-CNPs cell cytotoxicity was less than that of PTX-loaded Cremophor EL/ethanol and was excreted thorough kidneys.

Min et al have designed self-aggregate CPT-HGC nanoparticles by presenting 5β-cholanic acid to hydrophilic glycol chitosan with drug loading efficiency more than 80% which had a burst release less than 50%of drug during first 9h followed by prolonged release of CPT for up to 1 week. The CPT-HGC nanoparticles showed metronomic effect resulted in a sustained release for an extended time along with an enhanced localized targeting caused by the preservation of CPT active lacton ring from hydrolysis. The research group demonstrated 77.2% tumor volume reduction compared to control group after i.v. injection of 30 mg CPT/kg inside CPT-HGC nanoparticles towards MDA-MB231 breast cancer cells ($P<0.001$) while empty HGC nanoparticles revealed any noticeable cytotoxic effect on the mentioned cells. Another glycol chitosan-5β-cholanic acid (HGC) conjugate nanoparticle was designed to deliver Paclitaxel to MCF-7 breast cancer cells. After 20 days, measured tumor volume of PTX-HGC (50 mg/kg) treated reduced to about 15% of saline received samples with the less increase of body weight[26,27].

Chitosan/chitin-based hydrogel nanoparticles for lung cancer therapy

Lung cancer as the first cause of cancer death worldwide with about 1.6 million sacrifices (victims) in 2012 is comprised of two main histologic subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC)[28].

In a study, a self-assembled and pH-responsive nanogel was composed of glycol chitosan (GCS) as hydrophilic shell and 3-diethylaminopropyl (DEAP) groups as hydrophilic core (at physiological pH). The biodegradable and biocompatible GCS-g-DEAP nanogels had an average size of about 102 nm. Nanogel dissociation occurred in tumor extracellular pH as a result of DEAP protonation and the following reduction of core hydrophobicity. This process leaded to further drug (DOX) release at acidic conditions and also more DOX uptake by A549 cells proved by fluorescence images[29].

The effects of paclitaxel incorporated in a photocrosslinkable chitosan hydrogel having azide and lactose moieties (Az-CH-LA) on lewis lung cancer (3LL) are evaluated. Paclitaxel had similar release profile to its vehicle (Cremophor® EL and ethanol), specifically a fast release rate (about 35-45% in PBS) during the first day and a sustained release for the next 6 days. Researchers suggested that the half-releasing time of paclitaxel increased for 5h when the amount of Azide (p-azidebenzoic acid) get doubled.

The difference of inhibitory effect of paclitaxel between 3LL, HUVEC, HMVEC and fibroblast appeared to reflect inhibitions of tumor growth and angiogenesis without damage to surrounding connective tissues in vivo. The inhibition effect on tumor growth by the paclitaxel-incorporated Az–CH–LA hydrogel lasted a 14 days period, and subsequently the tumor in almost all mice grew again. Significant necrotic tumor tissue was induced in the paclitaxel-incorporated Az–CH–LA hydrogel treated tumor after 4 and 8 days, while the number of CD34 positive stained vessels were significantly less in the paclitaxel-incorporated Az–CH–LA hydrogel-treated tumors. In fact, paclitaxel-incorporated Az–CH– LA hydrogel significantly reduced the number of CD34 positive vessels when compared with other treatments, suggesting that the paclitaxel-incorporated Az–CH–LA hydrogel significantly inhibited angiogenesis in tumors

