Injectable hydrogels for anti-tumour treatment: a review

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Abstract: Injectable hydrogels have become the material of choice for the treatment of solid tumours based on their advantages in loading anti-tumour materials. This study reviews the main scientific research achievements on anti-tumour injectable hydrogels in recent years. The gel-forming mechanism of anti-tumour injectable hydrogels was listed, and the advantages and difficulties of each gel-forming mechanism were summarised. In addition, several current anti-tumour methods based on injectable hydrogels were discussed, including chemotherapy, hyperthermia-based therapy, catalytic therapy and immune therapy, as well as the integration of diagnosis and treatment to monitor the progress of cancer treatment. The anti-tumour mechanism and the advantages and disadvantages of various tumour treatments were analysed. Finally, the future development trend of injectable hydrogels for anti-tumour therapy was discussed.

1 Introduction

Malignant tumours are common and frequently occurring diseases that seriously threaten human health. The clinical efficiency of tumours is annually increasing, but the results are still not satisfying. At present, the number of deaths caused by tumours has surpassed that of cardiovascular diseases and become the number one cause of death in humans. Notably, humans have a long way to go to overcome malignant tumours [1, 2].

Intravenous anti-tumour drugs have a certain degree of anti-tumour effect and anti-tumour nano drug-delivery systems such as the liposome drug-delivery system, polymer micelle drug-delivery system and inorganic nano drug-delivery system, are proposed to increase the stability, bioavailability and targeting of drugs [3]. However, intravenous anti-tumour drugs often have high accumulation in the liver and spleen due to poor targeting, leading to a lack of drugs in the tumour and insufficient anti-tumour effects. In addition, the drug will produce certain toxic and side effects on normal cells and tissues due to the circulation of body fluids [4, 5]. To solve this problem, it is technically feasible to inject high-concentration anti-tumour drugs into the tumour. However, when drugs/nanoparticles are directly administered to tumours, the small particle size and high tumour osmotic pressure make it easy for them to leak along the path of the syringe needle. In addition, the retained drugs/nanoparticles cannot be fixed at the tumour site and can easily escape from the tumour tissue to adjacent tissues; thus, the tumour cannot be cured effectively [6]. Therefore, to achieve long-term and effective anti-tumour treatment, it will be necessary to fix the anti-tumour materials at the tumour site through a suitable carrier.

Hydrogels are one of the most popular carriers in the field of biomedicine because of its good biocompatibility, environmental responsiveness and simple preparation, especially as a drug sustained-release delivery system with excellent immune cell signalling [3] and high load capacity [7]. Drug/nanoparticle-loaded hydrogels have shown excellent therapeutic effects against melanoma, breast cancer and liver cancer [8]. The hydrogel-based anti-tumour treatments can be roughly divided into the following types: (i) chemotherapy – the targeted and sustainable release of anti-tumour drugs through the degradation of the hydrogel in vivo, (ii) thermotherapy – the thermal ablation of tumours through heat-generating nanoparticles by external stimulation such as light and magnetism, (iii) catalytic therapy – reactive oxygen species (ROSs) generation through photodynamic or chemodynamic anti-tumour therapies, (iv) immunity – immune microenvironment regulation through the use of immune drugs to treat metastatic tumours, (v) imaging-assisted therapy – monitoring the progress of tumour treatment by imaging to improve the efficiency of killing tumours. All of the above methods have been reported with a certain degree of success for anti-tumour treatment. However, the current hydrogel implants are pre-formed, which might involve large surgical incisions and cause slow wound healing and infection. Moreover, the hydrogel cannot completely fill the tumour surgical bed due to the irregular size and shape caused by surgical instruments. In addition, most pre-formed hydrogels are not biologically active and can easily cause rejection and local inflammation after implantation. Therefore, there are still limitations in the clinical application of pre-formed hydrogel loaded with anti-tumour drugs [9].

Recently, ‘injectable’ or ‘in situ gelling’ hydrogels have been intensively studied to achieve minimally invasive, seamless gap filling and fixed-point release [10]. The precursor solution mixed with the anti-tumour drug is injected into the tumour and solidified under the stimulation of external conditions (including light, heat, enzyme), to seamlessly fill the defects in the tumour. Anti-tumour drugs are fixed within the hydrogel through physical or chemical entanglement and released through the degradation of the hydrogel in vivo or external stimulation to achieve the anti-tumour effects [7, 11, 12]. Injectable hydrogel systems are able to achieve the locally sustained release of effective therapy agents, exhibiting great promise in overcoming many limitations related to the systemic toxicity and limited efficacy of current cancer therapy.

This report summarises the recent progress in anti-tumour injectable hydrogels, with an emphasis on the gelation methods of injectable hydrogel and anti-tumour mechanisms. We start with a brief overview of the gelation methods in tumour-specific micro-environments, including thermally induced gelation, photoinduced gelation, enzyme-induced gelation, metal coordination-induced gelation, self-assembly-induced gelation, click chemistry-induced gelation, Michael type addition reaction-induced gelation and Schiff-base reaction-induced gelation. Several anti-tumour
mechanisms are discussed in relation to the following aspects: (i) chemotherapy, (ii) thermotherapy, (iii) catalytic therapy and (iv) immunity therapy. In addition, imaging-assisted therapy has been explored to further progress the development of hydrogels. Finally, we propose several directions and guidelines for the design of injectable hydrogel for tumour therapy and conclude with a perspective discussion of the remaining opportunities and challenges.

2 Gelation methods of injectable hydrogel

Tumour tissues have a variety of unique microstructural and physical and chemical characteristics, including abnormal blood vessels, weak acidity, protease overexpression and hypoxia, when compared to normal tissues. Anti-tumour injectable hydrogels are usually designed by integrating modules that respond to the tumour microenvironment (TME) and cells into polymer networks. These networks undergo chemical structural transformation and microstructure rearrangement once stimulated by the TME, and then local solidification at the tumour site. Injection behaviour is highly dependent on the shear-thinning properties of the polymers [13]. The gelation of the injectable hydrogels has been designed on dynamic bond crosslinking, such as the hydrogen bond, polar bond, metal coordination [14, 15].

The method of forming injectable anti-tumour hydrogel can be roughly divided into thermally induced gelation, light-induced gelation, enzyme-induced gelation, metal coordination-induced gelation, self-assembly-induced gelation, click chemistry-induced gelation, Michael addition-induced gelation and Schiff-base reaction-induced gelation.

2.1 Thermally induced gelation

Thermally induced gelation is one of the most common types of gelation developed in recent years for local tumour therapy. Polymers with thermally gellable properties are often amphoteric, and a moderate balance of amphiphilicity or hydrophilicity is crucial in the temperature-stimulated sol–gel transition process. In the thermal gelation process, if the gelation temperature of the polymer is between the room temperature and body temperature, the system can mix with the drugs or materials at room temperature or lower, and then physically gel in situ under the body temperature after intratumoral injection based on the temperature difference between the in vitro and in vivo conditions. The experiment results showed that the PPP copolymers degraded gradually for up to 5 weeks and good biocompatibility was observed both in vitro and in vivo. Notably, heat-induced gel formation is based on weak physical bonds, and therefore the gel can degrade under the action of body fluids. Thermally induced PPP triblock copolymer hydrogels not only have the important biomaterial characteristics of degradability, but also have unique physical properties of polymers in a condensed state, and can be kept in water for several days to several months due to its slow degradation. However, diblock copolymer Pluronic® hydrogel has poor stability, which will collapse overnight when placed in a large amount of water. These results revealed that the ABA-type triblock copolymer has better biological stability than does the AB-type diblock copolymer. The triblock copolymer hydrogel has a higher crosslink density because it has two crosslinking points: the PEG bridge structure and hydrophobic tunnel. In contrast, two-block copolymer hydrogel only has a single crosslinking point. Therefore, the triblock copolymer network is more stable [20]. Thus, thermally induced triblock copolymer hydrogels are more superior based on the advantages of slow degradation, which could allow the long-term sustained release of anti-tumour drugs.

In summary, thermally induced hydrogels have a kind of physical crosslinking network, which is mostly composed of hydrophilic and hydrophobic polymers. There exist differences in the crosslink density of the hydrogel due to differences in the number of blocks and structure of the polymers.

2.2 Light-induced gelation

A light-induced gelation system utilises photoinitiators to trigger polymerisation and in situ gelation under light irradiation [21, 22]. Photoinitiated gelation can be divided into photodynamic-induced gelation and photocrosslinking-induced gelation. Photodynamic-induced gelation is a three-step process, comprising initiation, propagation and termination. In the initiation step, optical radiation excites the photoinitiator and the generation of free radicals. Then, the free radicals react with the photo-curable macromonomer to produce reactive species that can participate in propagation. Step-wise growth by crosslinking occurs during propagation. The termination step is characterised by the end of the crosslinking in the three-dimensional (3D) polymeric network [23]. The reported photosensitisers include Irgacure 2959 [24], chlorin e6 (Ce6) [25], infrared (IR) iodides [26], porphyrins [27], and aggregation-induced emission (AIE) photosensitisers [28], which are widely used in the field of light-induced gelation. Unfortunately, most photoinitiators are excited by short-wavelength light sources, such as ultraviolet (UV) light and visible light with limited tissue penetration depth [29, 30]. Therefore, it is necessary to select a photoinitiator that responds to near-IR (NIR) light to prepare injectable anti-tumour hydrogels to avoid poor gel formation due to insufficient tissue penetration.

Ce6, a photosensitiser widely used in photodynamic therapy (PDT), can respond to light of 660 nm wavelength and generate ROS, which can non-specifically destroy tumour cells, but also initiate the formation of hydrogel particles through free radical polymerisation [31]. Meng et al. [32] designed a light-triggered in situ gelation system that included Ce6-modified catalase (Ce6-CAT), PEG diacrylate (PEGDA), and imiquimod (R837)-loaded PLGA nanoparticles (RPNPs), in which PEGDA is used as a polymer matrix and RPNPs are used as immune adjuvants, expected to trigger a powerful synergistic anti-tumour effect by combining photodynamic and immunity. The gel formation process of this system can be described as follows. First, the Ce6-CAT/RPNPs/PEGDA precursor solution was
injected locally into the tumour and then the tumour is exposed to a 660 nm light-emitting diode. PEGDA was polymerised into gel by the local $\text{O}_2$ and $\text{O}_2^-$ generation with the participation of the Ce6-CAT photoinitiator under a red light (660 nm) irradiation (Fig. 1). After this light-triggered in situ gelation, CAT can be retained in the hydrogel by physical embedding, which can continuously relieve tumour hypoxia by triggering the endogenous $\text{H}_2\text{O}_2$ decomposition of the tumour to generate $\text{O}_2$, thereby improving the efficacy of PDT and reversing the immunosuppressive TME to facilitate anti-tumour immune attack. Subsequently, the tumour cell fragments generated after the death of immunogenic cells caused by PDT can be used as tumour-associated antigens, together with RPNPs as immune adjuvants, leading to strong anti-tumour immune responses.

