Hydrogel Drug Delivery Systems for Minimally Invasive Local Immunotherapy of Cancer

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Abstract

Although systemic immunotherapy has achieved durable responses and improved survival for certain patients and cancer types, low response rates and immune system-related systemic toxicities limit its overall impact. Intratumoral (intralesional) delivery of immunotherapy is a promising technique to combat mechanisms of tumor immune suppression within the tumor microenvironment and reduce systemic drug exposure and associated side effects. However, intratumoral injections are prone to variable tumor drug distribution and leakage into surrounding tissues, which can compromise efficacy and contribute to toxicity. Controlled release drug delivery systems such as in situ-forming hydrogels are promising vehicles for addressing these challenges by providing improved spatio-temporal control of locally administered immunotherapies with the goal of promoting systemic tumor-specific immune responses and abscopal effects. In this review we will discuss concepts, applications, and challenges in local delivery of immunotherapy using controlled release drug delivery systems with a focus on intratumorally injected hydrogel-based drug carriers.

Graphical Abstract

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Hydrogel-mediated Local Immunotherapy of Cancer
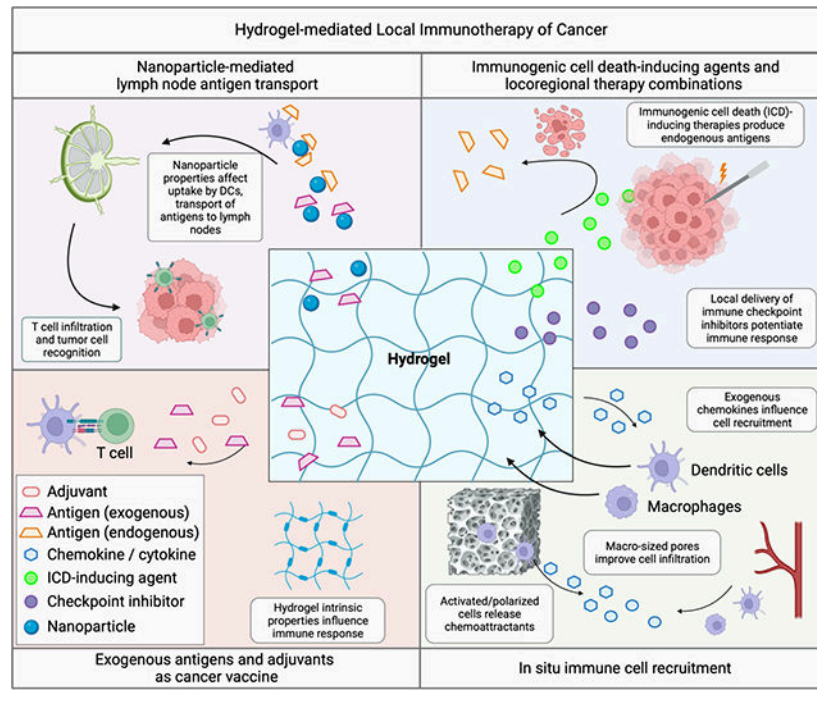
1. Introduction

Immunotherapy has transformed the treatment landscape of oncology by harnessing the body's immune system to combat cancer. This is exemplified by the success of immune checkpoint inhibitors (ICI), which have achieved durable responses in a subset of patients with advanced cancers. However, many tumors have limited immunogenicity and possess numerous mechanisms of immune suppression that contribute to poor response rates or eventual failure of immunotherapy [1]. Moreover, systemic immunotherapies commonly cause immune-related adverse events [2], which can be severe [3]. Therefore, strategies are needed to increase the percentage of patients that benefit from immunotherapy and improve its tolerability.

Intratumoral (intralesional) immunotherapy aims to potentiate systemic anti-tumor effects via local delivery of therapeutic agents that stimulate tumor antigen-specific immune responses or promote immuno-permissive conditions that increase a tumor's susceptibility to systemic ICI or other therapies. Immunotherapeutic agents delivered directly into a tumor via percutaneous injection dramatically increase intratumoral drug concentrations and reduce systemic drug exposure compared to systemic delivery. There are a variety of intratumoral immunotherapies undergoing clinical evaluation including Toll-like receptor (TLR) agonists [4, 5], stimulator of interferon gene (STING) agonists [6, 7], cytokines [8], and oncolytic viruses [9, 10]. However, leakage into adjacent tissues, rapid clearance, and variable distribution following intratumoral injection can contribute to toxicity and undermine treatment efficacy, potentially confounding analyses of treatment outcomes and jeopardizing clinical translation of intratumoral immunotherapies.

Hydrogels are a promising platform for enhancing the intratumoral delivery of immunotherapy. These biomaterials-based drug delivery systems consist of a semi-solid network of polymers with high water content that provide localized, sustained release of a variety of encapsulated drugs [11, 12]. Stimuli-responsive, in situ-forming hydrogels (ISFH) are of particular interest due to their ability to undergo a transition from liquid to semi-solid gel in response to physiologic stimuli (e.g., pH or temperature). This feature enables ISFH to be delivered via a syringe or catheter directly into a tumor, averting the need for surgical implantation. Encapsulation of immune-modulating agents within ISFH provides enhanced spatial and temporal control of local immunotherapy. In addition, ISFH can provide a three-dimensional scaffold-like microenvironment that attracts immune cells and fosters interactions between the immune system and entrapped tumor antigens or immunomodulators. Other local immunomodulation strategies include catheter-based intraarterial infusions with co-administration of embolics to induce tissue ischemia and necrosis as well as reduce washout of drug [13–15]. Recently, drug-eluting beads containing immunomodulators have been developed to provide sustained, local delivery of immunotherapy during transarterial immunoembolization [16, 17]. The extent to which these therapeutic paradigms impact the dynamic balance between treatment efficacy and systemic toxicity remains central to determining the success of local immunotherapy delivery strategies.

In this review we provide an overview of the determinants of drug distribution following intratumoral injection, highlight key properties of injectable ISFH, and define pathways, hurdles, and emerging opportunities for intratumoral, hydrogel-based delivery of immunotherapy.

1.1 The tumor immune microenvironment (TIME)

The tumor immune microenvironment (TIME) harbors multifaceted interactions between cancer cells, immune cells, stromal cells, and extracellular matrix components [18]. This heterogeneous intratumoral milieu possesses both anti-tumor immune activity and mechanisms of immune suppression that promote tumor development [19]. The hallmark of anti-tumor immunity is the presence of tumor-specific CD8+ T lymphocytes within the tumor microenvironment. So-called "inflamed" tumors are characterized by high levels of cytotoxic CD8+ T cells, while immune- "excluded" or "desert" phenotypes are characterized by T cells confined to the border of the tumor or absent from the tumor, respectively [20]. Immune-inflamed tumors are more likely to respond to ICI compared to immune-excluded or desert tumor phenotypes and are predictive of improved survival in some cancer types [21, 22].

Upon encountering specific tumor antigens, activated cytotoxic CD8+ and helper CD4+ Th1 T cells undergo extensive clonal proliferation and differentiation into various effector subtypes which can determine a tumor's fate. Tumors can induce a state of T cell exhaustion or anergy, characterized by reduced functionality and diminished cytokine production, limiting their ability to control tumor growth. Additionally, regulatory T cells (Tregs) may accumulate within the tumor, suppressing effector T-cell responses and promoting immune tolerance [23]. Manipulating the balance of T-cell phenotypes may be an effective approach

to enabling the immune system to combat tumors. Other cytotoxic cells, including NK cells, can promote tumor cell killing and immune cell recruitment. By contrast, myeloid derived suppressor cells (MDSC) are essential contributors to immunosuppressive conditions in the TIME [24].

Non-cellular components are crucial for the homeostasis of the TIME. Collagenous extracellular matrix (ECM), promoted by cancer-associated fibroblasts, can influence the migration, phenotype, and function of T cells [25, 26]. Moreover, dysfunctional tumor vasculature and connective tissue may reduce the infiltration of effector T cells and inhibit delivery of systemically administered therapeutic agents. Intratumoral delivery of ECM-modifying agents, e.g., collagenase or hyaluronidase, or antiangiogenic agents for vascular normalization, could alter the composition of the tumor microenvironment, improving distribution and penetration of drugs and immune cells, and reducing immunosuppression directly.

1.2   Rationale for intratumoral delivery of immunotherapy

Intratumoral drug delivery is a well-established technique that has generally been used to treat localized, superficial cancers, with limited rationale for treating locally advanced and metastatic tumors. However, the emergence of cancer immunotherapy and the recognition that local therapies can activate systemic adaptive immune responses has expanded the possibilities and potential indications for intratumoral drug delivery. In addition, advancements in interventional imaging guidance and minimally invasive devices have provided exquisite access to nearly all tissues within the body, which presents vast opportunities for selective tumor-localized delivery of immunotherapy [27].

Intratumoral delivery of immunotherapy has several advantages over systemic delivery. Since many immunotherapeutic agents, such as proinflammatory agents and adjuvants, possess a narrow systemic therapeutic index, local intratumoral delivery is appealing as it can substantially reduce systemic drug exposure and limit associated toxicities. This route of administration also results in higher drug concentrations in the tumor compared to an equivalent systemic dose. Moreover, intratumoral injections can circumvent some of the transport barriers associated with systemic drug delivery that impede drugs from reaching sequestered tumor cells distant from blood vessels [28]. Intratumoral injections can also enable precise targeting of spatially discrete tumor compartments using X-ray fluoroscopy, computed tomography (CT), cone beam CT, or ultrasound guidance (with or without magnetic resonance imaging (MRI) or positron emission tomography (PET) fusion imaging) for needle placement. Delivery of immunotherapy to cells within microenvironments not adequately reached by systemic therapy, such as hypoxic regions with limited blood supply, could enhance the robustness of the immune response.

