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Hydrogel-based platforms for site-specific doxorubicin release in cancer therapy

Chunbao Zang[1†], Yu Tian[2,3†], Yujing Tang[4†], Min Tang[5], Dingyi Yang[6], Fangfang Chen[5], Mohammadreza Ghaffarlou[7], Yanyang Tu[2*], Milad Ashrafizadeh[8,9*] and Yan Li[10*]

Abstract

Hydrogels are promising candidates for the delivery of therapeutics in the treatment of human cancers. Regarding to the biocomaptiiblity, high drug and encapsulation efficacy and adjustable physico-chemical features, the hydrogels have been widely utilized for the delivery of chemotherapy drugs. Doxorubicin (DOX) is one of the most common chemotherapy drugs used in cancer therapy through impairing topoisomerase II function and increasing oxidative damage. However, the tumor cells have developed resistance into DOX-mediated cytotoxic impacts, requiring the delivery systems to increase internalization and anti-cancer activity of this drug. The hydrogels can deliver DOX in a sustained manner to maximize its anti-cancer activity, improving cancer elimination and reduction in side effects and drug resistance. The natural-based hydrogels such as chitosan, alginate and gelatin hydrogels have shown favourable biocompatibility and degradability in DOX delivery for tumor suppression. The hydrogels are able to co-deliver DOX with other drugs or genes to enhance drug sensitivity and mediate polychemotherapy, synergistically suppressing cancer progression. The incorporation of nanoparticles in the structure of hydrogels can improve the sustained release of DOX and enhancing intracellular internalization, accelerating DOX's cytotoxicity. Furthermore, the stimuli-responsive hydrogels including pH-, redox- and thermo-sensitive platforms are able to improve the specific release of DOX at the tumor site. The DOX-loaded hydrogels can be further employed in the clinic for the treatment of cancer patients and improving efficacy of chemotherapy.

Highlights

- Hydrogels are 3-dimensional polymeric networks for therapeutic delivery.
- Doxorubicin (DOX) efficacy has been challenged with drug resistance development.
- Hydrogels can deliver DOX in a sustained manner to enhance its anti-cancer function.
- Hydrogels co-deliver drugs and genes in increasing DOX sensitivity.

†Chunbao Zang, Yu Tian and Yujing Tang contributed equally to this work.

*Correspondence:
Yanyang Tu
tufmmu@188.com
Milad Ashrafizadeh
dvm.milad1994@gmail.com
Yan Li
liyan0983@163.com

Full list of author information is available at the end of the article

- Natural-based hydrogels, nanocomposite-incorporated hydrogels and stimuli-responsive types improve DOX cytotoxicity.

Keywords Hydrogels, Cancer therapy, Doxorubicin, Chemotherapy, Drug delivery

Introduction

The presence of somatic mutations can result in the development of tumor and such mutations can be accumulated during the process of tumorigenesis with significant changes in both tumor-suppressing and –promoting factors [1]. The accumulation of these mutations occurs during the time and mutagens can be inherited or happen randomly. The number of mutations for the neoplastic transformation of a normal cell is different and according to the mathematical models, it can vary based on age and incidence [2]. In addition to understanding the underlying mechanisms involved in tumorigenesis and the efforts for cancer diagnosis, the treatment of cancer has been followed through the application of radiotherapy, chemotherapy and surgical resection. In the recent years, immunotherapy has been also introduced as an ideal mainstay for the tumor therapy [3]. Moreover, the alternative strategies have been deployed for the treatment of cancer by the application of nanostructures [4–6]. Currently, the gold standard treatment for cancer is chemotherapy. However, the chemotherapy has two major issues including side effects and resistance. Currently, the major focus is on the chemoresistance, adverse impacts can be alleviated by a number of strategies. The cancer chemoresistance has been considered as a main hurdle towards the appropriate cure of cancer patients [7]. In order to solve drug resistance, a strategy was the application of combination therapy using drugs with various mechanisms of actions, known as polychemotherapy that has been applied in the therapy of solid and haematological tumors [8–10]. As a result of promise provided by combinational therapy, various kinds of chemotherapy regimens were developed that they utilized various approaches in terms of dose intensity such as use in short periods or high concentration of chemotherapy drugs [11–13]. The resistance to chemotherapy can occur in two distinct strategies including intrinsic and acquired drug resistance. The intrinsic drug resistance is observed before chemotherapy and it is due to the presence of oncogenic factors to reduce efficacy of this strategy. On the other hand, acquired drug resistance is observed after chemotherapy to change the sensitive cancer cells into insensitive tumor cells. The acquired drug resistance can result from mutations during chemotherapy and upregulation of alternative survival pathways [14]. Furthermore, the high degree of molecular heterogeneity in the tumors is observed [15] that can cause resistance to therapy. Hence, it is a priority to develop novel types of strategies for overcoming chemoresistance in human cancers.

Regarding the aggressive nature of cancer, the heterogeneity of tumor microenvironment (TME) and the low efficacy of current chemotherapeutics, the researchers have emphasized on employment of nanostructures to reverse cancer drug resistance. The function of nanoparticles in the reversal of chemoresistance is versatile and they have demonstrated efficacy in the delivery of chemotherapeutics with nucleic acids [16], siRNA [17, 18] and miRNA [19] in boosting tumor suppression. In addition to genes, the nanoparticles are able to create a scaffold for the co-delivery of drugs in enhancing drug sensitivity in human cancers [20, 21]. The mutations in the cancer can cause drug insensitivity that one of them is mutant p53, while nanoparticles for the co-delivery of cisplatin and fluvastatin can mediate mutant p53 degradation to overcome drug resistance [22]. Furthermore, the nanoparticles are able to mediate TME remodelling such increasing M1 polarization of macrophages [23] that may be beneficial in overcoming chemoresistance. Other important features of nanoparticles include combination of hyperthermia and chemotherapy [24], stimuli-responsive materials [25] and surface-functionalized nanostructures [26] in decreasing drug resistance. In the recent years, hydrogels as 3-dimenstional materials have been introduced for the cancer therapy. Similar to nanoparticles and other kinds of (nano)materials, the hydrogels are able to provide sustained release of drugs, providing co-delivery with drugs or genes [27, 28], inducing phototherapy [29] and accelerating immunotherapy [30–34]. Regarding such importance of nanobiotechnology and hydrogels, the current manuscript has been dedicated to understanding the function of hydrogels in the prolonged delivery of doxorubicin (DOX) as a common chemotherapy drug for reducing drug resistance. The hydrogels can mediate co-delivery of DOX with genes or drugs. The stimuli-responsive hydrogels and natural-based hydrogels have been also used for DOX delivery in tumor inhibition. Such subjects are discussed in the current paper to improve the knowledge towards the function of hydrogels in potentiating cancer chemotherapy. Figure 1 schematically depicts the deployment of hydrogels in DOX delivery.

Search strategy

The papers used for the discussion were chosen from Googlescholar, Pubmed and Sciencedirect with the keywords of "Hydrogels + Biomedical application", "Doxorubicin + Resistance", "Hydrogels + Doxorubicin + Delivery".

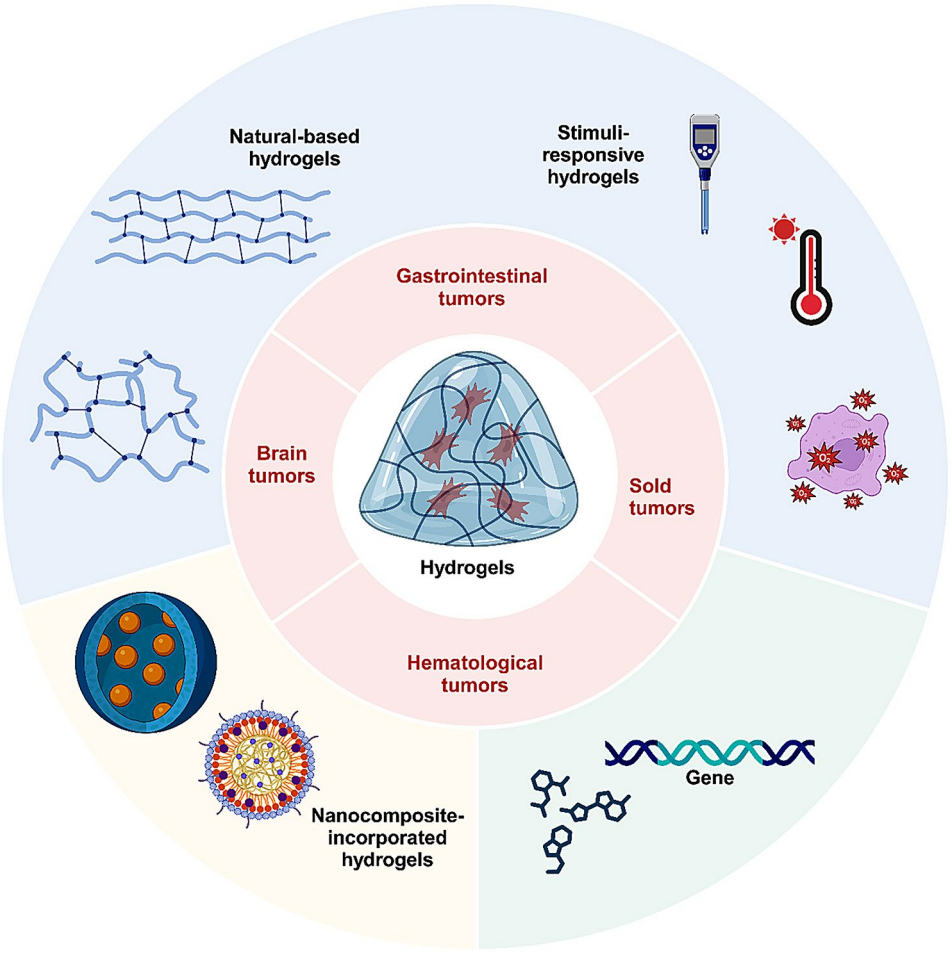

Fig. 1 The deployment of hydrogels in doxorubicin delivery. The hydrogels are able to co-deliver doxorubicin with other genes or drugs to mediate synergistic tumor suppression. Moreover, the stimuli-responsive hydrogels can increase the specific release of doxorubicin at the tumor site to enhance cancer suppression. The development of hydrogels from natural polymers including chitosan, gelatin and alginate can improve biocompatibility and biodegradability in the cancer therapy and doxorubicin release. Doxorubicin can be loaded on nanoparticles and incorporate into the hydrogels to improve sustained release of doxorubicin in cancer chemotherapy (Biorender.com)

We mainly selected the recent publications for discussing in the current paper.