According to reports, light-initiated gelation will consume the oxygen inside the tumour, and is not applicable under insufficient oxygen conditions. However, loading nanoparticles with photothermal conversion properties in hydrogel and synergistic thermosensitisers can promote gel formation without oxygen consumption. Meng et al. [33] prepared copper sulphide (CuS) 131I-PEGDA injectable hydrogel, using 131I-labelled CuS (CuS/ 131I) nanoparticles as photothermal-radiotherapy agents. PEGDA as the polymer matrix and light-2-azobis[2-2-imidazolin-2-yl]propane] dihydorchloride (AIPH) as a thermal initiator, to realise the combination of photothermal and brachytherapy for tumour treatment. The gel formation is described as follows: after local injection of the CuS/131I-PEGDA/AIPH solution into the tumour, the tumour site was irradiated with a 915-nm NIR laser. The CuS/ 131I nanoparticles generated heat under light conditions, increased the tumour temperature at a rapid rate, and initiated the polymerisation of PEGDA by activating the AIPH thermal initiator. The NIR-triggered PEGDA gelation allows CuS/131I to remain locally in the tumour for a long period of time, preventing radioactive leakage into normal organs. In addition, the tumour hypoxia signals were weakened because the local hyperthermia effect promoted intra-tumour blood flow by repeated NIR irradiation of the tumour, thereby producing a significant synergistic photothermal brachytherapy effect to eliminate the tumour.

Notably, light-induced gelation can be used to prepare in situ anti-tumour injectable hydrogels. However, the limited depth of light penetration, insufficient energy supply in the tumour and heating damage to the surrounding normal tissues, make light-induced gelation impractical in some cases. Therefore, to promote the light-induced gelation efficiency, it is important to choose a photosensitiser or heat-sensitive agent that can respond with high sensitivity to NIR light. At the same time, carrying oxygen or an intermediate that can produce oxygen to provide sufficient energy to photodynamic-induced gelation is also necessary.

### 2.3 Enzyme-induced gelation
In the past few decades, use of enzymatic crosslinking between existing polymers and the enzymatic polymerisation of monomers in gelation has attracted widespread attention. To improve biological applications, the idea of ‘green’ enzymatic polymerisation has become increasingly attractive due to its high catalytic efficiency, easy-to-control technology and environmentally friendly aspects. Examples of biofriendly enzymes include horseradish peroxidase, glucose oxidase (GOx) and laccase [34]. The utilisation of enzymes, particularly oxidases and peroxidases, for generating radicals via redox mechanisms is especially common for initiating radical-mediated polymerisation reactions, including vinyl chain-growth polymerisation, atom transfer radical polymerisation, thiol-ene step-growth polymerisation and polymerisation via an oxidative coupling.

According to reports, an N-hydroxyimide compound, N-hydroxy-5-norbornene-2,3-dicarboximide (HONB), can be readily reduced by β-D-glucose in the presence of GOx, which rapidly forms a carbon-centred radical. Su et al. [35] created an enzyme-reactive hydrogel by using the N-hydroxyimide–heparin conjugate, which can act as both an enzyme-mediated free radical initiator and enzyme-sensitive therapeutic carrier. Here, the enzyme-mediated redox initiation system involves GOx, N-hydroxyimide–heparin conjugate and glucose. This initiation system enables the rapid formation of carbon-centred free radicals, which can effectively initiate chain polymerisation at 37°C. The results of an in vitro experiment showed that the drug molecules that covalently anchored to the N-hydroxyimide–heparin conjugate hydrogel can be released in an enzymatic reaction because of the specific cleavage effect of heparanase on heparin. The drug-loaded gel can target cancer cells characterised by heparanase overexpression, which can minimise the adverse effects of premature drug release on normal cells.

Qiao et al. [36] designed a new hybrid nanogel with redox-responsive polymer gel shell and mesoporous silica nanoparticle (MSN) core through laccase-mediated free radical polymerisation. The polymer N,N-dimethylacrylamide (DMAA) acted as the gel network and N,N-bis(acryloyl) cystamine (BAC) with disulphides (S–S) acted as the responsive crosslinker. The laccase catalysed the reaction of N-hydroxysuccinimide (NHS) and molecular dioxygen can generate free radicals to initiate gelation. In the early stage of hydrogel synthesis, the hydrophilic anti-tumour drug DOX and the hydrophobic ultrasound (US) synergistic agent perfluorohexane (PFH) were encapsulated in the hydrogel networks and mesoporous channels of MSNs (MSNss-gel@PFH&DOX), respectively. In vivo experiments showed that MSNss-gel@PFH&DOX was activated to responsive increase DOX release after shedding hydrogel nanoshells in a reducing environment and enhanced US imaging after US-triggered temperature-sensitive PFH acoustic droplet evaporation when reaching the reducing environment in tumours.

For two main reasons, enzyme-mediated crosslinking is a widely used method in hydrogel preparation. First, the reaction is carried out under physiological conditions, which is a prerequisite for the formation of in situ injectable hydrogels. Second, the reaction is highly biocompatible. In addition, free radical species generated from enzymatic polymerisation either directly by the enzyme or indirectly via a secondary enzyme-derived product, which requires neither further thermal energy nor irradiation stimulus, unlike conventional free radical polymerisation.

### 2.4 Metal coordination-induced gelation
Metal coordination-induced gelation, also called ion crosslinking, is a method for crosslinking ionisable polymers using divalent and/or trivalent ions. The polymer has functional groups, such as carboxyl groups or hydroxyl groups. The metal ions can interact strongly with the functional groups of the polymer to promote the entanglement between the chains to form a 3D network structure. The commonly used polymers for anti-tumour injectable hydrogels are alginate (ALG) and pectin, whereas divalent metal ions (such as Ca$^{2+}$) are commonly used to crosslink the anion chain containing polycarboxylate [37].

The polysaccharide molecular chain will be crosslinked by the cation to form a network-like gel when divalent cation (Ca$^{2+}$ or...
Ba\(^{2+}\) was added to the sodium ALG solution. The gel can be used for anti-tumour therapy if anti-tumour drugs, enzymes, or cells are introduced. Zhao et al. [38] designed a multifunctional injectable hydrogel (AMD) based on Ca\(^{2+}\) and ALG, which is composed of ALG, MoS\(_2\)/Bi\(_2\)S\(_3\)-PEG (MBP) and DOX, used for tumour photothermal and chemotherapy. The combination of the α-L-guluronic acid block of ALG and Ca\(^{2+}\) enables AG/MBP/DOX solution to gel in vivo and in vitro. To avoid potential wound infection during tumour treatment, antibiotics (such as amoxicillin (AMX)) have also been successfully encapsulated in hydrogels. AMD hydrogel can act as a reservoir to control the release of encapsulated drug molecules (chemotherapy, DOX and anti-inflammatory drugs, AMX). In addition, the doped MBP nanosheets and DOX molecules give the composite hydrogel tumour photothermotherapy and chemotherapy abilities in vivo, respectively. The thermal conversion during NIR laser irradiation not only causes tumour aggregate necrosis but also triggers the release of DOX from the hydrogel matrix, thereby improving the efficiency of chemotherapy.

Chen et al. [39] reported a spray-based bioreactive immunotherapeutic fibrin gel to suppress local tumour recurrence. The fibrin gel is formed by the interaction of fibrinogen and thrombin. An equal volume of fibrinogen solution, aCD47@CaCO\(_3\) nanoparticles and thrombin were mixed to quickly form a fibrin gel when sprayed on the tumour site. The formation mechanism is that thrombin can convert fibrinogen into fibrin monomer and can also activate coagulation factor XIII to form activated XIIIa. XIIIa can catalyse the fibrin monomers to be connected to each other in a covalent bond to form a long chain of fibrin and can then be converted into a solid fibrin gel under Ca\(^{2+}\) crosslinking. Tumour treatment results showed that CaCO\(_3\) nanoparticles embedded in the gel matrix can help to release therapeutic drugs in a controlled manner by removing H\(^+\) and regulating the acidic and inflamed tumour resection environment, thereby promoting an anti-tumour immune response. In addition, the local release of aCD47 from CaCO\(_3\) nanoparticles blocked the ‘don’t eat me’ signal associated with cancer cells, allowing macrophages to clear cancer cells.

The preparation of anti-tumour injectable hydrogels by non-toxic metal ion crosslinking has been extensively studied; this method has a simple operation, a single type and is not toxic to the tissue. However, the hydrogel formed by metal coordination is unstable and easily degrades in the TME; thus, the long-term treatment of tumours cannot be achieved because of the accelerated drug release rate. Thus, increasing the crosslinking density of the hydrogel network by introducing dual networks to weaken the degradability of metal coordination hydrogels is a feasible solution.

2.5 Self-assembly-induced gelation

Self-assembled-induced hydrogels are formed with shear-thinning characteristics, which can achieve rapid in situ gelsations. The crosslinking of self-assembled hydrogels involves many weak interaction forces, and self-assembly can be achieved by adjusting the balance between attractive (e.g. electrostatic attraction, hydrogen bonding, hydrophobic forces and cohesive interaction) and opposing forces (e.g. electrostatic repulsion and solvation). Discretely, these forces are weak, but together, they help to form a stable structure [40]. The stable structure of the hydrogel will be destroyed under shear stress because of the dissociation of these dynamic physical forces. After the shear stress is eliminated, the hydrogel network will be rebuilt. Various types of shear-thinning gels have been reported, including colloidal systems that involve the self-assembly of oppositely charged micro/nanoparticles due to their excellent electrostatic attraction [41], peptide/protein-based systems [42], mingled hydrogel systems [43] and cyclodextrin (CD)-based hydrogels [44].

Qu et al. [45] developed a series of pH-sensitive polysaccharide-based hydrogels as a drug delivery system for the treatment of hepatocellular carcinoma. The hydrogel was prepared by mixing N-carboxyethyl CS (CEC) synthesised by Michael reaction and PEGDA terminated with benzaldehyde in an aqueous solution. DOX, as a water-soluble, small-molecule, anti-cancer drug, was wrapped in a hydrogel. The self-assembly of hydrogels was studied at the micro and macro levels. The hydrogel became thinner with continuous shaking, and the CEC/PEGDA hydrogel network showed rapid recovery after the shaking was stopped. The shear-thinning of CEC/PEGDA hydrogel is attributed to the dynamic covalent Schiff-base bond between the amine group from CEC and the benzaldehyde group from PEGDA. The hydrogel showed rapid self-curing without any external irradiation.

Poudel et al. [44] prepared a supramolecular hydrogel system (α-CD/M) by the host-guest interaction between the α-CD and PEG-block-poly(lactic acid) (PEG-b-PLA) micelles (M). The PEG chains are threaded inside the cavity of α-CD to form the inclusion complex, thus promoting the formation of α-CD/M hydrogel. DOX was encapsulated into the hydrogels via the micelles. Rheological studies indicated that the gelation kinetics and mechanical strength of the hydrogels could be modulated by the α-CD concentration. When the shear rate increases, the supramolecular hydrogel breaks down from a gel to sol state due to the disintegration of the inclusion complex between the PEG chains and α-CD, which greatly reduces the crosslinking density. Moreover, the viscosity of the α-CD/M hydrogel increased with the increase in α-CD concentration, suggesting that the viscosity can be easily altered by the α-CD concentration. Cell experiments showed that α-CD/M supramolecular hydrogel systems can better allow the long-term in vitro inhibition of tumour cells than that of free drugs, which can be attributed to the sustained release of the drug.

The self-assembling hydrogel can quickly form a gel at the tumour site without the need for external conditions based on the shear-thinning property when compared to light-induced gelation and heat-induced gelation. Therefore, self-assembling-induced gelation is beneficial for local tumour treatment based on the advantage of avoiding fluid leakage into the adjacent tissues or blood after injection into the tumour site. However, self-assembly hydrogels contain physically crosslinked polymerisation, which may lead to biological instability. Therefore, properly introducing chemical crosslinking in self-assembling hydrogel may make it feasible to obtain a physicochemical dual-crosslinked stable hydrogel, which can promote the biological stability of self-assembling hydrogel.