1.3   Determinants of drug distribution following intratumoral injection

1.3.1   Injection parameters—When a drug is injected into tissue, the tissue deforms around the needle tip creating a fluid-filled cavity [29, 30]. Fluid flows through the extracellular space at a velocity that increases with injection rate (pressure) and tissue permeability (hydraulic conductivity). When the applied pressure surpasses a threshold,

the tissue may rupture [31, 32], forming low-resistance channels that can result in non-uniform or off-target drug distribution, or rapid intravasation. Slower injection rates reduce the applied pressure, possibly mitigating the risk of tissue rupture and leakage [33, 34]. Similarly, multi-hole, or multi-pronged needles can reduce the applied pressure by distributing it over several points of fluid egress [35, 36]. Immediate outflow of injected drugs into systemic circulation has been observed even at injection volumes below the theoretical "hold-up" or fluid volume capacity of the tumor [37, 38]. This highlights a need for adequate calibration of injection parameters to maximize tumor coverage and minimize systemic drug exposure. However, optimal injection techniques and parameters, such as injection rate and volume as well as needle type, have yet to be defined, and are not universal. Indeed, various temporal, spatial, organ, and histological heterogeneities may influence this optimization across various clinical scenarios (Figure 1).

1.3.2   Drug physicochemical properties—The physiochemical properties (e.g., size, charge, and binding affinity) of a drug (or nanomedicine) influence its tissue transport and ultimate distribution following intratumoral injection [39–43]. Larger macromolecules or drug carriers have lower diffusivity, in accordance with the Stokes-Einstein relationship, and may be entrapped entirely within the ECM if they are large enough. Therefore, modification of the ECM, for example by delivery of enzymes that degrade hyaluronan or collagen, can increase diffusive transport [44]. Binding affinity to ECM components or cellular targets can also affect drug transport and retention in the tumor interstitium. Macromolecules with low binding affinity may penetrate further yet are cleared more rapidly compared to macromolecules with high binding affinity, such as targeted monoclonal antibodies [45–47]. For example, a preclinical study evaluated the effects of intratumoral injections of IL-2 fused to proteins of different sizes and collagen-binding affinity. The study demonstrated that higher molecular weight and collagen affinity resulted in greater tumor exposure and efficacy of IL-2 in a mouse tumor model [37].

1.3.3   Tumor biophysical and microenvironment properties—Drug distribution following intratumoral injection is influenced by the mechanical and hydraulic properties of the tissue (Figure 1). Tissue elasticity (stiffness) can influence its deformation during injection, which can affect convection and diffusion, and resistance to stress before mechanical failure [29, 48]. Tumors may be more susceptible to crack formation during injection into regions of necrosis, abnormal ECM, or other tissue defects [49, 50]. Tissue permeability affects the flow of fluid containing dissolved drug through tissue, with dense ECM and cellular compartments or desmoplasia contributing to reduced permeability and fluid flow. Since tumor properties can differ between tumor types or within different regions of the same tumor, use of a single universal injection strategy is therefore likely to yield inconsistent results.

1.4   Limitations of intratumoral drug delivery

Leakage of injected fluid into tissues surrounding the tumor or along the needle track ("backflow") [51] may compromise treatment efficacy or incur toxicity. A recent clinical study using CT imaging following injection of an iodinated immunotherapeutic agent in patients revealed heterogeneous intratumoral distribution and high inter-patient variability

[36] (Figure 2). In some cases, significant off-target drug deposition was observed requiring repeated injections. Moreover, multiple periodic injections may be needed due to rapid clearance of drug following a single injection, increasing bleeding risks and costs. Technical guidelines for optimal intratumoral injection, ideally developed using imaging confirmation of drug delivery, may expedite clinical translation of intratumoral immunotherapies. Unfortunately, most drugs cannot be seen using clinical imaging. Incorporation of immunotherapeutic agents into image-able controlled release drug delivery systems (DDS), such as image-able ISFH, may help to ensure adequate on-target drug delivery following intratumoral injection.

2.   Hydrogel drug delivery systems

DDS may be classified by the scale of the carrier (e.g., nano, micro, or macro), delivery route (e.g., intravenous (i.v.), intraarterial (i.a.), or intratumoral (i.t)), or pharmacologic paradigm (e.g., cell-cycle specific chemotherapy, or immunotherapy). Hydrogels are crosslinked macromolecular networks of hydrophilic polymers that contain a large proportion of water. These biomaterials are of particular interest for intratumoral drug delivery due to their ability to provide sustained localized release of entrapped drugs [11, 12, 52]. Hydrogels can be generated from natural (e.g., alginate) or synthetic (e.g., poloxamers) polymers that are chemically or physically crosslinked via covalent or non-covalent bonds, respectively, and may be permanent or degradable at predictable or calibrated rates [11, 12]. Drug-loaded hydrogels can be delivered by surgical implantation of preformed gels, or by intratumoral or intraarterial injection using a syringe or catheter, respectively, followed by gelation in situ. In the following sections we will focus on injectable hydrogel-based DDS for minimally invasive intratumoral drug delivery.

2.1   In situ-forming hydrogels

Unlike preformed hydrogels that must be surgically implanted, *in situ*-forming hydrogels can be injected with a standard needle and syringe prior to undergoing a phase transition (sol-gel) in response to specific endogenous stimuli [53]. Common stimuli to initiate gelation are changes in temperature [54, 55], pH [56, 57], or ion concentration [58]. Alternatively, polymers that are water-insoluble may be injected as a liquid dissolved in a solvent and subsequently precipitate forming a semi-solid depot [59]. Numerous natural and synthetic materials have been investigated for generating in situ-forming hydrogels including alginate [60–62], chitosan [63], polypeptides [64], PEG-conjugates [55], and hyaluronic acid [65]. For example, alginate can be injected as a liquid and undergo ionic crosslinking in the presence of physiologic concentrations of calcium ions (Ca^{2+}) which can interact with carboxylate groups on different polymer chains resulting in the formation of an insoluble gel [66]. In another example, poloxamers, comprised of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) and two hydrophilic chains of polyoxyethylene (poly(ethylene oxide), possess temperature dependent self-assembling behavior [67]. Aqueous solutions of poloxamers are liquid at room temperature and form a gel in a reversible manner at elevated temperatures [68]. Alternatively, shear-thinning, or "self-healing", materials can be used that undergo a reduction in viscosity when passed

through a needle, and regain their original viscosity once the applied shear stress has ceased [52, 69].

2.2 Drug loading and release

A variety of drugs can be loaded into hydrogels by solubilization and mixing with the hydrogel precursor solution. Upon gelation, the drug is physically entrapped within the hydrogel and is released by diffusion through the hydrogel polymer network (Figure 3). In general, larger macromolecular drugs, such as proteins, have lower diffusion coefficients and permeate more slowly through the hydrogel compared to small-molecule drugs [70]. The rate of release is determined in part by the size of the space between polymer chains, known as the hydrogel "mesh size", which can be influenced by polymer concentration or degree of crosslinking, and hydrodynamic radius of the drug [71–73]. Hydrogel degradation or swelling can alter the mesh size, triggering or altering the rate of drug release [74, 75] and may be initiated under normal physiologic conditions or by environmental changes (e.g., change in pH). Non-covalent interactions between the drug and the polymer network, for example electrostatic interactions, can influence the rate and extent of drug release with stronger affinity between drug and polymer resulting in slower or reduced release from the hydrogel [76]. For rapidly diffusing small-molecule drugs, burst release may be mitigated by tethering the drug to the polymer via a covalent bond that is cleavable by hydrolysis or specific enzymes [77]. Alternatively, drug release can be controlled by entrapment or formation of drug-loaded nanoparticles within the hydrogel. In this scenario, drug release from the hydrogel may depend on the rate of drug release from the nanoparticles, or release of the nanoparticles from the hydrogel [78]. Therefore, hydrogels may be designed to obtain desired rates of drug release through careful tailoring of their physical and chemical properties.

2.3 Determinants of intratumoral distribution and drug delivery

Intravenous drug delivery is limited by poor tumor penetration caused by tumor hypoperfusion, dense ECM, and elevated interstitial fluid pressure, which may leave substantial regions of tumor insufficiently treated (Figure 4A) [79]. Direct intratumoral drug delivery may circumvent some of these barriers, however drug distribution can be heterogeneous and prone to leakage (Figure 4 B). Tailoring hydrogel properties can enable spatial and temporal control of intratumoral drug delivery including more predictable localization at the injection site and controlled, prolonged drug release kinetics (Figure 4C). Several hydrogel characteristics, described below, are important determinants of hydrogel intratumoral distribution and drug delivery.

2.3.1 Viscosity—The injectability of a hydrogel-drug precursor solution depends on how much pressure is required to push it through a syringe or catheter within a given timeframe. The injection pressure depends on the solution viscosity, needle or catheter geometry (i.e. length and diameter), and flow rate, in accordance with the Hagen-Poiseuille equation, which describes flow through a cylinder [80]. Low viscosity or shear-thinning solutions require less injection pressure and are desirable for their ease of delivery [81]. A low viscosity solution may permeate through tissue to a greater extent than high viscosity solutions, according to Darcy's Law, resulting in more dispersed depot formation. However,

if the viscosity is very low, the resulting hydrogel distribution will approximate that of an injection of free drug. By contrast, high viscosity solutions may permeate tissue to a lesser extent, resulting in depot formation more proximal to the needle tip [29]. Therefore, rheological characterization is critical during the development of injectable in situ-forming hydrogels including testing of handling (e.g., injection pressure) and tissue distribution studies in bench and animal models using needles and catheters of relevant geometry to ensure translation of results to humans.

2.3.2 Gelation rate—Rapidly gelling hydrogels may result in gel formation that is proximal to the needle tip compared to slow gelling hydrogels that have more time to permeate through tissue, given the same viscosity, tissue properties, and injection parameters. Rapidly gelling temperature-responsive materials may be prone to gel formation inside the needle resulting in a blockage depending on the residence time of the precursor solution within the indwelling portion of the needle. Therefore, the gelation rate should be optimized for handling to provide adequate ease of delivery and desired tissue distribution.

