REVIEW

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Injectable hydrogels as emerging drug-delivery platforms for tumor therapy

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Tumor therapy continues to be a prominent field within biomedical research. The development of various drug carriers has been propelled by concerns surrounding the side effects and targeting efficacy of various chemotherapeutic drugs and other therapeutic agents. These carriers strive to enhance drug concentration at tumor sites, minimize systemic side effects, and improve therapeutic outcomes. Among the reported delivery systems, injectable hydrogels have emerged as an emerging candidate for the *in vivo* delivery of chemotherapeutic drugs due to their minimal invasive drug delivery properties. This review systematically summarizes the composition and preparation methodologies of injectable hydrogels and further highlights the delivery mechanisms of diverse drugs using these hydrogels for tumor therapy, along with an in-depth discussion on the optimized therapeutic efficiency of drugs encapsulated within the hydrogels. The work concludes by providing a dynamic forward-looking perspective on the potential challenges and possible solutions of the *in situ* injectable hydrogels for non-surgical and real-time diagnostic applications.

1. Introduction

Tumors are a significant health concern and the second leading cause of death globally, affecting millions of people worldwide each year. Different types of cancer affect various body tissues, but they all share the common feature of abnormal cell proliferation due to genetic and epigenetic alterations.[1] Despite the success of current therapies, such as surgery, chemotherapy, immunotherapy, radiation therapy, and targeted therapy, in treating patients, these methods are often associated with a variety of serious side effects. Additionally, patients may experience cancer recurrence due to metastasis, as well as damage to normal organs.[2]

In recent decades, nano-drug delivery systems (NDDS) have provided a promising approach for controlled and targeted drug delivery and are a promising strategy for cancer treatment.[3] Nanocarriers, including liposomes, polymeric micelles, dendritic macromolecules, carbon nanotubes, gold nanoparticles, *etc.*, have the ability to recognize tumor cells and deliver therapeutic agents in a fixed ratio, causing little harm to healthy cells. All these nano-systems offer advantages such as tumor-targeted drug delivery, reduced systemic side effects, prolonged plasma circulation, and the ability to carry larger

drug payloads while preventing recognition by efflux pumps.[4,5] However, these conventional nano drug-carrying systems often suffer from several drawbacks, including poor targeting, premature release, and the need for frequent injections during the delivery process. Therefore, there is a pressing need for the development of a novel drug delivery system that can act as a "drug reservoir" and deliver directly to the tumor site for controlled on-demand drug delivery.[6,7]

Hydrogel is a class of polymer materials with a three-dimensional network structure, which is widely used in the fields of biomaterials, environmental engineering, and biomedicine.[8–10] Nanoparticles are considered drug formulations with good biocompatibility and biodegradability. Compared to conventional nanomaterials, hydrogels are capable of designing hydrogel drug-carrying systems with stimulus-responsive properties, taking into account the complex tissue-specific physiology at the macroscopic scale in combination with dynamic biophysical/biochemical stimuli. Hydrogels can meet a variety of clinical needs, such as drug and cell delivery, tissue repair and regeneration, bio-adhesion, and immunomodulation.[11] Injectable hydrogels, in particular, are becoming more and more widely used in the field of tissue engineering and drug delivery due to their tunable physical and chemical properties, controlled degradation, high water content, and the ability to achieve delivery in a minimally invasive manner.

Our group has made significant contributions to the field of injectable hydrogels in recent years, particularly in the context of osteoporosis and myocardial repair treatment. For

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example, Wei *et al.* report a double crosslinked peptide-based hydrogel that fosters cartilage regeneration through a feed-back-regulated mechanism. This hydrogel integrates multiple supramolecular interactions, including host–guest interactions and extensive hydrogen bonding networks, endowing the hydrogel with superior self-healing properties and mechanical robustness for synergistically enhanced lubrication and compressive properties.[12] Additionally, our group has developed a reactive oxygen species (ROS)-responsive hydrogel fabricated by mixing poly-3-amino-4-methoxybenzoicacid with TK-NH$_2$-modified gelatin (PAMB-G-TK) and 4-arm-PEG-succinimidyl glutarate ester (4-arm-PEG-SG) for topical application, designed to directly target the mitochondria within damaged cardiomyocytes. This approach regulates, in a feedback-driven manner, the release of the encapsulated liposomes and the depletion of excess ROS, thereby improving the activity of cardiomyocytes and enhancing myocardial infarction (MI) therapy.[13] These studies provide a foundation for understanding and advancing researches on the applications of injectable hydrogels in a broad biomedical field.

Based on this, injectable therapeutic hydrogels are gaining increasing attention as non-invasive multifunctional platforms for traceable nanomedicine. With the rapid development of injectable hydrogels, many reviews have elaborated on the uses of injectable therapeutic hydrogels in myocardial repair[14] and tissue regeneration.[15] In the context of therapeutic applications in oncology, a large number of research on injectable hydrogels have been summarized. However, most of the published papers focus on showcasing a single therapeutic aspect of injectable hydrogels for cancer therapy. There is a lack of reviews, to our knowledge, providing a comprehensive summary of injectable hydrogels used for diverse and combinatory therapeutic effects, which includes elegant integration with chemotherapy, gene therapy, radiotherapy, phototherapy, immunotherapy, and combined drug therapies within a three-dimension hydrogel network. Besides this key uniqueness of this review relative to the published data, the intrinsic properties of hydrogels are emphasized by highlighting their drug-loading capabilities and distinctive structural modulation features. Last but not least, in-depth discussion and instructive prospects on the existing challenges and possible solutions, and clinical translation potentials of injectable hydrogels are made to promote a well understanding of injectable hydrogels from a fresh perspective (Fig. 1).

2. Brief introduction to hydrogels

Injectable hydrogels should not only be stable, economical, and easy to fabricate, but also should be formulated with injectable drugs to promote hydrogel formation, enable uniform and consistent drug loading, and facilitate drug release.[18–20] These hydrogels come with numerous advantages. (i) High water absorption capacity. The molecular structure of hydrogels contains a large number of hydrogen bonds, which are able to form strong interaction forces with water mole-
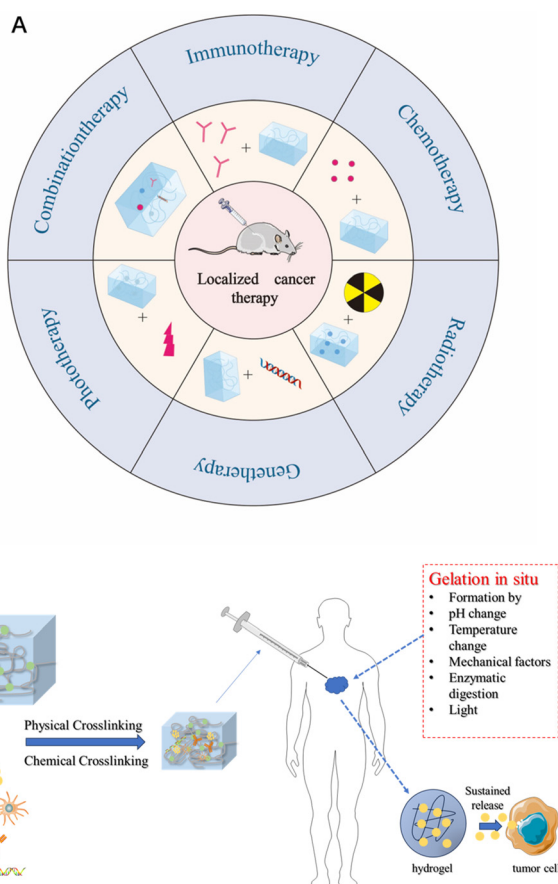

Fig. 1 (A) Schematic diagram of a hydrogel as a drug delivery platform for generating antitumor effects. (B) The role of hydrogels in tumor therapy.

cules. At the same time, small molecule water-soluble solutes penetrate easily in the hydrogel, which enables the loaded substance to remain active for a long time and ensures the continuous prolonged release of the drug in the body. (ii) Soft nature. The hydrogel can maintain a certain shape, and its meticulous structure prevents the leakage of the drug and also hinders the degradation of unstable macromolecules such as recombinant proteins and monoclonal antibodies. (iii) Commendable biocompatibility. Hydrogels are aggregates of water molecules and networks of hydrophilic polymers, and hydrogel formulations made from materials that can be absorbed and metabolized by the body have good biocompatibility and low cytotoxicity. The soft hydrophilic surface makes it easy to adhere to tissues, while its similarity to body tissues reduces its immunogenicity.[17,21] Additionally, the degradability makes it easy to metabolize and facilitates the complete removal of the carrier polymer from the body after the release of the drug. (iv) Controlled release. Injectable hydrogels are ideal drug delivery systems with the potential for non-invasive drug delivery,[16,22–25] and *in vivo* gel–sol transition enables localized drug delivery avoiding the problems of systemic drug delivery and increasing the local drug concentration.[25,26] It can spatially and temporally control the release of loaded thera-

peutic agents and prolong their action by modulating physical or chemical properties. For oncology therapy, injectable hydrogel formulations loaded with anticancer drugs can be easily prepared without loss of anticancer drugs and can be delivered directly to and accumulate in specific tumor sites, thereby minimizing drug accumulation in other organs, enhancing therapeutic efficacy and minimizing adverse drug reactions to normal tissues.[27,28] Hydrogels can also be used *in situ* to form bionic scaffolds for tissue regeneration while providing sustained release of encapsulated drugs.

