Hydrogels for RNA delivery

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RNA-based therapeutics have shown tremendous promise in disease intervention at the genetic level, and some have been approved for clinical use, including the recent COVID-19 messenger RNA vaccines. The clinical success of RNA therapy is largely dependent on the use of chemical modification, ligand conjugation or non-viral nanoparticles to improve RNA stability and facilitate intracellular delivery. Unlike molecular-level or nanoscale approaches, macroscopic hydrogels are soft, water-swollen three-dimensional structures that possess remarkable features such as biodegradability, tunable physiochemical properties and injectability, and recently they have attracted enormous attention for use in RNA therapy. Specifically, hydrogels can be engineered to exert precise spatiotemporal control over the release of RNA therapeutics, potentially minimizing systemic toxicity and enhancing in vivo efficacy. This Review provides a comprehensive overview of hydrogel loading of RNAs and hydrogel design for controlled release, highlights their biomedical applications and offers our perspectives on the opportunities and challenges in this exciting field of RNA delivery.

Nucleic-acid-based therapies, such as DNA, antisense oligonucleotides (ASOs), small interfering RNAs (siRNA) and messenger RNAs (mRNA), have been widely used in diverse biomedical applications. As a type of nucleic acid essential for all known life, RNA molecules play numerous regulatory roles, such as instructing protein expression and modulating targeted genes[1–3]. So far, several RNA therapeutics, mainly siRNA and mRNA, have been clinically approved for different diseases (Table 1), with many others in clinical trials. mRNA helps the body make its own missing, defective or functional exogenous proteins (for example, antigens)[4], while siRNA reduces the expression of endogenously expressed proteins or pathological proteins[5]. Additionally, microRNAs (miRNAs) and other non-coding RNAs have also been explored for regulating gene expression at the post-transcriptional level[6].

Despite the considerable therapeutic potential of RNAs, limitations on their in vivo delivery have been reported, including enzymatic susceptibility, extracellular and cellular barriers, and difficulties in trafficking to the subcellular compartment where the cargo will be active[1]. Therefore, the majority of clinical-stage RNA therapies are based on chemical modification (for example, phosphorothioate linkage), ligand conjugation (for example, N-acetylgalactosamine (GalNAC)) or non-viral nanoparticle (NP) delivery (for example, lipid NP)[7]. Specifically, chemical modification improves enzymatic and metabolic stability[8], and ligand conjugation improves delivery to specific organs and cell types[9]. Finally, NPs protect encapsulated RNA and improve pharmacokinetics and endosomal escape[10]. However, these delivery methods have their own limitations, with further improvements needed for transfection efficiency[11], organ/cell delivery specificity[12], RNA stability[13] and circumventing immune activation[14], which may require the development of entirely different categories of delivery systems. Along these lines, substantial efforts have recently been made

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Table 1 | RNA therapeutics approved for clinical use

Product name	RNA type	Carrier	Target	Administration route	Indication
Onpattro	siRNA	Lipid nanoparticle	TTR	Intravenous infusion	Polyneuropathy of hATTR amyloidosis
Givlaari	siRNA	GalNAC conjugation	5-Aminolevulinic acid synthase	Subcutaneous injection	Acute hepatic porphyria
Oxlumo	siRNA	GalNAC conjugation	Hydroxyacid oxidase 1	Subcutaneous injection	Primary hyperoxaluria type 1
Leqvio	siRNA	GalNAC conjugation	PCSK9	Subcutaneous injection	Primary hypercholesterolemia or mixed dyslipidaemia
Amvuttra	siRNA	GalNAC conjugation	TTR	Subcutaneous injection	Polyneuropathy of hATTR amyloidosis
Comirnaty	mRNA	Lipid nanoparticle	SARS-CoV-2 spike protein	Intramuscular injection	COVID-19
Spikevax	mRNA	Lipid nanoparticle	SARS-CoV-2 spike protein	Intramuscular injection	COVID-19

hATTR, hereditary transthyretin-mediated amyloidosis; PCSK9, proprotein convertase subtilisin/kexin type 9; TTR, transthyretin.

to explore the use of macroscale hydrogels for RNA-based therapeutics delivery, as well as a variety of biomedical applications ranging from gene silencing and protein replacement to immunomodulation (Fig. 1)[15–40].