2.6 Click chemistry-induced gelation

Click chemistry, a synthesis route of generating polymer networks by joining small modular entities, was firstly proposed by Sharpless, a Nobel Prize winner in chemistry [45]. At present, click chemistry can be roughly divided into the following four types: copper(I)-catalysed azide–alkyne cycloaddition, Diels–Alder (DA) cycloaddition, thiol-ene addition and azide–nitrite addition [46, 47]. The preparation of hydrogels by click chemistry has the advantages of fast and efficient gelation, modularity and few side effects. Although click chemistry has many advantages in preparing hydrogels, it still has problems to overcome. In particular, the catalysts are often needed in the click chemical reactions due to a high thermodynamic driving force (>83.7 kJ/mol) [48]. Among these, Cu(I)-catalysed azide–alkyne cycloaddition has been widely used in the preparation of hydrogel networks, due to its reaction specificity. After the products are formed, good functional group tolerance. Reithofer et al. [49] reported a kind of oxaliplatin-derived hybrid peptide hydrogel prepared by click chemistry for localised therapy. Specifically, 2-(3-azidopropyl)-2-methylmalonic acid was used as a dicarboxylic ligand to link with oxaliplatin and form an oxaliplatin analogue; the azide functional group on the oxaliplatin analogue was then linked to the alkyl-functionalised peptide via a Cu(I) catalysed 1,3-dipolar cycloaddition reaction. The ability of these short peptide assembly units in the click chemistry to form an antiparallel mechanism, allows the functionalisation of the termini of the peptides without interfering with the self-assembling
residue. Therefore, the functionalised peptide would still be able to assemble by itself or when mixed with its parent peptide, forming hybrid hydrogels. Through extensive in vitro and in vivo evaluations, the oxaliplatin-peptide conjugates displayed significantly lower systemic toxicity and higher localisation in the target tissue compared to those in the free drug.

Cu-catalysed azide–alkyne cycloaddition is commonly used for its specificity, high yield, less toxic byproducts and reaction under physiological condition. However, the use of Cu as a catalyst is detrimental for use in biological tissue because Cu(I) ions create oxidative stress in cells. Therefore, the use of Cu should be avoided in click reactions. Research has found that ring tension can promote the Cu-free catalysed azide–alkyne cycloaddition reaction, and therefore it is not necessary to introduce metal ion catalysis during the click chemistry reaction. Fan et al. [50] reported a new class of biocompatible and biodegradable polysaccharide hydrogels derived from CS and hyaluronan (HA) via metal-free click chemistry, without the addition of a Cu catalyst. For the metal-free click reaction, CS and HA were modified with oxanorbor-nadiene (CS-OB) and 11-azido-3,6,9-tetraoxaundecan-1-amine (HA-AA), respectively (Figs. 2a and b).

The gelation is attributed to the triazole ring formation between OB and azido groups of polysaccharide derivatives (Fig. 2c). The potential of metal-free hydrogel as a cell scaffold was demonstrated by the in vitro encapsulation of human adipose-derived stem cells (ASCs) within the gel matrix. Cell culture showed that this metal-free hydrogel supports the survival and proliferation of ASCs.

In summary, there will be broad potential application in the field of biomedicine because metal-ion-free click chemistry not only retains the advantages of click chemistry but also solves the problem of biological toxicity. However, the efficiency of the click reaction will be reduced to a certain extent due to metal-ion-free catalysis. Therefore, it is urgent to improve the efficiency of the click chemical reaction, that is, reduce the thermodynamic driving force in the reaction process.

2.7 Michael addition reaction-induced gelation

Michael addition is usually called 1,4-type addition, that is, nucleophiles such as mercaptans or amines added to unsaturated carbonyl compounds (such as aldehydes and ketones) are thermodynamically favourable under physiological conditions. Thus, amines, thiols, cyanide ions, halide ions and alcohols are commonly used nucleophiles. However, halide ions and cyanide ions are inherently toxic and should be avoided in biomedical applications. Generally, polymers containing primary, secondary and tertiary amines are used for the preparation of Michael addition reaction-induced hydrogel. In addition, the thiols are the preferred nucleophiles for the preparation of injectable hydrogels, because the Michael addition of thiols proceeds spontaneously under physiological conditions [51]. Other nucleophiles, such as vinyl sulfone, α,β-unsaturated esters and maleimide, are usually conjugated to the polymer backbone.

Pati et al. [52] prepared DOX-loaded PEGDA/polyamidoamines (PAMAM) injectable network hydrogels with double stimulus–response to pH and reduced environment for tumour treatment. PEGDA/PAMAM hydrogel was obtained by aza-Michael addition reaction between the diacrylate in PEGDA and amide bond of PAMAM under physiological conditions. The metabolic activity of HeLa cells increased over time, indicating the biocompatibility/non-cytotoxicity of the synthetic PEGDA/PAMAM injectable hydrogel. In addition, PEGDA/PAMAM hydrogel showed excellent degradability in tumours with lower pH and higher dithiothreitol (DTT) concentration, since PEGDA contains ‘acetel’ as a pH-sensitive group and a ‘disulphide bond’ as a reducible bond, which results in the sustained release of DOX in the PEGDA/PAMAM hydrogel.

Moon et al. [53] described a packaging material of PEG-based hydrogel based on the thiol-Michael addition reaction for vaginal brachytherapy. The thiol-Michael addition reaction involved the catalytic addition of a thiolate to an electron-deficient olefin via a base or nucleophilic catalyst. Here, the PEG-based hydrogel was formed by ethoxylated trimethylhydride tri-(3-diol propionate) and PEGDA (PEGDA670) through the thiol-Michael addition reaction under the action of a mild base (NaHCO₃). When culturing vaginal macrophages, the hydrogel did not cause significant IL-8 upregulation or cytotoxicity, indicating a lack of immunogenicity. Preliminary image-guided studies revealed high amenability of the hydrogel materials to brachytherapy procedures.

In summary, the Michael addition reaction does not require the addition of any external stimulation or initiators that are harmful to the cells. The reaction conditions are mild, and the hydrogel can react quickly without other byproducts under the physiological conditions of the human body. Therefore, the Michael addition reaction is beneficial for the synthesis of anti-tumour injectable hydrogels.

2.8 Schiff-base reaction-induced gelation

Schiff base mainly refers to a class of organic compounds containing imine or methylamine characteristic groups (–RC=–N–), which are usually formed by the condensation of amine and an active carbonyl group. The Schiff-base reaction mechanism can be described as the nucleophilic addition reaction of carbonyl-containing aldehydes, ketones and primary amines. As a nucleophilic reagent, a nitrogen atom with a lone electron pair in an amine compound attacks a positively charged carbon atom on the carbonyl group to complete a nucleophilic addition reaction and forms an intermediate α-hydroxymine compound, which is then further dehydrated to form a Schiff base. [54] The Schiff-base reaction has many advantages, including design simplicity, reaction efficiency and preparation safety. Thus, it has been widely used in recent years to design injectable hydrogels with a 3D network structure. In addition to this, the extracellular matrix in solid tumours with acidic and pH responses has been one of the most frequently considered issues in drug delivery for cancer treatment. The acid-labile Schiff base can be cleaved by hydrolysis, and the stability of the bond decreases with decreasing pH. Therefore, the use of injectable hydrogels with a Schiff base as a drug-delivery system is beneficial for tumour treatment.

Shi et al. [55] developed a new type of injectable pH-sensitive hydrogel using modified CS and ALG, by providing DOX for
tumour treatment. This self-crosslinking hydrogel is prepared based on a Schiff-base interaction. First, DOX and succinate CS (S-chi) were conjugated by Schiff-base reaction between the keto group in DOX and the amine group in S-chi. Then, the hydrogel was formed by covalent bonded mediated by Schiff-base reaction between the aldehyde group of the activated ALG and the amine group in S-chi. The acid sensitivity of the Schiff base endows the hydrogel with a highly sensitive DOX drug release at a lower pH value. In vivo experiments were carried out in mice with the MDA-MB-231 xenograft breast tumour model, and the results showed that the injectable hydrogel effectively inhibited tumour growth.

Wu et al. [56] reported a class of crosslinked physical and chemical composite injectable nanogels (nPCGs) for the joint delivery of DOX and the protein cytokines recombinant human interleukin-2 (IL-2) and recombinant human interferon-gamma (IFN-γ). The nPCGs are designed through a quick gelation induced by the ionic crosslinking of 4-arm PEG-b-poly-(L-glutamic acid) (PPLG) and hydroxpropyl CS/4-arm PEG-b-poly(L-lysine) (HPCS/PPLL) (Fig. 3(a, b and d)), followed by the formation of covalent bonds via a Schiff-base reaction of the oxidised cholesterol-bearing dextran nanogels with HPCS/PPLL, which results in increased hydrogel moduli and improved stability (Fig. 3(c)). This nPCG, which contains DOX, IL-2 and IFN-γ, showed a synergistic anti-cancer efficacy through the regulation of apoptosis-related genes in the Janus kinase signal transducer and activator of transcription (STAT) pathway and mitochondrial pathways in xenograft tumour-bearing mice.

In summary, the hydrogel prepared by Schiff-base reaction has a stable network structure and good biocompatibility, and can be used as an excellent biomedical carrier. In addition, the hydrogel is easily cleavable in the TME to achieve drug release due to the acid sensitivity of the Schiff base, which can avoid drug leakage into normal tissues with neutral or weak alkaline pH. Therefore, anti-tumour injectable hydrogels designed by Schiff-base reaction are very advantageous for achieving effective tumour treatment.

2.9 Composite gelation

Composite hydrogels by incorporation of nanoparticles have been developed to enhance the various properties of hydrogels, such as strength and drug encapsulation efficiency. Wu et al. [57] designed a kind of nanocomposite hydrogels (CDDP/Pept-AlgNP/irinotecan (IRN)) on the basis of supramolecular peptide hydrogels and ALG nanoparticles (AlgNPs), with two drugs cisplatin (CDDP) and IRN-packed separately in the co-assembled domains of the nanocomposite. The nanocomposite hydrogel was prepared by a double crosslinking process, with the CDDP-reinforced peptide hydrogel as the first crosslinker and IRN-loaded AlgNP (AlgNP/IRN) as the second crosslinker (Fig. 4). Specifically, CDDP formed coordination bonds with carboxyl residues of peptides and reinforced the self-assembled peptide conjugates through non-covalent interactions. Next, AlgNPs were incorporated in the hydrogel matrix to serve as the inner domain, which not only further improved the mechanical properties of hydrogels through electrostatic interactions but also greatly facilitated the encapsulation of IRN as a secondary drug. In vitro drug release experiments demonstrated that a fast release of CDDP occurred prior to a tunable release of IRN, thereby achieving temporal control on drug dosing in combination therapy. In vivo experiments show that the CDDP/Pept-AlgNP/IRN hydrogel enhanced the synergism of CDDP and IRN in inhibiting the growth of cancerous cell A549, plausibly through caveolae and clathrin-mediated endocytosis of CDDP and AlgNP/IRN, respectively.