3. Hydrogel drug loading method

Injectable hydrogels are capable of encapsulating drugs *via* multiple mechanisms, including physical encapsulation, chemical bonding, swelling, and cross-linking *etc*. During the physical encapsulation process, drugs are adsorbed onto hydrogels *via* various supramolecular forces, such as electrostatic interactions, hydrogen bonding, and van der Waals forces between the drug molecules and the hydrogel matrix. For example, Abraham *et al.* developed cationic hydrogels based on fluorenylmethyloxycarbonyl-modified phenylalanine (Fmoc–Phe) for the encapsulation and controlled release of diclofenac. The charge reversal property of this hydrogel at a physiological pH promotes functional pain management.[29] Sato *et al.* prepared poly(acrylamide) and poly(dimethylacrylamide) gels with surface significantly adsorbed submicron silica particles solely by van der Waals forces. Despite of a Hamaker constant much smaller in water than that in air, the van der Waals attraction between the particles and the gel is sufficient to facilitate adsorption.[30]

The relatively straightforward physical drug encapsulation is inevitably associated with a rapid drug release profile. Contrary to physical encapsulation, a chemical bonding process generally offers greater stability and a better controlled drug release behavior due to dynamic drug conjugation. During this process, the drug molecules undergo a chemical reaction with the reactive sites of hydrogels, resulting in the formation of covalent or ionic bonds for stable drug entrapment within the hydrogel matrix. Popescu *et al.* developed an amphiphilic, pH-sensitive hydrogel conjugated with a triblock copolypeptide *via* an imine bond generated by a Schiff base reaction between the N-terminus of the peptide and benzaldehyde of the hydrogel. The coupling and decoupling of the benzoic acid–imine dynamic covalent bond induces a pH-responsive reversible sol–gel transition for accelerated drug release.[31] Werner *et al.* engineered star PEG–heparin hydrogel networks that are crosslinked through Michael-type addition reactions. The incorporated MMP-cleavable peptides facilitate localized controlled VEGF delivery. Enzymatic cleavage was significantly accelerated when the gels' accessibility to proteases increased in contrast to the slow non-enzymatic hydrolysis and degradation of ester bonds. Consequently, the combination of protease-sensitive and -insensitive cleavage sites can enhance

the degradation of bio-responsive gel materials for promoted endothelial cell morphogenesis.[32]

Hydrogels possess an exceptional swelling capacity due to high water absorption and entrapment. Upon drug dissolution in water, the hydrogel undergoes simultaneous swelling and drug encapsulation *via* water absorption. This swelling-based encapsulation mechanism facilitates a sustained drug release profile but is generally limited to hydrophilic drug species. Mayte *et al.* developed chitosan-based covalent hydrogels through the Mannich reaction between the amino groups of chitosan and the hydroxymethyl groups of tetrakis(hydroxymethyl)phosphonium chloride as a crosslinking agent. The resulting hydrogels exhibited pH-sensitive second-order swelling kinetics and enabled a sustained release of the encapsulated camptothecin over 48 hours.[33]

Additionally, utilization of crosslinking agents generates an interconnected polymer network within the hydrogel for drug encapsulation. For instance, a phosphate crosslinker, bis[2-methacryloyloxy] ethyl phosphate (BMEP) was employed in the construction of Eswaramma's dual-responsive hydrogel networks (PPAD) composed of pectin and poly((2-dimethylamino) ethyl methacrylate). These hydrogel networks successfully employed for the encapsulation of the anticancer drug 5-fluorouracil (5-FU).[34] Yan *et al.* developed a straightforward and adaptable strategy for fabricating low hysteresis hydrogels by employing porous cationic polymers (PCPs) with adjustable counteranions as dynamic physical cross-linkers. The PCPs interpenetrated and became entangled with the polymer chain segments to form the hydrogels' topological structure. The reactive centers abundant in the PCPs effectively immobilized the polymer chains for inhibited segment slippage and minimized energy dissipation and enhanced elasticity.[35]

4. Response mode of injectable hydrogel

The loading of drugs on hydrogels can be divided into physical and chemical, physical binding is loading the drug after encapsulation and adsorption through hydrophobic interactions, coordination, *etc*., and chemical binding usually involves connecting the drug with the carrier hydrogel through bonds that can be enzymatically cleaved or hydrolyzed, and then releasing the drug after the bonds are enzymatically cleaved or hydrolyzed *in vivo*. Injectable hydrogels are also prepared in a very similar way to drug loading methods, which can be categorized into chemically cross-linked and physically cross-linked hydrogels, depending on the type of cross-linking used.[36,37] On the one hand, physical cross-linking is transient cross-linking that relies on physical interactions, such as ionic, hydrogen, and hydrophobic bonding, as well as inter- and intra-physical interactions. Therefore, physically cross-linked hydrogels usually be used to design delivery systems with self-healing and stress-responsive features. On the other hand, chemical cross-linking, produces chemical reactions, such as covalent bonding to build a persistent three-dimensional

Fig. 2 Construction strategy and response mechanism for injectable hydrogels.

network through light, enzymes, and clicking cross-linkers. Chemically cross-linked hydrogels are structurally stable and permanent.[38,39] The following section will focus on injectable hydrogels formed by physical and chemical methods (Fig. 2).

4.1. Physical cross-linking method

The relatively weak nature of physical cross-linking allows its reversible transformation back to solution when external conditions fluctuate. This flexibility facilitates the formation of hydrogel matrices that can adapt according to different biological conditions.[40,41] Moreover, physically cross-linked hydrogels eliminate the need for cross-linkers or chemical modifications.[42,43]

4.1.1. Polymer chain entanglement. Hydrogels consist of numerous tightly intertwined polymer chains, with tension transferred among these chains along their chain length. These polymers create hydrogels with high ductility, strength, and fatigue resistance. Upon immersion in water, the polymers dissolve into an equilibrium state, forming the hydrogel matrix. Yan *et al.* devised a method for designing hydrogels with low hysteresis, and high toughness by leveraging interactions between porous cationic polymers (PCPs) and entangled polymer chains. This approach allows for tunable counterions, enabling reversible bonding between the PCP framework/polymer segments (polyacrylamide, PAAm) and counterions/PAAm.[44]

4.1.2. Electrostatic interactions. Electrostatic interactions, a common type of physical bond, involve the interactions between two opposite charges, such as positive and negative electrolyte groups. The intensity of the electrostatic interactions primarily depends on the total charge density within the polyelectrolyte; a higher molecular weight directly corre-

lates with an increased total charge density, and consequently, strengthens the electrostatic interactions. Additionally, multiple stimuli such as pH changes, thermal and mechanical factors, and external electric reduction can also cause electrostatic interactions.[43] Biopolymers such as chitosan, pectin, cellulose, and polygalacturonate can chelate divalent cations (*e.g.*, Ca^{2+}, Mg^{2+}, Ba^{2+}) or trivalent cations (*e.g.*, Al^{3+}, Fe^{3+}) of carboxylates on the adjacent biopolymer chains to form a tight network, resulting in a metal alginate hydrogel with improved mechanical properties. For example, Qian *et al.* introduced a programmable electrostatic interaction into hydrogels based on the above properties and prepared a dual-network hydrogel structure, the structure is a composite hydrogel formed by immersing pre-dispersed chitosan in a solution of silicotungstic acid, as the small molecules of silicotungstic acid diffuse into the hydrogel, the amine groups in the chitosan molecules are protonated with a positive charge, and cross-link with the negatively charged silicotungstic acid clusters to form multivalent electrostatic interactions.[45] Wei *et al.* developed an integrated "all-in-one" *in situ* double-crosslinked conductive hydrogel that exhibits favorable handling characteristics *via* chemical covalent bonding and ionic interactions, which possessed self-healing capabilities and enhanced the mechanical properties by adapting to the dynamic myocardial systole–diastole cycle.[46]

4.1.3. Hydrogen bonding. A hydrogen bond functions as a single-atom non-covalent bond with fragile chemical properties. It primarily serves as a link between molecules, allowing for the creation of a molecular network structure through intermolecular bonding. This network enhances the stability of weak bonding connections and modulates configurations within molecules to alter the substances' chemical properties.

Hydrogen-bonded cross-linked hydrogel has many advantages: (i) superior water absorption. Due to the large number of hydrogen bonds in its molecular structure, these hydrogen bonds can form a strong interaction force with water molecules, thereby forming a three-dimensional network structure inside the gel. (ii) Excellent biocompatibility made possible by the water-like structure, which enables the hydrogel to bond closely with water molecules in living organisms. This characteristic makes hydrogen-bonded hydrogel widely used in the medical field. (iii) Outstanding mechanical properties. Its molecular structure contains a large number of hydrogen bonds that can form a meticulous cross-linking network structure, providing the gel with satisfactory strength and toughness that finds use in the preparation of high-strength, high-toughness materials, such as high-strength fibers, and high-strength composite materials. (iv) Exceptional controllability. Hydrogen bonds are able to form specific interaction forces with other molecules, which can be used to change the function of the hydrogel by adjusting the molecular structure. This property makes hydrogen-bonded cross-linked hydrogels superior and controllable, and they shine in the biomedical field.

Utilizing the above advantages of hydrogen bonding, Chen successfully prepared a high-strength, injectable supramolecular hydrogel by constructing a multi-hydrogen bonding system. It is self-assembled from a single nucleoside molecular gelling agent (2-amino-2′-fluoro-2′-deoxyadenosine (2-FA)) using distilled water/phosphate-buffered saline as solvent. The remarkable energy storage modulus, reaching 1 MPa at a concentration of 5.0 wt%, positions it as the strongest supramolecular hydrogel containing an ultra-low molecular weight (MW < 300) gelling agent.[47] Inspired by the slow transformation of mineral precursors during biomineralization, Li proposed a general endogenous gelation method for tannic acid-macromolecule hydrogels. The method controls the elimination or slow generation of hydrogen bonding interactions between tannins and macromolecules by regulating the pH value of the system. In order to prepare the hydrogel precursor, the pH of the tannin solution is first increased before combining the components. This process ensures that the phenolic hydroxyl groups transition into a phenol-negative ion state, which inhibits the formation of a sufficient quantity of hydrogen bonds to establish a crosslinked network when combined with the macromolecules. Subsequently, gluconolactone is added to the solution. As gluconolactone has the ability to gradually release hydrogen ions, this allows for the slow generation of hydrogen bonding interactions.[48] The disintegration conditions of hydrogels formed through hydrogen bonding primarily depend on the hydrogen bonds' interaction with surrounding water molecules, the forces exerted on the crosslinked interconnecting chains, and external loads strong enough to disrupt the crosslinks, and change the network's microstructure, leading to drug release.

4.1.4. Hydrophobic interactions. Hydrophobic interactions are important non-bonding interactions that favor the solubility of hydrophilic structures in water under certain conditions, as well as the solubility of amphiphilic substances in aqueous solutions. It has been reported that the surface structure of some hydrogels is related to hydrophobic groups, and the response relationship of the gel to environmental changes (pH, temperature, solvent, etc.) or the cycle of drug release is also altered by hydrophobic interactions induced by the hydrophobic groups. The conformational rearrangement of water molecules leads to hydrophobic interactions when the two hydrophobic substances are in close proximity, which adds a new driving force to the solubilization-an absorption equilibrium of the gels. Hydrophobic interactions may exhibit attraction over a longer range than other reversible interactions, such as ionic and hydrogen bonding, and thus may confer superior tensile properties to hydrogel materials. For example, by introducing cellulose nanocrystals or hydrophobized cellulose nanocrystals into polymers that are physically cross-linked through hydrophobic interactions, Lu et al. have designed novel dual physically crosslinked (DPC) hydrogels with excellent tensile strength, ultra-high elongation, and reparability.[49]

The hydrophobic interaction-driven gelation mainly exhibits thermo-responsive behavior.[50] Poly(N,N-diethylacrylamide) (PDEA) is a thermosresponsive polymer that can be used as a trigger for the formation of thermally reversible gels. Michael et al. synthesized ABA copolymers with the structure PDEA-b-poly(ethylene glycol) (PEG)-b-PDEA, yielding four block copolymers of PDEA and PEG with different molecular weights and high molecular weight PEG blocks to form thermally reversible gels with predominantly similar solid behavior.[51] Finally, A20–B10–A20 was selected as the most promising thermoreversible gel. It can be made thermoreversible by controlling the concentration (30% w/v) and ionic strength (0.3 m NaCl) and temperature control below 37 °C, making it possible that gelation occurs upon contact with the internal temperature of the human body.