Hydrogels are composed of a water-swollen three-dimensional network that recapitulates the intrinsic properties of the native extracellular matrix (ECM), making them useful for applications in tissue engineering, drug delivery, cellular morphogenesis, among others[41,42]. The unique physicochemical features of hydrogels allow the maintenance of RNA biological activity, the retention and sustained release of RNA as local delivery carriers (for example, injectable systems), and the delivery of high concentrations of payloads to a target site in an on-demand/pulsatile manner via stimuli-responsive strategies (Fig. 2)[43,44]. Thus, hydrogels could improve RNA stability, reduce unnecessary loss of therapeutics associated with systemic delivery, mitigate undesirable off-target toxicities and avoid the necessity of multiple doses. All of these make hydrogels an appealing system for RNA-based therapeutics delivery, complementary to the clinical-stage platforms mentioned above.

Compelling evidence supporting the application of hydrogels for delivery of RNAs and other nucleic acids has been gathered ever since the first use of polymer pellets for sustained nucleic acid release in 1976[45]. This Review aims to summarize RNA-loading strategies in hydrogels, discuss the design of hydrogels for controlled delivery of RNAs, highlight recent progress in biomedical applications, and provide perspectives on the challenges and opportunities in this blossoming research field.

RNA loading in hydrogels

RNA can be loaded into hydrogels either by direct inclusion of the naked RNA or by encapsulating RNA nanocarriers (Fig. 3). The loading of naked RNA largely depends on the physicochemical interactions between the RNA and the hydrogel network. As a comparison, nanocarriers may offer improved RNA bioactivity, better controllability of RNA release and targeting of specific cells, while the loading is dependent on the interactions of nanocarriers with the hydrogel.

Naked RNAs

Multiple strategies have been developed for loading naked RNAs into hydrogels, such as electrostatic interaction, covalent conjugation, host–guest interaction or combinations thereof (Fig. 4). Here, we introduce each strategy and the associated hydrogels, and discuss their features for delivery of naked RNA.

Ionic bond. Cationic/ionizable polymers and lipids, the most widely studied non-viral delivery materials, can interact with negatively charged biomolecules, making ionic bonds a simple and robust method for the encapsulation of RNAs and other nucleic acids in hydrogels[46]. However, synthetic polycations may cause moderate-to-high toxicity, mainly due to their high positive charge. Further, synthetic cationic polymers generally have a low molecular weight and a highly branched structure, which may limit their potential applications in hydrogel formation. Thus, conjugation of synthetic and natural polycations to hydrogels or the use of hydrogels solely based on natural polycations emerged to address these concerns[37,47]. For example, several different biodegradable polymers and fabrication systems were studied for localized delivery of naked siRNA: calcium crosslinked alginate, photocrosslinked alginate and acid-solubilized collagen[47]. siRNA was rapidly released within a week from the highly negatively charged alginate hydrogels, but required over two weeks to be released from the collagen hydrogel due to the effect of the amine groups. The incorporation of positively charged polyethylenimine (PEI) or chitosan further delayed siRNA release. Moreover, ionic bonds are susceptible to pH changes, which may hinder the sustained release of the loaded biomolecules. Factors such as the number and type of charge groups (for example, primary, secondary and quaternary amino groups and amidine groups) per single polymer can also help determine the ultimate release profile.

Hydrogen bond. Neutrally charged polymers such as polyvinyl alcohol (PVA) can bind to nucleic acids through the interaction of the positive hydrogen atom, which establishes an electrostatic link with electronegative acceptor atoms[48]. Such polymers can be further modified with certain chemical moieties to increase intermolecular hydrogen bonding interactions between the hydrogel and the RNAs. Similarly, negatively charged polysaccharides such as alginate and hyaluronic acid (HA) have been explored to promote the controlled release of RNAs, which can also be modified with additional moieties to increase the number of hydrogen bonds to the encapsulated nucleic acids. For example, HA-PVA hydrogels were shown to release siRNA in vitro more slowly than PVA hydrogels, which was attributed to a higher number of hydrogen bonds between the siRNA and the HA backbone[49]. It is important to emphasize that hydrogen bonds are primarily weak electrostatic interactions, which may not induce strong binding of RNAs to the hydrogels. Consequently, hydrogen and ionic bonds are sometimes combined to boost interactions between the hydrogel and RNAs, through the addition of cationic molecules or polymers to the hydrogel formula[39,50].