2.3.3 Drug release kinetics—The rate of drug release is an important consideration in the design of in situ-forming hydrogels for drug delivery. Rapid drug release from the hydrogel will result in tissue and plasma pharmacokinetics that approximate those of free drug. This may necessitate repeat injections to compensate for rapid clearance of drug from the tumor, and dose adjustments depending on the extent of systemic exposure and toxicity. In general, prolonged drug release from the hydrogel is desired to reduce peak plasma drug concentrations and associated systemic toxicity (Figure 5). Moreover, sustained drug delivery with a single intratumoral injection of drug-loaded hydrogel may alleviate the need for repeat or metronomic dosing, common to clinical immunotherapy dosing algorithms [82]. Biphasic and triggered drug release have also been studied, which may have special significance for the timelines inherent to the cancer immunity cycle [83, 84].

To assess drug release (elution) from hydrogels, the hydrogel precursor solution containing a known amount of drug is placed into a mold prior to gelation to achieve consistent sample morphology as the size and shape of the drug-loaded hydrogel may influence the rate of drug release. Subsequently the drug-loaded hydrogel is submerged in a release medium, such as a buffer solution, and incubated under controlled conditions that mimic the intended physiological environment. Alternatively, in situ-forming hydrogel precursor solutions may be placed inside a dialysis membrane cassette of fixed morphology and submerged in an environment with conditions appropriate to stimulate gelation. At predetermined time points, samples are collected from the release medium and the drug concentration measured using appropriate analytical techniques like high-performance liquid chromatography (HPLC) or UV-visible spectrophotometry. Sink conditions should be maintained throughout the experiment to avoid the influence of release medium saturation on the rate of drug release.

3 Hydrogel-mediated immunomodulation

The goal of immunotherapy is to generate sustained anti-tumor immunity by potentiating the steps of the cancer immunity cycle [85]. Local delivery of immunotherapy using hydrogels can effectively promote various stages of the cancer immunity cycle (Figure 6), which

starts with the release of tumor antigens and begins anew upon immune system recognition and destruction of cancer cells. Hydrogels can encapsulate tumor-associated antigens or neoantigens and release them gradually, providing a steady supply to antigen-presenting cells (APCs), such as dendritic cells (DC). Alternatively, hydrogel-mediated delivery of cytotoxic agents that induce ICD can initiate antigen release in situ, whereby the hydrogel may additionally serve as an antigen-adsorbing "reservoir", prolonging immune stimulation and enhancing DC recruitment. Hydrogels can also encapsulate immunomodulating agents, including adjuvants and cytokines, and release them in a controlled manner, priming the immune system via activation and maturation of APCs. The three-dimensional structure of hydrogels supports the infiltration of T cells and other immune effectors, enabling creation of an immunologic niche to support immunomodulation of immune cells lured by chemoattractants released from the hydrogel. In addition, hydrogel-mediated local delivery of immune checkpoint inhibitors or other co-inhibitory molecules may combat tumor immunosuppression and promote T cell function.

The following sections describe emerging applications for intratumoral delivery of immunotherapy, which commonly consists of small molecules, nucleic acids, proteins, antibodies, oncolytic viruses, or immune cells, using injectable hydrogels (Figure 7). Nano- and micro-particle DDS for immunotherapy have been recently reviewed [86–88], as have endovascular hydrogel embolics, with relevance to local drug delivery [89, 90]. A selection of preclinical studies demonstrating the use of in situ-forming hydrogels for delivery of intratumoral immunotherapy can be found in Table 1.

3.1 Intrinsic immunogenicity

When a biomaterial, such as a hydrogel, is injected or implanted in the body it triggers a series of responses collectively known as a foreign body reaction that is influenced by the biomaterial's composition, surface properties, size, shape, and the specific tissue or organ in which it resides. Proteins that become adsorbed to the biomaterial surface influence subsequent interactions with the immune system [91, 92]. The composition of this protein layer is affected by biomaterial properties including hydrophilicity/hydrophobicity (wettability) [93], charge [94], and topography [95].

Biomaterials with intrinsic immunomodulating properties possess structural or chemical features that can activate or suppress specific immune responses without pharmacologic agents [96, 97]. For example, cationic polymers such as polyethylenimine (PEI) and cationic dextran were reported to convert tumor associated macrophages (TAM) and MDSC polarization from immunosuppressive to antitumor phenotypes via TLR-4 dependent pathway, promote proinflammatory cytokines, and achieve anti-tumor immunity in murine tumor models [98, 99]. Soluble PEI was also found to increase the expressions of CD86 and MHC II on bone-marrow-derived dendritic cells as well as the production of pro-inflammatory cytokines TNF-alpha and IL-6 [100]. DCs grown on different polymer films demonstrated differences in the relative expression of various costimulatory molecules and MHC class II molecules suggestive of inherent adjuvant effects of some biomaterials [101]. Moreover, DCs exposed to different biomaterials and subsequently cultured with autologous T-cells differentially affected T cell phenotype and polarization, depending on

the biomaterial [102]. Degradation of biomaterials can also influence their immunogenicity since polymer properties may change during the degradation process [103] including by formation of particles that are known to have immunostimulatory effects that depend on particle size [104] and shape [105].

Immune reactions to foreign materials such as hydrogels can result in excessive inflammation and fibrotic encapsulation. Judicious tailoring of hydrogel properties may be required to strike a balance between surface passivation, for example by attachment of hydrophilic, protein-repelling polymer chains (e.g., polyethylene oxide), and bioactivity via specific interactions with proteins, antigens, and immune cells that promote anti-tumor immune responses.

3.2 Cancer vaccines

Cancer vaccines typically consist of tumor antigens and adjuvants that induce tumor-specific immune responses and anti-tumor immunologic memory [106]. A selection of recent studies demonstrating the use of ISFH for delivery of cancer vaccines can be found in Table 2. After injection (usually into subcutaneous tissue), tumor antigens are captured by endogenous DCs, processed, and presented to T cells in the lymph nodes resulting in T cell priming and activation [85, 107]. Activated T cells circulate throughout the body, infiltrate tumors, and recognize and destroy tumor cells, ultimately propagating the cancer immunity cycle. Successful therapeutic vaccination requires tumor antigens that possess sufficient immunogenicity (variety and tumor specificity) and a strategy to overcome mechanisms of tumor immunosuppression. Hydrogels can provide prolonged simultaneous delivery of antigens – including peptides [108, 109], nucleic acids [63, 110], and whole-tumor cell lysates [55, 64, 111] – and adjuvants, mimicking priming and boosting of conventional multi-injection protocols [112, 113]. They can also be administered in conjunction with systemic ICI or other immune-modulating agents to combat immune suppression (ideal timing and sequence has yet to be established). In a representative example, a preclinical study reported incorporation of a Hepa1–6 liver cancer-specific neoantigen, a toll-like receptor 9 (CpG-ODN) agonist, and stimulator of interferon genes (STING) agonist (2, 3-cyclic-GMP-AMP (cGAMP)) into a silk-hydrogel with sustained release kinetics [113]. In contrast to intradermal injection of free components, subcutaneous inoculation with immunotherapeutic-hydrogels achieved long-term prophylactic and therapeutic activity against Hepa1–6 orthotopic tumors in mice. Combining immunotherapeutic-hydrogel delivery and i.p. injection of TIM-3 antibody injection prevented pulmonary metastasis in orthotopic liver tumors and established long-term survival and protection against tumor rechallenge. Other cancer vaccination strategies include delivery of autologous DCs [114] or intratumoral delivery of immunogenic cell death (ICD)-inducing chemical agents or other locoregional ablative therapies (LRT) to increase antigen availability in situ [115].

3.3 Delivery of ICD-inducing agents

Certain chemotherapeutic agents, such as doxorubicin, can induce ICD resulting in release of tumor neoantigens and DAMPs which may potentiate tumor-specific immune responses [116–118]. To minimize systemic toxicities, ICD-inducing chemotherapy and immune adjuvants can be delivered locally using in situ-forming hydrogels to form an in situ cancer

vaccine that may be combined with other immunomodulatory agents including checkpoint inhibitors [57, 74, 75, 109, 119–122]. For example, in situ-forming hydrogels have been used for sustained local delivery of chemotherapy (e.g. doxorubicin, epirubicin) with immune-modulating agents such as Toll-Like receptor agonists [123], vascular disrupting agents [124], enzymes [74], and systemic ICI, an approach that has been shown to control the growth of treated and distant tumors in mouse models [119, 123]. Moreover, hydrogels may be designed as antigen "sponges" or "reservoirs" that adsorb antigens following ICD-inducing therapies to prolong antigen residence time, provide protection from degradation, and recruit DCs. Similarly, nanoparticles released from hydrogels may adsorb and traffic tumor antigens to lymph nodes after ICD-inducing ablative procedures [125].

3.4 Lymph node targeting

Tumor-draining lymph nodes are the primary location for DC antigen presentation and T-cell activation [126], although tertiary lymphatic structures adjacent to a tumor ablation zone or antigen source may also function as surrogate lymphoid organs [127]. As such, there is great interest in developing minimally invasive drug delivery systems that target lymph nodes for delivery of antigens and immune-modulating agents. Nanoparticles containing immune-modulating agents and antigens may be encapsulated within hydrogels (nanogels) and slowly released into the tumor as a means for increasing lymph node localization [63, 108, 125]. Antigen stability is preserved within the nanoparticle and transport to the lymph node is ameliorated compared to free antigens that can be degraded or quickly cleared from tumors [63, 108, 110, 125]. Nanoparticles with small diameters ($\sim < 200$ nm) enter lymph nodes via lymphatic drainage while larger particles generally remain entrapped within the ECM and may be degraded or trafficked to lymph nodes by DCs [128–130]. Other physicochemical properties, including charge [131] and composition [132], can also affect the route and extent of lymph node accumulation [133]. Established needle- and catheter-based techniques that deliver ethiodized oil to map the lymph system by lymphangiography have potential implications for delivery of immunotherapy [134]. For example, intratumoral injection of a Pickering emulsion of ethiodized oil and PLGA nanoparticles containing anti-CTLA-4 has been demonstrated in a preclinical model [135].