These above-mentioned nonpolar hydrophobic moieties aggregated together have strong hydrophobic interactions and can form several hydrophobic microregions (or micelles), which can accommodate small organic molecules of drugs under specific conditions and release them slowly under other specific conditions, overcoming the problem of rapid release of drugs from gels in the swelling state.[40]

4.1.5. Metal coordination. The covalent attachment of ligand groups to the polymer backbone, together with the incorporation of metal ions, lead to the formation of cross-linking hydrogels via ligand–metal coordination interactions. The metal–ligand bonds are commonly reversible and serve as an effective chemical strategy for the preparation of injectable hydrogels with shear-thinning and self-healing properties. For example, Birkedal et al. developed a catechol-based hydrogel that allows for precise control over the degree of oxidative covalent cross-linking. Double cross-linked hydrogels with tunable stiffness were constructed by incorporating the oxidizable catechol analog, tannic acid, into an oxidation-resistant hydrogel matrix and further coordination of the dihydroxy functionality of 1-(2′-carboxyethyl)-2-methyl-3-hydroxy-4-pyridi-

none with trivalent metal ions. By varying the amount of tannic acid added, the stiffness of the hydrogel can be customized for specific applications while maintaining the self-healing capabilities of the coordinated chemical components.[52]

In summary, physically crosslinked hydrogels exhibit superior biocompatibility and biodegradability due to minimal use of any chemical reagents during the hydrogel preparation process, thus are environmental friendly with significantly reduced environmental pollution. They generally have the abilities to form three-dimensional interconnected structures without the aid of any additional cross-linking agents. These advantages make physically crosslinked hydrogels undergo easy administration *via* injection through a fine needle and rapid gelation post-injection.

However, as the physical interactions depend substantially on the inherent properties of the used biomaterials, certain characteristics of these materials, such as gelation time, *in vitro* and *in vivo* maintenance, and mechanical properties of hydrogels are actually inherent inflexible. Consequently, precise modulation of the *in vivo* properties of hydrogels may constitute a significant challenge.[53,54] Nevertheless, these drawbacks can be overcome by using biomaterials with different molecular weights and concentrations, as well as composite biomaterials, which generates hydrogels with diverse hardness, viscosities, rheological behaviors, swelling and disintegration properties, and biocompatibilities. Overall, injectable, *in situ* forming hydrogels prepared by physical interactions have been extensively used for intra-tumor injections.

4.2. Chemical crosslinking preparation

Chemical synthesis is another important and commonly used method for preparing hydrogels. Chemical cross-linking is the creation of irreversible covalent bonds through chemical reactions (*e.g.*, free radical polymerization and click chemistry) to build a durable polymer structure. Hydrogels formed by this method have strong covalent bonds that can be preserved structurally stable and permanent, only swelling and not dissolving.

A covalent bond is a permanent connection between two molecules. The formation of covalent bonds within and between chains of biomaterials can lead to permanent immobilization of biomaterials or changes in their physical properties.[55] These covalent bonds can be used to prepare injectable hydrogels through chemical reactions such as thermal response, pressure, pH change, light irradiation, click reaction, or enzymatic activity.

4.2.1. Temperature response. Among the various types of stimuli-responsive hydrogels, thermo-responsive hydrogels are the classic example of clinically approved formulations used as drug delivery vehicles due to the ubiquity of heat as an external stimulus throughout the body following administration. At room temperature, hydrogels commonly exhibit characteristics of low-viscosity sols, which transform into gels when raised to body temperature. These thermoresponsive hydrogels offer

potential as innovative materials capable of extending drug retention time at the administration site, thereby modifying the drug release patterns or altering its absorption rate within the body. Hirayama *et al.* developed a thermo-responsive injectable hydrogel for sustained release of drugs by utilizing the host–guest interactions between hydrophobically modified hydroxypropylmethylcellulose (HM-HPMC) and cyclodextrins (CD). The host–guest interaction enables the HM-HPMC hydrogel to transform into a low-viscosity sol. Under conditions approximating body temperature, the HM-HPMC/β-CD hydrogel solidifies into a gel as the host dissociates from the polymer's hydrophobic portion in response to this temperature.[56]

4.2.2. Pressure response. The mechanical properties of hydrogels are strongly dependent on crosslink density, volume fraction, and polymer chain interactions. By modulating these factors with external triggers, hydrogels can be induced to become either tough or soft depending on the conditions. The formation and breaking of covalent bonds play a crucial role in these factors. Hydrogels are soft materials that consist of cross-linked polymer chains forming a three-dimensional network structure that absorbs large amounts of solvent in response to osmotic pressure. Despite this, the hydrogel remains solid and possesses a certain level of mechanical properties. The elastic modulus of the hydrogel is determined by polymer volume fraction, solvent mass, and molar mass between the crosslinks. When the gel swells, both stiffness and toughness decrease. However, for low-swelling hydrogels (which are usually characterized by low polymer volume fractions and weak dissipation factors), physical interactions that allow reversible fracture under tension[57,58] can significantly increase the toughness of hydrogels. Gel plasticity also contributes significantly to hydrogel strengthening, which can be achieved by introducing structural domains that can only deform above a specific pressure level.

4.2.3. pH response. Injectable hydrogels prompt *in situ* gelation, responding to specific stimuli or experiencing biodegradation to regulate drug release. Beyond the parameters of drug preparation, considering the microenvironment of the target site bears significant value. Throughout the uncontrolled, escalating proliferation of tumor cells, the glycolytic metabolic pathway continually excretes lactic acid, causing a slightly acidic extracellular pH in the tumor. As a result, several strategies can be used to develop pH-responsive drug-release hydrogels. These include utilizing bonds that degrade in acidic pH environments, such as Schiff base bonds, and polymers with ionizable groups like amines: these groups may either gain or lose protons based on pH fluctuations. For instance, Tong *et al.* constructed a biocompatible, tunable, and injectable cellulose-based hydrogel embedded with pH-responsive double-block copolymer micelles to enable topical drug delivery with prolonged, stimulus-driven, and slow-release functionality for a drug delivery system that exhibits pH-triggered, prolonged, and slow-release characteristics.[59]

4.2.4. Light response. Light-curing hydrogels are cured by polymerization initiated by visible or ultraviolet light to form a three-dimensional network-like structure. A significant advan-

tage of light-curing hydrogel is the precise control over the shape of the hydrogel, including the height, shape (bar, circle, complex patterns), *etc*. This feature makes it suitable for constructing the precise structure of the human body to simulate the environment of the tissues, and especially suitable for the application in the human body tissue repair and the *in vivo* drug slow-release technology compared to the oxidation–reduction initiator preparation of hydrogel technology. Light-curing hydrogels also have the advantages of *in situ* polymerization and curing, short curing time, low reaction heat, and unaffected polymerization reaction by the ambient temperature. The preparation of light-curing hydrogels requires the addition of a photoinitiator, which generates free radicals by absorbing light energy to trigger the polymerization and curing of the hydrogel precursor liquid. For the light curing of medical hydrogel, the photoinitiator used should be biocompatible, non-toxic, and easily degradable. However, light irradiation (especially UV light) can lead to cytotoxicity and potential genetic mutations and few compounds are available as suitable solvents and non-cytotoxic activators (*i.e.*, photoinitiators) *in vivo*.[60,61] Photoreactive synthesis of hydrogels is generally recognized as a fast and practical method.[40,62] Various photosensitizing materials have been developed[63] and extensively studied in various biological fields.[64–68] Among them, azobenzene, spirogyra, *p*-nitrobenzyl, galactose, 7-diethyl-amino-4-thiocoumarinomethyl, cumin, and cinnamic acid contain anthracene or acrylate groups exhibit photosensitizing properties, facilitating fast and straightforward hydrogel formation *via* light irradiation. This functionality presents a great advantage especially during *in vivo* injections, as it enhances the precision over the control of the injection site.[61]

4.2.5. Click response. The click reaction is formed by a mixture of click group-modified biomaterials that offer several advantages, including selectivity, absence of by-products, no need for product purification, and the ability to be carried out in an aqueous solution. This allows for the rapid formation of hydrogels with tunable mechanical properties for *in vivo* applications.[69,70] In recent years, click-reactive materials (*e.g.*, formed *via* copper-catalyzed azide–alkyne cycloaddition or alkyne–azide reactions, Diels–Alder reactions, Schiff base formation, Michael addition, or thioenol addition) have been introduced into biomaterials with a variety of structures (*e.g.*, straight-chained, dendrimeric macromolecules, and network structures). For example, Liu *et al.* synthesized the thermosensitive copolymer F127@ChS (F127 crosslinked chondroitin sulfate) with a low gelation concentration at 37 °C *via* DA Click chemistry between F127-AMI (maleimide-based terminated F127) and ChS–furan (furfurylamine grafted chondroitin). The physical crosslinking of F127@ChS provided the rapid gelling behavior of the hydrogel, while the *in situ* DA click reaction that occurred between F127@ChS and PEG–AMI provided covalent cross-linking of the hydrogel system.[71] The click reaction between the click-reactive materials can rapidly form bioorthogonal cross-linked hydrogels in aqueous media as well as in physiological environments in the absence of catalysts or external energy[72] The Click group-modified biomaterials can readily facilitate covalent bond formation in injectable *in situ*-forming hydrogels within seconds. However, click reactions are potentially cytotoxic because they involve the use of copper. In addition, the duration of the click reaction may depend on the regional specificity of the click reagent.[73,74]

4.2.6. Enzyme response. Enzyme-mediated cross-linking can form hydrogels under physiological conditions with favorable mild reaction conditions. This method surpasses other traditional methods, such as physical cross-linking, by avoiding issues like low physical strength and poor stability of the prepared hydrogels. Furthermore, enzyme-catalyzed self-assembling hydrogels demonstrate advantages such as precise chemistry, regioselectivity, and the mild nature of the catalytic reaction. Consequently, injectable *in situ* hydrogels prepared through enzyme reactions are increasingly replacing those set by metal catalysts and light irradiation as alternatives. Active enzymes for injectable *in situ* hydrogel formation include laccase, horseradish peroxidase (HRP), glutamine transaminase (TGase), tyrosinase (Tyr), and lysine oxidase and catalase (H_2O_2).[75–78] Unlike smart responses such as pH and temperature, which disappear after the stimuli stop, enzyme-responsive materials retain their function after the enzyme disappears. In addition, numerous enzymes present in living organisms contribute to hydrogel degradation and thus facilitate slow or controlled drug release. The strong covalent bonds formed *via* enzyme-mediated cross-linking usually take less than 10 minutes, and the kinetics of hydrogel formation can be regulated by adjusting the enzyme concentration. Furthermore, the products of these reactions tend to be highly biocompatible, making them ideal for the preparation of injectable hydrogels. Future research should focus on novel materials for enzyme-responsive hydrogels and investigative studies on the hydrogel degradation mechanism to control drug release. This could lead to the development of hydrogel systems responsive to multiple enzymes and control the timing of multiple drug releases, thereby establishing a spatiotemporally controllable system.