Hydrogels: basics, synthetic strategies and oncological application

The hydrogels have shown potential in the absorption of water in large volume to swell because of hydrophilic groups including -NH_2, -COOH, -OH, -$CONH_2$, -CONH, and -SO_3H. The scaffold of hydrogels can be generated by crosslinked polymeric chains that its formation can sometimes happen as a result of crosslinked colloidal clusters [35]. The hydrogels display flexibility and softness due to their ability in water absorption [36]. The hydrogels can be developed from the natural or synthetic polymer chains [37–41]. The hydrogels have similar characteristics to the living tissues due to their large water volume, soft structure and porous structure. The hydrogels were first developed in 1960 by Wichterle and Lim through the synthesis of poly-2-hydroxyethyl methacrylate (PHEMA) hydrogel that was utilized as a filler for eye enucleation and contact lenses [42]. Since then, multiple experiments have investigated the efficacy of hydrogels for the drug delivery and release of bioactive molecules [43–46]. The introduction of hydrogels for tissue engineering was performed in 1990s [47–50]. The unique features of hydrogels including swelling rate, stiffness, degradation and mech size can be changed and determined by changing the ratio of hydrophilic and hydrophobic rations, the concentration of polymers and reactions conditions including temperature and container, among others [51–54]. The previous years have noted the growing deployment of hydrogels as implantable, injectable and sprayable structures for the tissues and organs [55, 56].

The synthesis of hydrogels is mainly based on utilization of hydrophilic monomers to generate a crosslinked

network for water absorbance. There is a phenomenon known as sol-gel transition that occurs during the transformation of polymeric mixtures from sol state into gel state [57]. There are two major modalities for the development of hydrogels such as chemical and physical crosslinking. The chemically synthesized hydrogels can be covalently crosslinked and there are a number of methods including grafting, radical polymerization, click chemistry, enzymatic reactions, thermo-gelation and radiation crosslinking. Furthermore, the addition of ions including Ca^{2+}, Mg^{2+}, Zn^{2+} can mediate the ionic bound formation in the polymeric precursors to stimulate gelation. On the other hand, natural-based hydrogels are mainly developed based on the self-assembly physical crosslinking mechanisms including altering the intermolecular interactions through ionic crosslinking, hydrophobic interactions and hydrogen bonded gels. All these processes are achieved by modifying the temperature of the hydrogel precursor, either by increasing it to 37 °C or by drastically decreasing it to between −20 and −80 °C [58]. Notably, the paramters can be altered or adjusted during the gelation process to finally obtain an appropriate hydrogel network [59].

In respect to the significant challenged faced in the tumor therapy, the experiments have focused on the application of alternative structures in tumor suppression. The thermosensitive hydrogels and ROS-sensitive nanogels can be loaded in composites to introduce a sequential drug release structure for inducing immunotherapy through delivery of LY3200882, increasing ROS levels, suppression of epithelial-mesenchymal transition (EMT) and reducing immune evasion [60]. The hydrogels are able to enhance tumor penetration [61] and in addition to prolonged drug delivery, they can induce ferroptosis [62]. The hydrogels utilized for the treatment of cancer can be isolated from natural sources such as alginate and collagen or guanosine and isoguanosine [63, 64]. The hydrogels are able to combine therapies such as mixing chemotherapy and phototherapy or chemotherapy and immunotherapy [65] to enhance tumor ablation [66]. The hydrogels can be also used as vaccines in cancer therapy. The $CaCO_3$ biomineralized hydrogel has been developed for the encapsulation of DC fusion cells (FP) to provide tumor-associated antigens in increasing cancer immunotherapy [67]. One of the most common uses of hydrogels is delivering chemotherapy drugs, such as paclitaxel and epirubicin, to prevent recurrence and metastasis [68]. In respect to such features of hydrogels in cancer therapy, the next sections will focus on the site-specific delivery of DOX by hydrogels.

Doxorubicin: an overview resistance

As an anthracycline compound, DOX uses four distinct mechanisms for exerting its anti-cancer activity: (A) The development of DNA intercalation through binding to DNA base pairs in impairing the structure of DNA and suppressing replication of cells; (B) Suppressing the function of topoisomerase II to inhibit the re-ligation of DNA strands, causing DNA breacks in enhancing apoptosis and errors in DNA synthesis; (C) Increase in the generation of ROS to mediate damages into the lipids, proteins and DNA; (D) The induction of DNA damage and promotion in oxidative damage can cause apoptosis by DOX. However, the previous experiments have shown that changes the molecular profile of cancer cells can cause resistance into DOX chemotherapy. There are different factors in each specific cancer for it. For instance, in osteosarcoma, the upregulation of METLL1 and WRD4 is observed to enhance proliferation and metastasis of tumor as well as providing the capacity for the emergence of DOX resistance [69]. More importantly, DLX2 has been shown to upregulate HOXC8 for suppressing CDH2, causing EMT and promoting DOX resistance [70]. In addition to the molecular pathways, a number of cells can participate in the drug resistance such as mesenchymal stromal cells that secrete hyaluronan for promoting DOX resistance in breast tumor [71]. Since DOX is commonly deployed in the tumor elimination and its insensitivity also occurs, the studies have focused on applying new strategies to reverse chemoresistance. In the recent years, the most popular strategy has been the employment of nanostructures for the delivery of DOX. Notably, the induction of hyperthermia by the nanoparticles can increase the potential of DOX in glioblastoma therapy [72]. Moreover, the dendritic cells can secrete extracellular vesicles and loading VEGF-A-siRNA and DOX can suppress angiogenesis in the treatment of glioma [73]. The liposomal nanoparticles can co-deliver bufalin and DOX to impair malignancy of breast tumor stem cells and suppress their self-renewal ability [74]. The delivery of ursolic acid and DOX by hyaluronic acid/dextran-based micelles to the mitochondria can enhance the potential of chemotherapy in reversing drug resistance [75]. Given these facts, using hydrogels for delivering DOX in cancer therapy is highly recommended.

Hydrogels in the delivery of doxorubicin

An overview of hydrogel function in doxorubicin chemotherapy

Overall, the use of hydrogels for the delivery of DOX can increase its accumulation in the tumor site and promotes intracellular accumulation. This improved the cytotoxic function of DOX. On the other hand, since DOX is released in a prolonged manner from hydrogels and low concentration of DOX is used, there is low chance for the

development of drug resistance. The peptide hydrogels have been applied for the delivery of DOX. The hydrogels and nanogels can be developed from FY3 peptides. The hydrogels were synthesized using solvent-switch strategy, while nanohydrogels were developed from top-down strategy using TWEEN®60 and SPAN®60 as stabilizing agents. The drug release from hydrogels was based on peptide composition. The DOX-loaded nanogels demonstrate low drug release (20–40%) over a period of 72 h. The DOX-loaded hydrogels and nanogels can reduce the viability of tumor cells up to 57%, while their biocompatibility is high [76]. This provides the fact that peptide hydrogels are beneficial for the delivery of DOX in cancer therapy. Ade-FFF nucleo-peptide hydrogels can deliver DOX to suppress tumor proliferation and mediate apoptosis through caspase-3 upregulation [77]. The DOX-loaded hydrogels demonstrate cytotoxicity against breast and cervical cancers [78]. The hydrogels can be synthesized through cross-linking of 8-arm PEG glyoxylic aldehyde and 8-arm PEG hydrazine using glyoxylic hydrazone linkages to covalently encapsulate DOX for the treatment of cancer. The sustained release of DOX was 81.33%, while this release was 42.87% at the physiological conditions. Moreover, such DOX-loaded hydrogels can decrease tumor growth up to 40.50% [79]. The hydrogels can be developed by the interactions among DOX and the peptide in the β-sheet conformation to mediate prolonged release of DOX in cancer therapy [80]. Notably, the DNA-based nanoparticles have been utilized for the delivery of cargo [81, 82]. The function of CpG DNA in immune induction can be improved by the development of highly branched structures including Y-shaped DNA [83] and dendrimer-like DNA [84]. Moreover, the ligation of structured DNA units including X-shaped DNA can develop DNA hydrogels [85]. In this regard, the X-DNA has been used as a building agent and by ligation, it can develop DNA hydrogels. These hydrogels can mediate the maturation of dendritic cells and they can suppress growth of tumor cells [86].