Wu et al. [58] fabricated a robust injectable thermoresponsive supramolecular poly(N-acryloyl glycine-amide-co-acrylamide) (PNAm) hydrogel bearing polydopamine (PDA)-coated gold nanoparticles (AuNPs) and DOX. The gelation mechanism was explained as follows. In the presence of photoinitiator IRGACURE 1173 and APS, AAm and NAGA was polymerised through UV irradiation and gelatinised after cooling to room temperature. The CDDP/Pept-AlgNP/IRN hydrogel acted as the second crosslinker, while the positively charged complex hydroxypropyl CS/4-arm PEG-b-poly(L-lysine) (HPCS/PPLL) and the simultaneous Schiff-base reaction between amino-containing HPCS/PPLL complexes and aldehyde group-bearing nanogels containing IL-2, IFN-γ and DOX, respectively, were involved in the formation of double-crosslinking networks of nPCG. The optical image showed that the fabricated dual-crosslinking nPCG (dox) was white and opaque. SEM images manifest that the micropores inside the hydrogel are all interconnected. Scale bar of the inset is 10 µm. [56]

**Fig. 3** Schematic concept of a nanogel-incorporated physical and chemical gel (nPCG) as a delayed-release biosystem (a) Negatively charged mixtures of a drug-loaded oxidised cholesterol-bearing dextran (OCDEX) nanogel solution containing 4-arm PEG-b-poly-(L-glutamic acid) (PPLG) (b) Positively charged complex hydroxypropyl CS/4-arm PEG-b-poly(L-lysine) (HPCS/PPLL) prepared in PBS at pH 7.4. (c) Drug-loaded hydrogels were formulated by the ionic crosslinking of negatively charged PPLG with positively charged polysaccharide/poly peptide composites (HPCS/PPLL) and the simultaneous Schiff-base reaction between amino-containing HPCS/PPLL complexes and aldehyde group-bearing nanogels containing IL-2, IFN-γ and DOX. (d) Schematic illustration for the formation of double-crosslinking networks of nPCG. (e) Optical image showing that the fabricated dual-crosslinking nPCG is white and opaque. SEM images manifest that the micropores inside the hydrogel are all interconnected. Scale bar of the inset is 10 µm. [56]

**Fig. 4** Schematic illustration of double-crosslinked CDDP/Pept-AlgNP/IRN nanocomposite hydrogel for differential release of CDDP and IRN in combination therapy. [57]
injected into the cavity of resected cancerous breasts of rats where gelation occurred rapidly while the temperature decreased to body temperature during four weeks of implantation, interval NIR light irradiation can mediate the photothermal effect of PDA-AuNPs and conceretly control DOX release and therefore collectively prevent the recurrence of breast cancer.

In summary, it is beneficial to construct composite hydrogels with functional nanoparticles, which not only enhances the mechanical performance of the hydrogel but also endows the hydrogel with multifunctions.

### 3 Anti-tumour mechanism of injectable hydrogel

Nanoparticles or molecules with anti-tumour effects were encapsulated in the hydrogel network through physical or chemical means (Table 1). The treatment of tumours can then be achieved by releasing anti-tumour drugs, generating free radicals, locally generating heat or adjusting the TME through the stimulation of the external environment. Anti-tumour mechanisms can be roughly divided into five categories: chemotherapy, hyperthermia-based therapy, catalytic therapy and immunity therapy. In addition, imaging-assisted therapy is important for monitoring the progress of tumour treatment through imaging.

#### 3.1 Chemotherapy

Chemotherapy uses chemical drugs released from hydrogel to prevent the proliferation, infiltration and metastasis of cancer cells until they are eventually killed. Currently widely used chemotherapeutic drugs include paclitaxel [53], docetaxel (DTX) [54], DOX [55], Gefitinib [56] and trocaine [60, 61]. The currently reported injectable chemotherapy hydrogels can achieve the targeted and sustained release of chemotherapeutic drugs and have excellent anti-tumour effects. However, it is impossible to cure solid tumours owing to the limited internal drug loading of the hydrogel. Therefore, it is necessary to improve the efficiency of the anti-tumour treatment of chemotherapeutic drugs, especially at the low dosage [61, 62].

According to reports, the electrostatic interaction of cation-functionalised chemotherapeutic drugs with negatively charged mucin in mucus and mucosal epithelial cells can significantly prolong the residence time of the drug at the cancer site and improve the anti-tumour effect by elevating the mucosal permeability [63, 64]. Ci et al. [65] prepared polyimide functionalised imatinib nanocrystals (NC@PDA–NH₂) using PDA coating technology, and the thermosensitive Pluronıc® F127 hydrogel was used as a carrier to slowly release NC@PDA–NH₂. It can be seen from the analysis of mucin adsorption that NC@PDA–NH₂ exhibits an enhanced interaction with mucin compared to that of unmodified nanocrystals and exhibits an extended residence time on the vaginal mucosa of isolated mice. Further, NC@PDA–NH₂ showed stronger intracellular uptake and greater cell growth inhibition than did NC or free imatinib due to the electrostatic interaction between the positive charge on NC@PDA–NH₂ and the negative charge on the cell membrane.

These enhanced anti-tumour phenomena are attributable to the positive zeta potential value of NC@PDA–NH₂ and the interaction between NC@PDA–NH₂ and mucin. Therefore, it can be concluded that the selective modification of chemotherapeutic drugs can improve the anti-tumour effect to a certain extent.

In the past few years, local chemotherapy induced by a single drug has achieved great success. However, a single drug strategy may fail to kill the cancer cells due to the intrinsic heterogeneity of the tumour tissues and the difference in cancer cell divisions or growth stages. Therefore, the synergistic combination of two or more chemotherapeutic drugs with different toxicity profiles and cytotoxic action mechanisms is highly desired for cancer chemotherapy. More importantly, such combination chemotherapies may also be able to delay the generation of so-called multidrug resistance (MDR), so as to remarkably improve the therapeutic effect [66–68].

Xie et al. [69] developed a dual drug-loaded magnetic hydrogel (DDMH), which is prepared by crosslinking CS with telechelic bifunctional PEG (DF–PEG–DF), where DOX and DTX are used for chemotherapy (Fig. 5). The release of chemotherapeutic agents DOX and DTX in hydrogels were statistically analysed to explore the advantages of dual drug loading compared to single drug-loaded hydrogel in terms of release behaviour. The results show that owing to the mutual repulsion between hydrophilic DOX and hydrophobic DTX, DOX + DTX hydrogel shows accelerated chemotherapeutic agent diffusion compared with single drug-loaded DOX hydrogel and DTX hydrogel within 30 days. Intratumoural administration of dual drug-loaded hydrogel is fast and the dose is high, thus showing an enhanced anti-tumour effect.

The dual drug load can enhance the chemotherapeutic activity to a certain extent, but multiple and high doses are still needed to obtain a satisfactory anti-tumour effect, which will result in high toxicity and MDR. Therefore, it may be more effective for tumour chemotherapy if the simultaneous release of dual drugs and controlled release of drugs can be achieved. Xie et al. [69] used PLGA-encapsulated DTX (DTX/PLGA) to replace free DTX in hydrogels and combined this with the magnetocaloric effect of iron oxide to achieve the controlled multi-stage release of DTX. The release of DTX is delayed relative to the free DOX because of the PLGA package, so the simultaneous release of dual drugs can be achieved. An in vivo anti-tumour assessment was performed on DDMH-injected mice with or without alternative magnetic field (AMF). The results showed that the mice injected with DDMH exposed to AMF for 10 min obtained excellent anti-tumour

| Table 1 | Gel-forming type and corresponding anti-tumour method of injectable hydrogel |
| --- | --- |
| Gel type | Compounds | Anti-tumour methods | Refs. |
| --- | --- | --- | --- |
| thermal | Pluronic® F-127; DOX; Fu | chemotherapy | [17] |
| light | PLGA–PEG–PLGA copolymers; DOX; PLK1shRNA/PEI-Lys complexes | chemotherapy | [18] |
| enzyme | GOx; heparin; glucose; HONB | PDT; immunity therapy | [32] |
| metal coordination | ALG; MoS₂/Bi₂S₃–PEG; DOX; Ca²⁺ | photothermal therapy | [38] |
| self-assembly | N-carboxyethyl CS; PEGDA terminated with benzaldehyde; DOX | immuno therapy | [39] |
| click chemistry | α-CD; PEG-block-PLA: DOX | chemotherapy | [44] |
| Michael addition | 2-(3-Azido(propyl)-1-ethylmalonic acid; oxalipatin; alkylne-functionalised peptide; Cull | chemotherapy | [49] |
| reaction | ethoxylated trimethyl formaldehyde tri-(3-diol propionate); PEGDA (PEGDA670); | vaginal brachytherapy | [53] |
| Schiff-base reaction | NaHCO₃ | chemotherapy | [55] |
| | sodium ALG; sodium metaperiodate; CS; succinic anhydride; DOX | immunotherapy; chemotherapy | [56] |
| | human IL-2; human IFN-γ; DOX;4-arm PEG–PPLG; hydroxypropyl CS/4-arm PEG-b-poly (L-lysine) (HPCS/PPLL); sodium ALG; CS; cliplatin; dopamine | photothermal therapy | [59] |

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activity after four days of treatment compared to that of the DOX + DTX/PLGA NPs hydrogel group without AMF. This is because a higher temperature will change the structure of PLGA nanoparticles and increase the diffusion rate of drug molecules in PLGA. Therefore, controlled drug release can be achieved by controlling magnetic heat generation, thereby achieving the asynchronous release of dual drugs and avoiding destructive tumour treatment due to drug resistance.

In summary, although chemotherapy has great advantages in cancer treatment, the drug resistance and cytotoxicity caused by chemotherapy still cannot be completely overcome. Injectable hydrogel with chemotherapy function can realise the local sustainable release of the drug, avoiding the systemic toxicity and undesirable therapeutic effects caused by the free drug. To improve the effects of chemotherapy, multiple drugs can be introduced simultaneously for synergistic treatment. In addition, the asynchronous release of the drugs and a controllable drug release rate can further improve the efficacy of chemotherapy.

3.2 Thermotherapy

Thermotherapy is the heating of physiological tissues, which can hinder the growth and differentiation of cells and induce apoptosis by changing the structure of many cells and function of enzyme proteins when the temperature continues to be higher than 42°C. Therefore, hyperthermia is physical therapy with great potential to treat tumours because cancer cells are more sensitive to temperature than are normal cells. Hyperthermia can be divided into many types according to different heat sources, including photothermal therapy, magnetocaloric therapy and microwave thermotherapy. However, the most popular tumour therapeutics are photothermal therapy and magnetocaloric therapy.

3.2.1 Photothermal therapy: Photothermal therapy is a treatment method that injects hydrogel with high photothermal conversion efficiency into the tumour tissue. The treatment converts light energy into heat energy under the irradiation of external light sources, generally, NIR light, to kill tumours. Usually, photothermal agents were introduced through simple loading or polymer linking in a hydrogel precursor. The gel with light-to-heat conversion properties is then formed at the tumour site under the internal or external stimulus. The gel fixed onto the tumour site and then induced local photothermal ablation under NIR light radiation. Many types of photothermal agents have been reported, such as inorganic nanomaterials (such as carbon nanotubes, graphene and copper-based nanoplatons) [70, 71], organic dyes [72], conductive polymers [such as polyaniline (PANI)] [73, 74].

Hsiao et al. [75] prepared an injectable CS derivative hydrogel (NMPA-CS) with a PANI side chain by local pH-driven gelation to verify the photothermal ablation effect of PANI and understand whether the steric stability of hydrogel can provide PANI with high anti-tumour activity. The structure of NMPA-CS is shown in Fig. 6. The gel has a 3D polymerisation network, which can provide a certain spatial stability to prevent the photothermal agent from leaking into adjacent tissues. The photothermal ablation efficacy of NMPA-CS in tumours was studied in the Hep3B tumour model. The illumination conditions were an 808 nm laser (0.5 W/cm²) and the illumination time was 5 min. The results showed that intratumoural injection with hollow gold nanospheres (HGNs) + NIR laser (HGN + NIR) significantly inhibited tumour growth after the first NIR treatment. However, the growth of the tumour continued afterwards, possibly due to the leakage of HGNs from the injection site of the tumour. In contrast, the mice treated with NMPA-CS + NIR showed effective tumour growth inhibition, as well as no signs of tumour growth. These are attributed to the spatial stability of the NMPA-CS hydrogel in the tumour, so that repeated photothermal treatment can be performed.