Chemical cross-linking imparts superior stability in mechanical properties and diverse morphologies, surpassing the aforementioned deficiencies of physically cross-linked hydrogels. However, it usually involve the use and generation of certain toxic residues and complicated chemical design and synthesis with compromised scalable production and translational potentials.

Physically or chemically cross-linked hydrogels contain various reversible bonds, including metal–ligand, Diels–Alder covalent, imine, and acyl hydrazone. Activation of these bonds, triggered by a specific external temperature or pH, enables the formation of self-healing hydrogels.[79] This category of hydrogels, primarily constituted by reversible chemical reactions, exhibits the unique ability to transiently fluidize under shear stress, subsequently restoring their original mechanical properties. Such self-healing hydrogels, often employed in the development of drug/cell delivery systems, can be injected in a localized, targeted, and minimally invasive manner through a narrow syringe without the necessity for invasive surgical procedures.

5. Delivery of chemotherapeutic agents

Chemotherapy, proven as a highly effective anti-cancer treatment, eliminates tumor cells primarily by obstructing DNA replication or protein synthesis. Several studies have shown that low doses and slow drug infusions are more effective than aggressive chemotherapy given in multiple high doses. Injectable hydrogels, which allow localized co-delivery of multiple drugs with different therapeutic mechanisms around tumors, have made significant advances in the treatment of cancer.[80] Controlled release of chemotherapeutic agents can be achieved by modulating the properties of hydrogels or external stimuli, including pH, temperature, enzymes, reactive oxygen species, and radiation. The application of drug delivery systems not only reduces the side effects of chemotherapeutic drugs in cancer cells but also improves the efficacy of cancer treatment via controlling drug release. Chemotherapeutic agents can be categorized according to their composition and therapeutic mechanism (Table 1).

Alkylating agents, often seen as early-discovered and diverse anti-neoplastic agents, fall under the chemotherapeutic agents' classification. Using Cisplatin (CDDP) as a classic alkylating agent example, its robust antitumor effects stem from its irreparable interactions with nuclear DNA. Moreover, CDDP can interact with other biomolecules such as thionin and RNA to inhibit or modulate signaling pathways accountable for mediating cell death. By affecting DNA and killing cells at different stages of division (inclusive of the G0 stage), it brings about diversified side effects such as severe nephrotoxic, neurotoxic, and ototoxic side effects. Despite the potential to encapsulate CDDP into nanoscale drug delivery formulations enhancing its physicochemical properties, the stability and cancer cell-specific targeting insufficiency in formulations need novel carrier exploration for CDDP-targeted delivery.[81] The cytotoxicity of the drug to other tissues is mainly reduced by restricting the drug release conditions. Lee et al. synthesized chondroitin sulfate nano gels bearing cisplatin (CS-nano gels) through a chelating ligand–metal ligand cross-linking reaction. These nano gels were incorporated into pH- and temperature-responsive bioabsorbable hydrogels based on poly(ethylene glycol)–poly(β-amino ester urethane) (PEG–PAEU) for tumor cell-specific delivery. CDDP was released gradually under physiological conditions (pH 7.4), while accelerated CDDP release was triggered upon shift to an acidic pH condition (pH 5.0), which can effectively increase the localized drug concentration in the TME for inhibiting tumor cell proliferation (Fig. 3A).[82] The fluorescein 5-thiosemicarbazone-labeled CS-nano gels, released from hydrogels, selectively bind to the A549 lung cancer cell line, without any affinity for NIH 3T3 cells lacking of overexpressed CD44 receptors. Therefore, an in vitro cytotoxicity assay demonstrated the potent inhibitory effect of CS-nano gels on the growth of A549 lung cancer cells. Such an intelligent delivery technique allowed better cisplatin targeting to the tumor site, thereby improving its bioavailability.

Table 1 Injectable hydrogels in the delivery of classical chemotherapeutic agents

| Classification of chemotherapy drugs	Mechanism of action	Representative drugs	Hydrogel matrix	Gelling mechanism	Release mechanisms	Cancer types	Tumor model (in vivo)	Ref.
Alkylating agent	DNA destruction	Cisplatinum	Poly(ethylene glycol)–poly(β-aminoester urethane) (PEG–PAEU)	Chelating ligand–metal coordination cross-coupling reactions	pH and temperature responsiveness	Lung cancer	A549	82
Anti-metabolite	Interrupts the S phase of the cell cycle	5-Fluorouracil (5-FU)	Biodegradable temperature-sensitive hydrogel (PDLLA–PEG–PDLLA, PLEL)	Responding to physiological temperatures	Temperature response	Stomach cancer	MKN45-Luc	84
Anthracycline	Inhibition of enzymes causing DNA replication	Doxorubicin (DOX)	Poly(acrylic acid-co-4-vinyl-phenylboronic acid) nanohydrogels (P(AA-co-4-VPBA) NG)	Reflux-precipitation polymerization	pH and redox stimuli-responsive	Breast cancer	MCF-7	86
Topoisomerase inhibitors	Inhibiting topoisomerase activity	Topotecan	Chitosan (CS) and β-glycerophosphate (β-GP)	Responding to physiological temperatures	Temperature response	Liver cancer	H22	88
Alkaloid	Termination of M phase of cell cycle	Paclitaxel	The methoxy poly(ethylene glycol)-b-poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone) [impact]	Hydrophobic interaction	Photothermal response	Breast cancer	4T1	89
Corticosteroids	Inhibits tumor cell production	Dexamethasone	Dexamethasone (Dex)-loaded microspheres (Dex-Ms) mixed with click-crosslinked hyaluronic acid (Cx-HA)	Click-crosslinked	—	—	FRTL-5	90
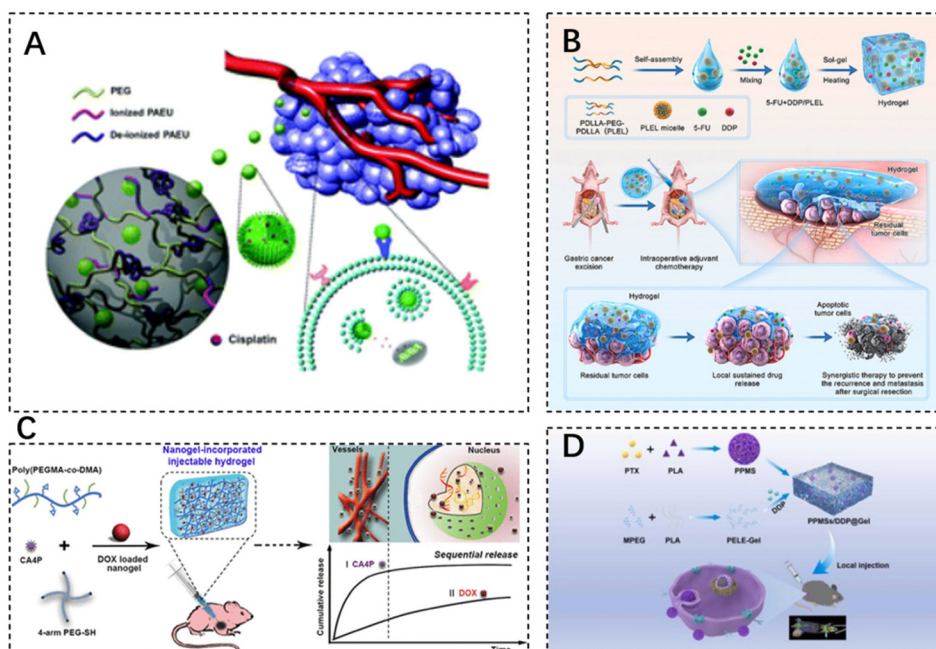
Fig. 3 The use of injectable hydrogels in chemotherapeutic drug delivery. (A) Chondroitin sulfate nanogels (CS-nanogels) with CDDP incorporated into pH- and temperature-responsive bioabsorbable poly(ethylene glycol)−poly(β-amino ester urethane) (PEG−PAEU) hydrogels for CDDP-specific delivery to cancer cells. Reproduced with permission from ref. 82. Copyright 2017, Royal Society of Chemistry. (B) Schematic diagram of sustained co-delivery of 5-fluorouracil (5-FU) and *cis*-platinum (DDP) *via* biodegradable thermo-sensitive hydrogel. Reproduced with permission from ref. 84. Copyright 2022, KeAi. (C) Preparation process of injectable Nanogel-Incorporated Hydrogel (NHG) for Sequential Local Delivery of CA4P and DOX. Reproduced with permission from ref. 86. Copyright 2018, American Chemical Society. (D) Construction of a dual-drug co-delivery hydrogel system (PPMSs/DDP@Gel) for *in situ* chemotherapy by co-embedding PPMSs and cisplatin (DDP) in a thermosensitive hydrogel. Reproduced with permission from ref. 87. Copyright 2023, Elsevier Masson SAS.

