Adv Polym Sci (2021) 288: 271–292
https://doi.org/10.1007/12_2021_96
© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
Published online: 6 May 2021

Chitosan-Based Theranostics for Cancer Therapy

A. S. Soubhagya and M. Prabaharan

Contents

Abstract Chitosan, a natural-based cationic polysaccharide, has a great potential to be utilized as drug delivery systems, tissue engineering scaffolds, and wound dressings due to its biocompatibility, bioactivity, biodegradability, antibacterial property, gelling behavior, cell adhesion, and proliferation abilities. Due to the presence of primary amino and hydroxyl groups, chitosan can be chemically modified or functionalized with other bioactive molecules easily to improve its physicochemical and biological properties required for advanced biomedical applications. In this context, considerable efforts have been made to conjugate chitosan and its derivatives with different types of photosensitizers/photothermal agents, quantum dots (QDs), bioactive molecules, metals, and metal oxides to develop theranostic agents for concurrent imaging and treatment of tumors. The chitosan-based theranostics were found to have better cellular imaging capability, tumor-targeted drug release, and multimodal therapeutic efficiencies. This chapter reviews the recent progress of chitosan-based theranostics, their properties, and applications in advanced cancer therapy.

A. S. Soubhagya and M. Prabaharan (✉)
Department of Chemistry, Hindustan Institute of Technology and Science, Chennai, India

Keywords Bioactive molecules · Cancer therapy · Chitosan · Quantum dots · Theranostic agents

1 Introduction

Cancer is a most divergent and lethal disorder that remains the second-leading cause of mortality worldwide [1]. The most commonly used cancer treatment methods are surgery, radiation therapy, and chemotherapy. These methods can be utilized individually or in a combined manner based on the type, stage, and location of cancer present in the body and the age, general health, and other factors of the patient. Although these methods have experienced many advancements in recent years, still their practical applications are not completely satisfied to suppress the cancer cells more effectively with minimum risk factors. Most of these treatment methods are non-specific to deliver the drug in the tumor site and hence show cytotoxicity to the normal cells and poor drug biodistribution in the diseased area. Moreover, these approaches are generally not sufficient to overcome biological barriers, to treat metastatic disease, and for monitoring, treating, and imaging cancer simultaneously [2].

In recent years, nanotechnology has been regarded as a promising tactic to deal with cancer and has been broadly exploited to expand the traditional cancer treatment methods [3, 4]. Nanotechnology plays a vital role in developing the theranostics for diagnosis, early detection, monitoring, and therapy of cancer [5]. These theranostics have therapeutic, diagnostic, and imaging properties. Moreover, they can carry an additional payload of drugs. Because of the large surface area to volume ratio, theranostic nanoparticles (NPs) can comprise various targeting ligands that provide specificity and high affinity for target cells. Due to the smaller size (<200 nm), theranostic NPs can preferentially accumulate in the cancer cells due to the enhanced permeation and retention (EPR) effect and may exhibit long blood circulation effects by avoiding recognition by the reticuloendothelial system (RES). Therefore, theranostic NPs with adequate drug loading and releasing ability would considerably enhance the efficacy of cancer therapy and potentially overcome the drawbacks related to conventional treatment methods.

So far, different types of NPs based on polymeric materials, ceramics, carbon nanotubes, and inorganic materials have been established as theranostics for cancer diagnosis and therapy [6]. Using surface modification, these NPs have been conjugated with a hydrophobic anticancer drug to improve its bioavailability. Moreover, they have been coated with hydrophilic and biocompatible polymers to improve their circulation in the bloodstream for a longer period and avoid recognition by the RES [7]. The theranostics NPs with a suitable size and surface modification can pass through the blood–brain barrier (BBB) and deliver therapeutic concentration of drugs in the brain for the treatment of central nervous system cancers [8]. Moreover, these NPs may have the benefits of overcoming multidrug resistance [9].

Chitosan is a cationic biopolymer, which is derived from chitin by the alkaline deacetylation process. In recent years, chitosan has been extensively considered as a promising biomaterial for the development of advanced theranostics for cancer therapy due to its desired characteristics such as biocompatibility, biodegradability, gel-forming ability, the capability to conjugate imaging agents, biomarkers, ligands, and therapeutic agents. The chitosan-based NPs with imaging, targeting, and therapeutic efficiencies have been proved to be a potential material for cancer theranostics. In this chapter, the recent improvements of chitosan-based theranostics such as glycol chitosan loaded with photosensitizers/photothermal agents, chitosan loaded with QDs, chitosan-noble metal conjugates, chitosan-based magnetic NPs, chitosan-based hybrid NPs, and chitosan-multimodal nanocomposites in cancer therapy have been reviewed in detail.

2 Theranostics Based on Chitosan and Its Derivatives

An ideal theranostic material must have a diagnosis, imaging, and therapeutic abilities instantaneously. A variety of multifunctional NPs containing therapeutic molecules, imaging agents, and targeting ligand have been developed for the early detection and inhibition of tumors. Due to the occurrence of targeting molecule, such NPs can reach the target-site more specifically and release the drug in a controlled manner and thereby avoid the unwanted side effects. The imaging agent loaded with NPs can be utilized to image the tumor cells and thereby monitor the progress of the treatment. In recent years, the NPs based on polymers, noble metals, metal oxides, and ceramics have been largely considered as theranostics for the simultaneous imaging and therapy of various diseases [10, 11]. Among the polymers, chitosan-based materials have been shown the potential to be used for the developments of advanced theranostic systems with various other functional materials for the diagnosis, imaging, and therapy of cancer because of their favorable characteristics such as biocompatibility, nontoxicity, biodegradability, bioactivity, non-toxicity, and lower adverse effects [12].

2.1 Chitosan Loaded with Photosensitizers/Photothermal Agents

Photodynamic therapy (PDT) is one of the important approaches for cancer treatment, where photosensitizers play a major role to create reactive oxygen species (ROS), namely singlet oxygen and hydroxyl radicals in presence of light with a suitable wavelength [13]. These ROS can kill various biological objects in the surrounding medium and hence are measured as a promising candidate for the abolition of tumor cells. The photosensitizers accumulated in the tumor sites can also produce an intense fluorescence signal under light irradiation, which can be

used as imaging agents for imaging and diagnostic applications [14]. However, the usage of photosensitizers as a therapeutic agent is highly limited due to their nonspecific phototoxicity, less water solubility,and ineffective tumor-targeting ability. The limitations of photosensitizers can be overcome by encapsulating them into chitosan-based tumor-targeted drug delivery carriers.

Glycol chitosan is a water-soluble derivative of chitosan. It is prepared by reacting chitosan with hydrophobic glycol molecules. Due to its amphiphilic nature, glycol chitosan can be self-assembled to NPs for the delivery of therapeutic and imaging agents in the tumor site. The glycol chitosan NPs loaded with photosensitizer and anti-cancer drugs have been considered as a promising candidate for imaging and therapy of cancer because of their biocompatibility, cationic nature, improved EPR effect, and functional groups for surface modification and bioconjugation. Lee et al. [15] developed glycol chitosan-based NPs loaded with protoporphyrin IX (PpIX) as a photosensitizer for PDT and imaging of cancer. The PpIX-loaded glycol chitosan NPs presented the improved tumor-targeting ability compared to free photosensitizer when treated with SCC7 tumor-bearing mice. Also, amphiphilic self-assembled NPs were prepared using the glycol chitosan conjugated with chlorin e6 (Ce6), a photosensitizer for PDT of cancer [16]. Since Ce6 generated ROS more effectively in the NPs, the Ce6-loaded glycol chitosan showed enhanced PDT in MDA-MB-231 breast cancer cells. In another approach, FITC-labeled glycol chitosan-Ce6 NPs and chitosan-Ce6 NPs were prepared and analyzed their biodistribution in vivo in the cancer cells [17]. Compared to free Ce6, chitosan-Ce6 NPs presented reduced in vitro cell viability and improved in vivo tumor phototoxicity, which confirms its potential for the PDT and imaging of cancer.

Shrestha and Kishen prepared rose Bengal-loaded chitosan and studied its antibiofilm efficiency on gram-positive and gram-negative bacteria using PDT [18]. Under the treatment of rose bengal-loaded chitosan, the shape of bacterial biofilm was disintegrated. This result indicated that rose bengal-loaded chitosan had improved antibiofilm activity compared to the control photosensor. Sun et al. [19] developed methylbenzene blue-loaded carboxymethyl chitosan NPs as a theranostic agent for cancer therapy. These NPs exhibited pH-dependent drug release behavior due to the presence of carboxymethyl chitosan. The prolonged controlled drug release from the NPs was observed at pH 7.4 medium compared to pH 5.5 medium. Under a laser light treatment, these NPs showed antibiofilm activity and inhibition of cancer cell growth in acidic conditions. Moreover, the developed NPs exhibited self-imaging ability and antitumor activity against the tumor mice model. Recently, Pandya et al. [20] prepared chitosan-tetraphenyl chlorin conjugate NPs loaded with mertansine or cabazitaxel as imaging contrast agents and for PDT of cancer. Due to the strong interaction between the drugs and photosensitizer, the chitosan-tetraphenyl chlorin conjugate NPs showed the improved drug loading capacity. These NPs presented the enhanced cytotoxicity and PDT effect on breast cancer cell lines, suggests their suitability for cancer theranostics.