3.5 In situ immune cell recruitment

Macroporous biomaterial scaffolds containing chemoattractants or inflammatory cytokines can serve as local microenvironments within which DC function may be modulated in situ [136–138]. These "cell-homing" hydrogels are designed to release chemokines, such as GM-CSF, TNF-alpha, and CCL21 to attract immature DCs [69, 139, 140]. Recruited DCs can infiltrate the porous hydrogels gaining access to a reservoir of antigens and adjuvants that initiate DC maturation, antigen-presentation, and priming of antigen-specific T cells. In one example, injectable porous alginate cryogels were loaded with irradiated melanoma tumor cells as antigen source, GM-CSF, a chemoattractant for recruiting dendritic cells, and CpG-ODN, a danger signal. Subcutaneous injection of the loaded hydrogel solution resulted in recruitment, hydrogel infiltration, and activation of DCs, and a strong antitumor response in a B16F10 mouse melanoma model [141]. In another example, injectable pore-forming alginate hydrogels enabled controlled release of GM-CSF, and epacadostat, an inhibitor of indoleamine 2, 3-dioxygenase for activating T cells. Peritumoral injection of the loaded

hydrogel resulted in recruitment of DCs, and significant DC infiltration of the hydrogels, as well as an increased CD8+/Treg ratio in the tumor [60].

3.6 Adoptive cell transfer

During adoptive cell transfer, a patient's immune cells are activated or genetically modified in vitro to improve their ability to recognize tumor cells once infused back into the patient. The efficacy of chimeric antigen receptor T (CAR-T) cells in treating hematologic cancers has raised the prospect of using this therapy to treat solid tumors, though several barriers remain. After systemic administration, CAR-T cells have difficulty localizing, infiltrating, proliferating, and maintaining adequate function within the immunosuppressive tumor microenvironment [142]. Locoregional delivery of immune cells using ISFH may increase intratumoral localization and retention of CAR-T cells by providing a cell scaffold capable of controlled cell deployment, as previously demonstrated using hydrogel implants [143, 144]. Moreover, ISFH could serve to protect cells during injection and subsequently provide an immunologic niche to improve cell viability via co-encapsulation of immune-modulating agents [145]. However, challenges such as achieving adequate viability and intratumoral distribution of CAR-T cells and potential role of local cell and drug delivery systems require further investigation.

4 Applications in interventional oncology

Interventional oncology employs medical imaging and interventional tools, such as needles, probes, and catheters, to provide direct, minimally invasive access to tumors using imaging guidance. The goal is to navigate selectively towards an identified therapeutic target and deliver therapy using intraprocedural feedback to make necessary adjustments to the treatment in real-time. Ultimately, this information may be used to optimize treatment in a tumor- and patient-specific fashion. In light of advancements in modern interventional oncology tools, almost every tissue in the human body can be reached via percutaneous needle placement or endovascular catheter for the purpose of LRT including drug delivery. Imaging guidance is crucial to achieving precise needle or catheter placement and is usually performed using computed tomography (CT), fluoroscopy, cone beam-CT (CBCT), and/or ultrasound (US). The ability to visualize or predict the spatial distribution of immunotherapeutic agents in vivo would also be beneficial for treatment guidance and assessment of treatment adequacy including early visualization of off-target drug deposition. However, most drugs cannot be seen using clinical imaging modalities such as CT or US. Therefore, image-able DDS such as ISFH could serve as surrogate markers of in vivo drug distribution enabling imaging confirmation of local delivery of immunotherapy.

4.1 Image-guided drug delivery

Soluble contrast agents can be incorporated into ISFH by mixing with the hydrogel precursor solution. The resulting image-able hydrogels could enable imaging confirmation of on-target delivery and tumor coverage. Radiopaque materials have been incorporated into injectable hydrogels for imaging with fluoroscopy or CT, including bismuth [146], iodinated compounds [147, 148], and gold nanoparticles [146, 149–151]. Micro- or nano-bubbles provide contrast on ultrasound due to the intrinsic hyperechogenic nature of air and may be

incorporated into hydrogels to impart US-image-ability or US-triggered drug delivery [152, 153]. Contrast agents entrapped within hydrogels are usually released over a period of hours to days, providing adequate time for assessment of delivery. Interestingly, it may be possible to correlate changes in image-ability, such as the diminution of X-ray attenuation over time on CT, with drug concentration remaining in the hydrogel, raising the potential for calibrated or prescribed drug dosimetry. Alternatively, contrast agents can be conjugated directly to the hydrogel polymer backbone if persistent image-ability is desired. For example, iodinated moieties have been incorporated into embolic drug-eluting microspheres thus enabling visualization of microsphere localization during transarterial chemoembolization [154–156], and correlation of radiopacity with drug concentration in embolized tissues [157, 158]. Image-able hydrogels may facilitate targeting of regions with identified susceptibilities to specific therapeutic agents or spatially discrete microenvironments using intraprocedural imaging for confirmation of delivery (Figure 8A).

4.2 Intratumoral immunotherapy

4.2.1 Percutaneous—Recently, new needle devices have been developed including multiple side hole needles (MSHN) (e.g., Profusion, Cook®, Regentec) and multipronged (deployable) injection needles (MPIN) (e.g., Quadra-Fuse, Rex Medical) (Figure 8B). A preclinical study demonstrated that intratumoral injections of soluble contrast into a rat flank model of hepatocellular carcinoma, viewed under live fluoroscopy, resulted in greater intravasation using a single end hold needle compared with MSHN, which demonstrated greater tumor localization. In addition, greater intratumoral retention of contrast on CT was observed when delivered with a hydrogel [35]. In another study, distribution of an X-ray and CT imageable thermosensitive hydrogel loaded with doxorubicin was evaluated using different needle types and injection techniques for intratumoral injection into ex vivo bovine liver [159]. More research is needed to better understand the influence of needle type, hydrogel properties, and injection parameters on intratumoral drug distribution and its implications for local delivery of immunotherapy.

4.2.2 Transcatheter—Catheter-based intraarterial infusions can achieve higher tumor drug concentrations, or improved localization of cellular immunotherapy, compared to intravenous administration. Yet, limited "first pass" extravasation and rapid washout may limit tumor drug concentrations and contribute to elevated systemic drug levels. Transarterial immunoembolization consists of targeted, catheter-based delivery of embolics and immunomodulators. This dual approach serves to obstruct blood flow to the tumor, leading to ischemia and necrosis as well as reduced washout of immunotherapy. In an early example, a low-virulence pathogen, thrombin, and fibrinogen were mixed with ethiodized oil (Lipiodol®) and injected into tumor arteries to treat hepatocellular carcinoma [15]. Immunoembolization was later performed by delivering GM-CSF emulsified in Lipiodol into hepatic arteries to treat uveal melanoma prior to injection of gelatin sponge particles [160]. Recently, immunomodulating drugs have been loaded into drug-eluting "immunobeads" to exploit combined and potential synergistic effects of embolization and sustained local delivery of immunotherapy [16, 17]. In situ-forming hydrogels are an intriguing delivery platform for combined embolization and controlled intraarterial delivery of immune-modulating agents due to their potential distal vascular penetration. However,

optimization of hydrogel properties such as the rate of gelation is required to achieve a balance between adequate handling (e.g., ease of flow through the catheter) and desired vascular distribution.

New microcatheters for endovascular therapies have been developed that may further enhance tumor-selective targeting of intra-arterial immunotherapy including multiple catheter designs intended to minimize reflux of drugs or embolics leading to off target delivery. Microcatheters can also be used in the systemic and portal venous systems as well as lymphatics. Microcatheters with small side holes near the tip create a fluid barrier that helps prevent non-target embolization that can occur with reflux [161, 162]. A catheter delivery system with an anti- reflux valve (TriSalus/Surefire) has also been used to deliver CAR-T cells for hepatic infusions with the ability to increase forward delivery pressure while minimizing reflux [163]. Additionally, inflatable balloon catheters and microcatheters (Occlusafe Terumo; Sniper balloon occlusion microcatheter, Embolx) are designed to prevent reflux but also modulate pressure in target vasculature potentially increasing deposition or vascular penetration of embolic materials and drugs in target lesions [164–167]. A clinical study found increased delivery of microspheres to tumors using microvalve infusion catheters compared to standard end hole catheters [168]. However, more research is needed to better understand the performance implications of different catheter designs for intraarterial delivery of embolics and drugs including hydrogels and immunotherapeutic agents, respectively.

4.3   Locoregional therapy – immunotherapy combinations

Minimally invasive LRT such as chemical, thermal, or non-thermal ablative therapies have demonstrated an ability to induce the release of tumor antigens and DAMPs via induction of ICD [169–172]. Consequently, LRT can lead to the activation of innate and adaptive immune responses, attracting immune cells such as dendritic cells, macrophages, and T lymphocytes into the tumor microenvironment. Antigens captured by APCs following LRT can potentiate systemic anti-tumor immune responses [173, 174]. A variety of tumor ablation modalities have demonstrated the ability to generate tumor-specific immune responses including radiation [175], chemoembolization [176, 177], RFA [169–172], MWA [178], cryoablation [179, 180], therapeutic thermal or mechanical ultrasound [181], photothermal therapy [182, 183], and pulsed electrical fields or irreversible electroporation (IRE) [184]. Unfortunately, the abscopal effect induced by ablative therapies is often too weak to achieve a detectable or significant therapeutic response. Thus, there is growing interest in combining immunotherapy with LRT to enhance systemic antitumor effects [185, 186]. For thermal therapies, a region of sublethal hyperthermia at the rim of the ablation zone, called the transitional zone, is host to immune activity that may be an ideal target for local delivery of immunotherapy (Figure 8C). A summary of preclinical studies demonstrating combined locoregional ablative therapies and in situ-forming hydrogels for delivery of immunotherapy can be found in Table 3.