5-Fluorouracil (5-FU) is a type of antimetabolite that operates by interrupting the S-phase of tumor cells. It is often used as a first-line chemotherapeutic agent for rectal adenocarcinomas. However, 5-FU presents cardiotoxicity risks, including coronary vasospasm, coronary thrombosis, cardiomyopathy, and sudden cardiac death.[83] Qian *et al.* designed a platinum (DDP) and 5-FU co-delivery system based on *cis*-biodegradable temperature-sensitive hydrogel (PDLLA–PEG–PDLLA, PLEL) for intraoperative adjuvant combination chemotherapy of gastric cancer. This hydrogel system presents a specific sol–gel phase transition in response to physiological temperature and exhibits sustained drug release *in vitro* and *in vivo*. As evidenced, strong synergistic cell proliferation inhibition and apoptosis promotion effects of hydrogels were observed on gastric cancer MKN45-luc cells. Dual drug-loaded hydrogel formulations showed superior antitumor effects compared to single drug-loaded analogues and the free drug combination, thus assisting in side effect minimization in a peritoneal metastasis model of gastric cancer (Fig. 3B).[84]

Doxorubicin (DOX), an anthracycline antibiotic example, stands out as a widely-used model cancer drug for drug delivery studies. It comprises glycosides, which contain a tetracyclic ring and a sugar portion. The drug can prevent DNA replication and RNA transcription by embedding into DNA and inhibiting DNA and RNA polymerase reactions. DOX (Adriamycin) has several cytotoxic effects, cardiotoxicity, myelosuppression, and alopecia.[85] The current application of DOX is used in combination with

chemotherapy to reduce toxicity and improve efficacy. Toward this end, Wang *et al.* designed a dual drug delivery system based on nanogel-doped injectable hydrogel (NHG) for sequential topical delivery of combretastatin A4 phosphate (CA4P) and adriamycin (DOX) toward combined antiangiogenic and anticancer therapy. Injectable hydrogels were prepared to load and rapidly release the hydrophilic drug CA4P, while pH- and redox-stimulation-responsive nanohydrogels were incorporated into the injectable hydrogels *via* pH-responsive borate bonds to maintain long-term DOX delivery. Such an approach offers universal drug carriers for local dual drug delivery offering sequential release behavior through simple injections (Fig. 3C).[86] DOX–CA4P@NHG significantly reduced cell viability compared to single-agent-loaded hydrogels by approximately 23% and 14% after 48 and 72 hours of incubation, respectively, which confirmed that the co-administration could greatly improve the therapeutic efficiency.

Porous polypropylene oxide (PLA) microspheres (PPMSs) loaded with paclitaxel (PTX) constructed *via* a modified double emulsion solvent evaporation method, along with cisplatin (DDP), were co-embedded in a thermosensitive hydrogel (PPMSs/DDP@Gel) for *in situ* chemotherapy, which is used for the treatment of melanoma by intra-tumor injection delivery. The system allowed the slow release of both drugs and exhibited good temperature-sensitive properties (Fig. 3D).[87]

Injectable hydrogels have been shown to be more suitable for intra-tumor drug therapy than traditional single carriers,

and they have great potential for oncology chemotherapy by acting as a companion to drugs for enhancing treatment efficiency.

6. Delivery of gene drugs

6.1. DNA hydrogels

DNA hydrogel is a hydrophilic material that exists between fluid and solid states and is composed of DNA. It can be designed to take on different shapes and sizes and is divided into two categories: single-component DNA hydrogel and multi-component DNA hydrogel.[91–94] Single-component DNA hydrogels are formed by using DNA as the only component, backbone, or cross-linking agent, either chemically (chemical bonding as the cross-linking point) or physically (non-covalent interactions such as hydrogen bonding, van der Waals' forces, or entanglement of DNA molecules between the strands, *etc.*). Multicomponent DNA hydrogels are usually constructed by DNA-mediated cross-linking of other materials, including biocompatible polymers such as polyacrylamide, chitosan, and polyethylene glycol,[95] as well as liposomes,[96] black phosphorus quantum dots,[97] carbon dots,[98] silica nanoparticles[99] nanoparticles, metal nanoparticles,[100] and other nanoparticles. Due to the high loading capacity, excellent mechanical stability, and viscoelasticity of hydrogels, effective encapsulation of biologically active molecules (*e.g.*, nucleotides, proteins, antibodies, and drugs) can be realized while their biological activities can be preserved.[101–103] DNA hydrogels, which use nucleic acid aptamers as specific detection elements, can serve as a new class of visualization platforms based on the DNA base complementary pairing principle and nucleic acid aptamer–target molecule interactions.

DNA is a polymer with a large number of negative charges, and its bases have the characteristics of aromatic ring structure, while DNA can be modified with many functional groups,

such as acrylate, amino, carboxyl, and thiols, *etc*. These unique properties make DNA can be combined with some polymers or nano-materials through covalent interactions, electrostatic force, hydrogen bonding, van der Waals force, *etc.*, which further expands the application of DNA hydrogel.[104,105] The reversible nature of the conformational changes between DNA can realize the reversible regulation of the hydrogel solution state and gel state. Through the encapsulation of color-developing substances and signal-amplifying enzymes, the DNA–hydrogel will change the state of the soul according to the response to target molecules, specifically releasing the stored signal substances and realizing the output of visual signals.

DNA structure-based nanoreactors are a promising biomaterial for antitumor therapy due to their inherent biodegradability, biocompatibility, and tunable multifunctionality. He *et al.* designed a smart DNA nano hydrogel that can target cancer cells, control size, respond to pH, and load glucose oxidase (GOx). GOx was successfully encapsulated in a DNA nanohydrogel by hybridizing two X-shaped DNA monomers and DNA junctions assembled together to form a DNA nano hydrogel. The DNA junctions were engineered with i-motif sequences and modified with ferrocene (Fc). In an acidic tumor microenvironment, the DNA nano hydrogel can be broken down to release GOx, which can oxidize intratumoral glucose to produce gluconic acid and H_2O_2. The generated H_2O_2 is catalyzed by Fc to induce the production of toxic hydroxyl radicals (–OH), which can effectively kill cancer cells (Fig. 4A).[106] The cytotoxicity assay of DNA nanohydrogels showed elevated cell mortality following Gel–GoxFc incubation, confirming its superior cell-killing capacity. In addition, Gel–Gox–Fc exhibited a total apoptosis rate of 51%.

6.2. Other gene drugs in hydrogels

Gene therapy is an ideal method for the treatment of diseases related to genetic abnormalities, which can be achieved by
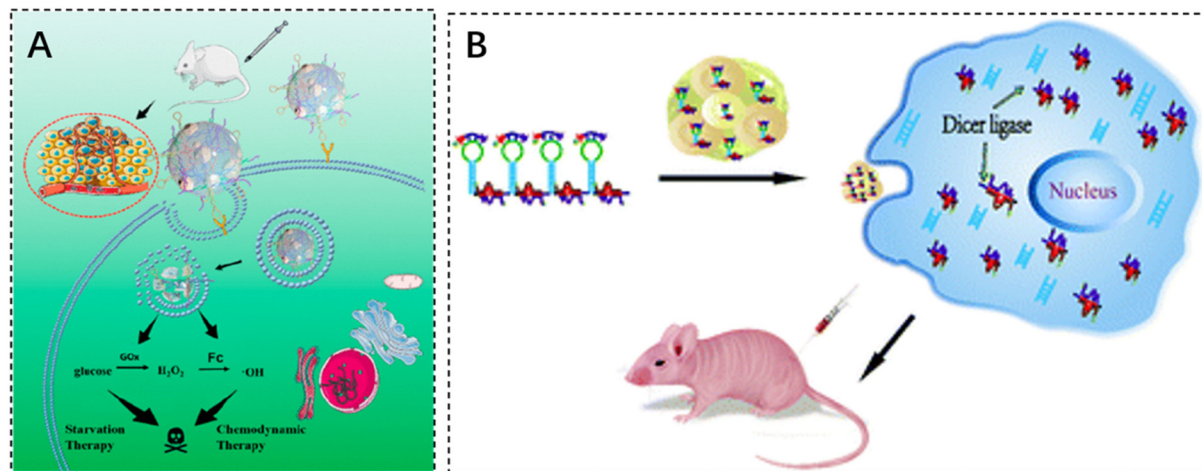

Fig. 4 The application of injectable hydrogels in gene therapy. (A) Schematic illustration of a self-assembled DNA nano hydrogel and application of the antitumor combination therapy. Reproduced with permission from ref. 106. Copyright 2021, American Chemical Society. (B) mRNA nanoparticles bound to CXCL9-loaded polyethylene glycol (PEG) hydrogels. Reproduced with permission from ref. 107. Copyright 2022, Springer Nature.

inserting molecules such as synthetic mRNA, cyclic RNA, and self-amplifying RNA (saRNA) to achieve upregulation of gene expression, which are usually made into the form of liposomal nanoparticles, polymer nanoparticles, inorganic nanoparticles and other forms of delivery into the body, but the problems of easy degradation, poor targeting, and low efficiency of entry into the target cells continue to hinder the effective gene therapy.

Maryam Rahman designed the HCM vaccine by combining mRNA nanoparticles with a polyethylene glycol (PEG) hydrogel loaded with the chemokine CXC ligand 9 (CXCL9). The HCM vaccine delivers a large mRNA payload *via* subcutaneous injection and recruits a diverse population of immune cells to produce significant anti-tumor efficacy (Fig. 4B).[107] In addition, Zhang *et al.* developed a novel RNA-triple helix-hydrogel for the treatment of triple-negative breast cancer (TNBC) by doping RNA-triple helix and siRNA double-stranded bodies of CXCR4 into the same RNA nanoparticles without the addition of synthetic polycationic reagents. The RNA-triple helix consists of a tumor suppressor miRNA (miRNA-205) and an oncomiR inhibitor (miRNA-221), both of which have shown excellent performance in synergistically eliminating tumors.[108] Following incubation with the RNA-triple-helix hydrogel for 36 hours, MDA-MB-231 cells exhibited a cell viability of mere 15%. Comparative analysis revealed that the RNA-triple-helix hydrogel outperformed free miRNA and RNA transcripts in silencing efficiency.

7. Other therapeutic agent delivery

7.1. For radiopharmaceutical delivery

Radiation therapy is a highly effective mainstream cancer treatment that kills cancer cells by targeting beams of high-energy radiation such as X-rays and gamma-rays to the tumor (external beam radiation therapy) or by introducing radioisotopes into the tumor through a catheter or other systemic route of administration. The main challenges of intense radiation therapy are the damage caused to adjacent normal tissues in the path of the radiation beam, the reduced response of tumors to radiation therapy, the resistance of cancer cells to radiation therapy, and the rapid elimination of radioisotope reagents. Hydrogels can be used as reservoirs of radioisotope reagents for targeted radiation therapy. Nanomaterials (*e.g.*, radiosensitizers, such as nanoparticles containing high-Z elements) are used in the development of cancer drug carriers to focus radiation on tumors and overcome radiation resistance, thereby greatly improving the efficacy and safety of radiation therapy.[109–111]

Liu *et al.* developed a radioiodinated thermosensitive supramolecular hydrogel for brachytherapy. An iodine 131 (^{131}I)-labeled injectable thermosensitive methoxy poly(ethylene glycol)-*b*-poly tyrosine hydrogel (denoted as PETyr-^{131}I) was prepared. This thermosensitive supramolecular hydrogel platform holds potential for 3D conformal radionuclide delivery and serves as a potential radiosensitizer due to its proximity to

the primary tumor lesion or the postoperative cavity. The SmacN7-R9 peptide combined with PETyr-I 131 radiation demonstrated a higher radiosensitization ratio (SER) compared to the PETyr-I 131 radiation alone, suggesting the radiosensitizing effect of SmacN7-R9 peptide on HepG2 cells strongly result in synergistic therapeutic outcomes and reduced radiation-related side effects[112] (Fig. 5).