Melanoma is one of the malignant diseases of skin tissue emanated from melanocytes and it is an aggressive tumor [87, 88]. In spite of using surgical resection, there is relapse of melanoma due to the incomplete resection [89]. The introduction of immunotherapy including PD-1/PD-L1 blockade antibodies can improve the melanoma therapy [90–92], but a few of cancer patients (20–40%) respond to immunotherapy. Therefore, the application of chemotherapy is still promising in melanoma suppression. Therefore, the hydrogels have been developed based on the Schiff-base linkages between N-succinyl chitosan (SC) and oxidized dextran (OD) to deliver DOX. The alteration of molar ratio of amino groups to aldehyde groups (NH2/CHO) of precursor solutions can affect the gelation time and mechanical features of the synthesized hydrogels. Regarding the injectable shear-shinning feature of the hydrogels, they demonstrated self-healing property. The hydrogels released DOX in a prolonged manner in response to pH and suppressed melanoma progression. Moreover, the hydrogels increased M1 polarization of macrophages in melanoma immunotherapy (Fig. 2) [93].

Co-delivery of doxorubicin and drugs by hydrogels

Upon the development of drug resistance, one of the potential mechanisms for the reversal of this phenomenon was the combination therapy. Although the combination therapy and application of drugs with various mechanisms of actions have been beneficial in drug sensitivity, the issue of drug resistance has not been solved completely due to the poor pharmacokinetic profile of the drugs. Therefore, hydrogels have been utilized as platforms for the co-delivery of drugs to potentiate cancer chemotherapy. The hydrogels have been developed from poly (lactide-co- glycolide)-poly (ethylene glycol)-poly(lactide-co-glycolide) (PLGA-PEG-PLGA) triblock copolymer to deliver cisplatin and DOX in osteosarcoma therapy. These polymeric hydrogels showed sol-gel transition at a suitable temperature and their degradation occurs in PBS as well as demonstrating favourable biocompatibility. The hydrogels can provide the sustained release of cisplatin and DOX to promote proliferation inhibition. In addition to proliferation suppression, this co-delivery by hydrogels induced apoptosis and promoted necrosis [94]. It is suggested to develop hydrogels in a way to induce sequential release of cargos. The self-assembled hydrogels have been prepared from tris(aminoethyl)amine (TREN) and phenylalanine. The addition of monovalent anions including $H_2PO_4^-$ and HSO_4^- can disrupt the hydrogels, whereas other anions including Cl^-, HPO_4^{2-}, CO_3^{2-}, HCO_3^- or SO_4^{2-} demonstrate no impact on gel stability. $H_2PO_4^-$ anions can mediate a nanofiber-to-nanoglobule morphological alterations in the self-assemblies of TREN. There was slow reformation of nanofibers upon ageing, showing the reversibility of the anion-gelator interaction. These hydrogels with sensitivity to anion and pH can provide the co-delivery of DOX and propranolol and mediate the sequential release to enhance its anti-cancer activity [95]. Notably, the hydrogels have been explored for the delivery of more than two drugs in cancer chemotherapy. In this case, PLGA-PEG-PLGA hydrogels have encapsulated DOX, cisplatin and methotrexate to synergistically suppress osteosarcoma. The peritumoral injection of hydrogels in mice showed the tumor inhibition for 16 days and enhance apoptosis. On the other hand, the hydrogels did not demonstrate toxicity on the other vital organs of body, highlighting their biocompatibility [96]. Another method is the development of hydrogels according to the

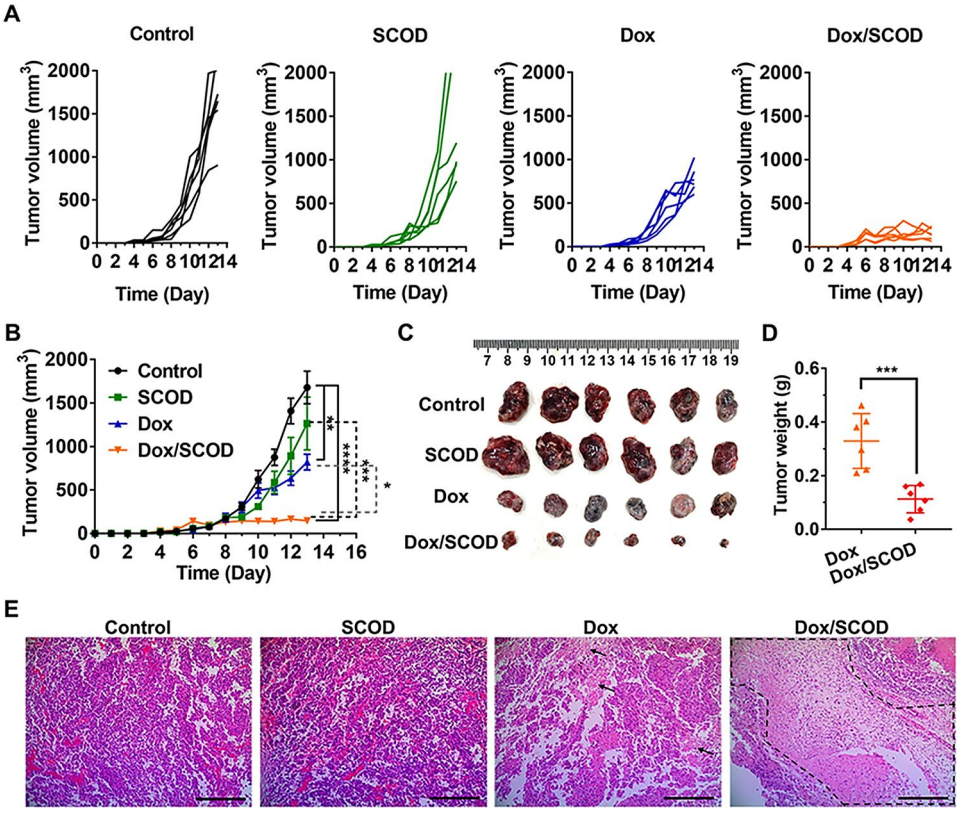

Fig. 2 Evaluating the anti-cancer activity of Dox/SCOD hydrogels. (**A** and **B**) The tmor volume is compared in the different groups, showing that treatment can decrease tumor volume that is more evident in Dox/SCOD group compared to Dox or SCOD alone. This treatment decreased tumro volume from day 13 onward, exerting better anti-cancer function. (**C**) The photographs of the excised tumors demonstrate the better potential of Dox compared to SCOD in decreasing the size of tumors. However, the highest anti-cancer activity is related to the Dox/SCOD, significantly decreasing the size of tumor. (**D**) Compared to Dox alone, the tumor weight demonstrates significant reduction upon application of Dox/SCOD (decrease in tumor weight from 0.3 g to less than 0.2 g). (**E**) The H&E staining demonstrated the presence of apoptosis and necrosis, highlighted with black dotted lines. Reprinted with permission from Elsevier [93]

host-guest interactions among a hyperbranched polyglycerol derivative and α-cyclodextrin to mediate prolonged delivery of DOX and camptothecin in cancer therapy [97]. The hydrogels have been significantly applied for local cancer therapy and such application becomes more prominent by the prolonged release of drug from hydrogels. The hydrogels comprised of N-isopropylacrylamide, cellulose, citric acid, and ceric ammonium nitrate have been shown to significantly make delay in the release of cargoes, being only % of DOX and 3% of niclosamide released after 1 week [98].

Indomethacin (IND) is a non-steroidal anti-inflammatory drug which has been significantly used for the alleviation of inflammation, pain and fever [99]. The combination of DOX and IDN exerts a synergistic anti-cancer impact and IND downregulates MRP1 to promote accumulation of DOX within cells and promote anti-cancer function [100, 101]. There have been a number of reports using oligosaccharide formulations and dextran micelles, highlighting the fact that IND can promote anti-cancer potential of DOX [100, 102]. In line with this, hydrogels have been prepared from CS-dextran phosphate carbamate to co-deliver DOX and IND. This hydrogel system can provide the prolonged release of DOX and delayed release of IND to improve anti-cancer function of DOX [103].

The delivery of DOX along with natural products can provide new insights in the treatment of cancer. Celastrol is a natural compound that has poor pharmacokinetic profile. Therefore, hydrogels have been used for the co-delivery of DOX and celastrol in enhancing apoptosis and decreasing proliferation of cancer cells [104]. Another natural compound that can be co-delivered with DOX in cancer therapy is curcumin. Notably, the self-assembled peptide hydrogels have been shown to co-deliver DOX and curcumin in the suppression of head and neck cancer through induction of apoptosis and cell cycle arrest [105]. It is suggested to first develop DOX-loaded hydrogels and then, load another drug on them to promote drug loading and encapsulation efficiency. In this regard, DOX has been grafted into oxidized pectin (pec-Ald) to develop self-healing hydrogels and subsequent loading

of limonin. The synthesis of hydrogel was based on the P(NIPAM195-co-AH54) cross-linking and the hydrazone bond and such hydrogels displayed self-healing property. It was shown that these hydrogels can be degraded by enzyme due to the pectin composition and they suppressed lung cancer [106].