In a study[30], chitosan-based luminescent/magnetic hybrid nanoparticles made of chitosan, superparamagnetic iron oxide and CdTe quantum dots (QDs) with ratios of 1/32/12 (chitosan/QD/MNP, wt/wt/wt) were conjugated with tetrapeptides (GFFG and LGPV) and folate orderly (CLMNPs-tetrapeptide-FA) with the mean size of 150 to 190 nm to specifically target CPT to A549 cancer cells and enhanced uptake by them through folate-receptor-mediated endocytosis mechanism. Besides ligand targeting, CLMNPs-tetrapeptide-FA copolymers could also be targeted to tumor tissues and even cells in vivo by an external stimulant based on magnetic targeting. Their mean photoluminescence (PL) intensity per microscopic field was about 50 folds higher than that of control group. CLMNPs-GFFG-FA and CLMNPs-LGPV-FA had CPT loading efficiencies of 8.6 and 1.1 wt %, correspondingly. CPT-loaded CLMNPs-GFFG-FA and CPT-loaded CLMNPs-LGPV-FA had 55% and 69% release rate, respectively at pH 5.3 after 28h while the corresponding value at pH 7.4 was 46% for CPT-loaded CLMNPs-GFFG-FA and 57% for CPT-loaded CLMNPs-LGPV-FA. These differences in the amount of drug release at different pH, exhibit the drug delivery systems pH responsibility. They also showed an initial fast release (15% of CPT was released from CPT-loaded CLMNPs-GFFG-FA and 25% from CPT-loaded CLMNPs-LGPV-FA within 1 h). Since CPT-loaded CLMNPs-GFFG-FA drug loading efficiency was more than that of CPT-loaded CLMNPs-LGPV-FA, it had remarkably more absolute release quantity.

CPT-loaded CLMNPs-GFFG-FA and CPT-loaded CLMNPs-LGPV-FA at concentration of 1000 µg/mL caused only 1.66 and 1.75% of hemolysis, respectively. Prothrombin time (PT) which indicates extrinsic

coagulation pathway and activated-partial-thromboplastin time (APTT) exhibiting intrinsic one were at normal ranges of 0.8-1.2 and 25.4-38.4 s at different concentrations of copolymers CPT-loaded CLMNPs-GFFG-FA/CLMNPs-LGPV-FA up to 1000 μg/mL which shows that CPT-loaded copolymers did not influence on coagulation pathways. The results show their good blood biocompatibility.

CPT-loaded copolymers exhibited more cytotoxicity in lung cancer A549 cell lines compared to normal human hepatocytes L02 cell lines in vitro because of their active targeting by folate moieties. They also showed good cell compatibility (75% of L02 cells viability up to the concentration of 500 μg/mL)

CPT-loaded CLMNPs-GFFG-FA had more anticancer activity against A549 cells (about 50% cell viability reduction at the concentration of 200 μg/mL) compared to CPT-loaded CLMNPs-LGPV-FA (30% cell viability reduction at the same concentration) due to their more absolute release quantity.

Two glycol chitosan nanoparticles modified by hydrophobic cholanic acid moieties (HGC-NPs) were designed for targeted delivery of cisplatin (CDDP) and DTX to A549 lung cancer cells with the range size of 300-500 nm. The average tumor volume in DTX-HGC nanoparticles treated mice was about half of those treated with free DTX(30 mg/kg) after 35 days and approximately 20% compared to control group. Free glycol chitosan revealed any noticeable toxicity toward cancerous cells. CDDP-HGC nanoparticles with about 80% drug loading efficiency were tested on Squamous cell carcinoma (SCC7) cells beside A549. The growth ratio of SCC7 tumor volume among mice injected with CDDP10 wt%-HGC was about 75% slower than saline treated group. Notably, cell viabilities of SCC7 decreased 35.6% and 24.8% by CDDP 10 wt%-HGC and free CDDP at 10 μg/ml After 72 h incubation, respectively. The decreased toxicity of CDDP loaded in HGC-NPs compare to free drug could be because of late release of drug from nanoparticle[31,32].

Chitin-poly(caprolactone) composite nanogels (chitin-PCLCNGs) was designed for delivery of Dox to A549, too, that reached attractive results. When Dox entrapped into free chitin-PCLCNGs (70±20 nm) with efficiency about 80%, the average size of nanogel measured around 240±20 nm. In the environment with pH lower than chitin nanogel pKa (6.1), enhancement in the swelling ratio was observed. When the concentration of Dox reach 120μg/mL in chitin-PCLCNGs, only about one fifth of cells were viable without any risk of hemolysis[33].