7.2. For delivery of immunotherapeutic agents

Immune checkpoint inhibitors are effective means to prevent tumors from escaping immune surveillance. In recent years, the combination of immune checkpoint inhibitors with other tumor treatments has become a hot research topic in the field of tumor therapy. However, the adverse toxicity of many immunotherapeutic agents to T cells, natural killer (NK) cells, and dendritic cells (DC) limits their integration with immunotherapy.[113] Some researchers are exploring new ways to deliver these drugs. Cui *et al.* designed and synthesized a peptide-based supramolecular filament (SF) hydrogel as a versatile carrier for local delivery of three immunomodulators with different mechanisms of action and different molecular weights, including αPD1 antibody, IL-15 cytokine, and STING agonist (CDA). It was demonstrated that intratumoral injection of SF solutions containing aPD1, IL-15, or CDA triggered hydrogelation, and the resulting hydrogel served as a scaffold reservoir for sustained release and response of immunotherapeutic agents in response to MMP-2, resulting in enhanced antitumor activity and reduced side effects (Fig. 6A).[114]

Immunotherapy, the most revolutionary anticancer strategy, faces major obstacles in achieving desirable outcomes in pan-
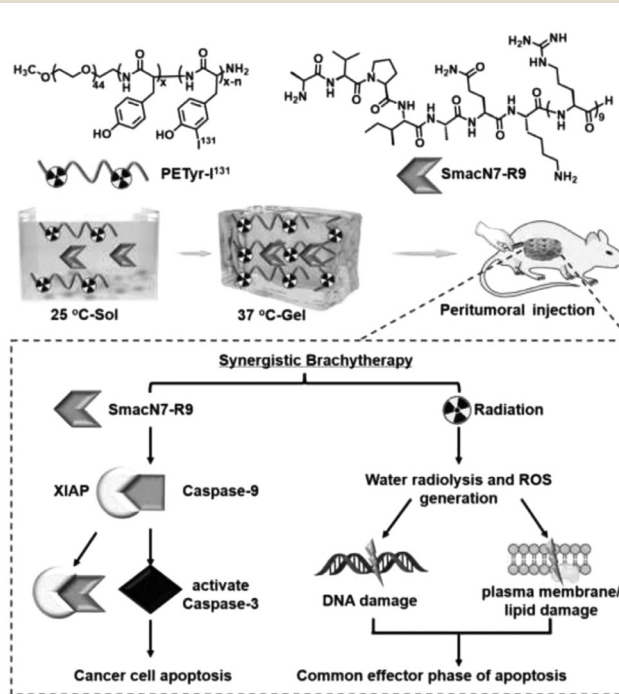

Fig. 5 The use of injectable hydrogels in radiopharmaceutical delivery. Reproduced with permission from ref. 112. Copyright 2020, Elsevier B.V.
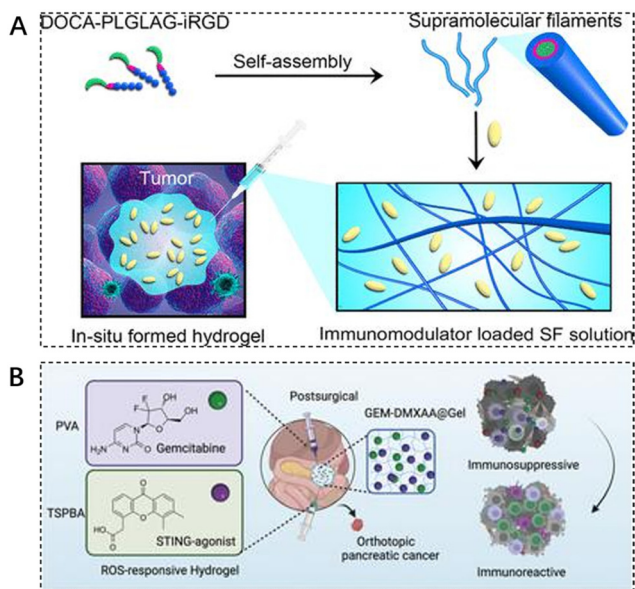
Fig. 6 The use of injectable hydrogels in immunotherapy. (A) Schematic illustration of localized immunomodulator delivery using a DOCA−PLGLAG−iRGD supramolecular hydrogel for MMP-2-responsive drug release and tumor microenvironment regulation. Reproduced with permission from ref. 114. Copyright 2023, American Chemical Society. (B) Enhanced immunotherapy of pancreatic ductal adenocarcinoma with STING agonist and gemcitabine for *in situ* formation of ROS-responsive hydrogels. Reproduced with permission from ref. 115. Copyright 2023, Wiley.

creatic ductal adenocarcinoma (PDAC) due to the highly immunosuppressive tumor microenvironment (TME). The traditional first-line chemotherapeutic agent gemcitabine (GEM) in the treatment of PDAC is insufficient to achieve durable efficacy when used alone. Liang *et al.* designed a reactive oxygen-degradable hydrogel system, denoted GEM–STING@Gel, to combine gemcitabine and the interferon gene-stimulating agonist DMXAA (5,6-dimethylxanthenone-4-acetic acid) co-delivered to the tumor site. It synergistically activates innate immunity and promotes cytotoxic T lymphocyte infiltration at the tumor site, thereby modulating immunosuppressive TME. The advantages of this integrated strategy combining chemotherapy, immunotherapy, and biomaterials-based hydrogels can be seen in the improved therapeutic efficacy and excellent biosafety (Fig. 6B).[115]

Hydrogels can elicit therapeutic effects by precisely delivering immune cells, such as dendritic cells (DC), directly to the tumor site. DCs stand out as the most functional antigen-presenting cells in the body. They possess the unique ability to mobilize other tumor-killing cells throughout the entire immune process, particularly in the presence of invading foreign entities, such as cancer cells. A DC vaccine could be designed to use the patients' own DCs to activate the general immunity for attacking tumor cells. As a proof-of-concept, Yi *et al.* developed a CaCO$_3$-biomineralized hydrogel DC vaccine by immobilizing the membrane proteins of 4T1 cells-DCs fusion cells (FP) into the biomineralized silk fibroin hydrogel.

The FP-contained biomineralized hydrogel vaccine, SH@FP@CaCO$_3$ exhibits improved immunogenicity by presenting a wide variety of tumor-associated antigens (TAAs) and facilitating long-term protein release for dendritic cell maturation and T cell activation. Additionally, the incorporated CaCO$_3$ contributes to an increased pH level in the tumor microenvironment (TME), promoting the polarization of M2 to M1-type macrophages, which in turn reverses the immune-inhibitory microenvironment and alleviates the immunosuppressive effects on T cells. The outstanding immune activation effects of the biomineralized hydrogel vaccine including simultaneously enhancing immunogenicity and reversing the immunosuppressive TME represent a promising strategy for advancing cancer immunotherapy.[116]

Acquired cell therapy (ACT) represents a promising strategy for cancer treatment, wherein immune cells are harvested from the patients, engineered with receptors to target and destroy cancer cells, subsequently expanded *in vitro*, and reinfused into the patients for therapeutic intent. Hydrogels can facilitate the direct and precise delivery of immune cells to tumor sites, eliciting a therapeutic response. Grosskopf *et al.* developed a self-assembled, injectable biomaterials platform for CAR-T cell delivery utilizing polymer-nanoparticle (PNP) hydrogels. These hydrogels employ highly scalable chemistries and are formulated under gentle conditions that enable straightforward encapsulation of CAR-T cells and cytokines, while their shear-thinning properties and injectability afford minimally invasive locoregional delivery of the therapeutic cells to tumors. These injectable hydrogels can create a localized inflammatory microenvironment that extends the exposure of CAR-T cells and cytokines, thus enhancing localized CAR-T cell proliferation and anti-tumor efficacy.[117]

7.3. For delivery of phototherapeutic agents

The development of photothermal therapy (PTT) and photodynamic therapy (PDT) has also led to advances in cancer treatments that minimize damage to normal tissue. Photothermal therapy utilizes photothermal reagents that absorb external light energy and convert it into heat, which is then used to kill cancer cells. These agents are biocompatible, have a high photothermal conversion efficiency, and are able to accumulate at the target site, allowing for selective and non-invasive treatment. The volume of the surgical site, the duration of treatment, and the temperature reached during treatment should be carefully controlled to ensure optimal efficacy and to avoid damage to surrounding normal tissues.

Photothermal therapy usually requires a local temperature (*i.e.*, local hyperthermia) of less than 44 °C, while maintaining a body temperature of less than 42 °C to minimize side effects. UV and visible light have the potential to cause severe burns. Therefore, near-infrared (NIR) light with wavelengths in the range of 650–950 nm is used in most cases. Shen *et al.* noted that injectable thermosensitive photothermal network hydrogels (PNT-gels) were designed by guest self-assembly of photothermal conjugated polymers and β-cyclodextrin. The conjugated polymer backbone can directly convert incident light
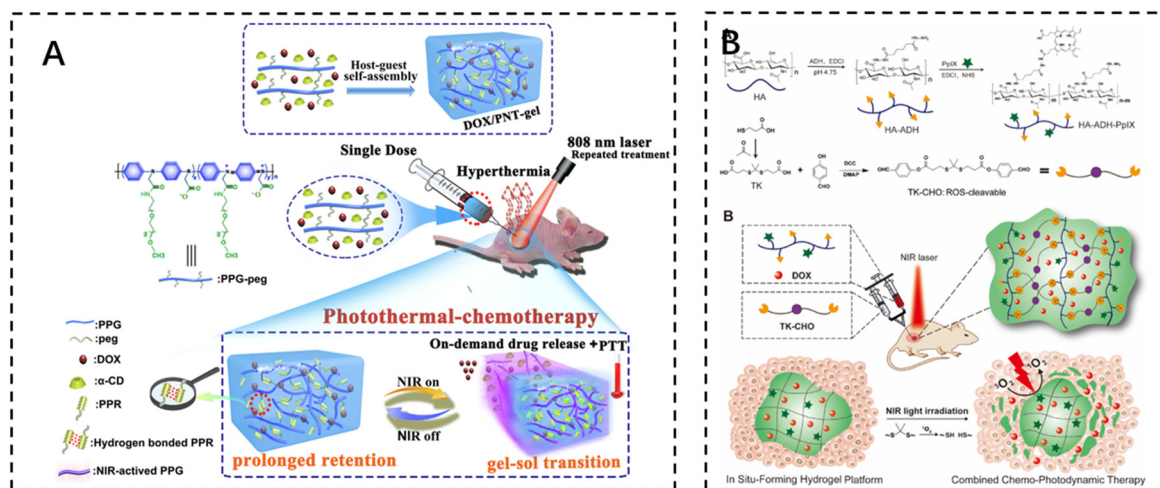
Fig. 7 The use of injectable hydrogels in phototherapy. (A) Schematic illustration of the fabrication of the supramolecular PNT-gel and its application for repeated photothermal-chemotherapy *via* one-dose injection followed by multiple treatments. Reproduced with permission from ref. 118. Copyright 2019, Elsevierc. (B) Schematic illustrations depicting the synthesis of the HA−ADH−PpIX conjugate and the ROS-cleavable TK-CHO, as well as the formation of an injectable NIR light-triggered ROS degradable hydrogel for combined chemo-photodynamic therapy with light-tunable on-demand drug release. Reproduced with permission from ref. 121. Copyright 2020, Elsevierc.

into heat, resulting in a PNT-gel with high photothermal conversion efficiency and photothermal stability. Remote controlled on-demand release of adriamycin (DOX) was achieved by photothermal-induced gel–sol transition. Since the backbone of the hydrogel absorbs NIR light and mediates the photothermal conversion itself, the PNT gel has the advantage of prolonged retention time, and NIR treatments can be repeated after a single intratumoral injection of this hydrogel. Under repeated NIR laser irradiation, synergistic photothermal chemotherapy mediated by the PNT-gel almost completely eradicated 4T1 breast cancer. The gel system integrates a multifunctional therapeutic platform with inherent photothermal properties and reversible stimulus responsiveness for on-demand delivery and a combination of photothermal chemotherapies[118] (Fig. 7A).