The degradability of the hydrogel is important for the anti-tumour activity based on the reduced inflammation; the degradation products should be non-toxic to the body. Shao et al. [76] combined black phosphorus (BP) nanosheets with thermosensitive hydrogels to produce a new photothermal therapy system for cancer postoperative treatment. Here, as a reagent for loading BP nanosheets, poly(D,L-lactide) (PDLLA)–PEG–PDLLA (PLEL) is a typical biodegradable and biocompatible temperature-sensitive hydrogel. As shown in Fig. 7a, the polymer chains of PLEL can self-assemble into core-shell like micelles in aqueous solution. The hydrophobic PDLLA constitutes the core and the hydrated PEG forms a hydrophilic shell. BP nanosheets act as bridges between polymer micelles owing to their larger surface areas. In addition, BP@PLEL has photothermal sensitivity to NIR radiation and can form a physically crosslinked gel structure when the temperature is increased (Figs. 7b and c). In vivo and in vitro analyses showed that BP@PLEL hydrogel has excellent biocompatibility and biodegradability. BP nanosheets and PLEL are both biodegradable, and all degradation products are safe, small molecules (phosphates, phosphonates, carbon dioxide and water) that can be harmlessly discharged from the body after treatment. Further, an in vivo photothermal therapy postoperative treatment strategy was established for BP@PLEL hydrogel. After the tumour was removed, the entire wound area was irradiated with the 808 nm laser (0.5 W/cm²) and sprayed with BP@PLEL hydrogel. The IR thermal imager test results showed that the temperature of the BP@PLEL group increased rapidly to 39.4°C in the first 5 s of NIR irradiation, and the tumour temperature reached 58.2°C within 30 s of irradiation, which was sufficient for the photothermal ablation of cancer cells. Furthermore, the physical signs of mice showed that the tumour was completely cured without recurrence within 16 days, and the mice could survive for more than two months without death.

Mussel-inspired PDA has become a candidate for photothermal agents due to its excellent biocompatibility and biodegradability; it shows strong absorption in the NIR region (700–1100 nm) and can effectively convert absorbed light into heat. Therefore,
PDA-containing materials have been considered to have great potential in the field of tumour therapy [77]. Luo et al. [59] prepared an injectable hydrogel containing cisplatin (DDP) and PDA-modified nano-hydroxyapatite through a Schiff-base reaction between the aldehyde group on oxidised sodium ALG and the amino group on CS (Fig. 8). The hydrogel exhibits the sustained release of DDP since DDP is immobilised by the rich functional groups on PDA. In addition, the hydrogel showed excellent photothermal effects when exposed to the NIR laser (808 nm) owing to the strong absorption of PDA in the NIR region. Cell experiments revealed that hydrogels can effectively ablate tumour cells (4T1 cells) in vitro and inhibit tumour growth in vivo.

PDA can not only be used for photothermal treatment in response to NIR light but can also provide hydrogels with robust and strong adhesion to different types of surfaces via covalent binding, such as Michael addition and/or Schiff-base reactions and non-covalent binding, including metal coordination or chelating, hydrogen bonding, π–π stacking and quinhydrone charge-transfer complexation [58]. Inspired by the adhesion of mussels, Lu’s group designed a series of hydrogels with good adhesion using the dynamic conversion of phenolic hydroxyl groups and quinone groups of dopamine. Designing an injectable anti-tumour hydrogel with adhesion is possible if the dynamic phenol–quinone conversion is introduced [78–80]. The hydrogel adheres to the tumour site, which may enhance the anti-tumour effect.

In summary, research on injectable photothermal anti-tumour hydrogel has been popular in recent years because of its simple operation, fast curative effect and general lack of side effects. The reported photosensitisers that can generate heat for tumour ablation are abundant, and they should be improved to penetrate a certain depth of tissue to achieve a good therapeutic effect in tumour treatment. In addition, in photothermal tumour treatment, the local temperature is difficult to control owing to the faster photothermal conversion efficiency, and will cause damage to normal tissues if the local temperature is too high. Therefore, the selection of photosensitisers with NIR response and controllable light-to-heat conversion sensitivity are the most important aspects for the local treatment of tumours. Finally, endowing hydrogel with tissue adhesiveness may improve the anti-tumour effect of photothermal therapy.

3.2.2 Magnetotherapy: Magnetic induction hyperthermia is considered one of the most promising methods of anti-tumour magnetotherapy. Specifically, magnetic materials generate heat in the alternating-current magnetic field (ACMF) due to the hysteresis, eddy-current and relaxation effects, which cause the temperature of the tumour area to rise, thereby treating the tumour. The cells undergo inflammatory and coagulative necrosis to achieve anti-tumour activity by generating high temperatures (>50°C) through high-concentration magnetic nanoparticles (MNPs), known as magnetic thermal ablation (MTA). MTA has been clinically applied to tumour treatment with good therapeutic effects, but it is accompanied by potential side effects, such as high heat conduction, that might damage normal tissues near the tumour. Therefore, to alleviate this side effect, mild magnetic hyperthermia (MHT) has become the preferred magnetotherapy for anti-tumour treatment in recent years. However, the thermal energy (40–46°C) induced by single magnetotherapy is not enough to kill the tumour through apoptosis-mediated cell death. To solve this problem, a combination with other methods or multiple MHT has been proposed to achieve magnetic ablation of the tumour [81–83]. Injectable hydrogels can load MNPs and achieve long-term intratumoural fixation, which is advantageous for multiple magnetocaloric therapies.

The most popular magnetic nanomaterials used for magnetocaloric therapy are iron oxide and zinc oxide. Zhang et al. [84] reported injectable and biodegradable superparamagnetic iron oxide nanoparticle-loaded nanocapsule hydrogels (SPION-NHs), which were used for multiple magnetic therapy against tumours (Fig. 9a). The gel was assembled by the hydrophobic interaction between SPIONs and poly(organophosphazene) and transformed into a hydrogel upon body temperature stimulation. Magnetic resonance imaging (MRI) showed that after a single injection of SPION–NHs, the retention time of SPIONs in the tumour was >3 weeks, and therefore multiple MHT can be achieved (Fig. 9b). In vivo treatment results showed that complete tumour regression was observed using multiple MHT at moderate temperatures and excellent anti-cancer effects were observed in vivo after the fourth MHT cycle, and the surrounding healthy tissue was not seriously damaged.
The local orientation of MNPs can further improve the magnetocaloric anti-tumour effects of MNPs. Based on this, Wu et al. [85] proposed an intelligent injectable thermosensitive magnetic nanoemulsion hydrogel (MNH) containing zinc ferrite MNPs, PEGDA, indocyanine green (ICG) and silicone oil droplets, used for the local magnetothermal ablation of tumours. PEGDA, as a well-known synthetic polymer, has been widely used in biomedical applications. As a terminal group of PEGDA, acrylate can act as a bridging agent and insert into the oil/water droplet interface with increasing temperature under the action of an ACMF, thus causing strong attraction force between droplets, leading to nanoemulsion gelation. Here, the MNPs achieve local orientation within the hydrogel owing to the effect of the ACMF, and the excellent magnetic induction heat of MNH under ACMF conditions was evaluated by the specific absorption ratio (SAR) value. MNH and PEGylated Zn ferrite MNP caused temperature increases of ∼80 and 61°C, with SAR values of 872 and 583 W/g Fe, respectively, under the same Fe concentrations when ACMF (410 kHz and 1.8 kA/m) was applied for 15 min. The greater magnetic heat generation performance of the MNH was due to the local orientation of MNP under ACMF and heat accumulation in the nanoemulsion gel network. The local orientation of MNPs enhances the magnetic interaction between nanoparticles and further enhances the heat generation effect of MNH. The in vivo experiment results show that the MNH-implanted tumours shrank significantly and almost disappeared after three days of feeding after the magnetic field was introduced (410 kHz, 1.8 kA/m). The black scar on the original tumour site was completely cured, and no death or tumour recurrence was observed in the next three months.

In summary, the magnetocaloric anti-tumour injectable hydrogel can achieve multiple local magnetocaloric treatments, avoiding the side effect of insufficient or excessive temperature caused by a single magnetocaloric treatment. In addition, anti-tumour activity can be improved by adjusting the magnetocaloric conversion efficiency under ACMF. Therefore, the magnetocaloric anti-tumour injectable hydrogel has potential for use in clinical treatment.

3.3 Catalytic therapy

Catalytic anti-tumour therapy is a method that consumes internally enriched compounds to generate ROS for anti-tumour effects. ROS, including the superoxide anion (O$_2^-$), singlet oxygen (O$_2^*$) and hydroxyl radical (•OH), play an important role in cell proliferation and homeostasis [86]. In contrast, when the levels of ROS in cells are high, they can destroy various biomolecules, such as proteins and DNA, resulting in cell death [87–89]. According to the different methods of initiating catalysis, catalytic therapy can be divided into PDT and chemodynamic therapy (CDT).

3.3.1 Photodynamic therapy: PDT is a method of treating tumours by activating the photosensitiser with a specific wavelength. Specifically, it transfers electrons to the surrounding oxygen to generate highly active singlet oxygen after the photosensitiser is activated. Singlet oxygen reacts with the nearby biological macromolecules and kills the tumour cells [90, 91]. When the free photosensitiser is injected into the tumour site, it will overlap or enter the adjacent site due to the small particle size, and then reduce the efficiency of the tumour treatment. Increasing the concentration of the photosensitiser may improve the photodynamic anti-tumour effect. However, photosensitisers may agglomerate in the aqueous solution when the concentration is raised, which would result in the quenching of fluorescence during the photodynamic anti-tumour process and reduced ROS generation. Injectable hydrogels, as 3D network polymers, can not only avoid the accumulation of photosensitisers due to the higher consistency but also avoid the outflow of photosensitisers and achieve the local long-term release of ROS. Thus, they are feasible carriers for achieving long-term photodynamic anti-tumour effects.

Xia et al. [92] encapsulated the water-soluble photosensitisier mesoporous tetrakis (1-methylpyridin-4-yl) porphin (TMPyP) in an injectable hydrogel for photodynamic tumour therapy. The hydrogel consisted of telechelic PEG terminated with ethylene glycol C5 and dibenzaldehyde, which was formed by using the Schiff base linkages between the amine groups of glycol and aldehyde groups of dibenzaldehyde-terminated telechelic PEG (or diacryl-functionalised PEG and DEF-PEG), synthesised by the esterification of hydroxyl-terminated PEG with 4-formylbenzoic acid. In vivo PDT was performed after administration in the tumour. The light condition was λ = 532 nm; the power was 10 mW/cm$^2$ and the time was 30 min. The results showed that PDT using TMPyP-loaded hydrogel can ablate tumours more effectively than that of free TMPyP. The specific performance is as follows: (i) more singlet oxygen was generated under the same laser irradiation conditions, (ii) a longer tumour retention time was observed due to the low fluidity of the hydrogel, (iii) enhanced fluorescence intensity was observed since the weakened self-quenching effect of TMPyP. Generally, the reason for the fluorescence quenching of free TMPyP can be attributed to the aggregation of TMPyP forming through π–π interactions because the free TMPyP molecules are more likely to collide with each other in a low-viscosity environment (such as water). Inside the hydrogel network, it is difficult for the fluorescent molecules to rotate in a high-viscosity environment. The 3D structure of the hydrogel helps to stabilise the TMPyP molecules in the hydrogel matrix, thereby reducing the rotation of the PS molecules and the intermolecular collisions, thereby improving the FL strength and the generation of ROS, and thus achieving a more favourable photodynamic anti-tumour effect.