Chitosan/chitin-based hydrogel nanoparticles for glioblastoma cancer therapy

Chitosan nanogels were conjugated with folic acid and then anionic gold nanoparticles (AuCOOH) were encapsulated in nanogels for dual loading of miR-218 mimics and Temozolomide. Temozolomide release behavior in BPS9 and cytoplasm of U87MG cells were approximately identical. Mimics AuCOOH_miR-218 mimics@FACS_Temozolomide had 80% more accumulation on tumor site compared to AuCOOH_miR-218 suggesting high potential of FA for specific targeting. The 50% inhibitory concentration (IC50) values of Temozolomide carried with the biocompatible nanogel on U87MG cells was 6.166 μM but its cytotoxicity remarkably enhanced by co-treatment of miR-218 mimics (2fold reduction of cancer cells viability). Tumor weight of the group treated with AuCOOH_miR-218 mimics@FACS_Temozolomide decreased by 1/40. It also had no serious effect on normal cells after 4 weeks after administration[34].

Chitosan/chitin-based hydrogel nanoparticles for prostate cancer therapy

Wang et al have designed a near infrared (NIR) triggered release system based on chitosan-modified graphene nanogel (CGN). CGN was made from chitosan-modified chemically reduced graphene oxide (CRGO), N-isopropylacrylamide and PEG diacrylate and DOX was then loaded. (Dry CGN's loading capacity was 48 wt%). Fluorescence images exhibited that DOX was gathered in nucleus of irradiated cells while it was found in the cytoplasm of non-irradiated cells suggesting the thermo-sensitive profile of DOX-CGN. DOX-CGN did not have a comparable cytotoxicity in TRAMP-C1 cells to free drug at 37 ℃, while it had a significant cytotoxicity ($p > 0.05$) at 42 ℃ with the 50% inhibitory concentration (IC50) values of less than 1 μM. DOX-CGN effect on LLC1 cells was also evaluated which cancer cells viability were 51.31 % when DOX-CGN was irradiated with NIR[35].

Chitosan/chitin-based hydrogel nanoparticles for bladder cancer therapy

In a study it was synthesized a magnetic thermosensitive hydrogel nanoparticle composed of chitosan (CS), b-glycerophosphate (GP) and Fe3O4 magnetic nanoparticle (Fe3O4-MNP) for intravesical and sustained delivery of Bacillus Calmettee-Guérin (BCG) to superficial bladder cancer cells. The results of frozen section examination showed that the gel revealed porous microstructures with different chamber diameters and distributions. Researchers demonstrated a considerable difference between the average tumor volume per rat for a treatment group with 1 mg/0.1 ml free drug (group 2) and a treatment group with 1 mg BCG plus 0.1 ml Fe3O4eBCGeCS/GP composition (group 4) (1.82 _ 0.48mm3 versus 0.53 _ 0.27 mm3, $P < 0.05$). A significant increase in urinary cytokines (interleukin-2 (IL-2) and interferon γ (IFN-γ)) for both group 2 and group 4 was observed.(area under the curve (AUC) values of IL-2 and IFN-γ for group 4 were 149.3 _ 8.06 pg/ml, $P < 0.01$ and 373.47 _ 40.53 pg/ml, $P < 0.05$, respectively)[36].

Chitosan/chitin-based hydrogel nanoparticles for hepatoma cancer therapy

A novel thermosensitive nanogel was made to treat hepatic cancer in an experiment done in 2013. Researchers prepared a chitosan-based thermosensitive hydrogel with the interaction between N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride and glycerophosphate which contained doxorubicin-loaded liposomes (DOX-LP/HTCC-GP). They indicated that the drug EE and formation of gel would not change if the liposomes disperse in hydrogel. The doxorubicin entrapment efficacy was more than 90% and its release

was about 22% in 9 days. The antitumor activity of DOX-LP/HTCC-GP was tested in H22-bearing mice suggesting that the MST was 35 days and one-fifth mice survived for more than 60 days[37].

In another study, galactosylated chitosan-graft-poly (N-isopropylacrylamide) (Gal-CS-g-PNIPAm) polymers were used as the base of galactose-functionalized pH-sensitive nanogels. Gal-CS-g-PNIPAm nanogels were then loaded with oridonin (ORI), the liver anti-cancer drug used in traditional medicine of Chinese. XRD measurement revealed high crystallinity of Gal-CS-g-PNIPAm. Researchers chose GC-1, GC-2, and GC-3 polymers with 7.26, 11.95, and 14.06% of levels of galactose substitution to investigate their features.