More importantly, some synthetic complexes have been also co-delivered with DOX by hydrogels in cancer therapy. The host-guest complexes between pH-responsive micelle derived poly(ethylene glycol) chains and α-cyclodextrin can result in the development of hydrogels to co-deliver hydrophilic 8-hydroxyquinoline glycoconjugate and hydrophobic DOX. These hydrogels showed fast drug release profile in the acidic pH and along with prolonged release of cargo, the hydrogels are able to suppress tumor proliferation in a synergistic manner [107]. Hence, the hydrogels are promising structures for the co-delivery in cancer suppression [108, 109].

In spite of significant efforts in this field for the co-delivery of DOX and drugs in cancer therapy, there are a number of perspectives that should be considered for the future studies:

A) It is suggested to use other kinds of polymers including PLGA and PNIPAM to improve the drug loading and encapsulation efficiency of the hydrogels. In this case, the natural polymers can be also embedded for improving biocompatibility and biodegradability.
B) Other kinds of synthetic methods including cryopolymerization, click chemistry, and photo-crosslinking to improve structural integrity and encapsulation potential of hydrogels.
C) The stimuli-responsive hydrogel for the co-delivery have been investigated only in a few experiments and more studies are required that can be obtained through application of polymers including polyacrylic acid (PAA).
D) It is encouraged to use layer-by-layer techniques for improving the sequential drug release by multi-layered hydrogels.
E) It is suggested to utilize the biodegradable polymers including PCL, PLGA and gelatin to monitor degradation rate of hydrogels.
F) The gold nanoparticles, silver nanoparticles and silica nanostructures loaded with different drugs can be embedded in the hydrogels for improving their potential in cancer therapy.
G) The application of electrospinning technique can lead to the development of nanofibrous hydrogel scaffolds for improving drug loading and adjusted release of DOX.
H) The microfluidic synthesis and 3D printing can be utilized as continuous production techniques for the development of scalable hydrogels in DOX delivery.
I) The green chemistry methods can be utilized for the development of hydrogels to improve their biocompatibility and increase potential in DOX delivery.

Co-delivery of doxorubicin and genes by hydrogels

In respect to the development of drug resistance, the current strategies have focused on the application of hydrogels for the delivery of DOX as chemotherapy drug with other genes. The genes can be targeted for a specific pathway in the treatment of cancer. This pathway may be responsible for the development of drug resistance in human cancers. Therefore, it is suggested to use hydrogels for the co-delivery of genes and DOX in cancer chemotherapy. Moreover, the application of hydrogels allows the encapsulation of gene not only for improving its accumulation at the tumor site, but also protection against the enzymatic degradation. Therefore, this can significantly improve the potential of genes in cancer therapy and sensitizing them to the function of DOX in cancer chemotherapy. In this regard, the hydrogels have been developed from methacrylated glycol chitosan (MGC) and then, they have been loaded with DNA and DOX. These hydrogels are able to provide prolonged release of the cargo and deliver them into the tumor site for the induction of immune cells in cancer immunotherapy [110].

One of the oncogenic factors responsible for the progression of tumor cells is PLK1. Therefore, the downregulation of PLK1 can increase response of tumor cells to DOX chemotherapy. In this way, shRNA can be used to suppress PLK1. However, shRNA has sensitivity to enzymatic degradation and there is a need for the development of nano-scale delivery systems. Therefore, thermosensitive PLGA-PEG-PLGA hydrogels have been developed to co-deliver PLK1-shRNA and DOX for the complete inhibition of tumor within 16 days and it can mediate apoptosis [111].

In spite of the development of hydrogels for the delivery of genes, there is still a long way for the co-delivery with DOX. First of all, siRNA is significantly applied for the downregulation of oncogenic factors. In spite of the importance of siRNA in the cancer therapy, this genetic tool is sensitive to the degradation. On the other hand, siRNA has been used along with DOX for the treatment of cancer [112–114]. Therefore, the co-loading of siRNA with hydrogels can significantly improve the potential of these structures in cancer therapy. Moreover, CRISPR/Cas9 has been utilized in the treatment of human cancers [115–118]. Loading CRISPR along with DOX on the hydrogels can significantly improve the response

of cancer cells to chemotherapy. More attention should be paid into the regulation of cell death mechanisms by genetic tools delivered by hydrogels to enhance DOX sensitivity.

Nanoparticle-embedded hydrogels

One of the notable advances in the application of hydrogels in cancer therapy is loading other kinds of nanostructures in the hydrogel networks to maximize the process of delivery and promote tumor suppression. This strategy has been also widely used for the DOX delivery to improve cancer removal. Moreover, the hydrogels can be developed based nanoparticles, known as nanocomposite hydrogels. In this way, injectable hydrogels have been synthesized from cellulose nanocrystals (CNCs) using amphiphilic copolymers, poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(ε-caprolactone-co-lactide (PCLA). The mechanical features and physicochemical properties of hydrogels have been improved through incorporating CNCs with amphiphilic PCLA copolymers. CNCs) played a unique role in physically reinforcing the PCLA copolymers' micelle network by forming intermicellar bridges. The rod-like CNCs embedded PLCA micelles at low temperature were able to transform into a stable viscoelastic hydrogel network at physiological temperature. DOX was loaded into hydrogels through hydrophobic and hydrogen bonding interaction. The injection of these self-healing hydrogels into mice led to the adjusted biodegradation with high biocompatibility lacking toxic impact on the implantation site or surrounding tissues. These hydrogels suppressed tumor growth through delivery of DOX [119]. Another method for the development of hydrogels from CNCs is that poly (acrylic acid) (PAA) is grafted onto CNCs and then, their doping with magnesium oxide (MgO) is followed. The nanocomposites demonstrated loading efficineyc of 79% for DOX and they were able to suppress cancer progression through the release of DOX in response to pH that was 53.7% in 24 h [120]. The incorporation of nanoparticles into hydrogels can improve the potential of DOX in cancer therapy. An example is loading DOX and ginsenoside Rg3 on chitosan (CS) and cell-penetrating peptide (R6F3)-based nanostructures to load into thermosensitive hydrogels. The Rg3-embedded nanostructures facilitated DOX-mediated immunogenic cell death. Such hydrogels demonstrated high potential in cancer immunotherapy in breast tumor and their combination with PD-L1 blockage, enhanced the number of memory T cells and reduced PD-L1 enrichment [121].

Until now, multiple kinds of radiosensitizers have been developed [122–125] and among them, a significant interest has been made towards gold nanostructures that can absorb X-rays and provide the concentration of radiation absorption. The experimental evidences have highlighted the role of gold nanostructures as radiosensitizier [126, 127]. Regarding this, thermosensitive hydrogels have been developed from Pluronic F127 for the delivery of gold nanostructures and DOX. This hydrogel has been comprised of 22% F127 and hydrogels released gold nanoparticles and DOX in a prolonged manner. These hydrogels were able to suppress progression of melanoma and hepatocellular carcinoma. Moreover, they caused chemotherapy through DOX delivery and function of gold nanostructures upon irradiation. These hydrogels reduced tumor size and suppressed proliferation of cancer [128].

The nanocomposite hydrogels are promising candidates for the development of several therapeutic strategies. Notably, the NIR- and pH-sensitive nanocomposite hydrogels with injectable feature have been developed based on sodium alginate-graft-dopamine (SD) and biomimetic polydopamine-Fe(III)-doxorubicin nanostructures. These hydrogels are able to exert anti-cancer activity and decrease the adverse impacts of DOX. Moreover, the nanoparticles can change light into hear for the elimination of tumor cells (melanoma). The hypoxia in the TME can be ameliorated by the hydrogels through degradation of endogenous hydrogen peroxide (H_2O_2) into oxygen (O_2) [129]. In another method, the combination of agarose hydrogel with DOX-loaded iron-gallic acid nanostructures has been performed to treat osteosarcoma. These hydrogels are able to enhance local temperature due to the response to NIR irradiation. Hence, they cased photothermal therapy and the degradation of agarose hydrogels released the DOX-embedded nanostructures. Then, DOX enhances hydrogen peroxide generation in promoting ROS levels via Fenton reaction, mediating apoptosis [130].

The current studies provided valuable insights regarding the application of nanocomposite-loaded hydrogels in DOX delivery and cancer removal. The synthesis of such hydrogels is complex and it maybe challenging for the precise control of their properties for future application in clinical studies. Hence, the scalability of the nanocomposite-incorporated hydrogels should be considered. There is also additional concern regarding the storage and transportation of the nanocomposite-embedded hydrogels. The physico-chemical features of hydrogels can be affected by the factors including temperature, pH and humidity. The experiments are also suggested to load other kinds of nanostructures including graphene oxide, carbon nanotubes, and metal-organic frameworks (MOFs) to improve the features of hydrogels including drug loading, release profiles, and therapeutic efficacy. One aspect that has been ignored in the present studies is the functionalization of nanoparticles, especially functionalization with ligands to improve their targeting ability. Moreover, it is encouraged to load genes on the

nanoparticles loaded in the hydrogels to improve their anti-cancer activity.