Locally administered hydrogels may be used to carry LRT sensitizers in combination with immune-modulating agents to promote immune responses following LRT. For example, several studies demonstrated the use of hydrogels to deliver photo-thermal sensitizers via

intratumoral injection, including gold nanoparticles and indocyanine green, and immune adjuvants that were released upon heating with laser irradiation resulting in effective inhibition of tumor growth [125, 182, 183]. In an intriguing example, hydrogels with high protein adsorption capability containing manganese dioxide nanoparticles for enhancement of photothermal therapy were injected into tumors forming a "reservoir" of autologous antigens in situ. The authors found enhanced CD8+ T cell-mediated immune responses, inhibition of distant tumor growth, and protection against tumor rechallenge compared to a formulation with lower protein binding [187]. Hydrogels containing immune adjuvants and microenvironment modulators have also been injected into tumors prior to or following radiofrequency or microwave thermal ablation resulting in greater anti-tumor immune effects compared to ablation alone in mouse models [188–190]. However, the relative timing of LRT and delivery of immunotherapy, as well as the location and coverage of intratumoral immunotherapy injections, may be important determinants of local and systemic anti-tumor responses and necessitate further investigation.

5   Conclusions

Immunotherapy has demonstrated remarkable promise in its ability to harness the immune system to recognize and destroy tumors. Intratumoral immunotherapy may be used to boost the effects of systemic checkpoint inhibition, for example by converting "cold" ICI-unresponsive tumors to "hot", and as an alternative route of drug delivery with reduced toxicity and improved tumor targeting. Reaching the full potential of cancer immunotherapy will require maximizing anti-tumor immune activity and overcoming mechanisms of resistance, without significant systemic toxicity, across a diverse array of cancer types.

Intratumoral delivery of immunotherapy can reduce systemic drug exposure and potentiate systemic anti-tumor immunity, particularly when combined with systemic ICI. Intratumoral immunotherapy could also provide benefits as neoadjuvant therapy in patients with locally advanced and resectable cancers, potentially at lower cost and with fewer treatment cycles than systemic immunotherapy. However, intratumoral injections have encountered limitations stemming from rapid egress or washout of drugs from the injection site, which necessitates frequent dosing and can result in adverse systemic inflammatory effects. Hydrogels are well-suited to meet formulation and delivery challenges for a broad repertoire of immunotherapeutic agents and emerging delivery paradigms due to their adjustable physico-chemical properties, tunable drug release characteristics, and potential use for image-guided drug delivery.

Early hydrogels were engineered to be biologically inert, minimizing interactions with tissues, proteins, and immune cells to mitigate inflammation and prolong their longevity and function in the body. In some respects, the emergence of immunotherapy requires revisiting these past goals to generate hydrogels that serve not only as inert implants or drug carriers, but rather as active agents or adjuvants with intrinsic immunogenic properties. These biomaterials-based "immunogels" could themselves be tailored to modulate specific immune pathways or microenvironments, while simultaneously delivering immunotherapy with optimized timecourses, which may have important implications and advantages for stimulating immune responses with known temporal dependencies.

Improvements in next-generation sequencing and computational tools are enabling more detailed insights into the mutational landscape of tumors, providing improved strategies for immunomodulation. In situ forming-hydrogels are promising delivery platforms for cancer vaccines, providing enhanced localization and prolonged delivery of entrapped antigens and adjuvants. A more robust immune response may be achieved by in situ vaccination, whereby the immune system is exposed to a broad array of endogenous neoantigens via ICD-inducing locoregional ablative therapies. Under this framework, hydrogels could be used to deliver chemotherapy, or sensitizers to locoregional therapies, while simultaneously serving as antigen "sponges" or "reservoirs" [187]. These antigen-saturated hydrogels could also recruit DCs or other immune cells by releasing loaded chemoattractants and serving as tunable cell scaffolds, or immunomodulatory niches, for promoting immune cell integration, activation, or repolarization [60, 141]. Furthermore, nanoparticles entrapped within the hydrogel polymer network and released over time could increase lymph node drainage or uptake of encapsulated or adsorbed antigens by APCs for subsequent trafficking to lymph nodes or tertiary lymphoid structures and enhanced T cell priming [63, 108, 125].

Recent studies have mapped extensive intratumoral spatial variations in clonality using spatially resolved transcriptomics and in situ sequencing technologies [191, 192]. Intratumoral immunotherapy using image-able hydrogels may enable greater tumor coverage of immunotherapy with delivery confirmation or enable precise spatial targeting of regions with distinct immunologic activity or clonality. In the future, these regions may be identified using AI-enhanced predictive imaging or pre-treatment biopsies and analysis of transcriptomics and subsequently targeted for intratumoral immunotherapy under imaging guidance. However, much progress remains to be made towards optimizing injection techniques and parameters for various clinical scenarios to achieve more consistent and predictable intratumoral delivery of immunotherapy, a process likely to be facilitated by image-able injectable hydrogel DDS. Progress in these areas will require the use of translational tumor-bearing animal models to evaluate novel interventional tools and treatment algorithms. Importantly, multi-disciplinary collaborations in materials science, immunology, engineering, and medicine, will help ensure expeditious clinical translation of innovative intratumoral immunotherapies and drug delivery systems.