ORI-loaded nanogels had more cytotoxicity against HepG2 cells than that of non-galactosylated drug-loaded nanogels as a result of enhanced uptake of the drug into cancer cells due to targeting of asialoglycoprotein receptors upregulated on HepG2 cells surface.

Drug-loaded GC-1, GC-2 and GC-3 nanogels (GCN-1, GCN-2 and GCN-3) had ORI encapsulation efficiencies of 73.71, 55.05, and 69.76% and drug loading of 5.11, 3.56 and 4.38%, respectively. All three spherical and monodispersed drug-loaded nanogels with positive surface charges had mean particle size of 100 nm, approximately.

GCN-3 release rate within 2 h at pH 7.4, 6 and 5 was 66%, 81%, and 84%, respectively suggesting their pH responsibility. ORI-free nanogels did not show any cytotoxicity against HepG2 and MCF-7 cells up to the concentration of 5 mg/mL.

ORI-loaded nanogels showed more in vitro anticancer activity against HepG2 cells compared with drug-loaded nanogels without galactosylation (ORI-loaded CS-NG) and free ORI because of their enhanced uptake (The higher cytotoxicity might result from the enhanced internalization of nanoparticles via endocytosis or phagocytosis and the increased uptake of the nanogels via the receptor-mediated mechanism).

IC50 values of GCN-3, GCN-2 and GCN-1 in HepG2 cells at pH 6.5 9.09, 7.88, and 7.19 μg/mL which GCN-3 with the most level of galactose substitution had the highest antitumor activity. Their therapeutic efficacy increased as their environment pH decreased as a result of their pH dependent release profile.

ORI-entrapped Gal-CS-NG nanogels did not show a pH responsive cytotoxicity in MCF-7 cells.

Although they had lower IC50 values in MCF-7 cells in comparison with ORI-loaded CS-NG at pH 7.4, their antitumor activity decreased at lower pH and their values of IC50 was more than that of ORI-loaded CS-NG at pH 6.5[38].

In another study, pH responsive and spherical shaped chitin-poly(l-lactic acid) composite nanogels with the mean size of 90 ± 20 nm and zeta potential of +48mV were prepared by regeneration technique to specifically deliver DOX to HepG2 (human liver cancer) cell lines. DOX was both adsorbed on the surface of the nanogels and entrapped within them (DOX entrapment efficiency was 86%). Drug entrapment efficiency had a positive correlation with incubation time and drug amount.

DOX-loaded nanogels (DOX-chitin-PLA CNGs) had more swelling at acidic pH ratio compared to neutral pH leaded to more drug release. More detailed DOX-chitin-PLA CNGs had about 20% more drug release at pH of 5.3 than that of pH of 7.4 (81.24 versus 61.73%)

Their drug release profile was consist of two parts. At the first part a quick-linear drug release was occurred during first hours which was relevant to those DOX adsorbed on the nanogels surface. At the second section, sustained release was observed form the entrapped drugs.

DOX-chitin-PLA CNGs had 16 and 25% weight loss in the absence and presence of lysozyme, respectively during 5 days. After 30 days about three forth (73%) of them were degraded with lysozyme and 65% without it. Researchers demonstrated DOX-chitin-PLA CNGs blood compatibility by hemolysis and coagulation assays. Less than 5% of the samples were hemolyzed

Cell viability of HepG2 cells treated with DOX-chitin-PLA CNGs at the concentration of 48 μg/mL[39].

Cytotoxicity of pravastatin loaded chitosan nanoparticles (PRV/CSNPs) with mean size of 129.8 ± 10.5 - 270.4 ± 23.3 nm and entrapment efficiency range of 49.05-72.04% were appraised toward HepG2 liver cancer cells. Nanoparticles were prepared via ionic gelation method. Initial fast release prolonged for 6h and then sustained release in the range of 52─92% was observed after 48h. After incubation period 72h, tumor growth inhibition (TGI) was found to about 51% by drug loaded CSNPs compared to 38% for free PRV and 33.8% for plain CSNPs[40].