Natural-based hydrogels in doxorubicin delivery

As a natural polymer, alginate has been widely utilized for the synthesis of hydrogels because of biocompatibility and affordability [131]. Alginate has a anionic feature and I can be derived from marine brown algae, Pseudomonas, and Azotobacter bacteria [132], comprised of α-L-guluronic acid and β-D-mannuronic acid repeats. The ionic hydrogels can be synthesized from alginate containing divalent cations, i.e. Zn^{2+}, Ca^{2+}, and Ba^{2+}, since alginate is enriched in -COOH group [133]. However, a drawback to the ionically cross-linked alginate hydrogels is the poor drug loading capacity and low mechanical strength, restricting their application in drug delivery [134–137]. Hence, it is suggested to use covalent cross-linking strategy to improve the properties of alginate-based hydrogels [138]. The injectable and redox-sensitive hydrogels have been developed through inverse electron demand Diels-Alder reaction between alginate-norbornene and a water-soluble PEG based disulfide cross-linker. The cross-linker possessed PEG chain containing two disulfide bonds along with two terminal tetrazine groups. The developed hydrogels demonstrated favourable swelling rations, porous network and high drug loading (92%) along with favourable mechanical features. The presence of glutathione (10 mM) led to the release of 90% of DOX, while the release at physiological condition was low (less than 25%). In spite of high anti-cancer activity against tumor cells, their toxicity on fibroblast cells was partial (Fig. 3) [138]. Notably, the alginate-based hydrogels have demonstrated the potential for the delivery of DOX-loaded nanostructures (detailed description can be found in Sect. 4.4). The alginate-based hydrogels were prepared through ionic cross-linking at room-temperature that demonstrated favourable solid-like elastic features. These hydrogels were able to deliveer DOX-loaded PLGA nanoparticles and magnetite nanostructures along with a slow initial burst release, they delivered DOX in a prolonged manner to mediate thermo-chemotherapy of tumor due to the function of magnetite nanoparticles in the heat generation [139].

Another natural material is nanocellulose that has been comprised of the highly structured cellulose chains. The cellulose nanofibrils (CNFs) are elongated and flexible nanocelluloses with a width of 3–5 nm up to 20–50 nm and length of 500 nm to a few microns [140]. The intensive mechanical disintegration strategy is usually used for the synthesis of CNFs that is followed by chemical pretreatment to decrease the consumption of energy. The polyion complex hydrogels can be developed from CNFs. The dissolving pulp via subsequent periodate oxidation, chemical modification and microfluidization were used to develop CNFs. Upon the development of aldehyde contents, the chemical modifications were performed to react with aldehydes, finally generating anionic CNFs with carboxylic acid groups (DCC) and another one is the cationic CNF possessing quaternary ammonium groups (CDAC) through imination with Girard's reagent T. Increasing the time of periodate oxidation decreases the length of fibrils. The self-standing hydrogels can be developed from the combination of DCC and CDAC dispersions at pH 4 and 5. These hydrogels were able to deliver DOX and released the cargo in response to pH with high biocompatibility [141]. Moreover, the development of cellulose hydrogels for the delivery of DOX in cancer therapy can be mediated through

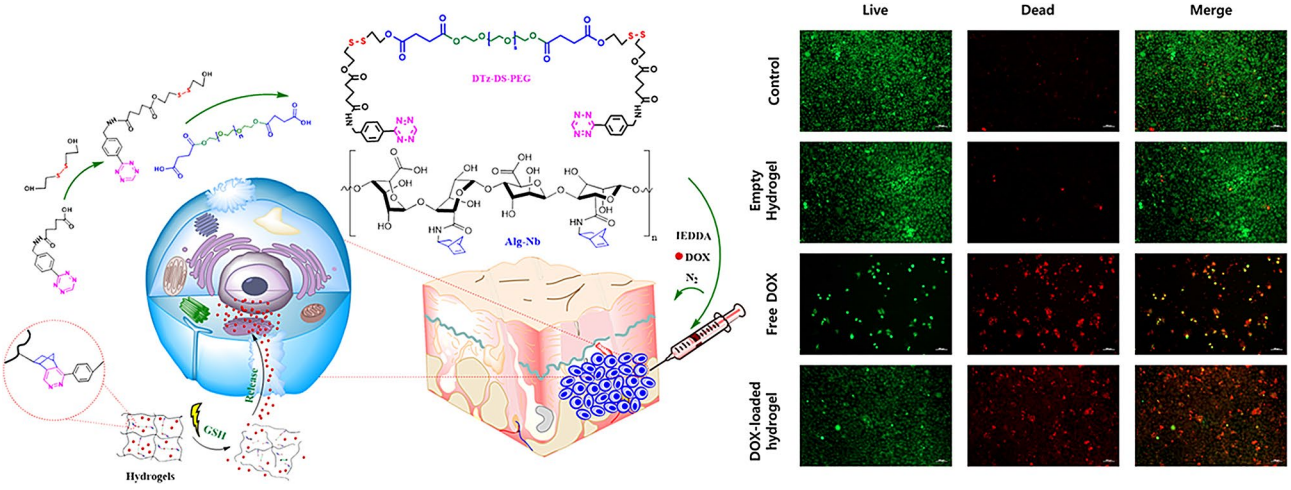

Fig. 3 Left side) The development of water-soluble disulfide cross-linker and alginate-based injectable and reduction-responsive hydrogels., Right side) Confocal laser scanning micrographs showing cytocompatibility of empty hydrogels in HeLa cells, and in vitro anti-cancer activity of free-DOX (40 µg) and DOX loaded-hydrogels (40 µg equi. of free-DOX). Cell viabilities were determined by calcein-AM/ethidium homodimer-1 assay (live/dead assay). Green color represents live cells, whereas, red color represents dead cells. Scale bars showing 100 µm. Reprinted with permission from Elsevier [138]

eversible ketoester-type acylhydrazone linkages, providing the hydrogels with self-healing feature and the ability to release cargo in response to pH and high biocompatibility [142].

The most common natural product for the synthesis of hydrogels in DOX delivery is chitosan (CS) [143]. CS has been widely used because of its biocompatibility, biodegradability and pH-responsive feature [144, 145]. In order to improve the mechanical strength of the hydrogels, it is suggested to load PEG into the hydrogels and monitor their degradation rate [146]. Therefore, the PEG-CS hydrogels have been developed for the co-delivery of DOX and sodium bicarbonate to improve drug delivery and mediate alkaline buffering of extracellular acidity. The low pH enhances the release of DOX from the hydrogels to suppress breast cancer (Fig. 4) [147]. Notably, the surface modification of CS-based hydrogels by a layer of alginate can improve the DOX release feature of hydrogels for 12 days [148]. Therefore, after the preparation of hydrogels from green sources, it is suggested to modify them with other green polymers to improve the property for the release of DOX in cancer therapy. A combination of CS and HA can be used for the development of hydrogels to co-deliver DOX and cisplatin. The hydrogels were synthesized from CS and then, modification with nitrosalicyl aldehyde and aldehyde HA was performed. These hydrogels delivered the drugs for lung cancer therapy and released the drug in response to pH [149].

A derivative of CS is glycol CS that possesses biocompatibility and biodegradability, and the water solubility can be improved by glycol groups, making it promising for drug delivery [150–153]. Moreover, glycol CS has been explored for the development of self-healing hydrogels. The self-healing hydrogels are able to recover their structure even upon damage. The self-healing hydrogels have been extensively investigated in terms of their synthesis method, the mechanism involved in self-healing and encapsulation of cells [154–158]. In this line, the studies have focused on the development of hydrogels based on glycol CS and telechelic difunctional poly(ethylene glycol) (DF-PEG). These self-healing hydrogels showed high drug loading for gemcitabine