PDT is a treatment that relies on high-oxidative levels; however, there are antioxidants, such as glutathione, in the tumour cells that prevent PDT. Therefore, it is necessary to consume the antioxidant inside the tumour to obtain a greater photodynamic therapeutic effect [87]. Liang et al. [93] synthesised an amphiphilic nanogel (HA–G@C60) by loading the hydrophobic photosensitiser C60 into gambogenic acid (GA)–grafted hyaluronic acid (HA–GA) through the hydrophobic GA–C60 interaction. C60 exists as a photosensitiser for PDT, and it can produce highly cytotoxic ROS after activation by laser irradiation, especially singlet oxygen ($^{1}\text{O}_2$) [31]. GA is a natural chemotherapeutic agent from Garcinia cambogia and has potent cytotoxic activity against various types of tumours [94, 95]. In addition, GA can consume glutathione (GSH) in cells and destroy redox homeostasis in cells [96, 97]. Among them, glutathione depletion will increase ROS levels, enhance PDT, and cause tumour cell death [98]. The experimental results showed that the PDT with the GA-sensitive pro-drug HA–GA, killing tumour cells while
consumer glutathione (GSH) in the cells, thereby weakening antioxidant levels and enhancing PDT.

PDT has significant effects on the treatment of tumours by generating ROS, which has similar efficacies as a PTT. The light absorption range of many photosensitisers is in the UV and visible light regions, which cannot penetrate tissues to provide treatment for deep-level tumours. Therefore, it is necessary to use photosensitisers with NIR light absorption to improve the PDT effect. In addition, PDT usually consumes oxygen inside the tumour to generate free radicals to kill the tumour; however, the tumour site often in a state of hypoxia, which cannot provide sufficient energy for PDT. Hence, the intratumoral delivery of oxygen or oxygen-producing intermediates may help to further improve the anti-tumour effect of PDT.

### 3.3.2 Chemodynamic therapy:
CDT can induce tumour cell apoptosis and realises efficient treatment based on the specific activation of the TME by catalysing H2O2 to produce hydroxyl radicals (•OH) and other strong oxidising active species [99]. Fenton reaction is the most commonly used CDT strategy in the anti-tumour field. The weak acidity and excessive H2O2 in the TME are necessary for the Fenton reaction. Functional nanomaterials based on transition metals are used as catalysts to trigger Fenton or Fenton-like reactions in tumour cells. Among these, transition metals, such as iron, copper, manganese and cobalt, have been proven to possess good Fenton catalytic activity.

It is well known that the effective generation of •OH through the Fenton reaction requires strict reaction conditions—such as an optimal pH of 3–4 and an appropriate H2O2 concentration—that are usually limited in the TME [100]. In the TME, the H2O2 concentration is 50–100 × 10^-6 M [101], and the pH is about 6.5–6.9 [102]. Therefore, increasing the content of H2O2 and lowering the pH of the TME is essential for triggering an effective Fenton reaction [103]. Yang et al. [104] prepared a new type of biocompatible Fe3O4–GOx nanogel (FGgel) using the host-guest self-assembly method. The gel was composed of β-CD-modified Fe3O4 (Fe3O4@β-CD) (host molecule), GOx and ferrocene (Fc)-linked poly(acrylic acid) (guest molecule), formed by the self-assembly of the host–guest interaction between the β-CD group and the Fc group. In the TME, H2O2 in the tumour oxidises Fe, causing GOx release by FGgel breakdown. Subsequently, the released GOx catalyses the glucose in the tumour to produce gluconic acid and H2O2. At the same time, under acidic conditions, Fe3O4 is used as a Fenton reagent to convert excessive H2O2 into highly toxic •OH, which ultimately induces tumour cell apoptosis.

In addition, improving the efficiency of the Fenton reaction in tumours by supplying external energy fields (light and electricity) is also a method of improving the chemical kinetics against tumours [105]. Hao et al. [106] prepared an injectable hydrogel containing a catalyst pair of GOx and gallic acid–ferrous iron (GA–Fe) nanocomposite for chemodynamic breast cancer therapy. The hydrogel was composed of DMAA and PEGDA, which was gelated by the radical polymerisation produced by Fenton reaction participate by GA–Fe. GA–Fe is a Fenton catalyst that responds to NIR light and can achieve the cascade production of •OH and rapid consumption of glucose. GA–Fe will rapidly increase the temperature of the hydrogel after NIR laser irradiation, which will simultaneously improve the catalytic efficiency of GA–Fe and GOx. The glucose in the tumour is quickly converted into H2O2 with the participation of GOx. Then, under the reduction of GA–Fe, H2O2 decomposes to produce hydroxyl radicals (•OH). The results of animal experiments showed that the enhanced glucose depletion and CDT were obtained, which effectively inhibited the growth of triple-negative 4T1 tumours in situ under NIR light exposure.

In summary, CDT is a potential strategy for tumour-specific treatment. It effectively avoids the difficulty of tissue penetration and exogenous factors that may cause normal cell death because it depends on endogenous chemical energy (H2O2) in the tumour instead of external energy sources, such as laser, US and magnetic fields. However, there are many obstacles, such as limited H2O2 concentration, inappropriate pH, reducing microenvironment, to performing CDT inside the tumour. Thus, it is feasible to improve CDT efficiency by introducing functional compounds in the hydrogel that can regulate the conditions of the TME, such as pH, H2O2 or response to external energy fields.

### 3.4 Immunity therapy

Tumour immunotherapy controls and clears the tumour by restarting and maintaining the tumour-immunity cycle to restore the normal anti-tumour immune response of the body. It has demonstrated strong anti-tumour activity in various tumour treatments, such as melanoma, non-small-cell lung cancer, kidney cancer and prostate cancer [107]. At present, tumour-related antigens have been proven effective in the treatment of various tumours, they have great potential for inhibiting cancer metastasis and recurrence, because they can activate the immune system of patients, allowing immune cells to recognise and eliminate uncontrolled tumour cells and effectively prevent immune escape [108, 109]. However, the weak immunogenicity and low-response rates are major challenges of tumour antigens. On this basis, the synergistic therapy of tumour-related antigens with immune adjuvants, immune checkpoint inhibitors, immune cells or small-molecule inhibitors provide new potential solutions to this problem [110]. A large number of studies have confirmed that malignant tumour cells can secrete cytokines and chemokines to recruit a variety of inhibitory immune cells into the TME, including tumour-associated macrophages (TAMs), regulatory T-cells and bone marrow-derived inhibitory cells [111–114]. Among these immunosuppressive cells, TAMs are the main component of the TME, and have two main types of polarisation: classically activated (M1) and alternatively activated (M2). M1-TAMs have the ability to clear foreign antigens and kill tumour cells and M2-TAMs suppress anti-tumour immune responses and promote tumour growth [115, 116]. IFN-γ, lipopolysaccharide, and other pro-inflammatory factors in the TME can induce M1 polarisation in TAMs, which can synthesise and secrete cytotoxic molecules, such as ROS, tumour necrosis factor α (TNF-α), and NO, promote HIF-1α secretion and cause tumour cell death. In addition, M2-TAMs can be converted into M1-TAMs to exert a lethal effect when subjected to certain external stimuli [117]. Dai et al. [118] constructed a melittin-(RADA)6 (MR52) hydrogel loaded with Ca2+/calmodulin-dependent protein kinase II (CAMKII) inhibitor KN93 (MR52-KN93 [MRK]). Melittin has high biological activity and can direct kill tumour cells. KN93 not only has direct tumour-killing activity but also macrophage reprogramming ability. Immunotherapy studies were carried out on a mouse melanoma model. The results showed that MRK hydrogel injection significantly increased the lethal effect on tumour cells and the level of immunogenic cell death, and also delayed the growth of subcutaneous melanoma tumours. The reasons are explained as follows: (i) KN93 directly kills tumour cells while regulating TAM reprogramming and differentiation into M1-TAMs, reducing the number of M2-TAMs in the tumour and (ii) KN93/melittin produces tumour fragments after killing the tumour. As tumour-related antigens, the fragments cause a large increase in mature dendritic cells, which leads to an increase in immune cytotoxic T-cells, providing effective treatment for melanoma.

Synthetic oligodeoxynucleotides with unmethylated cytosine—phosphate–guanosine (CpG) motifs are popular and inexpensive immune adjuvants; they can induce the best immune stimulation response in mammalian cells. CpG specifically recognise toll-like receptor 9 (TLR9), which is widely expressed in mouse and human plasmacytoid dendritic cells (DCs). The activated TLR9 can directly reduce the immunosuppressive environment and improve the anti-tumour response by triggering a signal cascade of innate and adaptive responses [119]. Yu et al. [120] used three biocompatible components: a synthetic oligodeoxynucleotide (CpG), radioisotope-labelled natural enzyme (131I-Cat), and natural polysaccharide (ALG), to synthesise an injectable...
131I-Cat/CpG/ALG hydrogel. When the 131I-Cat/CpG/ALG is injected locally into the tumour, the Ca\(^{2+}\) in the tumour can trigger rapid ALG gelation. 131I-Cat is used to consume tumour endogenous \(\text{H}_2\text{O}_2\) through Cat to generate oxygen; thus, effective radioscopy therapy (RIT) can be performed at a relatively lower radiation dose. Tumour residues generated by RIT, as tumour-related antigens, can activate the systemic immune response. To explore whether Cpg can promote systemic immunity after RIT, a subcutaneous rectal cancer model was established. Specifically, a colorectal CT26 tumour was inoculated on the left and right sides of each mouse. The right abdominal tumour, without direct treatment, was used as a control to evaluate the therapeutic effect. The results showed that the right tumours of mice injected intratumourally (left) with 131I-Cat/ALG showed fairly rapid growth. In contrast, 131I-Cat/CpG/ALG treatment can significantly reduce the growth of distant tumours, especially within the first 3 weeks. This indicated that the introduction of CpG through 131I-Cat/CpG/ALG can enhance the immunogenicity of tumour residues after RIT. To further enhance the systemic immune effect, CTLA-4 antibody was injected intravenously into mice after RIT treatment. The results of animal experiments show that the anti-tumour immune response obtained by 131I-Cat/CpG/ALG in combination with CTLA-4 checkpoint inhibitor produces a more significant systemic therapeutic effect, thereby effectively inhibiting distant tumour growth. Immune cell analysis results show that after treatment with 131I-Cat/CpG/ALG plus CTLA-4 antibodies, the infiltration rate of CD8\(^+\) CTL in distant tumours in mice was 3.85\%, which much higher than those of other methods, including 131I-Cat/ALG and anti-CTLA-4 or 131I-Cat/CpG/ALG-based RIT treatment only. In addition, after 131I-Cat/CpG/ALG plus anti-CTLA-4 treatment, the TNF-\(\alpha\) and IFN-\(\gamma\) contents in distant tumour cells were significantly increased. These results confirm the strong synergistic anti-tumour immune response of 131I-Cat-CpG/ALG combined with anti-CTLA-4.

In summary, hydrogel immunotherapy for tumours has become the most ideal method for eradicating tumours in recent years. Although applied locally, such injectable hydrogels can not only kill local tumours but also inhibit tumour metastasis and recurrence by regulating the immune microenvironment. Further, it is necessary to combine a variety of immunological agents for all-round coordination if an improved immune effect is expected. Moreover, combining other strategies, such as resection, chemotherapy, thermotherapy, or catalytic therapy, with immunotherapy may also be promising methods for improving cancer immunotherapy.

### 3.5 Imaging-assisted therapy

At present, many anti-tumour methods have been proposed. However, it is difficult to achieve the real-time visual monitoring of the treatment progress and the position and distribution of nanoparticles in the tumour. Therefore, developing an injectable hydrogel that integrates multimodal tumour imaging, tumour therapy, and therapeutic effect monitoring has become a research hotspot. Usually, the dual purpose of diagnosis and treatment can be achieved after a single administration when combining the imaging agent and therapeutic materials [121]. Currently reported intratumoural imaging methods to include fluorescence imaging [122], MRI [123], and US imaging [124].