Photothermal therapy (PTT) is another promising approach to destruct cancer cells by thermal energy. In PTT, near-infrared (NIR) light is engrossed and transformed to confined heat by the NPs, resulting in the demolition of the tumor cells [21]. Kumar et al. [22] developed polycaprolactone coated and IR

820 dye-loaded glycol chitosan composite for imaging and PTT of cancer cells. These composites retained their structural stability even after the exposure to laser light and presented the sustained release profile. Also, they exhibited the imaging capability, improved cellular uptake, and enhanced photothermal effect on breast cancer cells. Manivasagan et al. [23] fabricated chitosan-polypyrrole composite NPs for imaging-guided PTT of cancer. Because of the synergistic effects of chitosan and polypyrrole, these composites had improved biocompatibility, stability, and NIR absorbance. The in vivo study demonstrated that the prepared composite NPs had better antitumor activity against the tumor-bearing mice under NIR radiation. Recently, Lee et al. [24] prepared chitosan oligosaccharide lactate conjugated with ZW800–1 NIR fluorophore for imaging and targeted PTT of cancer. The HT-29 tumor-bearing mice treated with developed conjugate presented a considerable decrease in the volume of tumor after NIR laser irradiation. Further, chitosan oligosaccharide lactate-ZW800–1 conjugate showed light-triggered PTT and strong fluorescence in tumor sites.

2.2 Chitosan Loaded with QDs

The inorganic nanocrystals that belong to groups III–V and II–VI are known as QDs. Due to the fluorescent properties, QDs are considered an imaging agent in the field of cancer theranostics. The fluorescent properties of QDs can be altered by changing their size and configuration [25]. So far, considerable works have been done to develop multifunctional theranostics based on chitosan and its derivatives encapsulated with QDs for cancer therapy.

Tan et al. [26] prepared chitosan-QD composite surface modified with human epidermal growth factor receptor 2 (HER2) antibodies for the imaging and targeted delivery of si-RNA to MCF-7 and SKBR3 human breast cancer cell lines. Due to the presence of a greater number of HER2 receptors, more internalization of the chitosan-QD composite was found in SKBR3 cells compared to MCF-7 cells. When chitosan-QD was utilized to release HER2 si-RNA, HER2 gene expression levels were decreased to 80%. Also, Yuan et al. [27] prepared chitosan-ZnO QDs for imaging and tumor-targeted drug delivery. Due to the presence of chitosan, the prepared materials showed the improved drug loading efficiency and biocompatibility. Also, these materials presented the imaging ability by emitting the blue-light due to the existence of ZnO QDs. In another approach, curcumin-loaded *O*-carboxymethyl chitosan grafted with ZnO QDs was prepared for cancer therapy [28]. Since ZnO QDs were conjugated with chitosan, these materials presented enhanced stability for better drug release, imaging, and therapeutic efficiencies.

Ma et al. [29] developed carboxymethyl chitosan loaded with CdTe QDs. Due to the strong binding ability of carboxymethyl chitosan to CdTe QDs with Zn^{2+}, the developed materials presented an improved fluorescence property. These NPs accumulated more effectively at the tumor site of tumor-bearing mice after intravenous inoculation. Due to the biocompatibility and imaging ability, the developed NPs showed great potential for the imaging and therapy of cancer. In another study, a film

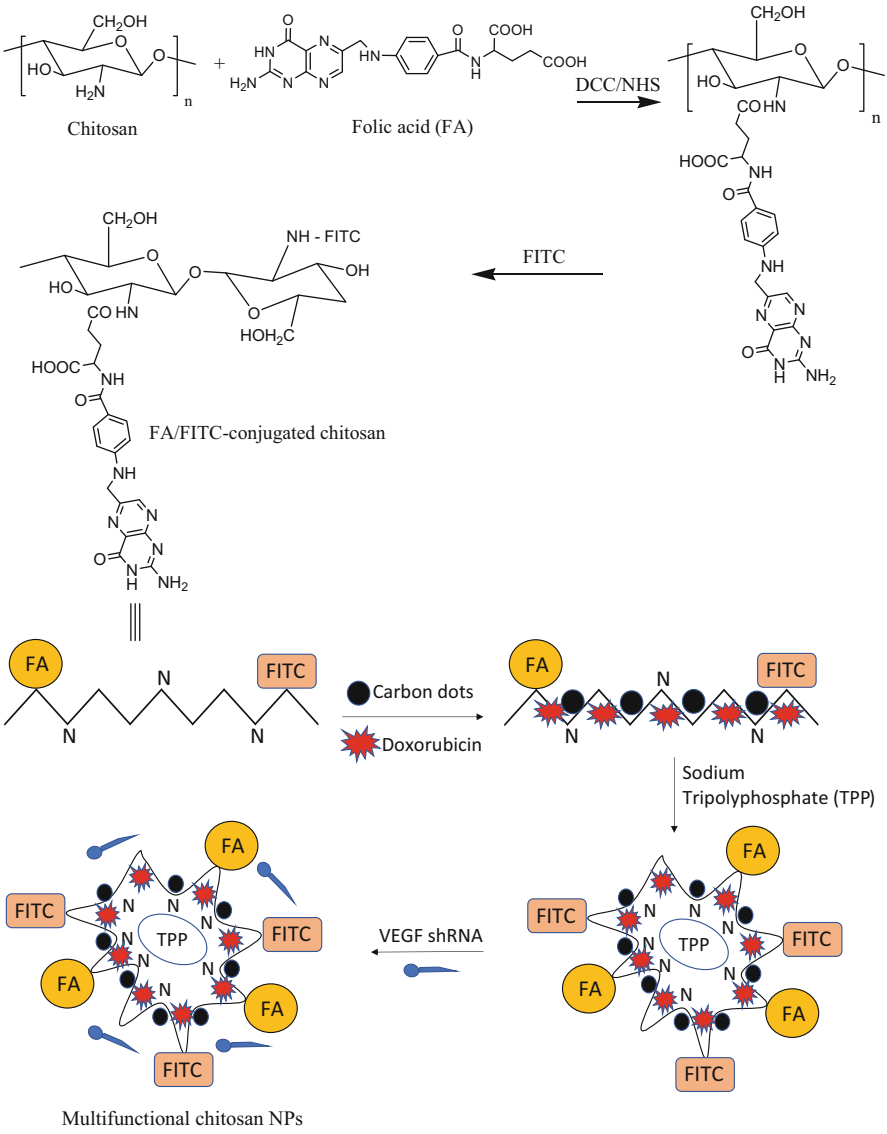

Fig. 1 Schematic representation of the preparation of multifunctional chitosan NPs

based on CdTe QDs functionalized with chitosan-*L*-cysteine was prepared. This film showed better imaging ability and antibacterial property due to the synergistic effect of CdTe QDs and chitosan-L-cysteine [30].

Multifunctional chitosan NPs containing folic acid (FA), FITC, doxorubicin (DOX), carbon QDs, and vascular endothelial growth factor (VEGF) shRNA were prepared as a theranostic agent for cancer therapy (Fig. 1) [31]. The average particle size and charge of these NPs were found to be 154 nm and 23.2 mV, respectively.

These NPs effectively delivered the VEGF shRNA into HeLa cells by shielding shRNA from degradation. The NPs presented the pH-responsive anti-cancer drug release profile. Due to the presence of FA, the prepared NPs exhibited improved cellular uptake by HeLa cells through folate-receptor-mediated endocytosis. The HeLa cells treated with multifunctional chitosan NPs presented reduced VEGF expression, minimal cell proliferation, and augmented cell apoptosis. Besides, these NPs confirmed outstanding fluorescence cellular imaging due to the presence of carbon QDs. In another study, Ding et al. [32] developed FA-conjugated carboxymethyl chitosan NPs containing QDs and Fe_3O_4 NPs for cellular imaging and tumor-targeted drug delivery. Due to the presence of FA, these NPs were successfully uptaken by tumor cells via the FA-receptor-mediated endocytosis mechanism. Moreover, they exhibited simultaneous fluorescence imaging and magnetic properties due to the synergistic effects of QDs and Fe_3O_4 NPs. Further, Lin et al. [33] fabricated nano-micelles based on pluronic-containing anticancer drug and ZnO/CdTe QDs. In this study, the surface of nano-micelles was altered by FA-conjugated chitosan to improve their tumor-targeting ability and blood circulation time. The in vivo study results disclosed that these NPs had controlled drug release and fluorescence cellular imaging capabilities.

Recently, Janus et al. [34] prepared chitosan-based carbon QDs doped with nitrogen for diagnosis, cellular imaging, and controlled drug delivery. The developed QDs presented photoluminescence property in visible light. The chitosan-based carbon QDs modified with amino acids such as lysine and glutamic acid showed a higher quantum yield. Biocompatibility studies demonstrated that the developed NPs had no cytotoxicity on human dermal fibroblasts.

In another approach, Yu et al. [35] designed FA-conjugated copper sulfide (CuS)-chitosan QDs for imaging-guided cancer therapy. These NPs presented adequate biocompatibility, increased cellular uptake, strong NIR light absorption, and improved photothermal efficiency. They also effectively targeted and amassed in the cancer cells within 60 min. Under laser light irradiation, these NPs potentially inhibited the growth rate of tumor cells.

2.3 Chitosan-Noble Metal Conjugates

In recent years, noble metals such as gold (Au), silver (Ag), and palladium (Pd) have received much importance as cancer theranostics due to their exceptional optical, electrical, and photothermal behaviors. The metal NPs encapsulated with chitosan and its derivatives have a large potential to be used for cancer imaging and therapy due to their excellent stability and multifunctional effects [36]. Chitosan can form complexation with metal ions through hydroxyl and amino groups present in the polymer chain and hence can improve the stability of metal NPs [37].