Covalent bond. Covalent conjugation of RNAs to the backbone of hydrogels allows for the homogeneous and predictable distribution of a large quantity of RNAs with minimal initial burst release. Although this method is very common in small-molecule drug delivery, there are very few examples of covalently binding RNAs[51]. For instance, siRNA was covalently tethered to the photocrosslinked
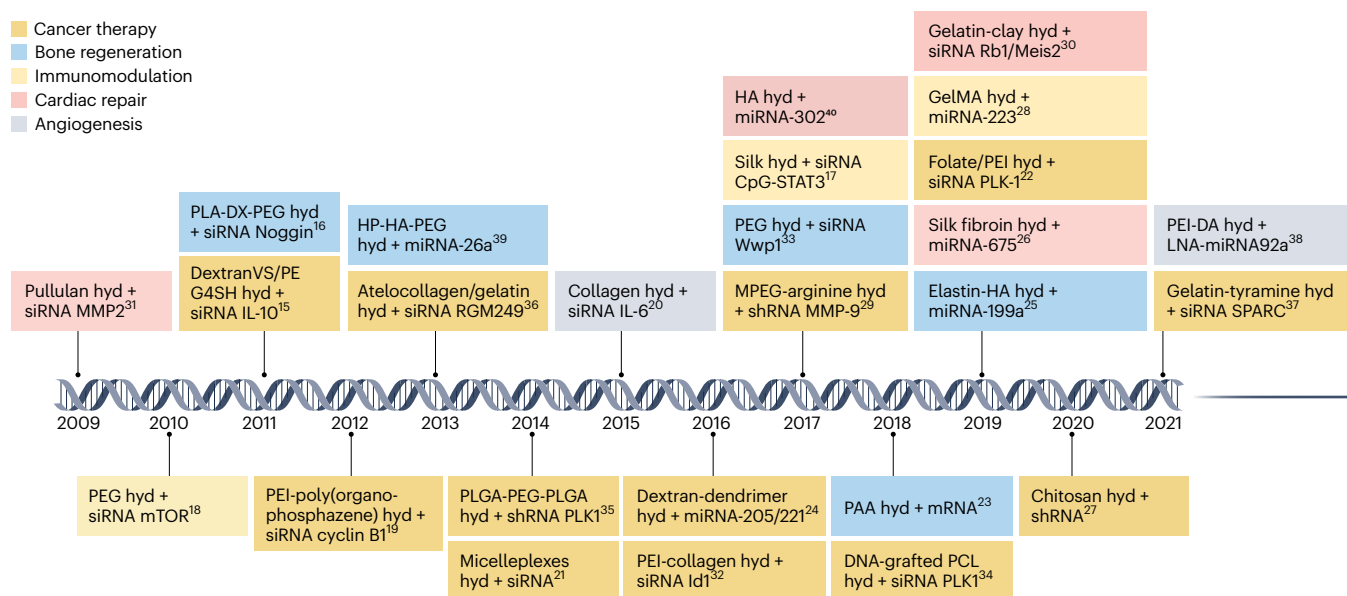
Fig. 1 | Timeline of recent preclinical studies of hydrogel-based RNA delivery. Coloured boxes indicate the type of biomedical application: cancer therapy (orange), bone regeneration (blue), immunomodulation (yellow), cardiac repair (red) and angiogenesis (grey). ACpG-STAT3, cytosine-phosphorothioate-guanine-signal transducer and activator of transcription 3; DextranVS, dextran vinylsulfone; GelMA, gelatin methacryloyl; HP-HA-PEG, a thiol-modified analogue of heparin-thiol-modified hyaluronan-poly(ethylene glycol) diacrylate; hyd, hydrogel; IL, interleukin; MPEG, methoxypolyethylene glycol; mTOR, mammalian target of rapamycin; PAA, polyacrylamide; PCL, poly(ε-caprolactone); PE, polyethylene; PEG4SH, tetra-thiolated polyethyleneglycol; PEI-DA, deoxycholic acid-modified polyethylenimine polymeric conjugates; PLA-DX-PEG, poly-D,L-lactic acid-p-dioxanone-polyethylene glycol block co-polymer; PLK, serine/threonine-protein kinase; Rb1/Meis2, retinoblastoma1/meis homeobox 2; RGM, RNA gene for miRNAs; SPARC, secreted protein acidic and rich in cysteine[15–40].
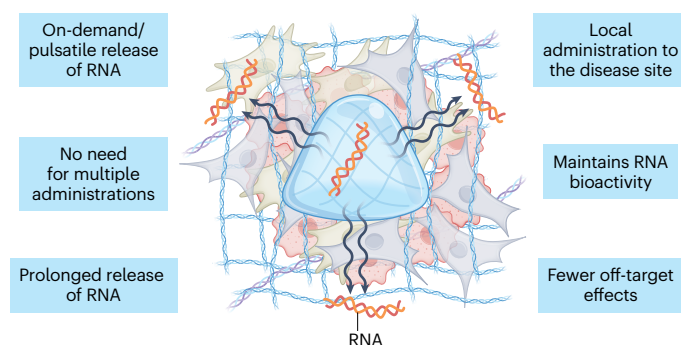