Acknowledgments

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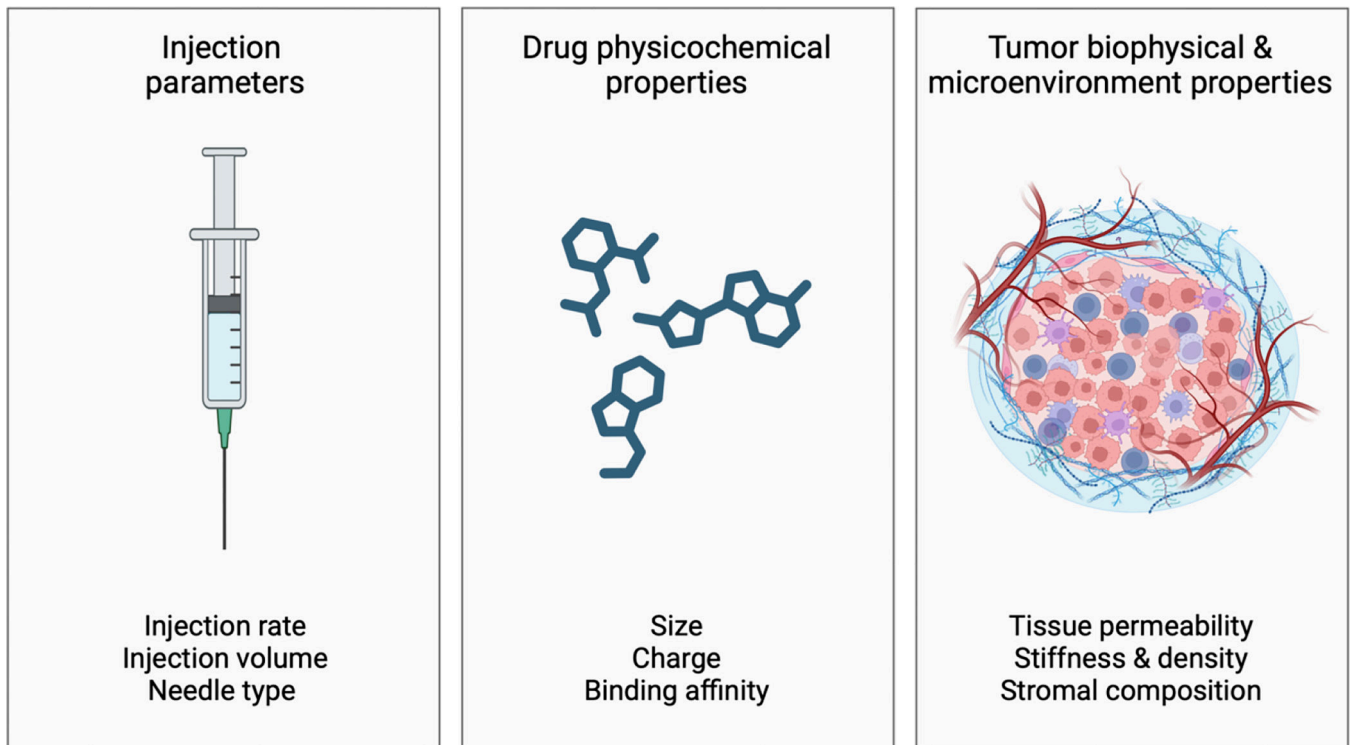
Figure 1.
Factors influencing intratumoral drug transport and distribution (i.t. injection). These include injection parameters, physicochemical properties of the injected drug and delivery system (e.g., hydrogels), and biophysical and microenvironment properties of the tumor.
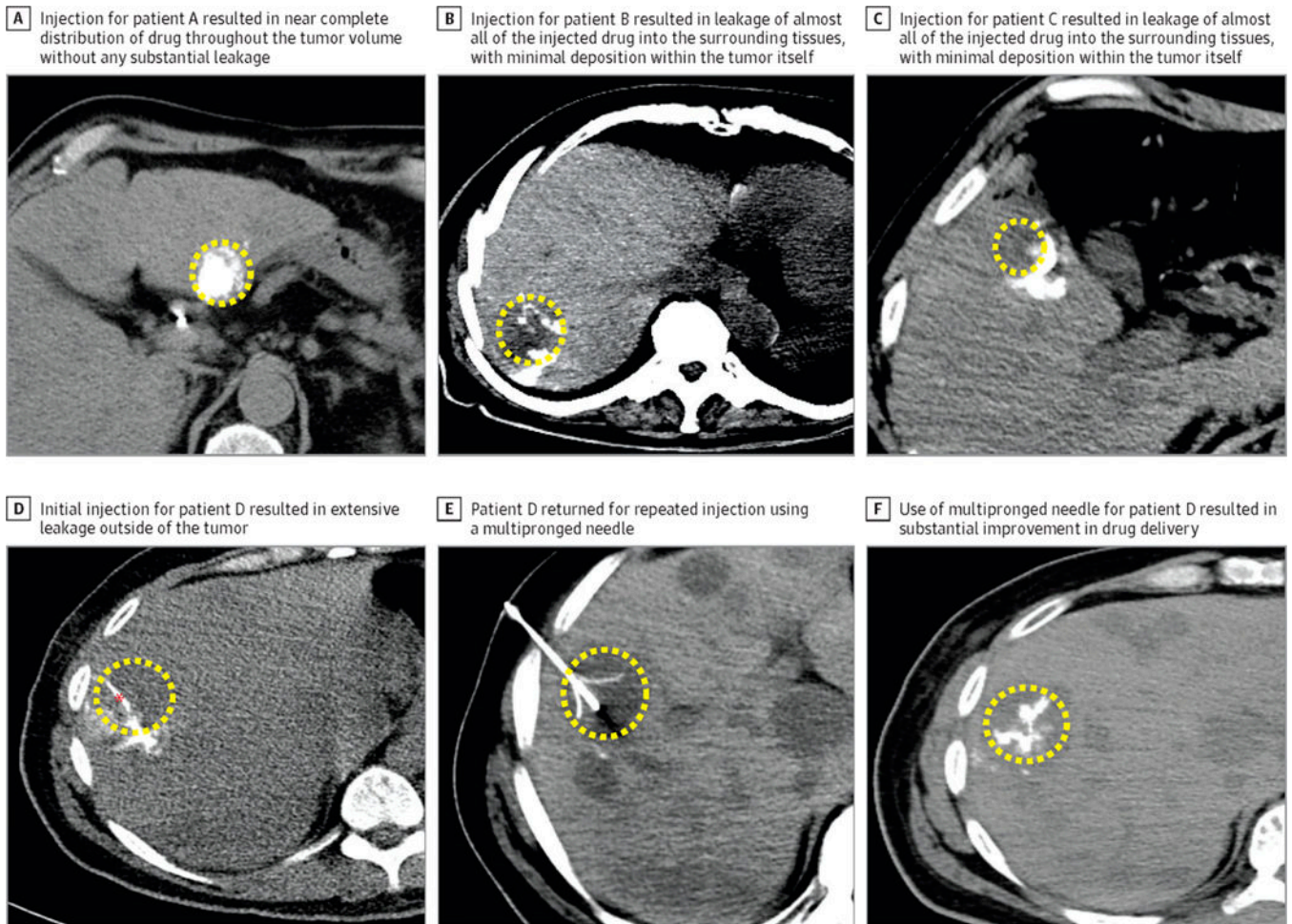
Figure 2.
(A-C) Example of variability in the distribution of a radiopaque drug, PV-10, following intratumoral injection into three patients as seen using intra-procedural non-contrast enhanced axial computed tomography. (D) Example of leakage of intratumorally injected drug from the tumor (yellow dashed ellipse) using a single end hole needle (red asterisk). (E) Deployment of a multipronged needle inside the tumor. (E, F) Intratumoral injection using multipronged needle demonstrating on-target delivery and heterogeneous intratumoral distribution [36]. Reproduced with permission from Elsevier.
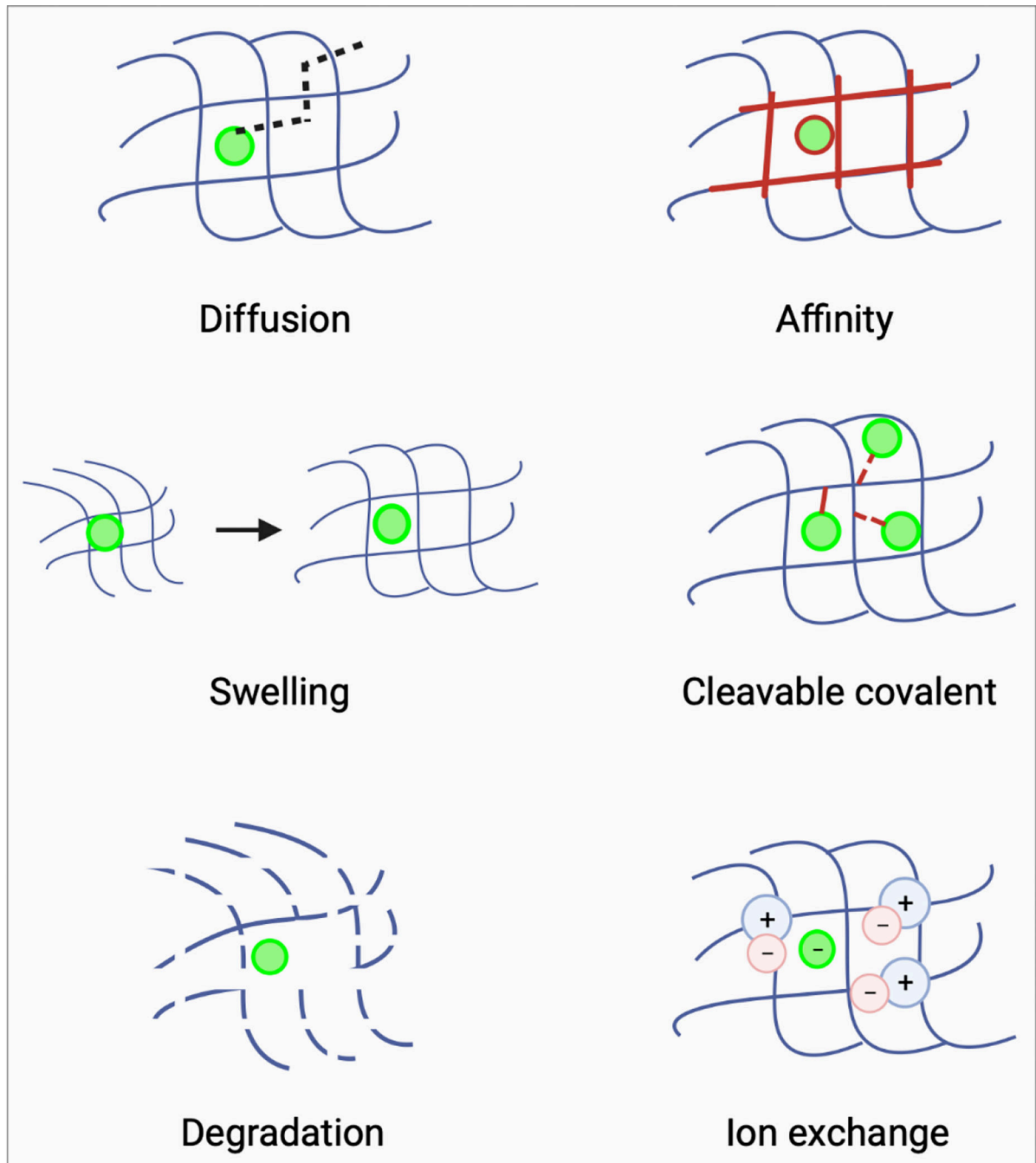
Figure 3.