Thangam et al. [38] developed the multifunctional chitosan NPs encapsulated with Ag and natural fluorescent protein, R-phycoerythrin for cancer therapy. The theranostic ability of these NPs was studied against MDA-MB-231 breast cancer

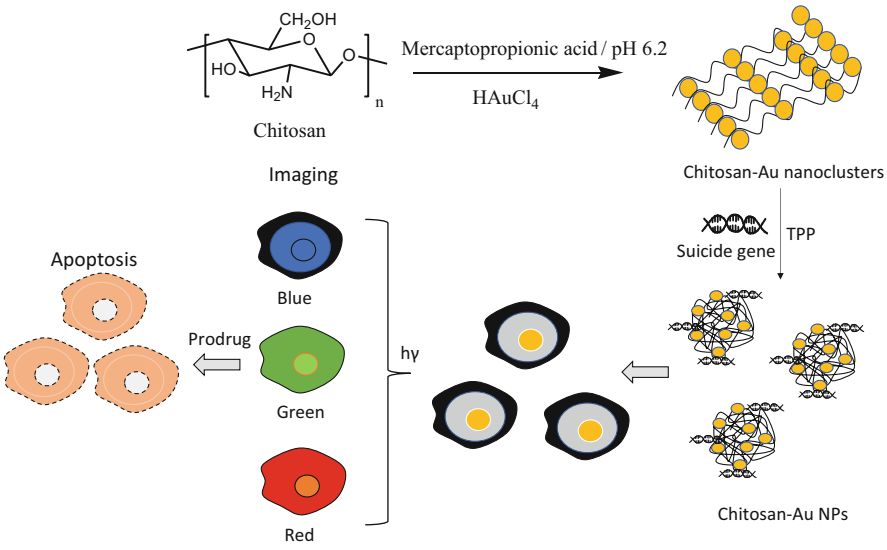

Fig. 2 Schematic representation of the preparation of fluorescence chitosan-Au NPs

cells. The developed NPs presented noticeably high cytotoxicity in the MDA-MB-231 cells, but less cytotoxicity in the normal cells. In recent years, Au NPs are largely utilized as a theranostic agent for cancer treatment because of their non-toxicity, high surface-to-volume ratio, excellent optical and photothermal properties [39]. The Au NPs encapsulated chitosan can have the improved drug loading and tumor-targeted drug delivery ability and imaging property.

Zhang et al. [40] developed chitosan-modified Au NPs for the radiotherapy of cancer. Due to the improved radiation sensitivity, the prepared NPs showed remarkable damage to tumor cells under X-ray irradiation. In another strategy, Sahoo et al. [41] developed chitosan-Au NPs for the apoptosis of cervical cancer cells (Fig. 2). Due to the fluorescence property of Au NPs, the developed NPs presented the optical imaging of cells without the usage of additional dyes. Also, as a radiosensitizer, the chitosan-Au NPs enhanced the efficiency of the radiotherapy. Yan et al. [42] prepared poly(vinyl alcohol)/chitosan nanofibers incorporated with Au NPs for delivery of the drug to cancer cells and imaging of cancer cells. Due to the surface plasmon resonance (SPR) property of Au NPs, the developed nanofibers can be used to attain image-guided therapies.

The Pd NPs coated with RGD peptide-linked chitosan oligosaccharide were developed for imaging and PTT of cancer cells (Fig. 3) [43]. These particles presented the selective accumulation in MDA-MB-231 breast cancer cells and improved photothermal effects under irradiation of 808-nm laser light at 2 W/cm^2 power density. Due to the coating of chitosan oligosaccharide, the NPs showed adequate biocompatibility, colloidal stability, and water dispersity. Since the developed NPs exhibit a good amplitude of photoacoustic signals, they facilitated the imaging of cancer cells using a photoacoustic tomography system.

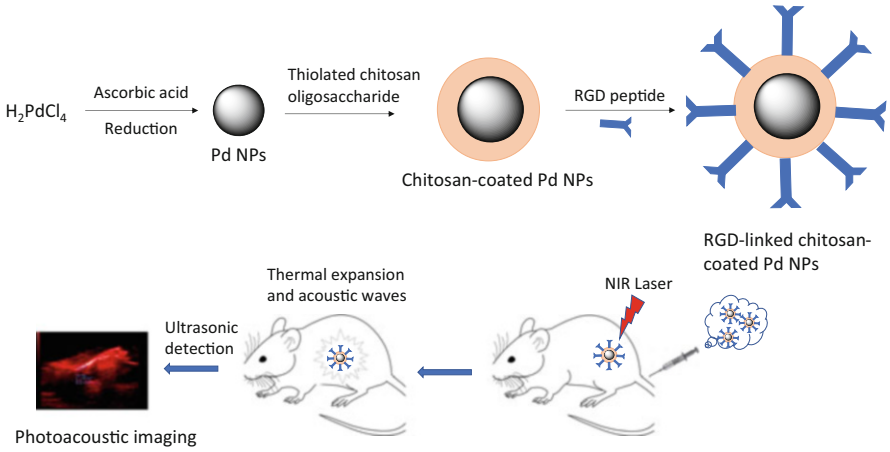

Fig. 3 Schematic illustration of the preparation of RGD-linked chitosan-coated Pd NPs, photothermal extirpation, and photoacoustic imaging of cancer tissue using them

Recently, Sun et al. [44] fabricated Au NPs coated with glycol-chitosan for cancer cell imaging and therapy. These NPs showed relatively improved cellular uptake by breast cancer cell lines and good photoacoustic signals in the in vitro studies. The improved photoacoustic signals could be due to the plasmon coupling effect of glycol-chitosan-coated Au NPs in cancer cells. Further, these NPs presented photoacoustic cancer cellular imaging without using any antibodies or surface alteration. Wang et al. [45] prepared core-shell composite NPs based on Fe_3O_4, chitosan, and Au NPs as cancer theranostics. These NPs had improved cytotoxicity against different types of human carcinoma cell lines. The developed core-shell composite NPs could be used for the imaging and therapy of pancreatic, gastric, and colon cancers. Further, Ma et al. [46] developed Au encapsulated chitosan NPs for cancer therapy. These NPs presented outstanding biocompatibility, improved colloidal stability, pH-dependent drug release property, high drug loading, and fluorescence imaging abilities.

2.4 Chitosan-Based Magnetic NPs

Among the inorganic nanomaterials, magnetic NPs have been extensively used in hyperthermia, drug delivery, magnetic resonance imaging (MRI), drug delivery, bioseparation, and catalysis due to their exceptional multifunctional characteristics [47, 48]. The magnetic NPs exhibit excellent superparamagnetic behavior when their size is reduced to <15 nm. In recent years, much interest has been given to chitosan-magnetic NPs conjugates for the tumor-targeted drug delivery and imaging modalities using an externally applied magnetic field [49]. Among the magnetic NPs, superparamagnetic iron oxide (Fe_3O_4) NPs are widely considered as a promising

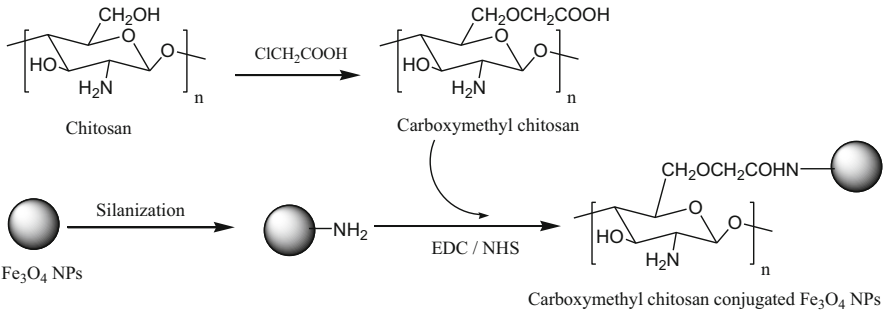

Fig. 4 Preparation of carboxymethyl chitosan conjugated Fe_3O_4 NPs

MRI contrast agent for cancer therapy because of their lesser toxicity and increased proton relaxation that results in a lesser detection limit [50]. Hence, superparamagnetic Fe_3O_4 NPs have been combined with chitosan as cancer theranostic agents. For instance, Lee et al. [51] prepared self-assembled chitosan-linoleic acid NPs encapsulated with Fe_3O_4 NPs as a contrast agent. These NPs were found to increase the colloidal stability of the Fe_3O_4 NPs. Moreover, they reduced the cytotoxicity and showed the targeted MRI of the cancer cells without imaging the normal cells. Maria et al. [52] prepared Fe_3O_4-loaded chitosan NPs for the sustained delivery of docetaxel and imaging of cancer cells. The results showed that the drug retains its efficiency during its loading into the NPs and hence NPs carried the drug into tumor cells effectively. Further, Shi et al. [53] prepared carboxymethyl chitosan conjugated Fe_3O_4 NPs as a contrast agent in MRI (Fig. 4). These NPs were efficiently taken up by the human mesenchymal stem cells (hMSCs) through endocytosis and showed minimal cytotoxicity as compared to the control medium. Due to high labeling competence, the developed Fe_3O_4 NPs detected <100 labeled cells by MRI. Santos et al. [54] synthesized multifunctional Fe_3O_4/chitosan-*L*-glutamic acid core-shell NPs for tumor-targeted drug delivery and hyperthermia usages. These NPs presented the improved drug loading and controlled drug release profile with a localized hyperthermia ability.