Fig. 2 | Advantages of hydrogels as a platform for RNA delivery. Hydrogels provide a unique strategy for local administration of RNA, overcoming some of the difficulties associated with systemic RNA delivery. They enable a localized, controlled and sustained delivery of high levels of payloads, while maintaining RNA biological activity. Off-target effects and the need for multiple payload administrations in systemic delivery may thus be avoided.

dextran hydrogels via Michael-addition chemistry. Upon hydrolytic degradation of ester and/or disulfide linkages, the profile release of siRNA was prolonged up to 10 days in comparison with the unbound compound that was released in the first 12 hours. By tailoring the degradable linkages or the amount of tethered siRNA in the hydrogel network is possible to control the cargo amount and its profile release. However, the conjugation of siRNA into the hydrogel network possesses additional technical complexity.

Hydrophobic interaction. Hydrophobic interactions occur through the formation of a clathrate cage, an ice-like matrix of water molecules formed through hydrogen bonds, around the hydrophobe[52]. The host–guest pairs are relatively easy to synthesize and can interact with biological molecules, being typically driven by molecule size and hydrophobicity. Guest–host pairs are widely used for injectable hydrogel fabrication mainly due to their dynamic bonds that, upon, can reform[44]. For example, cyclodextrin (CD) showed relatively low toxicity and high water solubility[29], enabling a range of hydrophobic guest molecules to be embedded in their interior cavities[53]. HA was modified with CD, as a host, or with adamantane, as a guest, which were self-assembled into injectable hydrogels. The HA assembly system enables the formation of complex CD-cholesterol-modified miR-302 interactions for miRNA local and sustained release[40]. The release of miRNA from the HA hydrogel network is measured over three weeks (in vitro studies), and it is faster than the erosion of the hydrogel. Thus, it is hypothesized that miRNAs diffuse from the hydrogel network, highlighting the crucial role of the anionic repulsion of HA to miRNA and the hydrogel guest–host interactions.

As an alternative approach, supramolecular hydrogels can also be formed by self-assembly of biocompatible small-molecule hydrogelators, which is triggered by intramolecular π–π stacking of hydrogelator molecules and RNAs. For example, polyethylene glycol (PEG) modified with ureido-pyrimidinone moieties (UPy-PEG) was able to dimerize in water, producing a fibrous supramolecular hydrogel[54]. siRNA and miRNA were covalently conjugated with cholesterol to directly interact with the hydrophobic core of the fibre, which fine-tunes siRNA and miRNA profile release. Otherwise, supramolecular hydrogelator molecules (for example, tetrazole and spiropyran) can be added to facilitate hydrophobic interactions with the hydrophobic side chains of amino acids within the encapsulated RNA structures[55]. However, in these hydrogels the ratio of hydrophobic moieties to hydrophilic regions must be adjusted carefully to preserve water-uptake capacity. It should also be noted that such hydrophobic interactions do not depend on RNA itself but on the hydrophobic modifier on RNA.