Photodynamic therapy (PDT) uses photosensitizers that, when exposed to light, trigger a photochemical reaction that produces cytotoxic reactive oxygen species (ROS). Cell death is then triggered through apoptosis, necrosis, or autophagy-related pathways. It can kill cancer cells directly, destroy tumor blood vessels, or induce an inflammatory response that leads to a host anti-tumor immune response.[119] Photosensitizers themselves are inefficient due to their low solubility in water and self-bursting effect. It has been reported that photosensitizers encapsulated in hydrogels exhibit enhanced fluorescence and single-linear oxygen generation due to the diminished self-burst effect in the network, which reduces collisions between photosensitizer molecules.[120] Zhao *et al.* developed an injectable and near-infrared (NIR)-triggered ROS-degradable hyaluronic acid hydrogel platform as a topical delivery vehicle for the photosensitizer protoporphyrin IX (PpIX) and the anticancer drug adriamycin (DOX) to achieve superior combined chemotherapy photodynamic therapy with on-

demand modulation of drug release. A large amount of ROS produced by the hydrogel under near-infrared light irradiation not only accomplishes an efficient PDT effect but also cleaves ROS-sensitive small-molecule cross-linkers for hydrogel degradation and subsequent on-demand DOX release[121] (Fig. 7B).

8. Combined drug delivery

Co-medication, the simultaneous or sequential use of two or more drugs, is often required for disease treatment due to various reasons. Reducing toxic side effects and maximizing the efficacy of the drug is the desired goal of the drug combination. Injectable hydrogels, as typical large-scale drug delivery carriers, can easily encapsulate a variety of therapeutic drugs by physical encapsulation, hydrophobic conjugation, and covalent binding, which significantly improves the *in vivo* anticancer effect. Various combination strategies based on injectable hydrogel platforms (*e.g.*, chemotherapy drug combinations,[122] chemotherapy and immunotherapy drug combinations[123,124] chemotherapy and photothermal chemotherapy,[125] chemotherapeutic drug and radioisotope combinations) have been developed and tested to enhance local antitumor effects (Table 2). Zhang *et al.* designed a novel injectable, self-crosslinked HA–SH hydrogel capable of simultaneously encapsulating multiple drugs (sorafenib, adriamycin, and metformin) simultaneously to enhance the chemotherapeutic effect. The hydrogel is relatively stable under physiological conditions and allows for rapid and direct release of the loaded drugs to the tumor site in a reductive tumor microenvironment. *In vitro*, antitumor activity and apoptosis assays have shown that hydrogels containing multiple drugs display significant synergistic effects on breast

Table 2 Injectable hydrogel in combination therapy

Type of combination therapy	Hydrogel's composition	Gelling mechanism	Drug composition	Strategies	Ref.
Multiple chemotherapy combinations	Injectable self-crosslinking HA–SH hydrogel	HA–SH polymers could self-crosslink to form the hydrogel by oxidizing free thiol groups into disulfide bonds.	Sorafenib, doxorubicin, and metformin	The combinational chemotherapy enhanced the sensitivities of tumor cells and promoted tumor cell apoptosis after peritumoral administration	122
Chemotherapy and immunotherapy combination	$PLGA_{1750}–PEG_{1500}–PLGA_{1750}$ thermosensitive hydrogel framework (PNP_{PTX} & MNP_{CpG}@gel)	Response to temperature	Glioma homing peptide modified paclitaxel targeting nanoparticles (PNP_{PTX}) and manipulated immunoadjuvant CpG targeting nanoparticles (MNP_{CpG})	The sustained-release PNP_{PTX} could target the residual infiltration of glioma cells and produce tumor antigens. Meanwhile, MNP_{CpG} targeted and activated the antigen-presenting cells, which enhanced the tumor antigen presentation ability and activated $CD8^+$ T and NK cells to reverse immunosuppression of the glioma microenvironment	127
Photothermal and chemotherapy combination	ICG–MTX@PP A gels	Heat it at a temperature above 45 °C or under 808 nm light to form a hydrogel	Near-infrared (NIR) phogeothermal reagents indocyanine green (ICG) and chemotherapy drugs Methotrexate (MTX)	ICG–MTX@PPA Gels can exhibit a sol–gel transition under photothermal stimulation, resulting in the accumulation at the tumor site and controlled release of nanomedicine. have the ability to NIR photothermal therapy and controlled release of the chemotherapy drug (MTX)	129
Chemotherapy and radiotherapy combination	Thermo-induced self-aggregation of poly (epsilon-caprolactone-*co*-1,4,8-trioxa[4.6]spiro-9-undecanone)-poly (ethylene glycol)-poly (epsilon-caprolactone-*co*-1,4,8-trioxa[4.6]spiro-9-undecanone) (PECT)	Response to temperature	Doxorubicin (DOX) and iodine-131 labeled hyaluronic acid ((131)I-HA)	Combination therapy significantly increased tumor growth inhibition efficiency while reducing drug-related side effects on major organs	135

cancer cells. After peritumor administration, combination chemotherapy enhanced tumor cell sensitivity to drugs and promoted apoptosis of tumor cells (Fig. 8A).[126]

Chemotherapy lacks specificity in killing cancer cells and also kills normal cells, which significantly impacts the immune system of tumor patients. Excessive chemotherapy can shorten the survival time of patients. The use of chemotherapy and cellular immunotherapy can effectively make up for the shortcomings, and the immune cells not only can kill and clear the tumor cells directly or indirectly but also can reduce the toxic side effects of chemotherapy and rebuild the immune system of patients. Glioma is currently the most malignant brain tumor. Due to the presence of blood–brain barrier (BBB) and tumor cell heterogeneity, systemic chemotherapy is not effective in the treatment of surgically resected gliomas, and may even impair the body's immune system. Xin *et al.* designed an *in situ* slow-release hydrogel delivery system for the local delivery of combined chemotherapeutic agents and immune-assisted treatment of gliomas with combined chemoimmunotherapy through the resection cavity. Glioma homing peptide-modified paclitaxel-targeted nanoparticles

(PNP_{PTX}) and mannitolized immunoadjuvant CpG-targeted nanoparticles (MNP_{CpG}) were embedded in a $PLGA_{1750}–PEG_{1500}–PLGA_{1750}$ thermosensitive hydrogel framework (PNP_{PTX} & MNP_{CpG}@Gel). *In vitro* and *in vivo* results demonstrated that the targeted nanoparticle-hydrogel hybrid system could be cross-linked into a gel drug reservoir when injected into the glioma resection cavity. Then, sustained release of PNP_{PTX} could target residual infiltrating glioma cells and produce tumor antigens. At the same time, MNPCpG targets and activates antigen-presenting cells to enhance tumor antigen presentation, activate $CD8^+$ T and NK cells, and reverse immunosuppression in the glioma microenvironment[127] (Fig. 8B).

Combined delivery of photothermal and chemotherapeutic agents is another approach. However, due to irregular tumor margins and blurred boundaries between normal and necrotic tissues, a single PTT could not achieve the desired therapeutic effect, and overheating would lead to severe inflammation of local tissues. An injectable composite hydrogel prepared from oxidized hyaluronic acid (OHA) and hydroxypropyl chitosan (HPCS) *via* imine bonding was used as a delivery substrate for
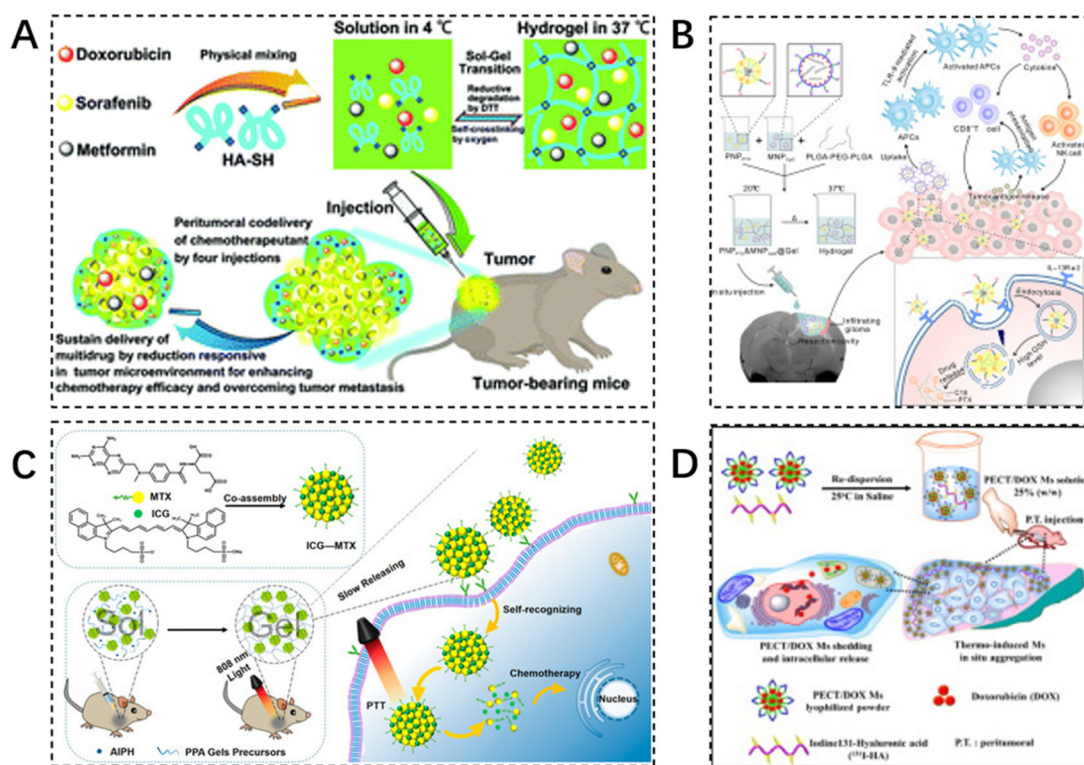
Fig. 8 Injectable hydrogels in combination therapy. (A) A novel injectable self-crosslinking HA−SH hydrogel is able to encapsulate multiple drugs (sorafenib, adriamycin, and metformin) simultaneously to enhance chemotherapy efficacy. Reproduced with permission from ref. 126. Copyright 2017, Royal Society of Chemistry. (B) Scheme design of PNP$_{PTX}$ & MNP$_{CpG}$@Gel for localized chemo-immunotherapy after surgical resection of glioma. Reproduced with permission from ref. 127. Copyright 2022, Elsevier B.V. (C) The preparation of ICG−MTX and ICG−MTX@PPA Gels and their application in tumor therapy. Reproduced with permission from ref. 129. Copyright 2022, Elsevier B.V. (D) The schematic diagram of design concept: formation of PECT/DOX MHg nanodrug depot as well as radionuclide reservoir for *in situ* chemoradiotherapy. Reproduced with permission from ref. 135. Copyright 2015, Elsevier B.V.