Fan et al. [55] developed multifunctional Fe_3O_4 NPs surface-modified with carboxymethyl chitosan and FA (Fig. 5). The results showed that the surface-modified Fe_3O_4 NPs decrease not only the cytotoxicity against the normal cells but also the detention of Fe_3O_4 NPs by macrophages. Due to the presence of FA, the established NPs were effectively taken up by FA-receptor positive cancer cells for MRI, drug targeting, and hyperthermia. Balan et al. [56] reported magnetic NPs comprised of N-palmitoyl chitosan and Fe_3O_4 NPs by ionic gelation method. The developed NPs had a mean size of ~150 nm and a zeta potential of 16.78 mV. In addition, they offered the drug loading ability, high value of magnetic saturation (54.59 emu/g), and superparamagnetic behavior required for cancer theranostics. In another approach, Wang et al. [57] developed magnetic graphene functionalized with chitosan for the simultaneous delivery of therapeutics and magnetic NPs to the tumor site. Because of the presence of chitosan, the developed magnetic graphene

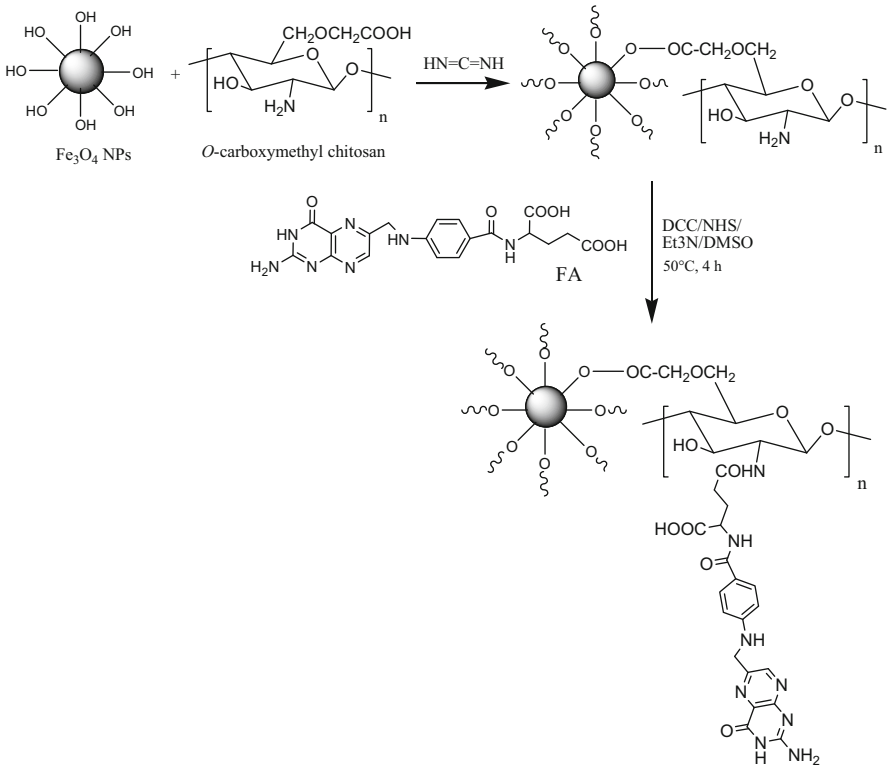

Fig. 5 Preparation of carboxymethyl chitosan and FA coated Fe_3O_4 NPs

sheets were found to be biocompatible, stable, and dispersed in the aqueous medium. These NPs presented the improved drug loading ability, pH-dependent drug release profile, cellular uptake by the A549 human lung cancer cells. Due to the enhanced cellular uptake, the cytotoxicity of the drug-loaded NPs towards the tumor cells was measured to be larger than that of free drug.

Also, Lim et al. [58] developed magnetic NPs based on *N*-naphthyl-*O*-dimethymaleoyl chitosan and Fe_3O_4 for effective cancer therapy. These NPs were found to have the capability of pH-dependent drug release and MRI abilities (Fig. 6). They showed an increased rate of drug release at acidic medium than that at alkaline medium, which could be desired for effective delivery of therapeutics in cancer sites.

Zhou et al. [59] prepared chitosan-coated Fe_3O_4 NPs with a collective ability of drug distribution and hyperthermia effect using a coacervation followed by a chemical cross-linking technique. In this study, FA-conjugated poly(ethylene glycol) (PEG) was functionalized on the surface of the chitosan-coated Fe_3O_4 NPs to expand the long blood circulation and targeting ability. Due to the presence of targeting ligand, the developed NPs showed higher cellular uptake by HeLa cells through folate-receptor-mediated endocytosis. Guanghui et al. [60] fabricated Fe_3O_4 and DOX-loaded chitosan NPs and found that the synthesized NPs could be suitable

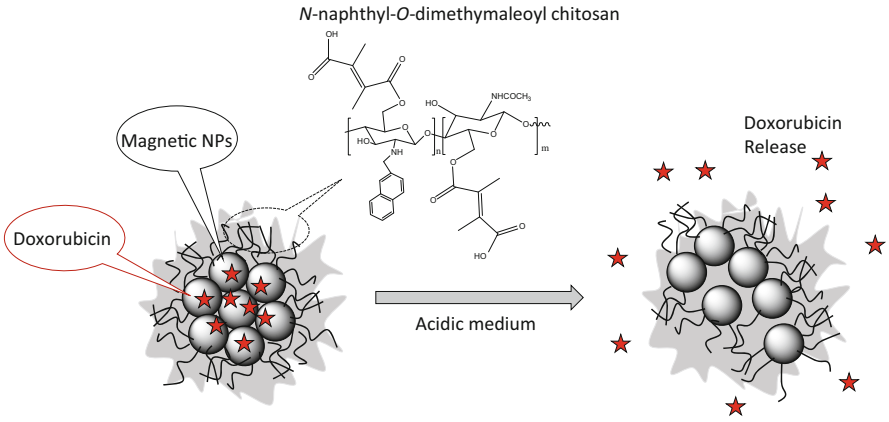

Fig. 6 Illustration of pH-dependent drug release behavior of magnetic *N*-naphthyl-*O*-dimethymaleoyl chitosan NPs

for MRI and delivery of therapeutic agents due to their imaging contrast ability and pH-sensitive properties, respectively. Further, Wang et al. [61] prepared multifunctional DOX-loaded Fe_3O_4–CdTe@SiO_2–carboxymethyl chitosan core-shell NPs. These NPs exhibited good biocompatibility, outstanding magnetic-guided tumor-targeting, and fluorescence labeling abilities. The nanobubbles encapsulated with magnetic NPs have also been considered as a promising candidate for cancer theranostics due to their long blood circulation ability and tumor-targetability. For instance, Yang et al. [62] developed carboxymethyl hexanoyl chitosan nanobubbles loaded with camptothecin and Fe_3O_4 NPs for the imaging and therapy of cancers. Due to the synergistic effect of carboxymethyl hexanoyl chitosan and Fe_3O_4 NPs, the developed nanobubbles demonstrated the increased cytotoxicity against the breast tumor cells and tumor-specific accumulation.

Li et al. [63] developed the multifunctional DOX-loaded Fe_3O_4 NPs blended with chitosan and graphene oxide by electrospray method (Fig. 7). These NPs had the particle size in the range of 100–1,100 μm. Due to the presence of Fe_3O_4 and graphene oxide, the multifunctional Fe_3O_4 NPs showed magnetic responsive behavior with improved drug loading efficiency and stimuli-responsive DOX release profile under NIR irradiation for the chemotherapy of cancer. In another study, chitosan NPs coated with Fe_3O_4 were reported for cancer theranostics [64]. These NPs presented the enhanced MRI signals, imaging of tumor cells, and tumor-specific drug delivery. The biodistribution study conducted using BALB/c mice demonstrated that the developed NPs mainly accumulated in kidneys and liver as they are the important organs that participated in the removal of Fe_3O_4 NPs. These results confirmed that Fe_3O_4-coated chitosan NPs are a promising material for the diagnosis, imaging, and therapy of liver cancer.

In recent years, gene silencing and RNA interference practices have received much interest in cancer therapy. However, due to the rapid disintegration of siRNA by endonucleases before cellular uptake, the usage of these methods is restricted. To

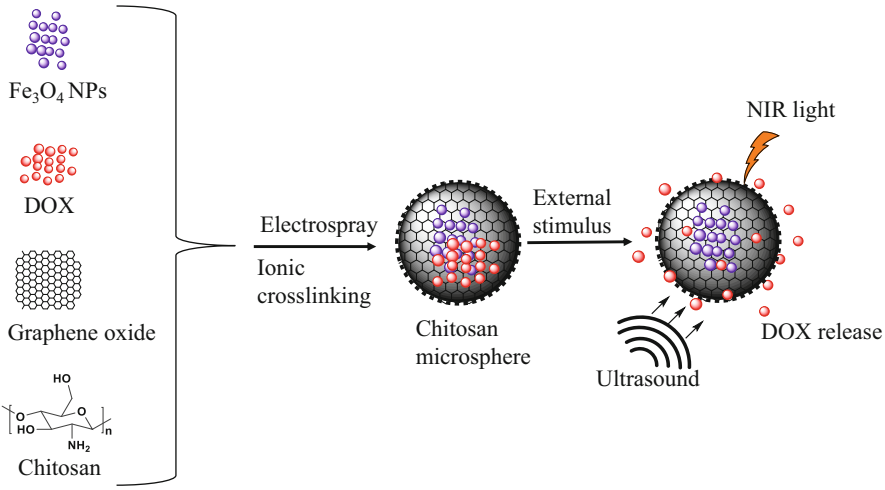

Fig. 7 Schematic representation for the preparation of DOX-loaded Fe3O4/chitosan/graphene oxide NPs and stimuli-responsive DOX release

overcome these drawbacks, Bruniaux et al. [65] developed chitosan functionalized with Fe_3O_4 NPs and siRNA for RNA interference and MRI contrast abilities. In this study, the coating of PEG and poly-L-arginine on the surface of NPs was found to improve their biocompatibility and siRNA transfection ability. Because of the existence of chitosan, the developed NPs presented the pH-dependent delivery of siRNA into the cells. Likewise, Israel et al. [66] reported chitosan-coated Fe_3O_4 NPs as nanocarriers for siRNA for gene silencing therapy.