Non-specific interaction. In some cases, RNA loading in hydrogels is merely mediated by non-specific interactions. The RNA release may therefore be governed simply by diffusion-controlled mechanisms,
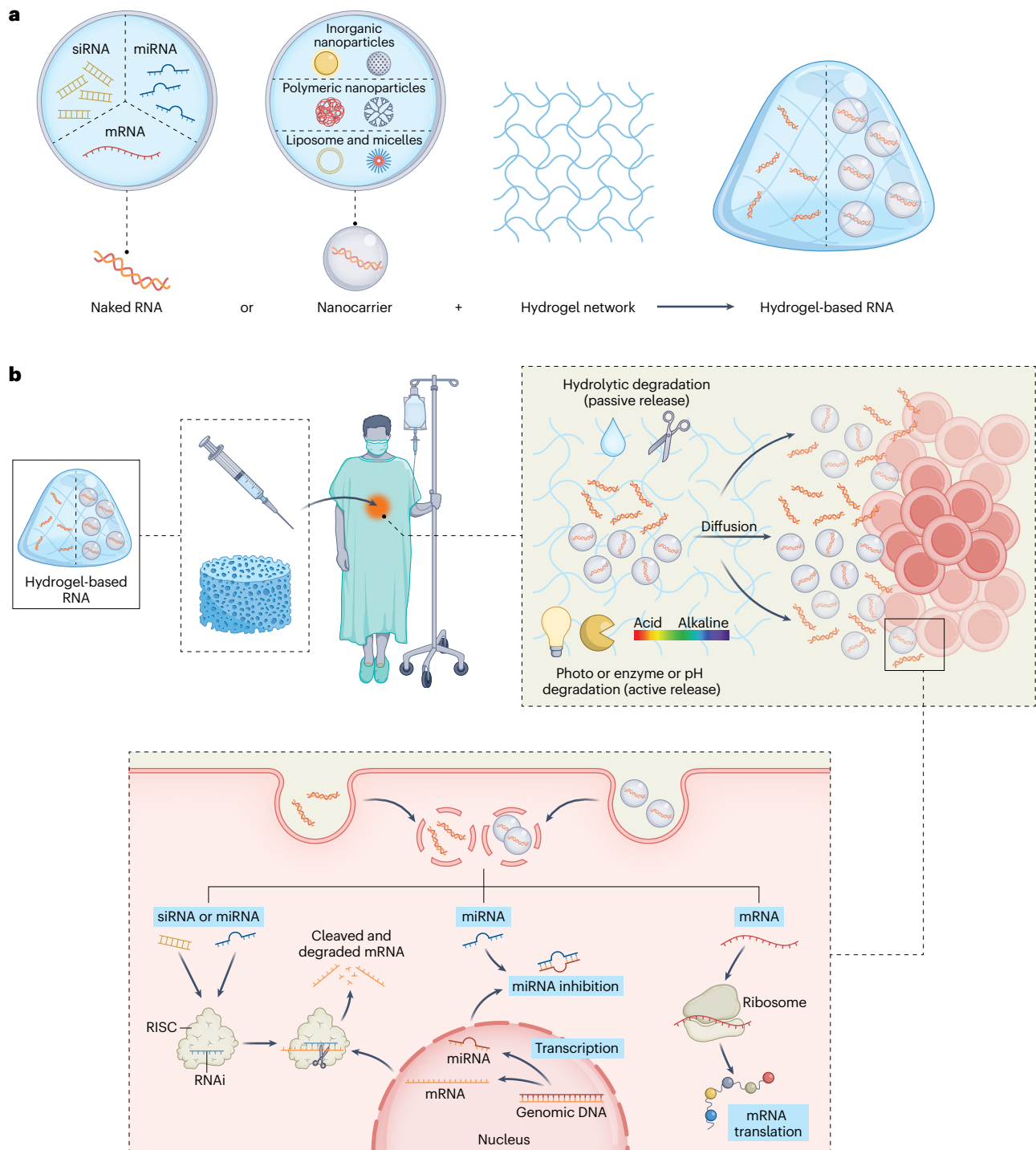
Fig. 3 | Functional hydrogels for RNA loading and delivery. a, RNA is loaded into hydrogel either with no manipulation (naked RNA) or by means of nanocarriers. **b**, RNA-loaded hydrogels can be used as implantable scaffolds or as injectable gels for local RNA delivery. The fine-tunable physical, biochemical and biological features of hydrogels allow the sustained and/or controllable release of RNA. Upon cellular entry (for example, via naked RNA or RNA-loaded nanocarriers), RNA reaches the proper subcellular compartment to initiate protein production/inhibition.

which are discussed in detail in the following section. For instance, one could increase the crosslinking density and consequently decrease the swelling and drug release rate, or reduce the macromolecular mesh size by altering the macromolecular structure and therefore extend the drug release time. This is often the case for thermo-sensitive hydrogels, such as poly(*N*-isopropylacrylamide)[56], which can be formed upon in vivo administration, instigated by the sol-to-gel phase transition. A major limitation associated with thermohydrogels is their lack of

biodegradability, which could be addressed by copolymerizing them with biodegradable polymers. The limited control over the RNA release profile may be further overcome by the incorporation of cationic polymers.