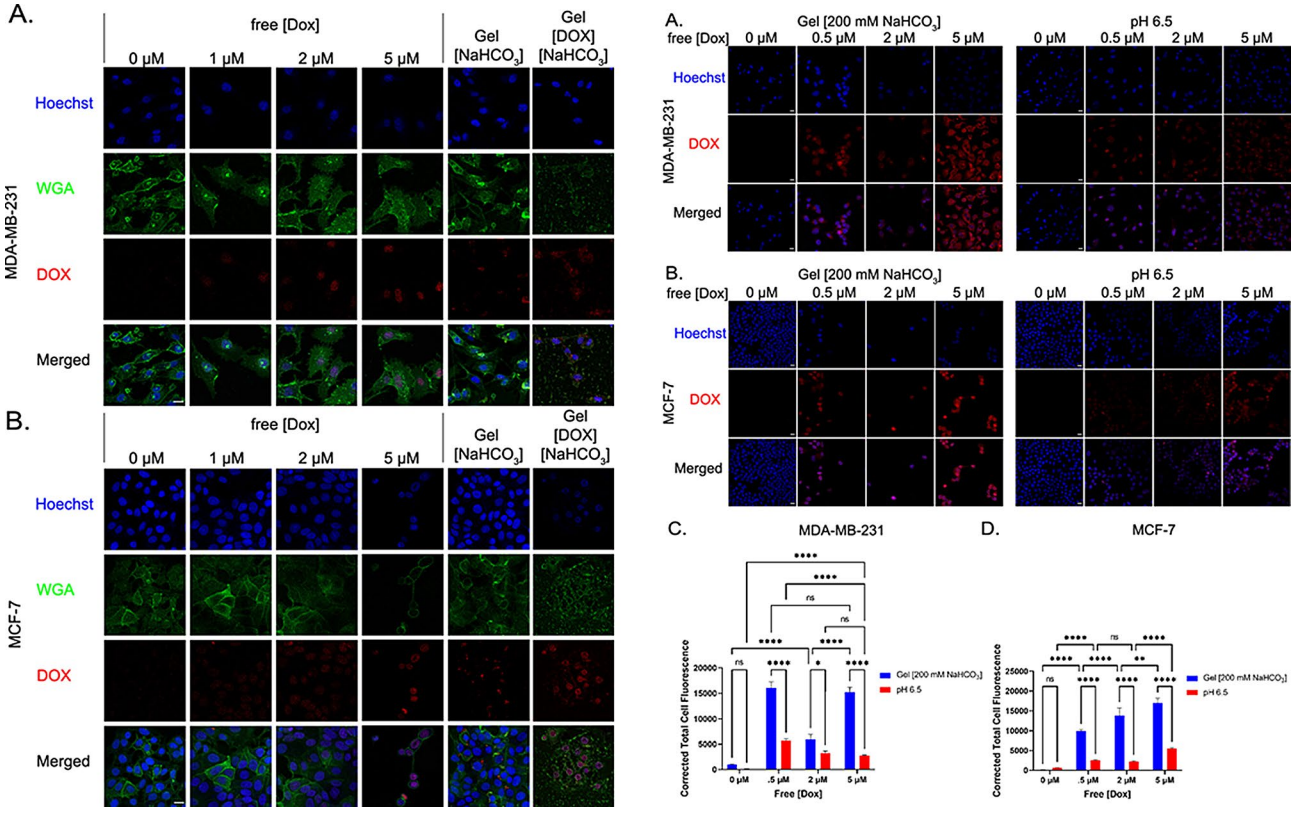

Fig. 4 Left side) (**A**, **B**) Evaluating the internalization of free doxorubicin (0–5 μM) and doxorubicin upon release from sodium bicarbonate-loaded chitosan-PEG hydrogels at low pH (6.5) for 48 h. The development of hydrogels was based using a sodium bicarbonate concentration of 100 mM and a doxorubicin concentration of 50 μM. This gives a doxorubicin concentration of 0.47 ± 0.05 μM in the cell solution over a 48 h period. This is afluorescence microscopy images of MDA-MB-231 and MCF-7 cells. Right side) (**A** and **B**) pH-dependent localization of doxorubicin in the nucleus and related confocal fluorescence images. (**C**, **D**) The tumor cells were treated with free doxorubicin (0–5 μM) and the fluorescent intensity of drug was evaluated in the nucleus. There was an increase in the nuclear accumulation of doxorubicin. Each value represents the mean ± SE ($n = 29–237$, MDA-MB-231) and mean ± SE ($n = 28–192$, MCF-7) with significance defined as * $p < 0.5$; *** $p < 0.001$, **** $p < 0.0001$. Reprinted with permission from ACS [147]

and DOX in the different concentration levels including 10%, 40% and 80%. The interaction of hydrogels and gemcitabine was based on van der Waals adsorption, while the interaction of hydrogels and DOX was based on hydrogen bonds and van der Waals adsorption [159]. Regarding this, such hydrogels can be utilized as potential structures for the drug delivery in cancer treatment.

Another natural biomaterial is gelatin with a number of benefits including biocompatibility, biodegradability, low immunogenicity, affordability, availability and the presence of functional groups providing the opportunity for its surface modification [160]. However, one of the problems of gelatin is its sensitivity into enzymatic degradation. Therefore, SMCM as a polysaccharide with chemical modification has been extensively utilized for the drug delivery. SMC has also shown biocompatibility, biodegradability and low immunogenicity [161]. Regarding this, the hydrogels have been synthesized from gelatin, sodium carboxymethyl cellulose, and gelatin/sodium carboxymethyl cellulose through a lyophilization technique to deliver DOX in the suppression of lung cancer [162].

Overall, the current studies provide significant findings regarding the development of hydrogels from green sources. However, there are a number of limitations that should be considered. In case of hydrogel synthesis from alginate, the ionically cross-linked alginate hydrogels may suffer from poor drug loading ability and low mechanical strength, decreasing their application in DOX delivery. Moreover, alginate-based hydrogel biocompatibility should be improved, as they demonstrated a little toxicity on fibroblasts. Regarding the development of hydrogels from nanocellulose, it should be noted that CNFs require extensive mechanical disintegration and chemical pretreatment that may be energy-consuming. In spite of PEG addition to CS hydrogels for improving their mechanical features, their inherent mechanical strength may be still a restriction. Furthermore, despite the function of alginate layer on CS hydrogels for improving their release profile, the overall control of DOX release and consistency require more evaluation. The glycol CS-based hydrogels possess favourable self-healing ability, but their long-term stability requires investigation. Regarding the gelatin-based hydrogels, the sensitivity to enzymatic degradation is a limitation. Although the combination of gelatin with sodium carboxymethyl cellulose can improve the features in DOX delivery, further changes in the degradation rate and release profile should be investigated. Finally, the biocompatibility of nature-based hydrogels should be evaluated in long-term.

Stimuli-responsive hydrogels

The TME has unique characteristics including alterations in temperature, pH, redox status and enzymatic activity. Therefore, the development of stimuli-responsive hydrogels can enhance tumor suppression. The most common type of hydrogels used for the DOX delivery is thermosensitive hydrogels. The thermosensitive hydrogels can be developed from poloxamer 407 (P407) and then, combined with DOX. The 25% of P407 demonstrated desirable gelation feature and the pore sizes were at the range of 30–180 μm. The release of DOX from hydrogels occurred within 120 h and they significantly improved the anti-cancer activity of DOX [163]. Another method for the development of hydrogels is combining DOX-CS conjugates, acrylated Pluronic and DOX at 37 degrees C. The presence of chitooligosaccharide-DOX conjugates diminishes the burst release of DOX from the hydrogels and 37 degrees C, it was shown that CS-DOX conjugates can be degraded into hydrophilic oligomers through reversed-phase chromatography. The intratumoral injection of these hydrogels suppresses the tumor growth and increases DOX activity against lung tumor [164].

In the recent years, an emphasis has been directed towards the development of multi-stimuli-responsive hydrogels. One of them is the development of hydrogels sensitive to light and temperature that can be synthesized from amphiphilic triblock copolymers, poly(N-isopropylacrylamide)-b-poly(4-acryloylmorpholine)-b-poly(2-((((2-nitrobenzyl)oxy) carbonyl) amino)ethyl methacrylate) (PNIPAM-b-PNAM-b-PNBOC) and they can deliver gemcitabine and DOX. The self-assembly of PNIPAM-b-PNAM-b-PNBOC triblock copolymers into polymers was performed and they comprised of hydrophobic photosensitive PNBOC cores, while the inner shel contained hydrophilic PNAM along with thermoresponsive PNIPAM coronas. The critical gelation temperature (CGT) relies on the composition and concentration of polymer that longer hydrophobic PNBOC block or a higher polymer concentration can diminish the CGT. The exposure to UV irradiation enhances CGT because of the PNBOC core. The reduction in temperature or UV irradiation can mediate gel-to-sol transition. The delivery of both DOX and gemcitabine by hydrogels can impair tumorigenesis and these hydrogels demonstrated response to temperature and irradiation (Fig. 5) [165].

Due to the competition in the TME for the proliferation and increased metabolism of tumor cells, the acidic byproducts are continuously produced that can reduce the pH of TME. As a result, the studies have focused on the development of pH-sensitive hydrogels in DOX delivery. The solid-phase synthesis method can be utilized for the development of pH-sensitive hydrogels based on P1 peptide that has affinity to surfactant-like peptides because of its hydrophobic tail and hydrophilic head. The biodegradable hydrogels can be self-assembled in neutral conditions from P1 peptide. These injectable

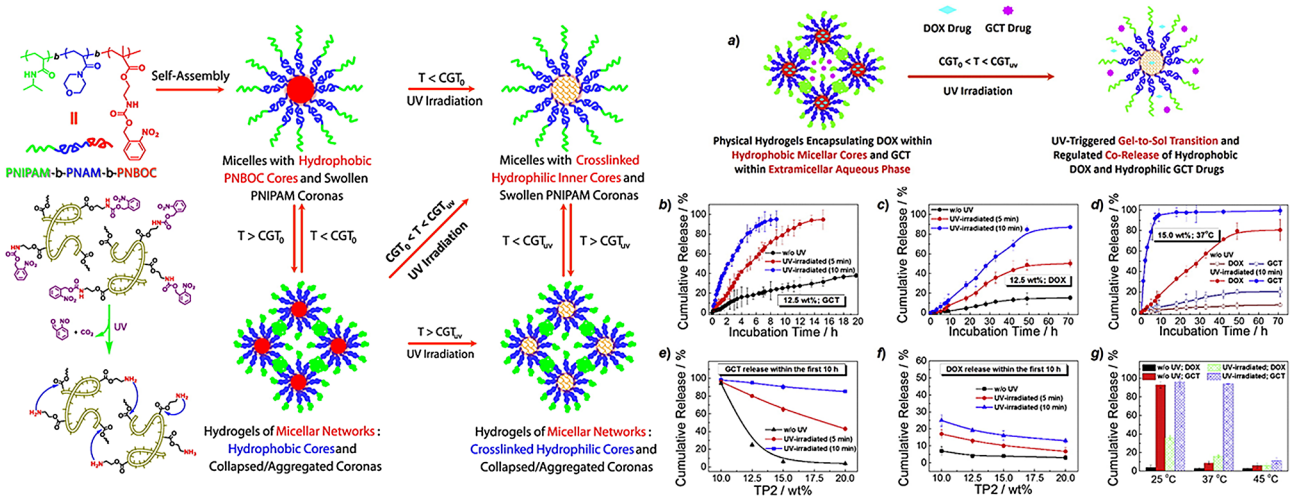

Fig. 5 The development of thermos- and light-responsive hydrogels for delivery of DOX and gemcitabine. Left side) A schematic representation for the design of thermo- and light-sensitive hydrogels in the co-delivery of doxorubicin and gemcitabine. Right side) (**a**) The presence of UV-triggered gel-to-sol transition and release of cargo in response to temperature. (**b**, **c**) The release profile of gemcitabine (**b**) and doxorubicin (**c**) from the hydrogels. (**e**, **f**) Cumulative release of gemcitabine and doxorubicin. (**d**) Release profile of cargo in presence or lack of 10 min UV irradiation. (**g**) The cumulative release in response to temperature for doxorubicin and gemcitabine within the first 10 h from the non-treated and UV-irradiated (10 min) hydrogels. Reprinted with permission from Elsevier [165]