He et al. [122] developed a silk fibroin (SF) nanofibre injectable SF/UCNP@NGO hydrogel system. UCNP@NGO composite micromaterials are prepared by combining \(\text{NaLuF}_4: \text{Er}^{3+}, \text{Yb}^{3+}\) upconversion nanoparticles (UCNP) with nanographene oxide (NGO) by ligand exchange. The composite material was then incorporated into an SF aqueous solution, and a hydrogel was prepared by incubating at 60\(^\circ\)C for 24 h. Anti-tumour activity was achieved through the combination of upconversion luminescence (UCL) imaging diagnosis and photothermal therapy. In vitro studies have shown that SF/UCNP@NGO hydrogel has excellent biocompatibility, and shows excellent UCL imaging performance and photothermal treatment effects under NIR laser irradiation; this may be attributed to the UCL characteristics of UCNPs and the light-to-heat conversion characteristics of NGOs. In vivo studies have shown that under the synergistic effect of NIR laser irradiation and SF/UCNP @ NGO hydrogel, tumour growth in 4T1 tumour-bearing mice is greatly suppressed.

Wu et al. [85] developed an injectable thermosensitive MNH, which can remove local tumours with precise magnetothermal ablation under the guidance of multimodal imaging. The liquid MNH consisted of ferrite zinc MNPs, PEGDA, ICG and silicone oil, which could quickly change into its solid-like state without the leakage after feeling the body temperature (Fig. 10a). MNPs generate heat to ablate tumours under the action of an ACMF. MNPs, ICG and o/w nanoemulsion act as high-performance contrast agents for MRI, NIR fluorescence (NIRF) imaging, and US imaging, respectively. The in situ injection process can be monitored in real time and dynamically by US imaging, and the magnetocaloric therapy process is guided and evaluated by MRI and NIRF imaging. In situ US imaging analysis showed that during the injection of MNH into the tumour centre by minimally invasive methods, the US signal strength in the body gradually increased, indicating that the injection site can be accurately located by US imaging (Fig. 10b). To confirm the location of MNH within the tumour, T2*-weighted magnetic resonance (MR) images were obtained before and after magnetocaloric therapy (Fig. 10c). MNH is a black spot in the tumour tissue, which stays in a fixed position for 10 min. After 2 h of hyperthermia, the black spots did not spread significantly. It shows that MNH stays in the centre of the tumour after being injected into the tumour tissue, and remains in the initial position of the tumour without spreading and penetration after an ACMF is applied. NIRF imaging is used to guide intratumoural injection and evaluate the fluorescence characteristics of MNH before and after magnetocaloric therapy (Fig. 10d). Five minutes after the injection, fluorescence imaging showed that MNH was accumulated in the tumour area by thermosensitive in situ gelsations. Two hours after ACMF, a NIRF signal expansion was observed in the tumour area, indicating the relatively stable gelation of MNH in the tumour tissue and the partial diffusion of small-molecule ICG under heat induction (Fig. 10e). This implies that ACMF has the potential to promote...
the spread of loaded anti-cancer drugs in tumour tissues. In summary, MNH can realise the integration of imaging and treatment.

In summary, imaging-assisted therapy can realise the integration of diagnosis and treatment of tumours by monitoring the progress of tumour treatment in real time by imaging. Moreover, imaging-assisted therapy can provide important pathological information, which will help doctors in making informed decisions about treatment strategies, including treatment time, drug selection, and dosage. Therefore, it is necessary to combine tumour treatments with imaging to monitor the therapeutic process.

3.6 Anti-tumour synergy therapy

Anti-tumour synergistic therapy refers to integrating multiple anti-tumour methods into a system to further improve the anti-tumour efficiency [125]. The currently reported synergy of multiple anti-tumour mechanisms included photothermal + magnetocaloric anti-tumour, thermal + ROS anti-tumour, thermal + immune anti-tumour, ROS + immune anti-tumour, and thermal + ROS + immune anti-tumour, and so on. The synergistic anti-tumour methods were not simply the superposition of a single anti-tumour method, but to achieve higher anti-tumour efficiency than single anti-tumour treatment. Here we reviewed several typical anti-tumour synergistic therapy by injectable hydrogels.

Puente et al. [126] provided an injectable CS hydrogel capable of releasing chemotherapy (temozolomide, TMZ) while retaining radioactive isotopes agents (iodine, I131), which was used as a vehicle for localised radiation and chemotherapy of brain cancer within the surgical cavity. The chemical cross-linking of CS with GA occurred through forming an imine group inside the hydrogel network. In vitro experiments show that the release of I131 was negligible over 42 days, whereas the TMZ was completely released over the first 48 h. I131 was completely retained in the tumour bed with negligible distribution in other tissues. The locally delivered drug accumulated in the tumour is 10-fold that of the drug delivered systemically. In vivo experiments show that the tumours were significantly decreased, and survival was improved in both treatment groups compared with the control group.

Zheng et al. [127] designed a temperature-sensitive injectable CS/MBP/DOX (CMD) hydrogel for safe and efficient hyperthermia and chemotherapy of colon cancer in vivo. Physically mixing CS solution and β-glycerophosphate (β-GP) led to sol-gel phase transition when the temperature was higher than 37°C due to the hydrogen bond, electrostatic attraction and hydrophobic interaction between CS and β-GP. The addition of the drug molecule DOX and the photothermal material MoS2/ Bi2S3-PEG (MBP) realises multifunctional in vivo tumour PTT and chemotherapy. Experimental results show that the CS/MBP/DOX (CMD) hydrogel exhibited a photothermal efficiency of 22.18 and 31.42% in the first and second NIR light (NIR I and NIR II) biowindows, respectively, at a low MBP concentration (0.5 mg/mL). Further, the release of the DOX from CMD hydrogel was controllable because the gel temperature could be governed by NIR laser irradiation.

In addition, synergy therapy that kills or inhibits local tumour growth with immunotherapy may achieve the goal of radical treatment of the tumour. Jiang et al. [128] incorporated multifunctional dendritic nanoparticles into a PLGA–PEG–PLGA triblock copolymers based thermosensitive injectable hydrogel matrix, so as to construct a localised drug-delivery system for combining chemotherapy and immunotherapy. The multifunctional dendritic nanoparticles were designed with dendritic scaffolds, which can provide a hydrophobic interior to load the anticancer drug, DOX, for chemotherapy. In addition, dendritic scaffolds were used to build arginine-rich molecules to provide the inducible nitric oxide synthase (iNOS) substrate, L-Arg, to M1 macrophages, which can produce the cytotoxic nitric oxide (NO) and subsequently induce tumour cell destruction through immunotherapy. In vivo experiments showed that this system had great efficacy in treating 4T1 cells-xenografted BALB/C mice (86.62% tumour growth inhibition).

In summary, the synergistic anti-tumour through the integration of two or more methods can greatly improve the anti-tumour efficiency, compared with a single anti-tumour method. Thus, the synergy of multiple anti-tumour through facile injectable hydrogels may be a promising research direction in the future.

3.7 Modified hydrogel for anti-tumour therapy

At present, most of anti-tumour hydrogels are loaded with anti-tumour drug/nanoparticle, which achieves the purpose of anti-tumour through drug release or external stimulation [129]. However, hydrogel itself could perform anti-tumour functions, in which the molecule with anti-tumour function is used as a component of hydrogel or integrated with the hydrogel network through grafting. Then, anti-tumour therapy can be achieved by the degradation of the hydrogel.

David et al. [130] developed a cyclic dinucleotide (CDN)-loaded multidomain peptide (MDP) hydrogel, named as STINGel. In this hydrogel, MDP had a sequence of K2(SL)6K2, while CDN was a promising stimulator of interferon genes (STING) agonist. MDP hydrogel was formed through electrostatic interactions between the CDN’s negative thiolphosphate linkages and the positive lysine residues at the peptide backbone. In vivo bioactivity tests showed that MO2-C6E67 tumours in wild type C57BL/6 mice were successfully rejected by a single injection of STINGel. In total, 60% of STINGel treated mice exhibited complete anti-tumour response and acquired immunity. All mice that were re-challenged with a secondary inoculation exhibited none tumour growth.

It is reported that heparin-based biomaterials exhibit significant inhibition of cancer cell metastasis. Based on this, heparin and poly(ε-caprolactone-co-lactide)-b-PEG-b-poly(ε-caprolactone-co-lactide) (PCLA–PEG–PCLA) were used to construct non-anticoagulant heparin prodrugs loaded in thermosensitive hydrogel for anti-metastasis treatment [131]. The heparin was conjugated with the polymer via esterification, and its sustained release was ensured by hydrolysis and polymeric biodegradation. The in vitro metabolism tests confirmed the biocompatibility of the hydrogels with HeLa cells and HaCaT cells. The in vivo anti-metastasis effects showed that this hydrogel by using as a peritumoural injection treatment can efficiently inhibit cell migration in vitro and reduce tumour metastasis to the vulnerable lungs in nude mice.

In summary, the hydrogels modified with anti-tumour function have simple compositions and exhibit excellent anti-tumour effects. However, this type of hydrogel needs to be degraded or hydrolysed to exert anti-tumour functions. Therefore, it is necessary to employ degradable polymers to participate in the gel formation when designing this type of injectable anti-tumour hydrogel.

4 Conclusions and future perspectives

In summary, we reviewed the advantages of injectable hydrogels in tumour treatment, the various gelation methods of injectable hydrogels, and the anti-tumour mechanisms. Although injectable hydrogels have achieved good anti-tumour effects, there are still enormous challenges in future clinical applications.

(i) The reported hydrogels have shown excellent anti-tumour effects, but the anti-tumour efficiencies of individual anti-tumour mechanisms are not satisfactory. The synergy of multiple anti-tumour mechanisms (e.g. photothermal + magnetocaloric anti-tumour, thermal + ROS anti-tumour, thermal + immune anti-tumour, ROS + immune anti-tumour and thermal + ROS + immune anti-tumour) may be a promising research direction in the future. Among them, the combination of immunotherapy and other therapies (e.g. photodynamic therapy) may be one of the most promising methods of eradicating systemic tumours. This is because tumour fragments are generated after the tumour is killed. These tumours
fragments, as tumour-specific antigens, activate the immune system and immune agents, such as immunoadjuvants and immunosuppres-
sants, further amplify the immune response, thereby eradicating tumour cells.

(ii) Hydrogels have been extensively studied and applied in the clinical treatment of tumours due to their good biocompatibility, environmental responsiveness and simple preparation. However, the tumour-treatment process often involves hydrogel rupture due to the twisting and rotation of the tumour treatment site, which causes therapeutic drug outflow and adverse therapeutic effects. To adapt to the strain and deformation caused by human movement, toughness and stretchability have become indispensable properties for the preparation of anti-tumour injectable hydrogels. Furthermore, to prevent the hydrogel from slipping on the tumour site and improve the anti-tumour treatment effect, good tissue adhesiveness is another issue that needs to be considered when designing anti-tumour hydrogels. The design and preparation of multifunctional hydrogels with the integration of good toughness, ductility and adhesion is the current research challenge.