RNA nanocarriers
The delivery of RNA nanocarriers (for example, liposomes/lipid NPs, polymeric NPs and inorganic nanomaterials) loaded within a hydrogel
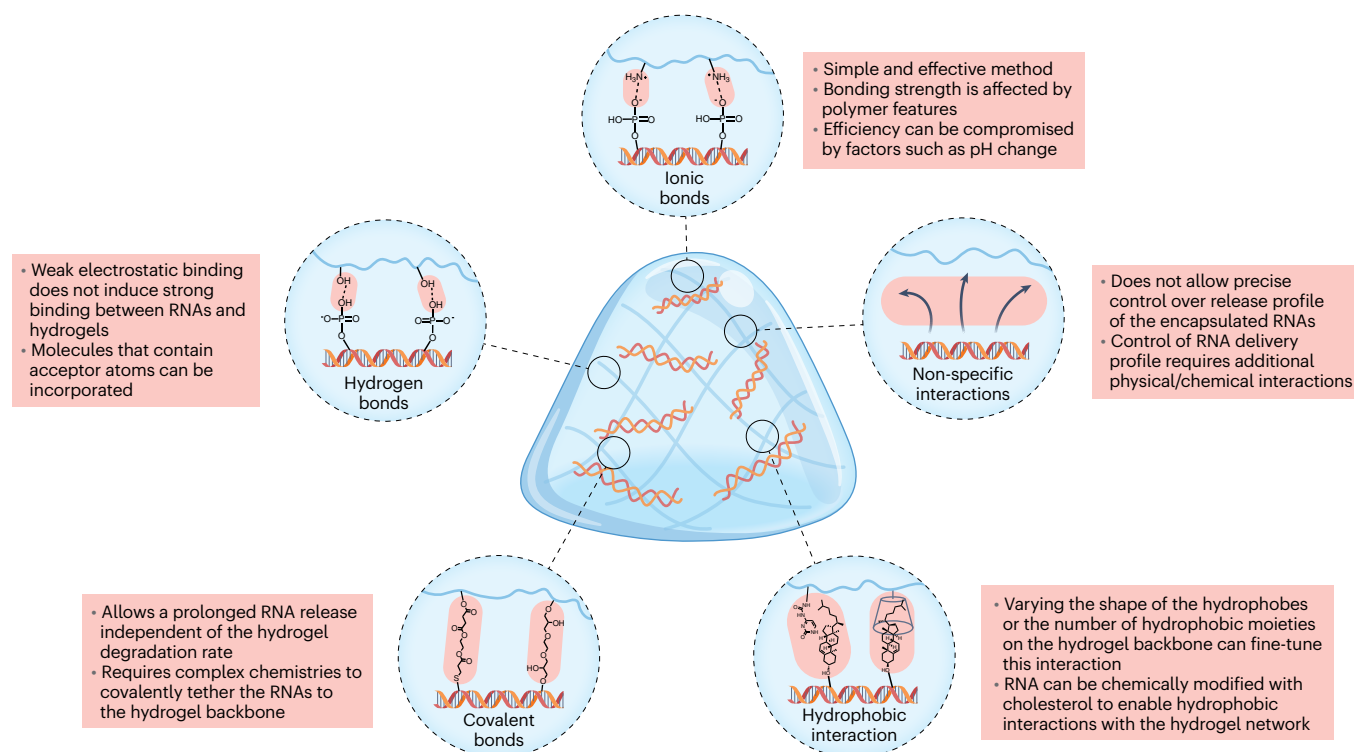
Fig. 4 | Strategies for loading naked RNA into a hydrogel network. RNA therapeutics can interact with hydrogel networks through ionic bonds between negatively charged RNA parts and positively charged hydrogel network parts; hydrogen bonds produced when positively charged hydrogen atoms come within a certain radius of an electronegative acceptor atom; covalent bonds that chemically link the RNA to polymer hydrogel chains; hydrophobic interactions that use modified RNA; and non-specific interactions.

network could avoid the chemical modification of both RNA and hydrogel polymers and improve loading, stability and transfection efficiency compared with naked RNA strategies (Fig. 3)[3]. Below, we outline the loading of several representative RNA nanocarriers in hydrogels.

Lipid nanocarrier. Cationic/ionizable lipids can be used in the form of either liposomes or RNA/lipid complexes, such as lipoplexes and niosomes[57]. Fibrin gels are capable of supporting cell migration while maintaining lentivirus activity[58]. Fibrin-based hydrogel surface was conjugated with siRNA-loaded lipofectamine to increase its cellular internalization levels and knock down antagonists (for example, noggin)[59]. Approximately 20% of free siRNA or siRNA complexed with lipofectamine was remained on the fibrin surface after 3 days, therefore indicating that the negative charge of fibrin does not appear to influence the surface retention of nanocomplexes. On the other hand, the use of injectable chitosan–alginate scaffolds containing mRNA–lipoplexes demonstrated the ability to induce an in vivo local transfection and increase antibody production and T-cell proliferation levels when compared with the systemic administration of only mRNA–lipoplexes[60]. Notably, there is still a limited number of published studies using RNA–liposome-loaded hydrogels, which might be explained by the thermodynamic instability of liposomes and their further aggregation in a charged hydrogel[61].