functional substances. In the gel medium, mesoporous poly-dopamine (MPDA) nanoparticles were doped as an efficient photothermal agent and reservoir of DOX, which could achieve good photothermal conversion performance and slow drug release, and showed good Hepa1-6 tumor inhibition *in vivo*.[128]

Based on the former drug delivery system in which the DOX-encapsulated nanoparticles could not specifically act on tumors, the targeted nanodrugs were designed to be combined with an injectable hydrogel for photothermal chemotherapy combination therapy. The targeted nanomedicine (ICG−MTX) was prepared by combining a near-infrared (NIR) photothermal reagent (ICG) and a chemotherapeutic drug (MTX). ICG−MTX was then mixed with a hydrogel precursor and a free radical initiator to obtain an injectable hydrogel precursor solution. Under near-infrared light irradiation, the precursor solution can release alkyl radicals, which promotes the transformation of the precursor solution from a liquid to a colloidal state, allowing the nanomedicine to be effectively retained at the tumor site and continue to be released from the hydrogel. Due to the targeted nature of MTX, the released ICG−MTX can target tumor cells and improve the accuracy of combined photothermal-chemical therapy[129] (Fig. 8C).

Radiation therapy, based on radioisotopes such as ^{131}I, [67]Cu, [188]Re, ^{90}Y and ^{177}Lu, has been widely used in the clinical treatment of many types of cancer, including lung, rectal, liver, and cervical cancers. During radiation therapy, healthy tissues are also exposed to radiation.[130–132] Many chemotherapeutic agents can be used as radiosensitizers to reduce radiation resistance and improve the efficacy of radiation therapy. However, their side effects still limit their clinical use.[133,134] To overcome these challenges, injectable hydrogels have been used as a flexible platform for co-delivery of chemotherapeutic agents and radioisotopes. By simultaneously delivering radioisotopes and chemotherapeutic drugs, injectable thermosensitive micellar hydrogels can not only be used as micellar drug reservoirs to locally deliver concentrated chemotherapeutic nano drugs but also immobilize the radioisotopes at the thermal focus of internal irradiation. For example, a hydrogel formed from adriamycin (DOX) and ^{131}I-labeled hyaluronic acid (^{131}I-HA) were used as model therapeutic agents. It exhibits a sol–gel transition near body temperature, which allows for the rapid intracellular release of DOX to internalize it, while ^{131}I can be stably immobilized at the injection site. The hydrogel allows for precise control of the dosage and ratio of

the combined therapeutic agent, resulting in the desired therapeutic effect by a single administration with reduced side effects (Fig. 8D).[135]

9. Clinical applications of injectable hydrogels in tumor therapy

Injectable hydrogels have received extensive attention for their applications in drug delivery and tissue engineering. Various hydrogel technologies have obtained regulatory approvals for healthcare applications, including cancer treatment, cosmetic plastic surgery, and spinal fusion. Cancer therapeutic hydrogels have been utilized to encapsulate and release small molecules and biologics, enabling sustained local therapeutic drug delivery in specific tumor sites. For example, Endo's Vantas, an FDA-approved subcutaneous hormone therapy, efficiently prevents the growth of testosterone-dependent prostate cancer cells by releasing gonadotropin-releasing hormone (GnRH) or luteinizing hormone-releasing hormone (LH–RH) through a cylindrical diffusion-controlled reservoir system. The long-term convenience offered by these hydrogel reservoirs greatly enhances patient acceptance and convenience through a single injection. Nevertheless, injectable hydrogels still need further consideration regarding their polymer-dependent, mechanical and solute transport properties, degradability, compatibility, and scale-up challenges.

10. Conclusions and outlook

The study of *in situ* hydrogel formation has recently gained attention due to its potential for local delivery of anticancer drugs *via* intra-tumor injection. This paper discusses the preparation of injectable hydrogels containing various anticancer drugs through chemical and physical interactions, as well as the injection therapy of these hydrogels within tumors, with a focus on the types of drugs delivered.

The greatest advantage of injectable hydrogels is their potential for non-invasive administration, which offers numerous benefits to both patients and healthcare providers. Unlike traditional drug delivery methods that involve invasive procedures such as injections or surgical implantation, injectable hydrogels can be easily administered through minimally invasive techniques such as subcutaneous or intramuscular injections. This non-invasive route eliminates the need for complex surgical procedures, reduces the risk of infections, and improves patient comfort. Moreover, injectable hydrogels can be formulated to be biodegradable, eliminating the need for subsequent removal surgeries. This ease of administration and patient comfort make injectable hydrogels an attractive option for drug delivery.

In addition, injectable hydrogels have the ability to enhance the stability of drug molecules and provide controlled release. The hydrogel matrix can protect the encapsulated drug from degradation, enzymatic activity, and harsh physiological conditions, thereby increasing its shelf life. Additionally, the porous structure of hydrogels allows for the controlled release of drugs over an extended period, ensuring sustained therapeutic levels in the body. This controlled release mechanism not only improves drug efficacy but also reduces the frequency of administration, leading to enhanced patient compliance.

Finally, injectable hydrogels offer the unique advantage of targeted and localized drug delivery. By incorporating specific ligands or functional groups into the hydrogel matrix, drugs can be selectively delivered to the desired site of action. This targeted delivery minimizes systemic exposure, reduces off-target effects, and enhances therapeutic outcomes. Furthermore, the injectability of hydrogels allows for precise placement of the drug-loaded hydrogel at the desired location, enabling localized treatment of diseases such as tumors or chronic wounds. This targeted and localized drug delivery approach has the potential to revolutionize the treatment of various diseases, making it a highly desirable feature of injectable hydrogels.

However, injectable hydrogels are fraught with challenges in cancer therapy and translation to the clinic. Firstly, designing hydrogels with both desirable mechanical properties and injectability is difficult. Hydrogels need to possess sufficient mechanical strength to provide structural integrity and support tissue regeneration, while also being injectable through minimally invasive procedures. Achieving this delicate balance is crucial for effective cancer therapy, as it enables the hydrogels to be easily delivered to the tumor site and exert their therapeutic effects. Researchers are actively exploring various strategies, such as incorporating reinforcing agents or crosslinking methods, to enhance the mechanical strength of hydrogels. Suo *et al.* proposes to prepare hydrogels that are much more entangled than cross-linked by using unusually small amounts of water, cross-linking agents, and initiators in the synthesis process.[136] Ordinary hydrogels are predominantly cross-linked and reflect a mesh-like topology, while highly entangled hydrogels have a fabric-like topology. The different topologies lead to different properties, and the dense entanglement allows the hydrogels to transfer tensile tension along the length of the polymer chain, dissipate elastic potential energy, and improve the tensile strength and toughness of the hydrogels.

Secondly, the controlled release of therapeutic agents from injectable hydrogels remains a challenge. Injectable hydrogels have the potential to encapsulate and deliver various anticancer drugs. However, achieving sustained and controlled release of these agents from the hydrogels is essential for optimal therapeutic outcomes. The release kinetics should be tailored to match the desired drug concentration and duration of treatment. Developing hydrogels that can release therapeutic agents in a controlled manner remains a complex task, requiring careful consideration of factors such as drug loading, hydrogel degradation, and diffusion properties.

Thirdly, the biocompatibility and biodegradability of injectable hydrogels are crucial factors for their successful translation to the clinic. Since most hydrogels are composed of

chemically synthesized polymers *via* organic solvents or cross-linking agents, degradation of the polymer matrix may trigger an inflammatory response. Therefore, the possible immunogenicity caused by hydrogels is also an issue of concern. The short-term biocompatibility of hydrogels can be evaluated using animal models, but there is no guarantee that the long-term biocompatibility will be the same, and the long-term safety of composite hydrogels and additives is still an important issue of concern. Ensuring the safety and efficacy of injectable hydrogels *in vivo* is a critical step towards their clinical translation.

Finally, the clinical translation of injectable hydrogels for cancer therapy is hindered by regulatory and manufacturing challenges. The development and approval of medical devices, including injectable hydrogels, require compliance with rigorous regulatory standards and guidelines. These standards ensure the safety, efficacy, and quality of the hydrogels, but they also add complexity and time to the translation process. Additionally, the scale-up manufacturing of injectable hydrogels to meet the demands of large-scale clinical trials and commercialization poses significant challenges. Ensuring batch-to-batch consistency, reproducibility, and cost-effectiveness are key considerations for successful clinical translation.

In conclusion, although there are still many risks and challenges associated with the large-scale application of hydrogels in cancer therapy, they also offer several advantages and broader perspectives than conventional therapeutic agents (Fig. 9). Future attention will also be more focused on highly controlled and precisely tunable injectable hydrogel formulations as well as on the study of release kinetics and triggering conditions of different hydrogels.

Fig. 9 Advantages and limitations of injectable hydrogels.

Author contributions

Yao Cheng & Haitao Zhang: Writing – original draft, writing – review & editing, writing – revision. Hua Wei & Cui-Yun Yu: Conceptualization, supervision, writing-review & editing writing revision.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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