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References

[1] Drumberg, V., Bitcouver, R., Rajchenbach, W., et al.: ‘Modulating cancer multidrug resistance by sertraline in combination with a nanomedicine’, Cancer Lett., 2014, 354, pp. 290–298.
[2] Xiong, L., Luo, Q.-J., Wang, Y., et al.: ‘An injectable drug-loaded hydrogel based on a supramolecular polymeric prodrug’, Chem. Commun., 2015, 51, pp. 14644–14647.
[3] Stuart, M.A.C., Huck, W.T.S., Genzer, J., et al.: ‘Emerging applications of stimulus-responsive polymer materials’, Nature Mater., 2010, 9, pp. 101–113.
[4] Li, Y.-P., Liu, T.-Y., Luo, Y., et al.: ‘A smart and versatile theranostic nanomedicine platform based on nanophosphoryn’, Nat. Commun., 2014, 5, pp. 4712–4726.
[5] Cheng, M., Wang, H., Zhang, Z., et al.: ‘Gold nanorod-embedded electropun fibrous membrane as a photothermal therapy platform’, ACS Appl. Mater. Interfaces, 2014, 6, pp. 15596–1575.
[6] Hayashi, K., Sakamoto, W., Yogo, T.: ‘Smart ferrofluid with quick gel transformation in tumors for MRI-guided local magnetic thermothermochemistry’, Adv. Funct. Mater., 2016, 26, pp. 1708–1718.
[7] Sang, Y.-J., Ren, J.-S., Qu, X.-G., et al.: ‘Construction of nanozyme-hydrogel for enhanced capture and elimination of bacteria’, Adv. Funct. Mater., 2019, 29, p. 1900518.
[8] Qu, Y., Chu, B.-Y., Peng, J.-R., et al.: ‘A biodegradable thermo-responsive hybrid hydrogel: therapeutic applications in preventing the post-operative recurrence of breast cancer’, NPJ Biother. Adv., 2015, 7, p. e207.
[9] Patenaude, M., Smeets, N., Hoare, T.: ‘Designing injectable, covalently cross-linked hydrogels for biomedical applications’, Macromol. Rapid Commun., 2014, 35, (6), pp. 598–617.
[10] Chao, Y., Chen, Q., Xu, J., et al.: ‘Light-triggered in situ gelation to enable robust photodynamic-immunotherapy by repeated stimulations’, Adv. Mater., 2019, 31, p. 1900927.
[11] Sanyal, A.: ‘Nanoengineered phase-change materials based on on-demand antibiotics release using thermo-sensitive hydrogel-based drug reservoir for combating bacterial infection’, Biomaterials, 2019, 188, pp. 83–95.
[12] Wang, H., Zou, Q., Boerman, O.-C., et al.: ‘Combined delivery of BMP-2 and bFGF from nanostructured colloidal gelatin gels and its effect on bone regeneration in vivo’, J. Control. Release, 2013, 166, (2), pp. 172–181.
[13] Solans, J., Alderighi, M., Barossi, M.-C., et al.: ‘Chemical and in vivo evaluations of a self-assembling amphiphilic peptide as an injectable hydrogel scaffold for biomedical applications’, J. Bioactive Compat. Polym., 2013, 28, (1), pp. 3–15.
[14] Zheng, Y.-L., Liang, Y.-Q., Zhang, D.-P., et al.: ‘Gelatin-based hydrogels blended with gelan as an injectable wound dressing’, ACS Omega, 2018, 3, pp. 4766–4775.
[15] Poudel, A.-J., He, F., Huang, L.-X., et al.: ‘Supramolecular hydrogels based on poly (ethylene glycol)-poly (lactic acid) block copolymer micelles and n-ε-cyclodextrin for potent injectable drug delivery system’, Carbohydr. Polym., 2018, 184, pp. 69–79.
[16] Rostovtsev, V.V., Green, L.G., Fokin, V.V., et al.: ‘A stepwise huisgen cycloaddition process: copper(i)-catalyzed regioselective 'ligation' of azides and terminal alkynes’, Chem. Rev., 2002, 102, pp. 35–45.
[17] Sanvila, A.: ‘Diels-Alder cycloaddition-cycloreversion: a powerful combo in materials design’, Macromol. Chem. Phys., 2010, 211, pp. 1417–1425.
[18] Khurana, P.-M., Rehman, S., Skerritt, W.: ‘Thiol–ene click hydrogels for therapeutic delivery’, ACS Biomater. Sci. Eng., 2016, 2, pp. 165–179.
[19] Sletten, E.-M., Bentzoni, C.-R.: ‘From mechanism to mouse: a tale of two orthogonal reactions’, Accounts Chem. Res., 2011, 44, (9), pp. 666–676.

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Zhu, H., Wang, Y., Hussain, A., et al.

Ci, L.-Q., Huang, Z.-G., Lv, F.-M., et al.

Fan, M., Ma, Y., Mao, J.-H., et al.

Reithofer, M.-R., Chan, K.-H., Lakshmanan, A., et al.

Wu, C., Liu, J., Zhai, Z., et al.

Zhang, C.-N., Wang, W., Liu, T., et al.

Lim, C.-K., Shin, J., Lee, Y.-D., et al.

Yang, J., Choi, J., Bang, D., et al.

Positively charged polypeptide nanogel

Convertible organic nanoparticles for

Anti Cancer Agents in Med.

Enhanced photodynamic therapy with an oxidation-regulated strategy for enhancing anti-tumor efficacy', Theranostics, 2018, 8, pp. 5059–5071

Duan, D., Zhang, B., Yao, J., et al.

Wang, X., Chen, W.-T.: 'Gamma-emitting agent that inhibits cell proliferation, angiogenesis and metastasis', Ant Cancer Agents in Med. Chem., 2012, 11, pp. 994–1000

Han, J., Lim, M., Bonhij, J., et al.

Wang, J.-Y., Zhou, Z.-W., Luo, R.-J., et al.

Enhanced fluorescence emission and singlet oxygen generation of photosensitizers embedded in injectable hydrogels for imaging-guided photodynamic cancer therapy', Biomacromolecules, 2017, 18, pp. 3073–3081

Bingang, H., Zhou, Z.-W., Luo, R.-J., et al.

Ding, W., Zhou, Z.-D., Cao, M., et al.

Yan, L., Wang, X., Chen, W.-T.: 'Copper(II)-graphitic carbon nitride triggered sequential nanocatalytic therapy in both tumor-suppressive and biotherapy platform for postsurgical treatment of cancer', Adv. Sci., 2018, 5, pp. 1700848

Wang, X.-Y., Zhang, J.-S., Wang, Y.-T., et al.

Han, L., Lu, X., Liu, K.-Z., et al.

Yan, L., Yan, Y.-H., Jiang, L.-L., et al.

Positively charged polypeptide nanogel

Biocompatible and biodegradable polymeric nanoparticles for long-lasting moisture and extreme temperature tolerance', Adv. Funct. Mater., 2018, 28, pp. 1704195

Han, L., Liu, K.-Z, Wang, M.-H., et al.: 'Mussel-inspired adhesive and tough hydrogel based on nanoclay confined dopamine polymerization', ACS Nano, 2017, 11, pp. 25561–25574

Shao, J.-D., Ruun, C.-S., Xie, H.-H., et al.

Black-phosphorus-incorporated hydrogel as a sprayable and bioresorbable material for postoperative treatment of cancer', Adv. Sci., 2018, 5, pp. 1700848

Wang, J.-S., Zhang, J.-S., Wang, Y.-T., et al.

Multi-responsive photothermal-chemotherapy with drug-loaded melanin-like nanoparticles for specific tumor ablation', Biomaterials, 2016, 81, pp. 114–124

Han, L., Lu, X., Liu, K.-Z., et al.: 'Mussel-inspired adhesive and tough hydrogel with long-lasting moisture and extreme temperature tolerance', Adv. Funct. Mater., 2018, 28, pp. 1704195

Han, L., Yan, L.-W., Wang, N., et al.: 'Transparent, adhesive, and conductive hydrogel for soft bioelectronics based on light-emitting polydopamine-doped polypropylene nanofibers', Chem. Mater., 2018, 30, pp. 5561–5572

Yao, D., Dong, H., Preus, C., et al.: 'Double-effector nanoparticles: a synergistic approach to apoptotic hyperthermia', Angew. Chemie Int. Ed., 2012, 51, pp. 12482–12485

Dong, D., Jeong, H., Noh, S.H., et al.: 'Magnetically triggered dual functional nanoparticles for resistance-free apoptotic hyperthermia', Angew. Chem., 2013, 52, pp. 13047–13051

Di Corato, R., Bealle, G., Kolosnjaji-Tabi, J., et al.: 'Combining magnetic hyperthermia and photodynamic therapy for tumor ablation with photosresponsive magnetic liposomes', ACS Nano, 2015, 9, pp. 2904–2916

Zhang, Z.-Q., Song, S.-C.: 'Thermosensitive/superparamagnetic iron oxide nanoparticle loaded nanocapsule hydrogels for multiple cancer therapeutics', Biomaterials, 2016, 106, pp. 219–233

Wu, H.-A., Song, L.-N., Chen, L., et al.: 'Injectable thermosensitive magnetic nanoemulsion hydrogel for multimodal-imaging-guided accurate thermoablative cancer therapy', Nanoscale, 2017, 9, pp. 16175–16182

Sahharwal, S.-S., Schumacker, P.T.: 'Metal-organic frameworks in cancer: initiators, amplifiers or an aches’ heel?', Nat. Rev. Cancer, 2014, 14, pp. 709–721

Zhou, Z., Song, J., Nie, J., et al.: 'Reactive oxygen species generating systems meeting challenges of photodynamic cancer therapy', Chem. Soc. Rev., 2016, 45, pp. 6597–6626

Zhou, Z., Song, J., Tian, R., et al.: 'Activatable singlet oxygen generation from lipid hyperoxide nanoparticles for cancer therapy', Angew. Chem. Int. Ed., 2017, 56, pp. 6492–6496

Liu, Y.-L., Ai, K.-J., Ji, X.-Y., et al.: 'Comprehensive insights into the multi-antioxidative mechanisms of melanin nanoparticles and their application to protect brain from injury in ischemic stroke', J. Am. Chem. Soc., 2017, 139, pp. 856–862

Master, A., Livingston, M., Sen Gupta, A.: 'Photodynamic nanomedicine in the treatment of solid tumors: perspectives and challenges', J. Control. Release, 2013, 168, pp. 98–105

Felsher, D.-W.: 'Cancer revoked: oncogenes as therapeutic targets', Nat. Rev. Cancer, 2003, 3, pp. 375–380

Xia, L.-Y., Zhang, X.-D., Cao, M., et al.: 'Enhanced fluorescence emission and singlet oxygen generation of photosensitizers embedded in injectable hydrogels for imaging-guided photodynamic cancer therapy', Biomacromolecules, 2017, 18, pp. 3073–3081

Bingang, H., Zhou, Z.-W., Luo, R.-J., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Wang, X., Chen, W.-T.: 'Gamma-emitting agent that inhibits cell proliferation, angiogenesis and metastasis', Anti Cancer Agents in Med. Chem., 2012, 11, pp. 994–1000

Pan, H., Michael, L., Bonhij, J., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Ding, W., Zhou, Z.-W., Luo, R.-J., et al.

Han, J., Lim, M., Bonhij, J., et al.

Ding, W., Zhou, Z.-W., Luo, R.-J., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.

Yan, L., Zhang, X.-D., Cao, M., et al.
Mantovani, A., Marchesi, F., Malesci, A., et al.

Hao, Y., Dong, Z.-L., Chen, M.-C., Lin, L.-S., Song, J.-B, Song, L., et al.

Rodell, C.-B., Ahmed, M.-S., Garris, C.-S., et al.

Togashi, Y., Shitara, K., Nishikawa, H.

Lauryn, E.-K., Ryan, M.-T.

Dai, X.-M., Meng, J.-S., Deng, S.-K., et al.

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