Polymer nanocarrier. Cationic polymers such as polyethylenimine (PEI) chitosan, and poly(L-lysine) (PLL) are often used to create polyplexes[62]. Unlike lipid-based nanocarriers, cationic polymers are completely soluble in water due to their general absence of hydrophobic moieties[46]. Moreover, cationic polymers have the ability to compress nucleic acids to a smaller size than cationic lipids. One potential disadvantage of RNA–polymer nanocarriers is that, for some soft and charged NPs, aggregation could occur during loading, which may limit the amount of RNA that can be loaded into the hydrogel[63]. Collagen-based hydrogels, which closely resemble natural ECM, have been used for local delivery of RNA nanocomplexes. One specific collagen hydrogel was capable of sustained delivery of siRNA/PEI nanocomplexes in vitro over 10 days[32]. However, the foreign proteins in collagen hydrogels might elicit a foreign body response, which could hinder their biological application[64]. Along these lines, HA-based hydrogels may be one of the preferred choices for RNA delivery[65]. Cyclooxygenase-engineered miRNA (COX-1 and COX-2) plasmids were loaded onto PLGA/PEI NP complexes, and then embedded in HA hydrogels[65]. A slower, more sustained release profile from the hydrogel was detected compared with plasmid/NP complexes. Possible interactions of polymeric nanocarriers with the hydrogel could affect the release rate. In fact, aggregation and deactivation of RNA-loaded NPs within hydrogels have been reported[63]. To address this, coating NPs with agarose[66], covalent attachment of NPs to the hydrogel backbone[67] or similar strategies may be applied in engineering such delivery systems.

Inorganic nanocarrier. Inorganic colloidal NPs (for example, gold, iron oxide, silica and quantum dots) have been utilized in RNA therapy mainly for their facile synthesis process and wide availability[3]. For example, PEI-based hydrogels were conjugated with siRNA-Au-Fe$_3$O$_4$ nanocapsule. The subcutaneous administration of nanocapsule hydrogel demonstrated a better tumour penetration and a higher blood circulation time compared with intravenous administration of nanocapsule only[22]. Certainly, AuNPs allow a wide variety of functionalizations via gold–thiol conjugation, being broadly used in multi-modal approaches[68]. Multifunctional quantum dot DNA hydrogels containing doxorubicin and siRNA reduced EGFR expression significantly more than siRNA alone[69]. Quantum dot DNA hydrogels are capable of functioning as a delivery vector without toxic transfection agents and have demonstrated high in vivo therapeutic efficacy against breast cancer.

The combination of inorganic core–hydrogel scaffolds with colloidal NPs introduces the possibility of new scaffold structures with different core size, charge, coatings and physical stretching/compression of hydrogels.

Hydrogel design for controlled RNA release

The profile release of RNA from the engineered hydrogel network, including the duration of RNA availability (short term versus long term) and the release pattern (continuous versus pulsatile), will strongly depend on the target application. As a result, multiple hydrogel designs have been employed to facilitate the controlled release of RNAs through either passive or active mechanisms. While passive mechanisms can enable continuous short- and long-term release, active mechanisms can yield pulsatile release patterns. In the following section, a comprehensive review of these mechanisms for controlled release of naked RNAs or RNA-nanocarriers from hydrogels is provided (Fig. 5).

Hydrogel for continuous RNA release

Continuous passive release from hydrogels is achieved by the individual action or the combination of factors like diffusion, hydrogel network degradation, and hydrogel swelling. On this account, passive release can be adjusted by engineering hydrogel features such as molecular weight, matrix concentration, crosslinking density, hydrophilicity and pore size distribution[33,70,71], in addition to the aforementioned hydrogel chemistries for interacting with RNAs.

Hydrolytically degradable functionalities, such as ester groups, have been incorporated into the hydrogel backbone as a means to control release rate[72]. To this end, based on thiol–ene interactions, an hydrogel formed in situ was made using a combination of eight-arm thiol-modified PEG (8-arm-PEG-SH) with either eight-arm acrylic-modified PEG (8-arm-PEG-A) that contains one ester group on each arm or eight-arm mono(2-acryloyloxyethyl) succinate-modified PEG (8-arm-PEG-MAES) that contains three hydrolysable ester groups on each arm[73]. The hydrogel with the higher ester linkages in the macromolecular networks (8-arm-PEG-MAES) exhibited faster swelling and degradation, which induces a faster release of RNA-PEI nanocomplexes ($85.09 \pm 2.43\%$ over 19 days) compared with the other two hydrogel formulations.

The release rate can be further tuned by adjusting the nanocarrier size and concentration[28]. Photocrosslinked DEX hydrogels (DEX-HEMA) were covalently functionalized with cationic linear PEI methacrylate (LPEI-GMA) via a biodegradable ester linkage[74]. siRNA electrostatically interacts with the cationic linear PEI. Thus, siRNA profile release was fine-tuned by the degradation rate of DEX-HEMA hydrogels via biodegradable ester linkages and the degree of siRNA/PEI interactions, which were achieved by controlling the hydrogel (8 and 12 wt%) and/or the nanocarrier (0, 5 and 10 μg) concentration. The hydrogels were able to release the siRNA over long time periods (9 to 17 days). Remarkably, the observed sustained profile release of RNA from hydrogel network over a long time period (weeks) can be detrimental to induce a significant physiological response. Indeed, the low amount of RNA that is released from the hydrogel network cannot be sufficient to promote a target effect that systemic administration (or several local administrations) can achieve.