Wang et. al. targeted human SMMC-7721 hepatocellular carcinoma cells by thermo-responsive PTX loaded Chitosan–Poly(N-Isopropylacrylamide-co-Acrylamide) nanogel, in vitro. As the designed nanogels showed some specific characteristics like more than 70% inhibition rate of SMMC-7721 cells proliferation by PTX nanogel compare to about 50% for PTX solution, acceptable compatibility of blank nanogels with experimented cells and also low hemolysis rate, they can be good carriers for cancer drug delivery. In addition to liver carcinoma PTX loaded chitosan nanoparticles tried against human HT-29 colon carcinoma cells, in vivo. No apparent body weight reduction of the tumor bearing mice beside of fixed tumor volume, even slight reduction ($p<0.01$), compare to become 3 fold in control group were important results[41].

Chitin/Chitosan-based hydrogel nanoparticles for skin cancer therapy

Malignancy of melanocytes with high risk of treatment-refractory was known as the most mortal type of skin cancer. After introducing of Curcumin (CUR) loaded chitin nanogels (CCNGs) to normal and cancerous skin cells, transdermal flux of CUR enhanced 4-fold in comparison to control curcumin solution. MTT assay showed high specific toxicity of CCNGs against A375 human melanoma cell lines such that the percentage of viability decreased to about 30% at the concentration of 1mg/ml and also high viability (>70%) of human dermal fibroblast cells (HDF) in response to CCNGs at the same concentration[42].

Chitosan/chitin-based hydrogel nanoparticles for colorectal cancer therapy

A photosensitizer drug meso-tetra (N- methyl-4-pyridyl) Porphine tetra tosylate (TMP) encapsulated in chitosan/alginate nanoparticles was fabricated in a study. TMP-Loaded Nanoparticles was conjugated with antibodies to actively target death targeting receptor 5 (DR5) existing on HCT116 cells to increase nanoparticles antitumor activity and their uptake and enhance TMP-loaded nanoparticles photocytotoxicity. Alginate-based hydrogel increased the likelihood of drug entrapment and facilitate effective drug loading by strong interacting with cationic TMP (negative enthalpy change (ΔH) and positive entropy change (ΔS)). Researchers also showed that changes in concentration of chitosan had a positive correlation with drug loading amount, particle size and PI value. EC50 of HCT116 cells treated with drug in dark situation was about 10 fold more than this value in light treating condition. (181 versus 17 μg/mL) indicating the drug activating role of light. TMP-loaded chitosan/alginate nanoparticles with 15 μg/mL concentration of TMP, 16 h prior exposure to red light (100 J/cm2) and 12 h incubation decreased HCT116 cells viability to less than 20%[43].

Chitosan/chitin-based hydrogel nanoparticles for cervical cancer therapy

In a study thermo sensitive poly(N-isopropylacrylamide) (PNIPAAm) was used to encapsulate/entrap+ amphiphilic chitosan coated with photo-thermal sensitive single-wall carbon nanotubes (CS/PNIPAAm@CNT) to form a spherical and multi responsive drug delivery system with mean diameter of 300 nm and narrow size distribution for delivery of DOX to HeLa cells with drug loading capacity and efficiency of about 43 wt% and 90%, respectively. NIR exposure, acidic pH (5 vs. 7.4) and higher temperature (40℃ vs. 25℃) all triggered release of the loaded drug from the biocompatible nanomaterials.

CNT could convert NIR light to heat resulted in the shrinkage of the PNIPAAm and boosted DOX release. DOX-loaded CS/PNIPAAm@CNT nanoparticles showed only 10% drug release at pH 7.4 and 25℃ through 72h while the corresponding value reached to 40% at pH 5.0 and 40 ℃. Furthermore, 30 s of NIR exposure could enhance the release rates.

DOX-loaded CS/PNIPAAm@CNT nanoparticles exhibited higher IC50 value compared to free DOX (~50 vs. 13 μg/ml) while after 10 min NIR irradiation they IC50 value dropped to 6 μg/ml.

DOX-loaded CS/PNIPAAm@CNT nanoparticles significantly inhibited the growth of Hela cells by 65% approximately after 10 min NIR irradiation while the cell growth inhibition was only about 20% without NIR exposure.

DOX-loaded CS/PNIPAAm@CNT nanoparticles irradiated by NIR exhibited more uptake by/cellular internalization into cytoplasm and nuclei of HeLa cells compared to free DOX after 24 h[44].