Olono et al. [67] fabricated the core-shell of Fe_3C/γ-Fe_2O_3 NPs encapsulated micelles based on sodium dodecyl sulfate and oleic acid stabilized with chitosan using the sonochemical synthesis method. These micelles showed an average size of 19.71 nm, a polydispersity index of 0.134 and a zeta potential of −41.5 mV. Due to their drug loading efficiency and imaging property, these nanocarriers could be an ideal theranostic agent for cancer therapy. Kumar and Srivastava [68] prepared FITC-linked polycaprolactone glycol chitosan IR 820 NPs for hyperthermia-induced cancer cell death. These NPs showed outstanding photostability for 5 min. The results confirmed that FITC-linked polycaprolactone glycol chitosan IR 820 NPs could be an effective theranostic material for image-guided PTT of cancer. Recently, Baktash et al. [69] prepared hybrid NPs based on chitosan grafted graphene oxide combined with Fe_3O_4 NPs as a pH-sensitive theranostic for cancer therapy (Fig. 8). The DOX-loaded NPs showed the increased rate of drug release at the acidic medium that could be ideal for tumor-targeted drug delivery. Due to the presence of Fe_3O_4 NPs and graphene oxide, the T2 contrast efficacy of the developed NPs was found to be improved. MTT study conducted with L929 cell lines showed the good biocompatibility of NPs, suggesting less contact of GO with the cell membrane due to the presence of chitosan. The results confirmed that the drug-loaded NPs

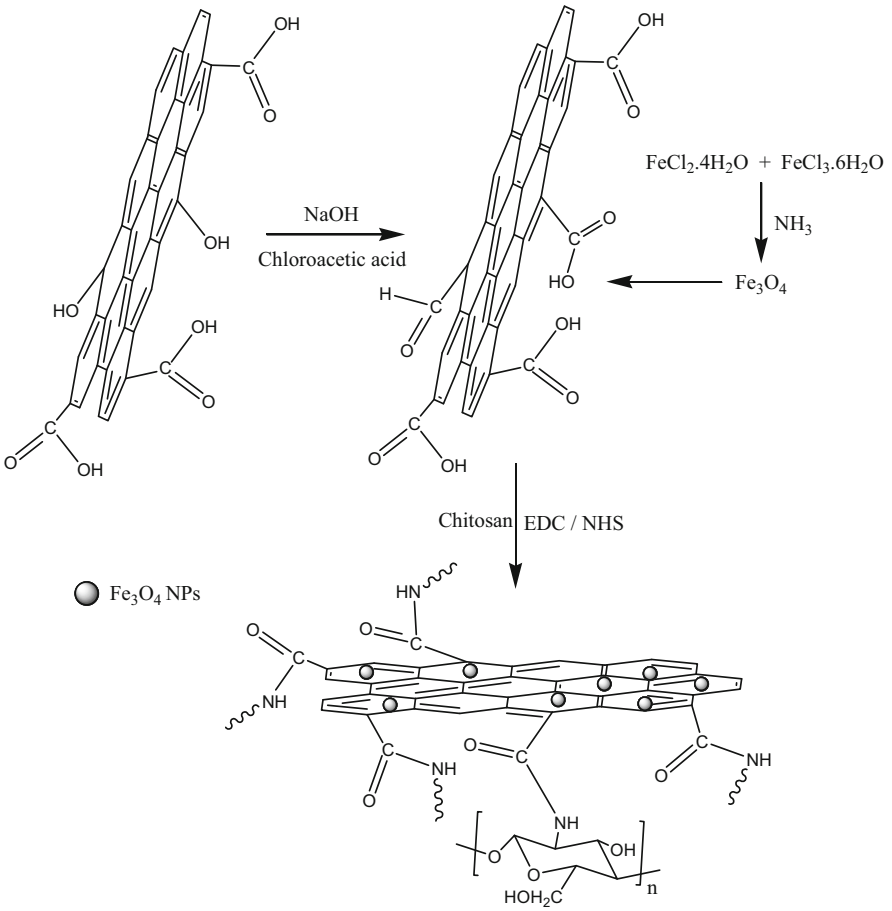

Fig. 8 Preparation of chitosan grafted graphene oxide loaded with Fe_3O_4 NPs

developed using low molecular weight chitosan presents improved cytotoxicity against the MCF7 cancer cells.

2.5 Chitosan-Based Hybrid NPs

In recent years, glycol chitosan-5β-cholanic acid conjugate has received much interest for its outstanding biocompatibility and tumor-specific biodistribution. The NPs based on glycol chitosan-5β-cholanic acid conjugate presented the selective accumulation in liver tumor tissues, suggesting that these NPs can evade the recognition by RES in the liver [70]. Further, these NPs were found to be extremely dispersed in the brain tumor, indicating the possibility of using them as cancer

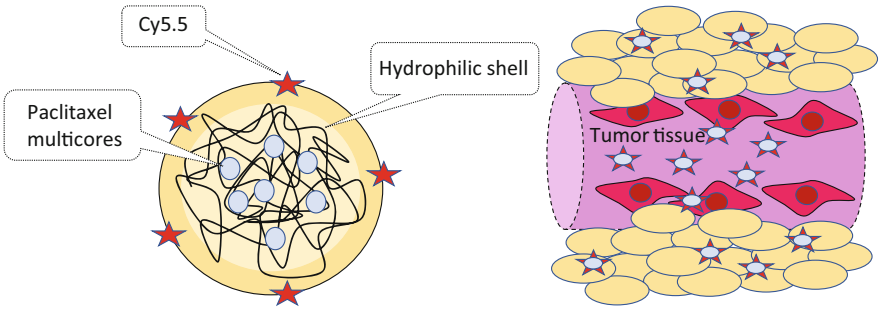

Fig. 9 Schematic diagram of paclitaxel-loaded chitosan NPs linked with Cy5.5 for imaging and therapy of cancer

theranostics [71]. Considering together, glycol chitosan-5β-cholanic acid NPs could be ideal for cancer theranostics irrespective of the location and type of the cancers. Similarly, Yoon et al. [72] developed glycol chitosan-based NPs as a carrier for si-RNA and chemotherapeutic agents and studied their physicochemical characteristics such as particle size, surface, and pH-sensitive properties.

Kim et al. [73] developed paclitaxel-loaded chitosan NPs linked with Cyanine-5.5 (Cy5.5) for imaging and therapy of cancer (Fig. 9). The developed NPs at the optimal amount of administration resulted in the increased rate of tumor cell death compared to free drugs and showed the imaging capability. Na et al. [74] also reported chitosan NPs loaded with paclitaxel and Cy5.5 for imaging and therapy of cancer. In another approach, Srinivasan et al. [75] developed chitosan-IR820 conjugates for imaging and hyperthermia of cancer cells. These conjugates showed hyperthermic cell growth inhibition in human sarcoma cancer cells, SKOV-3, and antibody upon exposure with laser light. The extent of cell growth inhibition due to hyperthermia was found to be higher in chitosan-IR820 conjugates than IR820 alone exist in cancer cells/antibody.

Carbon-based materials such as graphene and graphene oxide have the potential to be used in drug delivery, bioimaging, wound healing, and tissue engineering [76]. These materials can be suitable for the PTT of cancer cells due to their biocompatibility and functionalization ability [77]. Since carbon-based materials have layered sheet-like structure, they offer a high surface area for the increased adsorption of therapeutic agents. By considering this merit, Fu et al. [78] prepared GO-modified carboxymethyl chitosan and linked it to hyaluronic acid and FITC for tumor-targeted controlled delivery of DOX. The developed NPs presented a high drug loading ability along with the increased rate of drug release at acidic conditions (pH 5.8). Also, these NPs showed increased cellular uptake and thereby cancer cell apoptosis. Baghbani et al. [79] prepared the nanodroplets based on curcumin-loaded chitosan/perfluorohexane for ultrasound (US) imaging contrast and cancer therapy. Under US exposure, the nanodroplets presented the increased cytotoxicity against the 4 T1 human breast cancer cell lines, which confirmed their potential to be used in image-guided cancer therapy.

Recently, a nanodroplet has been developed for siRNA delivery using the microbubble transmission technique [80]. In this study, the core of nanodroplets was made up of perfluoropentane and magnetic NPs with a shell containing deoxycholic acid conjugated chitosan and siRNA. The nanodroplets were converted into microbubbles after exposure to US for a longer circulation time in the bloodstream. The magnetic NPs within the microbubbles were utilized to improve the localization via an externally applied magnetic field. The developed materials presented a four-fold decrease in the viability of human lung and breast cancer cells. In another study, DOX-loaded chitosan-Ag hybrid NPs were prepared and analyzed their physicochemical properties [81]. Due to the existence of chitosan, these NPs showed the pH-responsive controlled drug release behavior. Moreover, they showed enhanced cytotoxicity against subcutaneous tumors and cancer cell lines. Further, Zhang et al. [82] fabricated methylene blue-loaded hybrid PEG-chitosan/Fe_3O_4 NPs for imaging and combined PDT/PTT of cancer. Due to the formation of singlet oxygen, the prepared NPs effectively killed the cancer cells under NIR radiation. Also, they exhibited controlled photoexcitation to eradicate the tumor cells without affecting normal cells. These hybrid NPs could be considered for MRI and collective PDT/PTT of cancer.