Mechanisms of drug release from hydrogels. Drug release from hydrogels is governed by diffusion. However, interactions between drug and hydrogel (drug-hydrogel affinity) can alter the rate and extent of drug release. Similarly, hydrogel swelling or degradation may trigger or alter the kinetics of drug release. Drugs can be loaded into hydrogels and released using cleavable covalent bonds between drug and hydrogel or by ionic interaction between drug and hydrogel and subsequent displacement by ions in tissues.
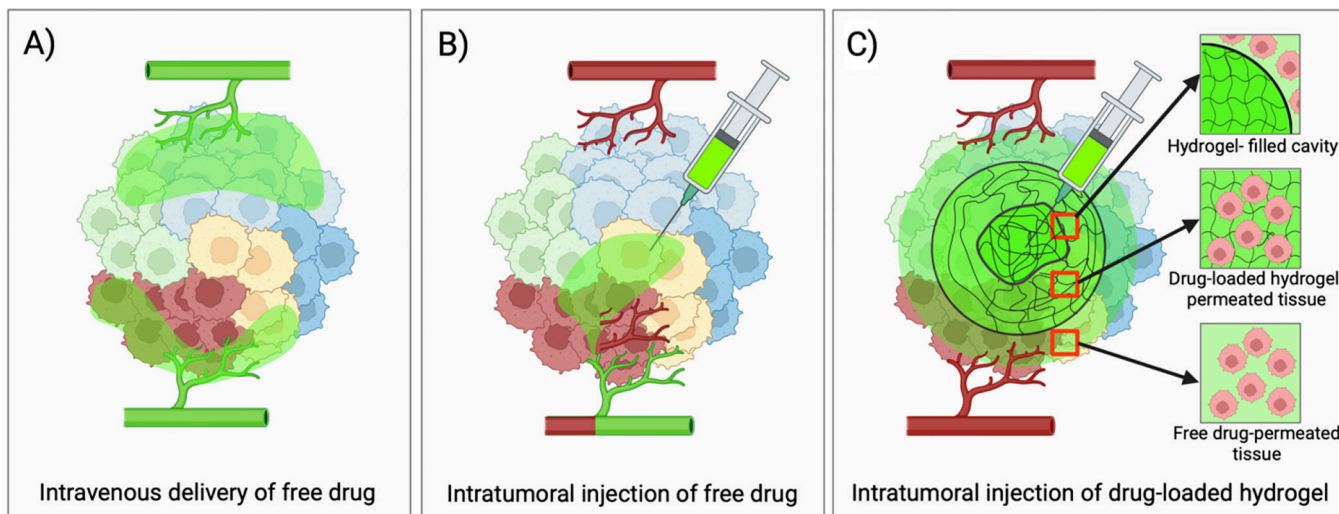
Figure 4.
Intratumoral drug distribution following systemic and intratumoral drug delivery. A) Systemic drug delivery is limited by poor penetration of the tumor interstitium resulting in insufficient treatment of cells distant from blood vessels. B) Intratumoral injections of free drug are prone to leakage, rapid clearance, and intravasation leading to incomplete and heterogenous tumor drug coverage. C) In contrast, intratumoral injections using in situ-forming hydrogels can improve on-target drug delivery and retention resulting in prolonged tumor drug exposure [59].
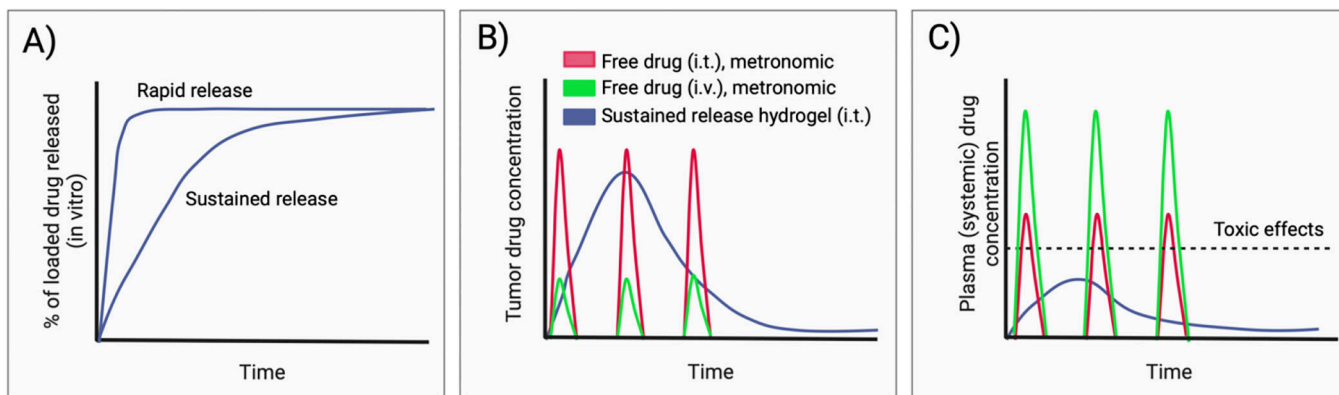
Figure 5.
Hydrogel drug release kinetics and the influence of hydrogels on tumor and plasma pharmacokinetics. A) Representation of sustained and rapid drug release kinetic profiles for drug-loaded hydrogels measured using an in vitro drug elution assay. B) Sustained drug release from hydrogels can achieve more prolonged exposure of the injected tumor to therapeutic drug concentrations, compared to free drug administered by intratumoral (i.t.) or intravenous (i.v.) injection. C) Sustained drug release from hydrogels reduces peak plasma concentrations and associated systemic toxicity compared to free drug following i.t. or i.v. administration.
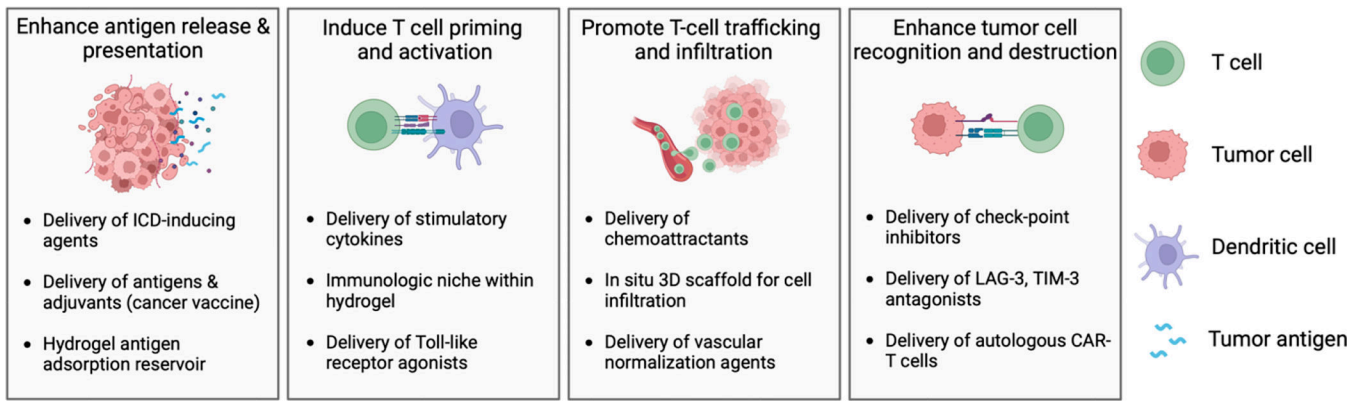
Figure 6.
Potential applications of in situ-forming hydrogels for local immunotherapy. ICD: immunogenic cell death.
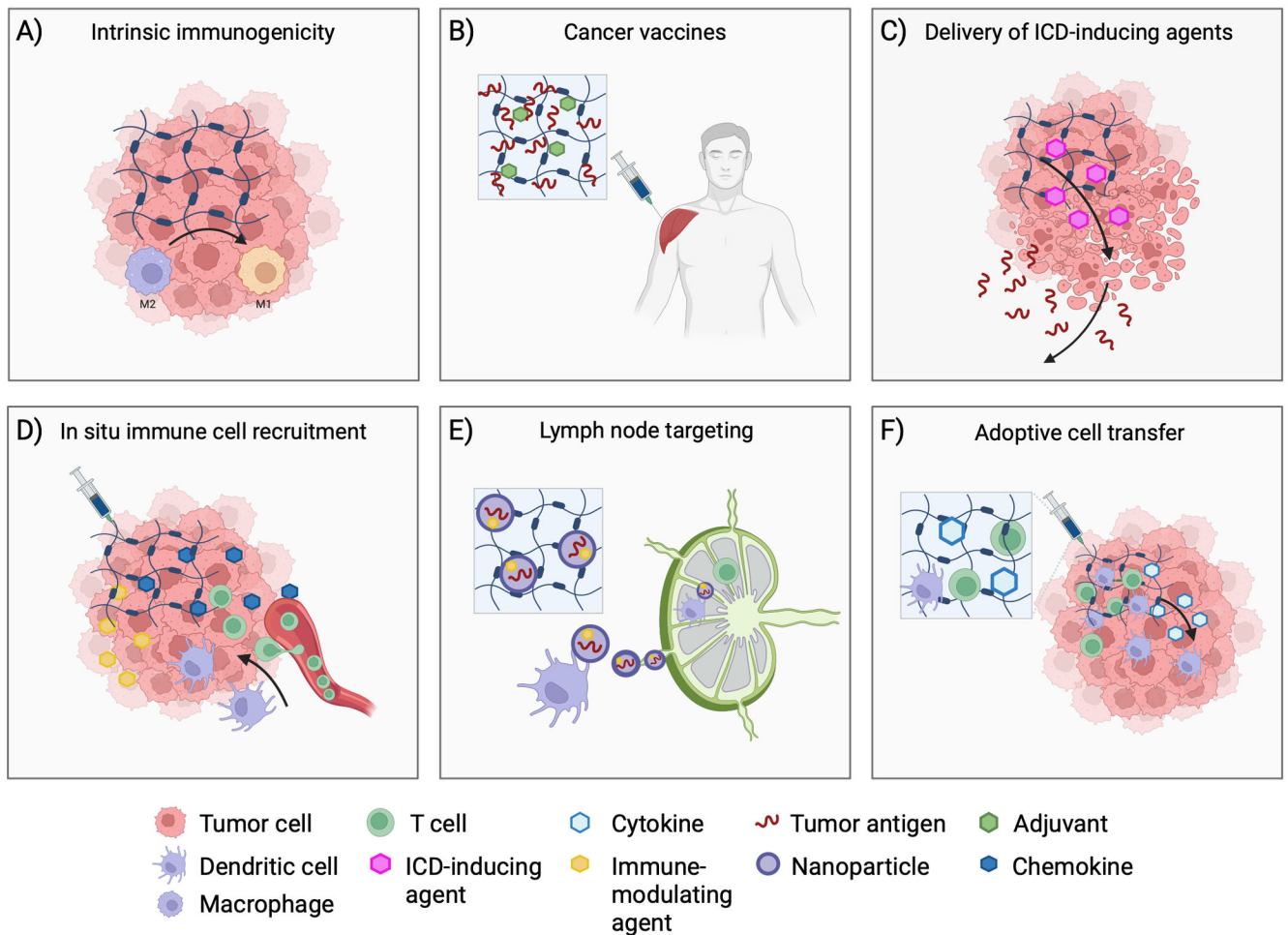
Figure 7.
Applications of hydrogels for local delivery of immunotherapy. A) Hydrogel properties, such as charge and surface topography, can influence immune responses including by converting tumor-associated macrophages from immunosuppressive (M2) to antitumor (M1) phenotypes. B) Hydrogels may be employed for delivery of antigens and adjuvants in the form of cancer vaccines that generate tumor-specific immune responses. C) Release of ICD-inducing agents (with or without ICI) from hydrogels delivered via intratumoral injection can result in release of tumor antigens that can potentiate tumor-specific immune responses. D) Hydrogels can be used to increase tumor immune cell infiltration by releasing chemoattractants. Inclusion of immune-modulating agents within the hydrogel may provide an immunologic niche for immune cell integration and immunomodulation within macroporous hydrogels. E) Nanoparticles loaded with antigens and immunomodulators may be released from hydrogels and shuttled to lymph nodes via dendritic cells. F) Hydrogels can also be used for autologous transfer of immune cells, such as CAR-T cells, providing protection during injection and potentially improving cell localization and viability compared to free injection of cells.
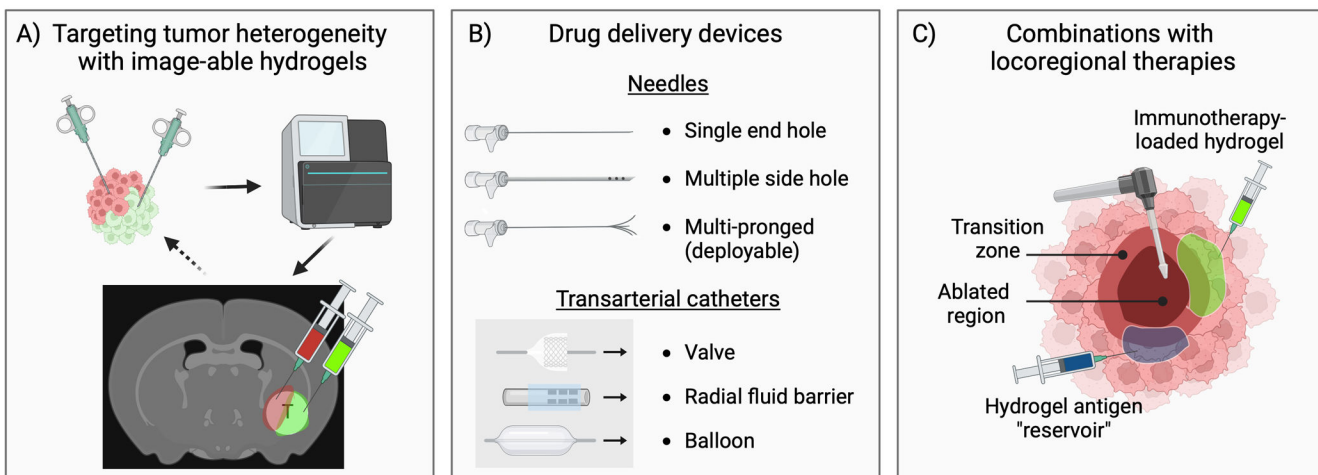
Figure 8.
Opportunities for innovation and advancements in intratumoral delivery of immunotherapy-loaded hydrogels ("immunogels"). A) Improvements in next-generation sequencing and computational tools are enabling more detailed insights into the mutational landscape of tumors. Image-able hydrogels may thus enable targeting of identified regions of clonal heterogeneity or discrete microenvironments within the tumor using intraprocedural imaging for dose estimation and verification of on-target delivery of multiple drugs. B) Innovation and evaluation of advanced needles and catheters may enable greater control of intratumoral drug delivery including immunogels. C) The ability to deliver immunogels to regions of increased immunologic activity stimulated by locoregional therapies (e.g., the transition zone following thermal ablation) may potentiate local and systemic (abscopal) anti-tumor effects. Injection of hydrogels with or without entrapped immunotherapy could serve as antigen "sponges" or "reservoirs" that adsorb tumor antigens released during ICD-inducing locoregional therapy, prolonging antigen tumor residence time and promoting uptake by antigen presenting cells.