Three formulations of chitosan nanoparticles (CS-NPs) were used to deliver doxorubicin to cellules of cervical HeLa tumor, including PEGylated, poloxamer-modified and non-modified CS-NPs. Dox encapsulation efficiency of NPs with size range of 170–211 nm and PDI values <0.24. The release of DOX from both CS-NPs and PEG-CS-NPs was more than 97% in pH of 6.6 and 5.4 mediums after about 4-6h and also both of them had similar amount release in physiological pH (around 75% at 24h) that illustrated ineffectiveness of PEGylation of this system on inhibiting drug release at tumor pH condition. Polox-DOX-CS-NPs show more accelerated drug release so that release amount of DOX at pH 7.4, 6.6 and 5.4 reached 100% after 8h, 5h and 3h, respectively. Moreover, stability of doxorubicin under UVA radiation improved after lyophilization of NPs, for example lyophilized PEG-DOX-CS-NPs showed 15-fold greater half-life compare to non-lyophilized ones (t1/2, 62.5h vs. 4.17h). Cell viability reduction of all formulations at concentration of 0.2 micro g/ml were constantly around 60% like free Dox, in vitro, whereas more than 85% of cells were viable after treating with unloaded CS-NPs even at the concentration of 200 micro g/ml[45].

CONCLUSION

Chitosan-based hydrogel nanoparticles have been wildly utilized for drug delivery purposes. Cancer chemotherapeutic agents were considerably the subject of novel drug delivery systems because of the wide challenges in management of the malignancies as the hard to threatening pathophysiologic conditions. Biocompatibility of chitosan as one of the well-known its properties made it suitable for cancer drug targeting. Beside the biocompatibility, high water absorptivity, flexibility and versatility are another good features of chitosan as a natural polymer used for the targeting purposes. The usage of chitosan nanogels in cancer therapy significantly improved in recent years. The various cancers were the target of chitosan nanogels. Also, modification of other delivery systems with chitosan were much reported. Passage the anticancer agents from biological barriers such as blood brain barrier due to the alteration of the intracellular trafficking pattern reported too[14, 46]. It means that the chitosan nanoparticles have played a more special role in cancer therapy during these years.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;144(5):646-74.
2. Walsh DA. Pathophysiological Mechanisms of Angiogenesis. Advances in Clinical Chemistry2007. p. 187-221.
3. Swift LP, Rephaeli A, Nudelman A, Phillips DR, Cutts SM. Doxorubicin-DNA adducts induce a non-topoisomerase II-mediated form of cell death. Cancer Research. 2006;66(9):4863-71.
4. Tanday S. Targeted biopsy boosts detection of high-risk prostate cancer. The Lancet Oncology. 2015;16(3):e107.
5. Xu R, Wang Q. Toward creation of a cancer drug toxicity knowledge base: Automatically extracting cancer drug-side effect relationships from the literature. Journal of the American Medical Informatics Association. 2014;21(1):90-6.