hydrogels displayed favourable biocompatibility and their release profile is higher in pH 5.8 compared to pH 7.4. These hydrogels increase the accumulation of DOX at the tumor site and boost anti-cancer activity [166]. Notably, the hydrogels can be developed in a way to respond to both pH and temperature for the delivery of DOX. The hydrogels were developed from thiolated chitosan (CSSH) and their gelation degree was estimated to be 37 °C. The release of cargo from hydrogels occurred at the acidic pH of TME due to the presence of disulfide bonds. The hydrogels delivered DOX and curcumin-embedded liposomal nanocarriers to promote therapeutic index and diminish adverse impacts. The gelation time of hydrogels was 8–12 min in normal conditions and the hydrogels released cargo in pH 5.5 compared to pH 7.4 in first 24 h that approximately 10% of DOX was released, while the release of curcumin was at 24–120 h that is maybe due to encapsulation by liposomes. These hydrogels can effectively reduce the progression of tumor cells [167]. The hydrogels derived from poly(N-isopropylacrylamide-co-itaconic acid) (PNIAAm-co-IA) and CS via ionic crosslinking using glycerophosphate (GP) also display thermo- and pH-responsive features in the DOX delivery for breast cancer therapy [168].

The NIR has shown high potential for the deepen penetration into the biological tissues (up to 3.2 cm) and it is a non-invasive manner with less scattering in the tissues [169]. The NIR-sensitive coumarin-based nanoscale delivery systems are promising due to the impact of coumarin on the high single/two-photon absorption [170]. The exposure of coumarin into the irradiation at the wavelengths of 310–800 nm can lead to the photo-dimerization [171]. In this regard, the NIR-sensitive hydrogels have been developed from hyaluronic acid and coumarin to deliver DOX. The cross-linking of coumarin and hyaluronic acid was performed by processing terminal tetrazine (Tz) groups. The hydrogels can be generated at the physiological conditions by the inverse electron demand Diels–Alder cross-linking reaction between Nb and Tz functionalities, while the hydrogels would be porous networks due to the release of N_2 gas. The hydrogels were injectable and NIR irradiation induced the release of DOX from hydrogels due to the presence of NIR-sensitive coumarin-ester cleavage and suppressed the tumorigenesis (Fig. 6) [172].

In spite of the development of thermosensitive hydrogels, there is still challenge regarding the precise and predictable release kinetics. The alterations in physiological temperature can change the drug release profile. It is also challenging to optimize the gelation temperature align with preserving stability and functionality. There is also initial burst release from thermosensitive hydrogels that may reduce the therapeutic index of DOX. The multifunctional hydrogel synthesis is complicated and it may be challenging in the clinical level. It should be ensured and investigated that how multi-functional hydrogels can be still stable in the physiological conditions with the various changes in the pH, temperature and light exposure. In case of light-responsive hydrogels, the penetration depth of the light should be evaluated. The pH-sensitive hydrogels may respond to pH alterations in the non-cancerous tissues or body fluids, affecting their release rate. Similarly, there is risk of initial burst release in pH-sensitive hydrogels that may endanger their therapeutic index.

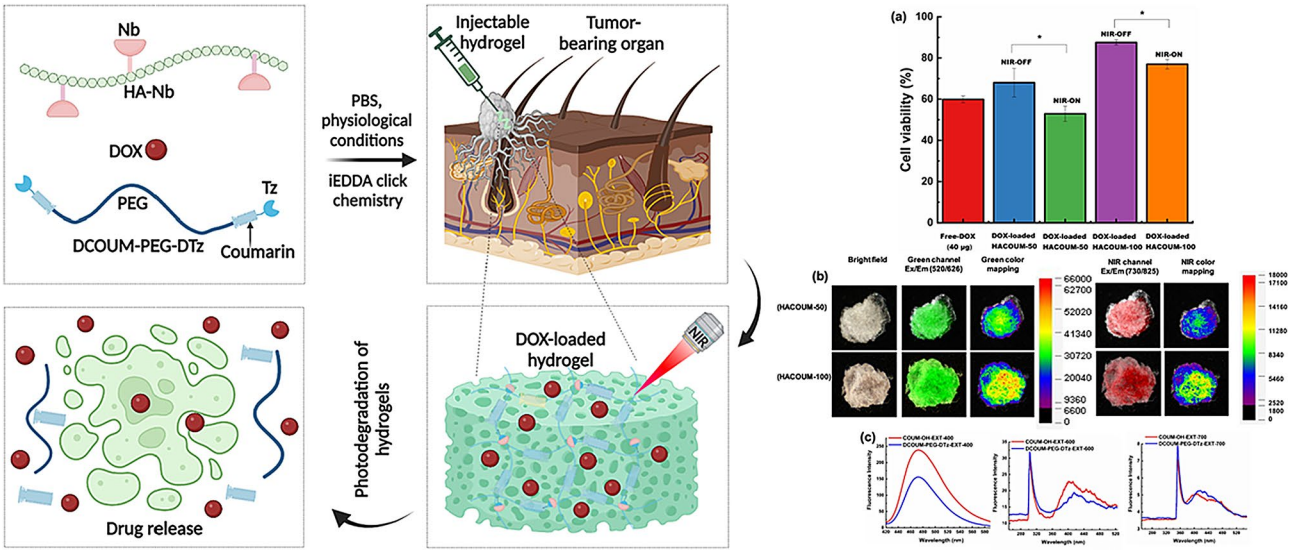

Fig. 6 Left side) A schematic design for the development of hydrogels and their potetnail in the cargo release in response to light. Right side) (**a**) The anti-cancer activity of doxorubicin-loaded hydrogels in vitro against BT-20 cells in the presence and lack of irradiation (4-watt, 5 min). Based on the figure, the application of irradiation causes more decrease in the viability of cells compared to the lack of irradiation. Moreover, DOX-loaded HACOUM-50 demonstrates better anti-cancer activity compared to DOX-loaded HACOUM-100, decreasing viability of cells to less than 60%. (**b**) FOBI images of hydrogels achieved after excitation at different wavelengths of light, and (**c**) fluorescence spectrophotometer curves of COUM-OH intermediate and DCOUM-PEG-DTz obtained after excitation at various wavelengths of light. Upon excitation at 400 nm, the hydrogels showed a favourable fluorescent emission peak at 473 nm. Upon excitation at 600 nm, there were two emission peaks including 303 and 401 nm. Reprinted with permission from Elsevier [172]

In spite of the potential of NIR light in the biological tissue penetration higher than visible light, the penetration is still poor that may reduce the therapeutic index of DOX-loaded NIR-responsive hydrogels. Table 1 summarizes the recent advances in the application of hydrogels for DOX delivery.

Conclusion, perspectives and challenges

The treatment of cancer is mainly dependent on the application of chemotherapeutics. However, the frequent application of chemotherapy drugs can lead to the changes in the molecular profile of cancer cells to trigger drug resistance. Moreover, a number of cancer cells are aggressive enough and demonstrate significant alterations in the molecular profile causing resistance before exposure to any chemotherapy drug. Therefore, there is high risk of chemoresistance and most of the tumor cells are able to trigger resistance into therapeutics. In spite of the application of polychemotherapy, the challenge is still available and therefore, gene therapy developed for combination therapy. Moreover, the immunotherapy or radiotherapy combination with chemotherapy were introduced for the suppression of tumors. However, there is still a need for the development of novel and more effective therapeutics for drug resistance. DOX is among the most common chemotherapeutics in tumor suppression. The solid and haematological tumors have demonstrated the resistance into DOX chemotherapy. Therefore, the materials and nanostructures have been developed for the delivery of DOX to not only improve the cytotoxicity of DOX, but also minimize drug resistance and side effects. Among the different kinds of platforms, materials and structures utilized in drug delivery, hydrogels have been significantly deployed in tumor suppression. Therefore, the present review focused on the role of hydrogels in DOX delivery. Noteworthy, hydrogels can encapsulate the DOX and release in the tumor site. The prolonged release of DOX by the hydrogels can significantly increase the anti-cancer activity. The nanocomposite-incorporated hydrogels provide better insights regarding the delivery of DOX, since the DOX-loaded nanoparticles can be released by hydrogels in the cancer site and then, the nanoparticles mediate the internalization of DOX in the tumor cells to trigger endosomal/lysosomal escape for increasing nuclear transfer of DOX in the intercalation with DNA and suppressing proliferation and mediating cell cycle arrest. In order to improve the biocompatibility and biodegradability of hydrogels, the hydrogels have been prepared from natural sources including CS. The hydrogels can provide a platform to co-deliver DOX with other drugs or genes to mediate chemotherapy/phototherapy or chemotherapy/immunotherapy in the suppression of tumors. A progress in the field of DOX delivery of the application stimuli-responsive hydrogels including thermo-, pH- and redox-sensitive hydrogels. Since there are several enzymes in the TME, a special attention should be also directed to the enzyme-responsive hydrogels in cancer therapy.