2.6 Chitosan-Based Multimodal Nanocomposites

Chitosan has been utilized as a matrix for encapsulating two or more theranostic NPs to obtain nanocomposites with improved theranostic capability. In this context, Lin et al. [83] developed the multi-modal nanocarriers based on chitosan functionalized with Fe_3O_4 NPs, methotrexate-PEG, and Cy5.5. In this study, methotrexate-PEG was used as a prodrug, which can target the tumor cells. These NPs presented higher accumulation in the target site, decreased adverse effects, sustained drug release, enhanced therapeutic efficacy, and higher cellular uptake compared to the control. Due to the presence of Fe_3O_4 NPs and Cy5.5, these nanocarriers could be used in multi-modal imaging and cancer therapy. Wang et al. [84] prepared multifunctional core-shell NPs based on Fe_3O_4/Au encapsulated chitosan as a photothermal and dual-imaging agent. The NPs showed outstanding magnetic properties, plasmonic activities, and fewer hemolytic effects. Due to the exceptional photothermal behavior of Fe_3O_4/Au NPs, the developed NPs could be ideal for PTT and simultaneous MRI and field imaging of cancer cells. Kim et al. [85] fabricated Cy5.5-labeled Au NPs coated with glycol chitosan and linked with fibrin-targeting peptides for CT imaging and therapy of cerebrovascular thrombi using a tissue plasminogen activator. Key et al. [86] prepared peptide-conjugated glycol chitosan NPs loaded with Fe_3O_4 and Cy5.5 for MRI/NIR fluorescence imaging and therapy of cancer. These NPs specifically accumulated in the tumor site without gathering in the normal tissues and presented multimodal cellular imaging capability.

Recently, Liu et al. [87] prepared DOX-loaded polyoxometalate NPs surface coated with mesoporous silica and FA–chitosan for multimodal imaging and

chemotherapy of cancer. These NPs presented upconverting luminescence under NIR light and CT imaging characteristics. Due to the generation of heat under laser light irradiation and the release of DOX, the prepared NPs showed their potential to be used for the combined PPT/chemotherapy of cancer. Because of the presence of FA-chitosan, they also exhibited the tumor-targeted pH-responsive drug release. Further, Choi et al. [88] developed glycol chitosan NPs containing iodine for US and computed tomography (CT) imaging of cancer tissues. In this study, diatrizoic acid-containing iodine was linked to glycol chitosan and then perfluoropentane was loaded into the resulting product to form a multimodal imaging agent. The prepared NPs showed effective accumulation and US/CT signals in the tumor site.

3 Summary

Over the last few decades, considerable efforts have been taken to utilize chitosan and its derivatives for the development of theranostics for effective imaging and therapy of cancer due to their desirable physiochemical and biological properties. Chitosan and its derivatives have been largely combined with photosensitizers/photothermal agents, QDs, noble metals, magnetic NPs, and other functional materials to fabricate the advanced cancer theranostics. Since photosensitizers-loaded chitosan selectively accumulates in the tumor site, they can be effectively utilized for PTT and fluorescence imaging of cancer cells. Due to the presence of tumor-targeting ability and imaging capability, the targeting ligand-linked chitosan NPs encapsulated with QDs and anticancer drugs demonstrated better cellular imaging and therapeutic efficacy at the tumor site. In recent years, chitosan-Au NPs conjugates are largely considered for the combined PTT and imaging of tumors. In this system, the Au NPs provided the optical and photothermal properties to chitosan-Au NPs conjugates, while chitosan offered better drug loading, controlled release, and tumor-targeting abilities. Due to the exceptional superparamagnetic and photothermal properties of Fe_3O_4 NPs, chitosan and its derivatives have been conjugated with Fe_3O_4 NPs to fabricate different types of chitosan-based magnetic NPs as theranostics for cancer therapy. These magnetic NPs could be a promising material for simultaneous MRI, PTT, and chemotherapy of cancer because of the synergistic effects of Fe_3O_4 NPs and chitosan-based materials. Further, chitosan-based materials conjugated with different fluorescent dyes and functional materials, namely Fe_3O_4 NPs, Au NPs, therapeutic agents, 5β-cholanic acid, PEG, and GO established their suitability for multimodal imaging and therapy of cancer. Overall, it is apparent that chitosan-based theranostics have a large potential to be utilized in real-time cancer therapy after the systematic evaluation of their cytotoxicity and immunotoxicity and completion of preclinical studies.

References

1. Nair JB, Joseph MM, Mohapatra S, Safeera M, Ghosh S, Sreelekha TT, Maiti KK (2016) A dual-targeting octaguanidine–doxorubicin conjugate transporter for inducing caspase-mediated apoptosis on folate-expressing cancer cells. Chem Med Chem 11(7):702–712
2. Peng N, Wu B, Wang L, He W, Ai Z, Zhang X, Wang Y, Fan L, Ye Q (2016) High drug loading and pH-responsive targeted nanocarriers from alginate-modified SPIONs for anti-tumor chemotherapy. Biomater Sci 4(12):1802–1813
3. Gonciar D, Mocan T, Matea CT, Zdrehus C, Mosteanu O, Mocan L, Pop T (2019) Nanotechnology in metastatic cancer treatment: current achievements and future research trends. J Cancer 10(6):1358–1369
4. Li Y, Zhang H (2019) Nanoparticle-based drug delivery systems for enhanced tumor-targeting treatment. J Biomed Nanotech 15(1):1–27
5. Teijeiro-Valino C, Novoa-Carballal R, Borrajo E, Vidal A, Alonso-Nocelo M, de la Fuente FM, Lopez-Casas PP, Hidalgo M, Csaba N, Alonso MJ (2019) A multifunctional drug nanocarrier for efficient anticancer therapy. J Control Release 294:154–164
6. Jahangirian H, Kalantari K, Izadiyan Z, Rafiee-Moghaddam R, Shameli K, Webster TJ (2019) A review of small molecules and drug delivery applications using gold and iron nanoparticles. Int J Nanomedicine 14:1633
7. Nunes SS, De Barros ALB (2015) The use of coating agents to enhance liposomes blood circulation time. J Mol Pharm Org Proc Res 3(1):e120
8. He Q, Liu J, Liang J, Liu X, Li W, Liu Z, Ding Z, Tuo D (2018) Towards improvements for penetrating the blood–brain barrier - recent progress from a material and pharmaceutical perspective. Cell 7(4):24
9. Jiang H, Guo D, Chen D, Wu Y, Jin X, Zhu X (2019) A new insight into the reversal of multidrug resistance in cancer by nanodrugs. Biomater Sci 7(8):3489–3496
10. Mirrahimi M, Abed Z, Beik J, Shiri I, Dezfuli AS, Mahabadi VP, Kamrava SK, Ghaznavi H, Shakeri-Zadeh A (2019) A thermo-responsive alginate nanogel platform co-loaded with gold nanoparticles and cisplatin for combined cancer chemo-photothermal therapy. Pharmacol Res 143:178–185
11. An J, Yang XQ, Cheng K, Song XL, Zhang L, Li C, Zhang XS, Xuan Y, Song YY, Fang BY, Hou XL (2017) In vivo computed tomography/photoacoustic imaging and NIR-triggered chemo–photothermal combined therapy based on a gold nanostar, mesoporous silica, and thermosensitive liposome-composited nanoprobe. ACS Appl Mater Interfaces 9 (48):41748–41759
12. Wang Y, Liu X, Deng G, Wang Q, Zhang L, Wang Q, Lu J (2017) Multifunctional PS@CS@Au–Fe_3O_4–FA nanocomposites for CT, MR and fluorescence imaging guided targeted-photothermal therapy of cancer cells. J Mater Chem B 5(22):4221–4232
13. Calavia PG, Bruce G, Pérez-García L, Russell DA (2018) Photosensitiser-gold nanoparticle conjugates for photodynamic therapy of cancer. Photochem Photobiol Sci 17(11):1534–1552
14. Simões JCS, Sarpaki S, Papadimitroulas P, Therrien B, Loudos G (2020) Conjugated photosensitizers for imaging and PDT in cancer research. J Med Chem 63(23):14119–14150
15. Lee SJ, Park K, Oh YK, Kwon SH, Her S, Kim IS, Choi K, Lee SJ, Kim H, Lee SG, Kim K, Kwon IC (2009) Tumor specificity and therapeutic efficacy of photosensitizer-encapsulated glycol chitosanbased nanoparticles in tumor-bearingmice. Biomaterials 30:2929–2939
16. Lim CK, Shin J, Kwon IC, Jeong SY, Kim S (2012) Iodinated photosensitizing chitosan: self-assembly into tumor-homing nanoparticles with enhanced singlet oxygen generation. Bioconjug Chem 23:1022–1028
17. Lee SJ, Koo H, Jeong H, Huh MS, Choi Y, Jeong SY, Byun E, Choi K, Kim K, Kwon IC (2011) Comparative study of photosensitizer loaded and conjugated glycol chitosan nanoparticles for cancer therapy. J Control Release 152:21–29
18. Shrestha A, Kishen A (2012) Polycationic chitosan-conjugated photosensitizer for antibacterial photodynamic therapy. Photochem Photobiol 88:577–583