6. Wu Q, Yang Z, Nie Y, Shi Y, Fan D. Multi-drug resistance in cancer chemotherapeutics: Mechanisms and lab approaches. Cancer Letters. 2014;347(2):159-66.
7. Ashrafi H, Amini M, Mohammadi-Samani S, Ghasemi Y, Azadi A, Tabandeh MR, et al. Nanostructure l-asparaginase-fatty acid bioconjugate: Synthesis, preformulation study and biological assessment. International Journal of Biological Macromolecules. 2013;62:180-7.
8. Ashrafi H, Azadi A. Chitosan-based hydrogel nanoparticle amazing behaviors during transmission electron microscopy. International Journal of Biological Macromolecules. 2016;84:31-4.
9. Azadi S, Ashrafi H, Azadi A. Mathematical modeling of drug release from swellable polymeric nanoparticles. Journal of Applied Pharmaceutical Science. 2017;7(4):125-33.
10. Hamidi M, Ashrafi H, Azadi A. Surface Functionalized Hydrogel Nanoparticles. Biomedical Materials and Diagnostic Devices: John Wiley and Sons; 2012. p. 191-213.
11. Hamidi M, Azadi A, Ashrafi H, Rafiei P, Mohamadi-Samani S. Taguchi orthogonal array design for the optimization of hydrogel nanoparticles for the intravenous delivery of small-molecule drugs. Journal of Applied Polymer Science. 2012;126(5).
12. Hamidi M, Azadi A, Rafiei P, Ashrafi H. A Pharmacokinetic Overview of Nanotechnology-Based Drug Delivery Systems: An ADME-Oriented Approach. Critical Reviews in Therapeutic Drug Carrier Systems. 2013;30(5):435-67.
13. Peppas NA, Khare AR. Preparation, structure and diffusional behavior of hydrogels in controlled release. Advanced Drug Delivery Reviews. 1993;11(1-2):1-35.
14. Azadi A, Rouini MR, Hamidi M. Neuropharmacokinetic evaluation of methotrexate-loaded chitosan nanogels. International Journal of Biological Macromolecules. 2015;79:326-35.
15. Hamidi M, Azadi A, Mohamadi-Samani S, Rafiei P, Ashrafi H. Valproate-Loaded hydrogel nanoparticles: Preparation and characterization. Journal of Applied Polymer Science. 2012;124(6):4686-93.
16. Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. Advanced Drug Delivery Reviews. 2008;60(15):1638-49.
17. Kato Y, Onishi H, Machida Y. Application of chitin and chitosan derivatives in the pharmaceutical field. Current Pharmaceutical Biotechnology. 2003;4(5):303-9.
18. Van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. Chitosan for mucosal vaccination. Advanced Drug Delivery Reviews. 2001;52(2):139-44.
19. Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science (Oxford). 2006;31(7):603-32.
20. Donepudi MS, Kondapalli K, Amos SJ, Venkanteshan P. Breast cancer statistics and markers. Journal of Cancer Research and Therapeutics. 2014;10(3):506-11.
21. Tobin NP, Harrell JC, Lövrot J, Egyhazi Brage S, Stolt MF, Carlsson L, et al. Molecular subtype and tumor characteristics of breast cancer metastases as assessed by gene expression significantly influence patient post-relapse survival. Annals of Oncology. 2015;26(1):81-8.
22. Tsai CH, Chiu JH, Yang CW, Wang JY, Tsai YF, Tseng LM, et al. Molecular characteristics of recurrent triple-negative breast cancer. Molecular Medicine Reports. 2015;12(5):7326-34.
23. Jain A, Thakur K, Sharma G, Kush P, Jain UK. Fabrication, characterization and cytotoxicity studies of ionically cross-linked docetaxel loaded chitosan nanoparticles. Carbohydrate Polymers. 2016;137:65-74.
24. Saboktakin MR, Tabatabaie RM, Maharramov A, Ramazanov MA. Synthesis and in vitro studies of biodegradable thiolated chitosan hydrogels for breast cancer therapy. International Journal of Biological Macromolecules. 2011;48(5):747-52.
25. Koo H, Min KH, Lee SC, Park JH, Park K, Jeong SY, et al. Enhanced drug-loading and therapeutic efficacy of hydrotropic oligomer-conjugated glycol chitosan nanoparticles for tumor-targeted paclitaxel delivery. Journal of Controlled Release. 2013;172(3):823-31.
26. Min KH, Park K, Kim Y-S, Bae SM, Lee S, Jo HG, et al. Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy. Journal of Controlled Release. 2008;127(3):208-18.
27. Kim J-H, Kim Y-S, Kim S, Park JH, Kim K, Choi K, et al. Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel. Journal of controlled release. 2006;111(1):228-34.
28. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 2015;136(5):E359-E86.
29. Oh NM, Oh KT, Baik HJ, Lee BR, Lee AH, Youn YS, et al. A self-organized 3-diethylaminopropyl-bearing glycol chitosan nanogel for tumor acidic pH targeting: in vitro evaluation. Colloids Surf B Biointerfaces. 2010;78(1):120-6.
30. Shen JM, Guan XM, Liu XY, Lan JF, Cheng T, Zhang HX. Luminescent/magnetic hybrid nanoparticles with folate-conjugated peptide composites for tumor-targeted drug delivery. Bioconjug Chem. 2012;23(5):1010-21.
31. Kim J-H, Kim Y-S, Park K, Lee S, Nam HY, Min KH, et al. Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. Journal of Controlled Release. 2008;127(1):41-9.
32. Hwang H-Y, Kim I-S, Kwon IC, Kim Y-H. Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. Journal of Controlled Release. 2008;128(1):23-31.
33. Arunraj T, Rejinold NS, Kumar NA, Jayakumar R. Doxorubicin-chitin-poly (caprolactone) composite