Table 1 A summary of the application of hydrogels in doxorubicin delivery

Vehicle	Cargo	Remark	Reference
Multi-sensitive hydrogels	Doxorubicin Indocyanine green	Development of hydrogels from hyaluronic acid and diselenide based cross-linker The generation of hydrogels according to the inverse electron demand Diels-Alder click chemistry Loading cargo in the porous structure of hydrogels High biocompatibility and release of cargo in response to pH and NIR irradiation	[173]
HPMA hydrogels	Doxorubicin	The linkages in the structure of hydrogels are sensitive to hydrolytic cleavage Suppression of tumor growth and increasing survival of animal model	[174]
HA-based hydrogels	Doxorubicin Urotensin II (hUII)	Reduction in the viability of tumor cells to less than 80%	[175]
Alginate and chitosan hydrogels	Doxorubicin Ciprofloxacin	The development of hydrogels from aldehyde-alginate (aAlg) and acrylic acid-chitosan (aCS) using Schiff base and ionic interactions Injectable hydrogel with self-healing features Elimination of tumor cells	[176]
Nanocomposite-incorporated hydrogels	Doxorubicin	The development of hydrogels from nitrogen-doped carbon quantum dots, docotubicin and hydroxyapatite Inhibition of breast cancer	[177]
Silk fibroin hydrogel	Doxorubicin Cy7	Co-delivery to exert synergistic impact Induction of chemotherapy and phototherapy Synthesis of hydrogels from silk fibroin	[178]
Cellulose-based hydrogels	Doxorubicin	The cellulose grafted hydrogel were loaded with doped calcium oxide nanocomposites to increase the potential for the adjusted release of doxorubicin in cancer therapy	[179]
GelMA hydrogels	Doxorubicin	The hydrogels were developed from GelMA and the ZIF-8@CeO2 nanosturctures were loaded to deliver and release doxorubicin for the removal of tumor cells and suppressing cancer relapse	[180]
Starch/PVA/g-C3N4 hydrogel	Doxorubicin	The release of cargo in a pH-sensitive manner and induction of apoptosis in breast cancer	[181]
Hydrogel microparticles	Doxorubicin	The development of hydrogels from carboxymethyl cellulose Prolonged release of cargo Biocomaptibility Tumor growth suppression	[182]
Cellulose hydrogels	Doxorubicin	Prolonged release of doxorubicin The injectable hydrogels demonstrated self-healing feature pH-sensitive release of cargo and suppression of tumorigenesis in vivo	[183]
Polypeptide hydrogels	Anti-PD-L1 Doxorubicin	The injectable hydrogels are able to co-deliver cargo for the induction of immunogenic cell death	[184]
Lipopeptide-based hydrogels	Doxorubicin	Permeable hydrogels capable of 80% of drug encapsulation The release of drug in the acidic pH	[185]
MXene-DNA hydrogel	Doxorubicin	Loading MXene sheets in the hydrogels can provide their photothermal impact Release of DOX for cancer chemotherapy High biocompatibility and injectable feature	[186]
Sodium deoxycholate hydrogel	Doxorubicin Resveratrol	pH-responsive feature The first rapid release of resveratrol and then release of doxorubicin Injectable feature Tumor growth suppression	[187]
Magnetic natural hydrogel	Doxorubicin	The development of hydrogels from alginate and gelatin Loading Fe_3O_4 nanostructures in the hydrogels with the size of 25 nm pH-responsive feature	[188]
Chitosan hydrogels	Doxorubicin	Porous and pH-sensitive hydrogels Reduction in the proliferation of breast cancer cells	[189]
Nanocomposite hydrogels	Doxorubicin Nox4 inhibitor	The development of hydrogels from carboxymethyl chitosan and tetrabasic polyethylene glycol Induction of immunogenic celkl death Suppression of cancer-associated fibroblasts by Nox4 inhibitor Preventing T cell exahaustion	[190]
PLGA-PEG-PLGA hydrogels	Doxorubicin	Loading doxorubicin and arginine-terminated nanoparticles containing KIAA1199 specific shRNA inside the hydrogels Synergistic cancer therapy Doxorubicin creates intercalation with DNA ShRNA reduces KIAA1199 levels to prevent tumor malignancy	[191]

Table 1 (continued)

Vehicle	Cargo	Remark	Reference
3D printed hydrogels	Copper-doxorubicin complexes	The development of hydrogel from Pluronic F127 and sodium alginate Copper ions and doxorubicin Burst release of doxorubicin and sustained release after that Induction of apoptosis and ferroptosis	[192]
PSBMA hydrogels	Doxorubicin STING agonist 2′,3′-cGAMP	The PSBMA hydrogels were loaded with doxorubicin-embedded copper peroxide nanostructures Loading STING agonist 2′,3′-cGAMP into hydrogels Induction of STING axis to promote IFN-related gene expression in the inhibition of immunosuppressive TME	[193]
Natural and polymeric hydrogels	Doxorubicin	The synthesis of hydrogels from benzylaldehyde functionalized polyethylene glycol, poly(N-isopropylacrylamide) functionalized chitosan and {Mo154 } Self-healing and injectable features Drug release in response to pH and NIR irradiation Combination of chemotherapy and phototheraml therapy	[194]
Thermosensitive hydrogels	Doxorubicin Imiquimod	The exposure to NIR can cause heat generation to release doxorubicin Immunogenic cell death induction to impair progression of metastatic tumors	[195]
Thermo/pH-sensitive hydrogels	Doxorubicin	The synthesis of hydrogels from tempo-oxidized cellulose nanofiber (TOCN), polyvinyl alcohol (PVA) and a polydopamine (PDA) Stimulation of chemotherapy and phototherapy Suppression of breast cancer	[196]
Heparin-based hydrogels	Doxorubicin	The development of hydrogels from heparin-β-cyclodextrin derivatives (Hep-β-CD), α-cyclodextrin (α-CD) and pluronic F-127 Self-healing feature The electrostatic interaction between heparin and doxorubicin	[197]
MOF-based hydrogels	Doxorubicin	pH- and ATP-sensitive release of cargo Elimination of tumor cells Prolonged release of cargo Suppressing cancer growth	[198]
Thermosensitive hydrogels	Doxorubicin	The development of injectable polypseudorotaxane-based supramolecular hydrogel using α-CD and the PEG chains of the pseudo-block copolymer Prolonged delivery of doxorubicin Uptake by tumor cells Exerting anti-cancer activity against the tumor cells that are resistant to chemotherapy	[199]

In the recent years, drug resistance has been the major reason for the therapy failure in clinical setting. Therefore, the patients would benefit from development of novel strategies. The DOX-loaded hydrogels are promising candidates in this case capable of site-specific delivery of DOX to the tumor site in improving its nuclear accumulation in cancer therapy and preventing drug resistance. However, the large-scale production of hydrogels should be considered. Moreover, the long-term biocompatibility of these hydrogels should be evaluated. The development of hydrogels from natural sources in improving their mechanical behaviour and strength should be improved. Both chemical and physical cross-linking can be utilized for the development of hydrogels in DOX delivery. For chemical cross-linking, the agents including glutaraldehyde, genipin, and carbodiimides can be used, while physical cross-linking based on hydrogen bonding, hydrophobic interactions, and ionic interactions are used and polymers such as chitosan, alginate and gelatin are utilized for this purpose.

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Consent for publication
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Competing interests
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Author details
[1]Department of Radiation Oncology, Division of Life Sciences and Medicine, The First Affiliated Hospital of USTC, University of Science and Technology of China, Hefei, Anhui 230031, China

[2]Research Center, The Huizhou Central People's Hospital, Guangdong Medical University, No. 41 Eling North Road, Huizhou, Guangdong, China
[3]School of Public Health, Benedictine University, Lisle, USA
[4]Department of General Surgery, Southwest Jiaotong University Affiliated Chengdu Third People's Hospital, Chengdu, China
[5]Department of Oncology, Chongqing General Hospital, Chongqing University, Chongqing 401120, China
[6]Department of Radiation Oncology, Chonging University Cancer Hospital; Chongqing Key Laboratory of Translational Research for Cancer Metastasis and Individualized Treatment, Chongqing University Cancer Hospital, Chongqing 400030, China
[7]Bioengineering Division, Institute of Science and Engineering, Hacettepe University, Ankara 06800, Turkey
[8]Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, PR China
[9]Department of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, Shandong 250000, China
[10]Department of Gastrointestinal Surgery, Changzhou Cancer Hospital, No.1 Huaide North Road, Changzhou, Chin, China

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