19. Sun L, Jiang W, Zhang H, Guo Y, Chen W, Jin Y, Chen H, Du K, Dai H, Ji J, Wang B (2019) Photosensitizer-loaded multifunctional chitosan nanoparticles for simultaneous in situ imaging, highly efficient bacterial biofilm eradication and tumor ablation. ACS Appl Mater Interfaces 11:2302–2316
20. Pandya AD, Øverbye A, Sahariah P, Gaware VS, Høgset H, Masson M, Høgset A, Mælandsmo GM, Skotland T, Sandvig K, Iversen TG (2020) Drug-loaded photosensitizer-chitosan nanoparticles for combinatorial chemo- and photodynamic therapy of cancer. Biomacromolecules 21:1489–1498
21. Doughty ACV, Hoover AR, Layton E, Murray CK, Howard EW, Chen WR (2019) Nanomaterial applications in photothermal therapy for cancer. Materials (Basel) 2(5):779
22. Kumar P, Srivastava R (2015) IR 820 stabilized multifunctional polycaprolactone glycol chitosan composite nanoparticles for cancer therapy. RSC Adv 5:56162–56170
23. Manivasagan P, Bui NQ, Bharathiraja S, Moorthy MS, Oh YO, Song K, Seo H, Yoon M, Oh J (2017) Multifunctional biocompatible chitosan-polypyrrole nanocomposites as novel agents for photoacoustic imaging-guided photothermal ablation of cancer. Sci Rep 7:43593
24. Lee S, Jo G, Jung JS, Yang DH, Hyun H (2020) Near-infra-red fluorescent chitosan oligosaccharide lactate for targeted cancer imaging and photothermal therapy. Artif Cells Nanomed Biotech 48:1144–1152
25. Kulkarni NS, Guererro Y, Gupta N, Muth A, Gupta V (2019) Exploring potential of quantum dots as dual modality for cancer therapy and diagnosis. J Drug Del Sci Technol 49:352–364
26. Tan WB, Jiang S, Zhang Y (2007) Quantum-dot based nanoparticles for targeted silencing of HER2/neu gene via RNA interference. Biomaterials 28:1565–1571
27. Yuan Q, Hein S, Misra RD (2010) New generation of chitosan encapsulated ZnO quantum dots loaded with drug: synthesis, characterization and in vitro drug delivery response. Acta Biomater 6:2732–2739
28. Upadhyaya L, Singh J, Agarwal V, Pandey AC, Verma SP, Das P, Tewari RP (2015) Efficient water soluble nanostructured ZnO grafted O-carboxymethyl chitosan/curcumin-nanocomposite for cancer therapy. Process Biochem 50:678–688
29. Ma Q, Lin ZH, Yang N, Li Y, Su XG (2014) A novel carboxymethyl chitosan–quantum dot-based intracellular probe for Zn^{2+} ion sensing in prostate cancer cells. Acta Biomater 10:868–874
30. Kumar H, Srivastava R, Dutta PK (2013) Highly luminescent chitosan-L-cysteine functionalized CdTe quantum dots film: synthesis and characterization. Carbohydr Polym 97:327–334
31. Yang H, Xu M, Li S, Shen X, Li T, Yan J, Zhang C, Wu C, Zeng H, Liu Y (2016) Chitosan hybrid nanoparticles as a theranostic platform for targeted doxorubicin/VEGF shRNA codelivery and dual-modality fluorescence imaging. RSC Adv 6:29685
32. Ding Y, Yin H, Chen R, Bai R, Chen C, Hao X, Shen S, Sun K, Liu F (2017) Carboxymethyl chitosan based nanocomposites containing chemically bonded quantum dots and magnetic nanoparticles. Appl Surf Sci:433
33. Lin YJ, Huang KB, Wu YC, Rani P, Lin HR (2019) Pluronic-chitosan-folate nano-micelles incorporated with quantum dots for anti-cancer drug therapy. Int J Polym Mater Polym Biomater 68:16
34. Janus L, Piatkowski M, Pragłowska JR, Bogdał D, Matysek D (2019) Chitosan-based carbon quantum dots for biomedical applications: synthesis and characterization. Nano 9:274
35. Yu W, Yu N, Wang Z, Li X, Song C, Jiang R, Geng P, Li M, Yin S, Chen Z (2019) Chitosan-mediated green synthesis and folic-acid modification of CuS quantum dots for photoacoustic imaging guided photothermal therapy of tumor. J Colloid Inter Sci 555:480–488
36. Mohammed MA, Syeda JTM, Wasan KM, Wasan EK (2017) An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharmaceutics 9(4):53
37. Detsi A, Kavetsou E, Kostopoulou I, Pitterou I, Rozaria A, Pontillo N, Tzani A, Christodoulou P, Siliachli A, Zoumpoulakis P (2020) Nanosystems for the encapsulation of natural products: the case of chitosan biopolymer as a matrix. Pharmaceutics 12:669

38. Thangam R, Sundarraj S, Vivek R, Suresh V, Sivasubramanian S, Paulpandi M, Karthick SV, Ragavi AS, Kannan S (2015) Theranostic potentials of multifunctional chitosan-silver-phycoerythrin nanocomposites against triple negative breast cancer cells. RSC Adv 5:12209–12223
39. Wang Z, Dong J, Zhao Q, Ying Y, Zhang L, Zou J, Jiang S (2020) Gold nanoparticle-mediated delivery of paclitaxel and nucleic acids for cancer therapy. Mol Med Rep 22:4475–4484
40. Zhang C, Huang P, Bao L, He M, Luo T, Gao G, Cui D (2011) Enhancement of gastric cell radiation sensitivity by chitosan-modified gold nanoparticles. J Nanosci Nanotechnol 11:9528–9535
41. Sahoo AK, Banerjee S, Ghosh SS, Chattopadhyay A (2013) Simultaneous RGB emitting Au nanoclusters in chitosan nanoparticles for anticancer gene theranostics. ACS Appl Mater Interfaces 6:712–724
42. Yan E, Cao M, Wang Y, Hao X, Pei S, Gao J, Wang Y, Zhang Z, Zhang D (2016) Gold nanorods contained polyvinyl alcohol/chitosan nanofiber matrix for cell imaging and drug delivery. Mater Sci Eng C 58:1090–1097
43. Bharathiraja S, Bui NQ, Manivasagan P, Moorthy MS, Mondal S, Seo H, Phuoc NT, Phan TTV, Kim H, Lee KD, Oh J (2018) Multimodal tumor-homing chitosan oligosaccharide coated biocompatible palladium nanoparticles for photo-based imaging and therapy. Sci Rep 8:500
44. Sun IC, Ahn CH, Kim K, Emelianov S (2019) Photoacoustic imaging of cancer cells with glycol-chitosan-coated gold nanoparticles as contrast agents. J Biomed Opt 24(12):1–5
45. Wang X, Almoallim HS, Cui Q, Alharbi SA, Yang H (2021) In situ decorated Au NPs on chitosan-encapsulated Fe_3O_4-NH_2 NPs as magnetic nanocomposite: investigation of its anti-colon carcinoma, anti-gastric cancer and anti-pancreatic cancer. Int J Biol Macromol 171:198–207
46. Ma K, Cheng Y, Wei X, Chen D, Zhao X, Jia P (2020) Gold embedded chitosan nanoparticles with cell membrane mimetic polymer coating for pH-sensitive controlled drug release and cellular fluorescence imaging. J Biomater Appl 35(7):088532822095259
47. Guo T, Lin M, Huang J, Zhou C, Tian W, Yu H, Jiang X, Ye J, Shi Y, Xiao Y, Bian X, Feng X (2018) The recent advances of magnetic nanoparticles in medicine. J Nanomater 2018:7805147
48. Natarajan S, Harini K, Gajula GP, Sarmento B, Petersen MTN, Thiagarajan V (2019) Multifunctional magnetic iron oxide nanoparticles: diverse synthetic approaches, surface modifications, cytotoxicity towards biomedical and industrial applications. BMC Mater 1:2
49. Farnaz A, Hoda J, Hossein A, Vaghari H, Anarjan N, Ahmadi O, Berenjian A (2017) Chitosan magnetic nanoparticles for drug delivery systems. Crit Rev Biotechnol 37:492–509
50. Nabavinia M, Huarac JB (2020) Recent progress in iron oxide nanoparticles as therapeutic magnetic agents for cancer treatment and tissue engineering. ACS Appl Bio Mater 3 (12):8172–8187
51. Lee CM, Jeong HJ, Kim SL, Jeong HJ, Kim EM, Park EH, Kim DW, Lim ST (2009) SPION-loaded chitosan–linoleic acid nanoparticles to target hepatocytes. Int J Pharm 371:163–169
52. Maria VL, Daniel T, Dolores T, Anxo V, Fernando D, Alonso MJ (2009) Highly efficient system to deliver taxanes into tumor cells: docetaxel-loaded chitosan oligomer colloidal carriers. Biomacromolecules 9:2186–2193
53. Shi Z, Neoh KG, Kang ET, Shuter B, Wang SC, Poh C, Wang W (2009) Carboxymethyl chitosan-modified superparamagnetic iron oxide nanoparticles for magnetic resonance imaging of stem cells. ACS Appl Mater Inter 1(2):328–335
54. Santos DP, Ruiz MA, Gallardo V, Zanoni MVB, Arias JL (2011) Multifunctional antitumor magnetite/chitosan-l-glutamic acid (core/shell) nanocomposites. J Nanopart Res 13:4311–4323
55. Fan C, Gao W, Chen Z, Fan H, Li M, Deng F, Chen Z (2011) Tumor selectivity of stealth multifunctionalized superparamagnetic iron oxide nanoparticles. Int J Pharm 404(1–2):180–190
56. Balan V, Butnaru M, Verestiuc L (2013) Synthesis and characterization of magnetic nanoparticles based on N-palmitoyl-chitosan with potential applications in cancer theranostics. In: The 4th IEEE international conference on E-health and bioengineering
57. Wang C, Ravi S, Garapati US, Das M, Howell M, Mallela JM, Alwarapan S, Mohapatra SS, Mohapatra S (2013) Multifunctional chitosan magnetic-graphene (CMG) nanoparticles: a