nanogel for drug delivery. International journal of biological macromolecules. 2013;62:35-43.
34. Fan L, Yang Q, Tan J, Qiao Y, Wang Q, He J, et al. Dual loading miR-218 mimics and Temozolomide using AuCOOH@FA-CS drug delivery system: Promising targeted anti-tumor drug delivery system with sequential release functions. Journal of Experimental and Clinical Cancer Research. 2015;34(1).
35. Wang C, Mallela J, Garapati US, Ravi S, Chinnasamy V, Girard Y, et al. A chitosan-modified graphene nanogel for noninvasive controlled drug release. Nanomedicine: Nanotechnology, Biology, and Medicine. 2013;9(7):903-11.
36. Zhang D, Sun P, Li P, Xue A, Zhang X, Zhang H, et al. A magnetic chitosan hydrogel for sustained and prolonged delivery of Bacillus Calmette-Guerin in the treatment of bladder cancer. Biomaterials. 2013;34(38):10258-66.
37. Wang W, Zhang P, Shan W, Gao J, Liang W. A novel chitosan-based thermosensitive hydrogel containing doxorubicin liposomes for topical cancer therapy. J Biomater Sci Polym Ed. 2013;24(14):1649-59.
38. Duan C, Gao J, Zhang D, Jia L, Liu Y, Zheng D, et al. Galactose-decorated pH-responsive nanogels for hepatoma-targeted delivery of oridonin. Biomacromolecules. 2011;12(12):4335-43.
39. Arunraj TR, Sanoj Rejinold N, Ashwin Kumar N, Jayakumar R. Bio-responsive chitin-poly(L-lactic acid) composite nanogels for liver cancer. Colloids Surf B Biointerfaces. 2014;113:394-402.
40. Badran MM, Harisa GI, AlQahtani SA, Alanazi FK, Zoheir KM. Pravastatin-loaded chitosan nanoparticles: Formulation, characterization and cytotoxicity studies. Journal of Drug Delivery Science and Technology. 2016;32:1-9.
41. Wang Y, Xu H, Wang J, Ge L, Zhu J. Development of a Thermally Responsive Nanogel Based on Chitosan–Poly (N- Isopropylacrylamide- co- Acrylamide) for Paclitaxel Delivery. Journal of pharmaceutical sciences. 2014;103(7):2012-21.
42. Mangalathillam S, Rejinold NS, Nair A, Lakshmanan V-K, Nair SV, Jayakumar R. Curcumin loaded chitin nanogels for skin cancer treatment via the transdermal route. Nanoscale. 2012;4(1):239-50.
43. Abdelghany SM, Schmid D, Deacon J, Jaworski J, Fay F, McLaughlin KM, et al. Enhanced antitumor activity of the photosensitizer meso-Tetra(N-methyl-4-pyridyl) porphine tetra tosylate through encapsulation in antibody-targeted chitosan/alginate nanoparticles. Biomacromolecules. 2013;14(2):302-10.
44. Qin Y, Chen J, Bi Y, Xu X, Zhou H, Gao J, et al. Near-infrared light remote-controlled intracellular anti-cancer drug delivery using thermo/pH sensitive nanovehicle. Acta Biomaterialia. 2015;17:201-9.
45. Scheeren LE, Nogueira DR, Macedo LB, Vinardell MP, Mitjans M, Infante MR, et al. PEGylated and poloxamer-modified chitosan nanoparticles incorporating a lysine-based surfactant for pH-triggered doxorubicin release. Colloids and Surfaces B: Biointerfaces. 2016;138:117-27.
46. Azadi A, Hamidi M, Khoshayand MR, Amini M, Rouini MR. Preparation and optimization of surface-treated methotrexate-loaded nanogels intended for brain delivery. Carbohydrate Polymers. 2012;90(1):462-71.