Table 1.

Preclinical study examples demonstrating the use of in injectable hydrogels for delivery of immunotherapy.

Hydrogel	Injection location	Gelation-stimuli	Immunotherapy	Other loaded molecules	Drug release stimulus	Tumor model	Citation
Alginate	peritumoral	Ionic interactions	R837	Doxorubicin	None	B16F 10	[123]
Alginate	i.t.	Ionic interactions	R837; anti-PD-L1 (i.v. or i.t.)	Doxorubicin or Oxaliplatin	None	CT26; 4T1	[119]
Alginate	peritumoral	Ionic interactions	GM-CSF; epacadostat	None	None	4T1	[60]
F127 (Pluronic)/polyethylene glycol (PEG)	intradermal (i.d.)	In situ crosslinking; body temperature	anti- CTLA-4 and anti-PD-1	None	None	4T1	[193]
Hyaluronic acid (modified)	i.t.	In situ mixing of components	OX40; anti-PD-1 (nanocomplex)	None	ROS-triggered depolymerization and hydrolysis	B16F 10	[194]
PEG-b-PLA (nanoparticle) + HPMC	peritumoral	Shear thinning	CD40 agonist antibody	None	None	B16F 10	[52]
Self-assembling camptothecin prodrug - nanotubes	s.c.	Ionic interactions	Anti-PD-1	Camptothecin	MMP activity	GL-261; CT 26	[195]
PCLA-PEG-PCLA PAMAM dendritic nanoparticle	i.t.	Temperature	Indoximod	Doxorubicin	pH (drug release from nanoparticle)	HeLa	[120]
PLGA-PEG-PLGA	i.t.	Temperature	CA4P (vascular disruptive agent)	Epirubicin	None	4T1	[124]
Silk-chitosan composite hydrogel with dibenzaldehyde-functionalized polyethylene glycol (DF-PEG)	peritumoral	Crosslinking in situ (two syringe compartments)	JQ1	Doxorubicin	pH (degradation of hydrogel)	4T1	[57]
Hyaluronic acid and poly(N-(3-aminopropyl)methacrylamide)-co-(N-[Tris(hydroxymethyl)methyl] a crylamide) (p(AMPA-THMA))-based hydrogel	peritumoral	Shear thinning	kynureninase	Doxorubicin	Degradation of hydrogel	4T1; B16F10	[74]
poly(ethylene glycol)-block-poly(γ-ethyl-$_L$-glutamate) (mPEG-b-PELG)	i.t	Temperature	Anti-PD-L1	Doxorubicin	None	B16F10	[121]
Hyaluronic acid-based supramolecular hydrogel	i.t	Temperature	^{D}PPA-1 peptide (high binding affinity to PD-L1)	Doxorubicin	None	CT26	[109]
Alginate	i.t	Ionic interactions	CpG	catalase labelled with the therapeutic ^{131}I radioisotope	None	4T1; CT26; PDX; VX2	[62]
Chitosan-based hydrogel	Injection at site of tumor resection	Temperature	Liposome/ protamine/RNA	None	None	KPC	[63]
poly(ethylene glycol)-block-polylactide (PEG-b-PLA) nanoparticles + HPMC	s.c.	Shear-thinning	DC cytokine CCL21	None	None	Tumor-free mice	[69]
Hyaluronic acid	s.c.	Reduction-responsive	PD-L1 inhibitor	rod-like nanohydroxyapatite	None	B16	[65]

Hydrogel	Injection location	Gelation-stimuli	Immunotherapy	Other loaded molecules	Drug release stimulus	Tumor model	Citation
Multidomain peptide (MDP)	Oral cavity injection	Self-assembling nanofiber matrix	Cyclic dinucleotide (CDN) (STING agonist)	None	None	MOC2-E6E7 tumor cells in maxillary oral vestibule	[196]
functional triblock copolymer comprising a central polyethylene glycol (PEG) block flanked by two polypeptide blocks	i.t.	temperature	aPD-L1	None	ROS-responsive hydrogel degradation	B16F 10	[197]
Low molecular weight polyethylenimine (LPEI) mixed with graphene oxide	s.c.	None	Ovalbuminen-coding mRNA; R848 (resiquimod)	None	Hydrogel degradation (transformation into nanoparticles for lymph node targeting)	B16-OVA	[110]
Poly-L-lysine-PEG	peritumoral	Shear thinning	R848 (resiquimod);	PP2 (Polyphyllin II)	None	MFC gastric tumors (s.c.)	[198]
melittin-(RADA)n hybrid peptide sequences	i.t.	Self-assembly	None	Ca2+/calmodulin-dependent protein kinase II (CAMKII) inhibitor, KN93	None	B16F10; H22 hepatoma i.p ascites model	[199]
Pluronic F-127	i.t.	temperature	None	NaHCO$_3$	None	MC38	[200]
Poly vinyl alcohol (PVA) + TSPBA, ROS- responsive hydrogel	peritumoral	Mixing of hydrogel precursors	IPI549 (PI3 kinase inhibitor); anti-PD-L1	None	None at early timepoints, ROS-induced polymer degradation	CT26, 4T1	[190]
Nanofiber hydrogel (betamethasone phosphate + calcium ions)	i.t.	Exposure of betamethasone phosphate (anti-inflammatory steroid drug) to Ca^{2+}	Anti-PD-L1 antibody	None	Hydrogel disassembly in vivo	Mouse CT26	[201]
mPEG-b-poly (γ-ethyl-l-glutamate) (mPEG-b-PELG)	peritumoral	Temperature	Interleukin-15	Cisplatin	None	C57BL/6 mice B16F0-RFP	[202]

Table 2.

Preclinical study examples demonstrating the use of in situ-forming hydrogels for delivery of cancer vaccines.

Hydrogel	Injection location	Gelation-stimuli	Antigen	Other loaded drugs/ immunotherapy	Tumor model	Citation
PEG crosslinked melittin-peptide	i.t.	Self-assembly	Ultrafiltered retentate from irradiated cells	Doxorubicin, melittin	B16F10; LLC; MC38	[64]
self-assembled poly(L-valine)	s.c.	Self-assembly	Tumor cell lysates	TLR3 agonist (poly(I:C)	B16	[203]
PEG-b-poly(L-alanine)	s.c.	Self-assembly	Tumor cell lysates	GM-CSF; Anti-CTL-A-4; anti-PD-1 (encapsulated in hydrogel)	B16F10; 4T-1	[111]
polymerized phenylboronic acid (pPBA)-based	Contralateral flank	Mixture of components from two syringes	Tumor cell lysate	Mannan	4T1	[204]
Silk hydrogel	s.c.	Ultrasound	Hepa1−6 liver cancer-specific neoantigen	TLR9 agonist (CPG-ODN); STING;	Hepa1−6	[113]
mPEG-OVApeptide-AuNPs + α-cyclodextrin	s.c.	Complexation between alpha-CD and PEG before injection. Shear thinning	OVApeptide	CPG	B16-OVA	[108]
PDLLA-PEG-PDLLA	s.c.	Temperature	Tumor cell lysates	GM-CSF, CpG-ODN	B16F10; CT26	[55]

Table 3.

Preclinical study examples demonstrating combinations of locoregional ablative therapies and local delivery of immunotherapy using in situ-forming hydrogels.

Hydrogel	Injection location	Gelation-stimuli	Immunotherapy	Ablative modality	Other loaded molecules	Tumor model	Citation
Alginate/ collagen	i.t.	Temperature	Poly I:C	Photothermal therapy	ICG	CT26	[182]
Alginate	i.t.	Ionic interactions	R837 (imiquimod)	MWA	None	4T1	[189]
Alginate	i.t.	Ionic interactions	CPG ODNs	Photothermal therapy	NIR-II photothermal nanoagent	4T1	[61]
α-cyclodextrin (CD)–based	i.t.	Time-dependent	Mannose (attached by disulfide bond) CPG	Photothermal therapy	Doxorubicin ICG	B16F10	[125]
PLGA-PEG-PLGA	i.t.	temperature	ROCK inhibitor, Y27632	RFA	None	B16F1x0	[188]