theranostic platform for tumor-targeted co-delivery of drugs, genes and MRI contrast agents. J Mater Chem B 1(35):4396–4405
58. Lim EK, Sajomsang W, Choi Y, Jang E, Lee H, Kang B, Kim E, Haam S, Suh JS, Chung SJ, Huh YM (2013) Chitosan-based intelligent theragnosis nanocomposites enable pH-sensitive drug release with MR-guided imaging for cancer therapy. Nanoscale Res Lett 8:467
59. Zhou S, Li Y, Cui F, Jia M, Yang X, Wang Y, Xie L, Zhang Q, Hou Z (2014) Development of multifunctional folate-poly (ethylene glycol)-chitosan-coated Fe3O4 nanoparticles for biomedical applications. Macromol Res 22:58–66
60. Guanghui Z, Jianzhi W, Xiaomen P, Yanfeng L, Xuemei Y, Ma Y (2014) Facile solvo thermal synthesis of mesostructured Fe_3O_4/chitosan nanoparticles as delivery vehicles for pH-responsive drug delivery and magnetic resonance imaging contrast agents. Chem Asian J 9:546–553
61. Wang G, Jin L, Dong Y, Niu L, Liu Y, Ren F, Su X (2014) Multifunctional Fe_3O_4–CdTe@SiO_2–carboxymethyl chitosan drug nanocarriers: synergistic effect toward magnetic targeted drug delivery and cell imaging. New J Chem 38:700–708
62. Yang PS, Tung FI, Chen HP, Liu TY, Lin YY (2014) A novel bubble-formingmaterial for preparing hydrophobic-agent-loaded bubbles with theranostic functionality. Acta Biomater 10:3762–3774
63. Li S, Xiao L, Deng H, Shi X, Cao Q (2017) Remote controlled drug release from multifunctional Fe3O4/GO/chitosan microspheres fabricated by an electrospray method. Colloid Surf B 151:354–362
64. Kania G, Sternak M, Jasztal A, Chlopicki S, Błazejczyk A, Nasulewicz-Goldeman A, Wietrzyk J, Jasinski K, Skórka T, Zapotoczny S, Nowakowska M (2018) Uptake and bioreactivity of charged chitosan-coated superparamagnetic nanoparticles as promising contrast agents for magnetic resonance imaging. Nanomedicine 14:131–140
65. Bruniaux J, Ben Djemaa S, Herve-Aubert K, Marchais H, Chourpa I, David S (2017) Stealth magnetic nanocarriers of siRNA as platform for breast cancer theranostics. Int J Pharm 532:660–668
66. Israel LL, Lellouche E, Greneche JM, Bechor M, Michaeli S, Lellouche JP (2016) Ultrasound-mediated surface engineering of theranostic magnetic nanoparticles: an effective one-pot functionalization process using mixed polymers for siRNA delivery. J Nanomed Nanotechnol 7:385/381–385/314
67. Sauceda-Oloño PY, Lucero-Acuña JA, Zavala-Rivera P (2018) Encapsulation of iron oxide nanoparticles type core-shell in chitosan as possible theranostic agent. Microsc Microanal 24:2018
68. Piyush K, Rohit S (2018) FITC conjugated polycaprolactone-glycol-chitosan nanoparticles containing the longwave emitting fluorophore IR 820 for in-vitro tracking of hyperthermia-induced cell death. BioRxiv. https://doi.org/10.1101/273748
69. Baktash MS, Zarrabi A, Avazverdi E, Reis NM (2020) Development and optimization of a new hybrid chitosan-grafted graphene oxide/magnetic nanoparticle system for theranostic applications. J Mol Liquids 322(24):114515
70. Na JH, Koo H, Lee S, Min KH, Park K, Yoo H, Lee SH, Park JH, Kwon IC, Jeong SY, Kim K (2011) Real-time and non-invasive optical imaging of tumor-targeting glycol chitosan nanoparticles in various tumor models. Biomaterials 32:5252–5261
71. Veiseh O, Sun C, Fang C, Bhattarai N, Gunn J, Kievit F, Du K, Pullar B, Lee D, Ellenbogen RG, Olson J, Zhang M (2009) Specific targeting of brain tumors with an optical/magnetic resonance imaging nanoprobe across the blood–brain barrier. Cancer Res 69:6200–6207
72. Yoon HY, Son S, Lee SJ, You DG, Yhee JY, Park JH, Swierczewska M, Lee S, Kwon IC, Kim SH, Kim K, Pomper MG (2014) Glycol chitosan nanoparticles as specialized cancer therapeutic vehicles: sequential delivery of doxorubicin and Bcl-2 siRNA. Sci Rep 4:6878
73. Kim K, Kim JH, Park H, Kim YS, Park K, Nam H, Lee S, Park JH, Park RW, Kim IS, Choi K, Kim SY, Park K, Kwon IC (2010) Tumor-homing multifunctional nanoparticles for cancer

theragnosis: simultaneous diagnosis, drug delivery, and therapeutic monitoring. J Control Release 146:219–227
74. Na JH, Koo H, Lee S, Min KH, Park K, Yoo H, Lee SH, Park JH, Kwon IC, Jeong SY, Kim K (2011) Real-time and noninvasive optical imaging of tumor-targeting glycol chitosan nanoparticles in various tumor models. Biomaterials 32:5252–5261
75. Srinivasan S, Manchanda R, Fernandez-Fernandez A, Lei T, McGoron AJ (2013) Near-infrared fluorescing IR820-chitosan conjugate for multifunctional cancer theranostic applications. J Photochem Photobiol B 119:52–59
76. Hoseini-Ghahfarokhi M, Mirkiani S, Mozaffari N, Abdolahi Sadatlu MA, Ghasemi A, Abbaspour S, Akbarian M, Farjadian F, Karimi M (2020) Applications of graphene and graphene oxide in smart drug/gene delivery: is the world still flat? Int J Nanomedicine 15:9469–9496
77. Wang C, Wang X, Chen Y, Fang Z (2020) In-vitro photothermal therapy using plant extract polyphenols functionalized graphene sheets for treatment of lung cancer. J Photochem Photobio B 204:111587
78. Fu G, Zhu L, Yang K, Zhuang R, Xie J, Zhang F (2016) Diffusion-weighted magnetic resonance imaging for therapy response monitoring and early treatment prediction of photothermal therapy. ACS Appl Mater Interfaces 8:5137–5147
79. Baghbani F, Chegeni M, Moztarzadeh F, Hadian-Ghazvini S, Raz M (2017) Novel ultrasound-responsive chitosan/perfluorohexane nanodroplets for image-guided smart delivery of an anti-cancer agent: curcumin. Mater Sci Eng C 74:186–193
80. Lee JY, Crake C, Teo B, Carugo D, Victor MS, Seth A, Stride E (2017) Ultrasound-enhanced siRNA delivery using magnetic nanoparticle-loaded chitosan-deoxycholic acid nanodroplets. Adv Healthcare Mater:6
81. Mohamed N (2020) Synthesis of hybrid chitosan silver nanoparticles loaded with doxorubicin with promising anti-cancer activity. BioNanoScience 10(3)
82. Zhang G, Gou H, Liu Y, Xi K, Jiang D, Jia X (2020) pH-responsive PEG-chitosan/iron oxide hybrid nanoassemblies for low-power-assisted PDT/PTT combination therapy. Nanomedicine (Lond) 5(11):1097–1112
83. Lin J, Li Y, Li Y, Wu H, Yu F, Zhou S, Xie L, Luo F, Lin C, Hou Z (2015) Drug/dye-loaded, multifunctional PEG-chitosan-iron oxide nanocomposites for methotraxate synergistically self-targeted cancer therapy and dual model imaging. ACS Appl Mater Interfaces 7:11908–11920
84. Wang X, Liu H, Chen D, Meng X, Liu T, Fu C, Hao N, Zhang Y, Wu X, Ren J, Tang F (2013) Multifunctional Fe_3O_4@ P(St/MAA)@chitosan@ Au core/shell nanoparticles for dual imaging and photothermal therapy. ACS Appl Mater Interfaces 5:4966–4971
85. Kim JY, Ryu JH, Schellingerhout D, Sun IC, Lee SK, Jeon S, Kim J, Kwon IC, Nahrendorf M, Ahn CH, Kim K, Kim DE (2015) Direct imaging of cerebral thromboemboli using computed tomography and fibrin-targeted gold nanoparticles. Theranostics 5:1098
86. Key J, Dhawan D, Cooper CL, Knapp DW, Kim K, Kwon IC, Choi K, Park K, Decuzzi P, Leary JF (2016) Multicomponent, peptide-targeted glycol chitosan nanoparticles containing ferrimagnetic iron oxide nanocubes for bladder cancer multimodal imaging. Int J Nanomedicine 11:4141–4155
87. Liu S, Li W, Gai S, Yang G, Zhong C, Dai Y, He F, Yang P, Suh YD (2019) A smart tumor microenvironment responsive nanoplatform based on upconversion nanoparticles for efficient multimodal imaging guided therapy. Biomater Sci 7(3):951–962
88. Choi D, Jeon S, You DG, Um W, Kim JY, Yoon HY, Chang H, Kim DE, Park JH, Kim H, Kim K (2018) Iodinated echogenic glycol chitosan nanoparticles for X-ray CT/US dual imaging of tumor. Nano 2(2):117–127