Hydrogels for stimuli-responsive RNA release

To address the issues associated with passive release, on-demand delivery of RNAs from the hydrogels has been achieved using active mechanisms of release. Active release is achieved in response to internal (for example, pH and enzyme) or external stimuli (for example, photoradiation), which can facilitate on-demand degradation of the hydrogel network and hence promote RNA release. The trigger of RNA release through external stimuli introduces additional control of RNA delivery at defined doses and during specific periods, providing an alternative to physician- or patient-delivered strategies.

pH-responsive release. pH-responsive hydrogels often contain Schiff base bonds, which are stable at pH 7.4 but disrupted in an acidic environment (for example, pH 6.8)[75]. Such hydrogels are suitable for on-demand release of RNA in diseases associated with an acidic tissue microenvironment (such as cancer and myocardial infarction (MI)). For instance, a pH-sensitive hydrogel was created based on a combination of miRNA-loaded amine-functionalized mesoporous silica NPs (MSNs), aldehyde-functionalized PEG (PEG_{CHO}) and α-CD[76]. The hydrogel fabrication relies on Schiff and hydrophobic interactions between PEG_{CHO}, α-CD and MSN. At a slightly acidic environment (pH 6.8), Schiff base bonds are cleaved to produce an aldehyde functional group, and MSN/miR-21-5p is released from the hydrogel (75% for 1 week in vitro) to the infarct region. While at pH 7.4, only 6% of MSN/siRNA nanocomplexes were released from the hydrogel. Interestingly, the amount of released RNA upon pH stimuli was effective as an MI treatment.

Enzyme-responsive release. Enzyme-sensitive hydrogels normally contain a polymeric network that is crosslinked with an enzyme-sensitive peptide linker[77]. In the presence of a certain enzyme (for example, matrix metallopeptidase 2 (MMP-2), protease, trypsin and lysozyme) the peptide linker is broken, which leads to the release of the entrapped RNA therapeutics from the hydrogels. Along these lines, HA-based hydrogels were formed through hydrozone bonds (that is, aldehyde-modified HA and hydrazide-modified HA) and protease degradable peptide crosslinkers[78]. Then, CD-modified HA was introduced in the hydrogel system to sequester cholesterol-modified siRNA, as previously described[40]. As expected, the hydrogel was eroded and siRNA targeting MMP2 was released in response to protease (for example, collagenase) levels for MI treatment[60]. In another study, an MMP-2 degradable hydrogel was loaded with tumour growth factor-β1 siRNA polyplexes, which were further adsorbed onto electrospun fibres[79]. High MMP-2 concentrations promoted faster release of polyplexes due to the MMP-2 substrate peptide degradation in the hydrogels, whereas the addition of MMP-2 had almost no influence on siRNA release from the hydrogels containing MMP-2-nondegradable crosslinkers.

Photo-triggered release. Light-responsive hydrogels offer on-demand spatial and temporal control. Generally, these hydrogels contain single or multiple photocleavable moieties (for example, nitrobenzyl-based linkers with ester or amide bonds) with variable degradation properties in response to light wavelength and intensity, as well as radiation time. While naked RNA is attached to the hydrogel network via photolabile bonds, the RNA nanocarriers are encapsulated in the hydrogel crosslinked by these photo-sensitive bonds. Photodegradable PEG-di(photolabile acrylate) (PEG-DPA) has been consistently used as a building block in these hydrogels[50]. Upon ultraviolet (UV) light exposure, ester groups linked to ortho-nitrobenzyl photolabile groups cleave into acetal and acidic moieties, promoting siRNA release. Photolabile hydrogels have been also prepared using Michael addition to control the release of siRNA-PEI nanocomplexes[80]. The photodegradable hydrogels with the lowest amount of photolabile moieties showed an increase in the hydrolytic degradation rate of ester bonds, which enhances siRNA therapeutics release. In addition, the siRNA profile release from photolabile hydrogels was also affected by UV light exposure. Remarkably, the selective delivery of miRNA was achieved by using PEG-based hydrogels via a copper-free click reaction and conjugated with UV-cleavable Chol-miR-26a, which allows control over miRNA release by tailoring UV irradiation time and UV intensity.

Altogether, proper design of hydrogels with photo-degradable linkers can achieve on-demand release of RNA therapeutics upon UV radiation. However, due to the low penetration of UV light, these hydrogels may not be suitable for use in deep tissues.
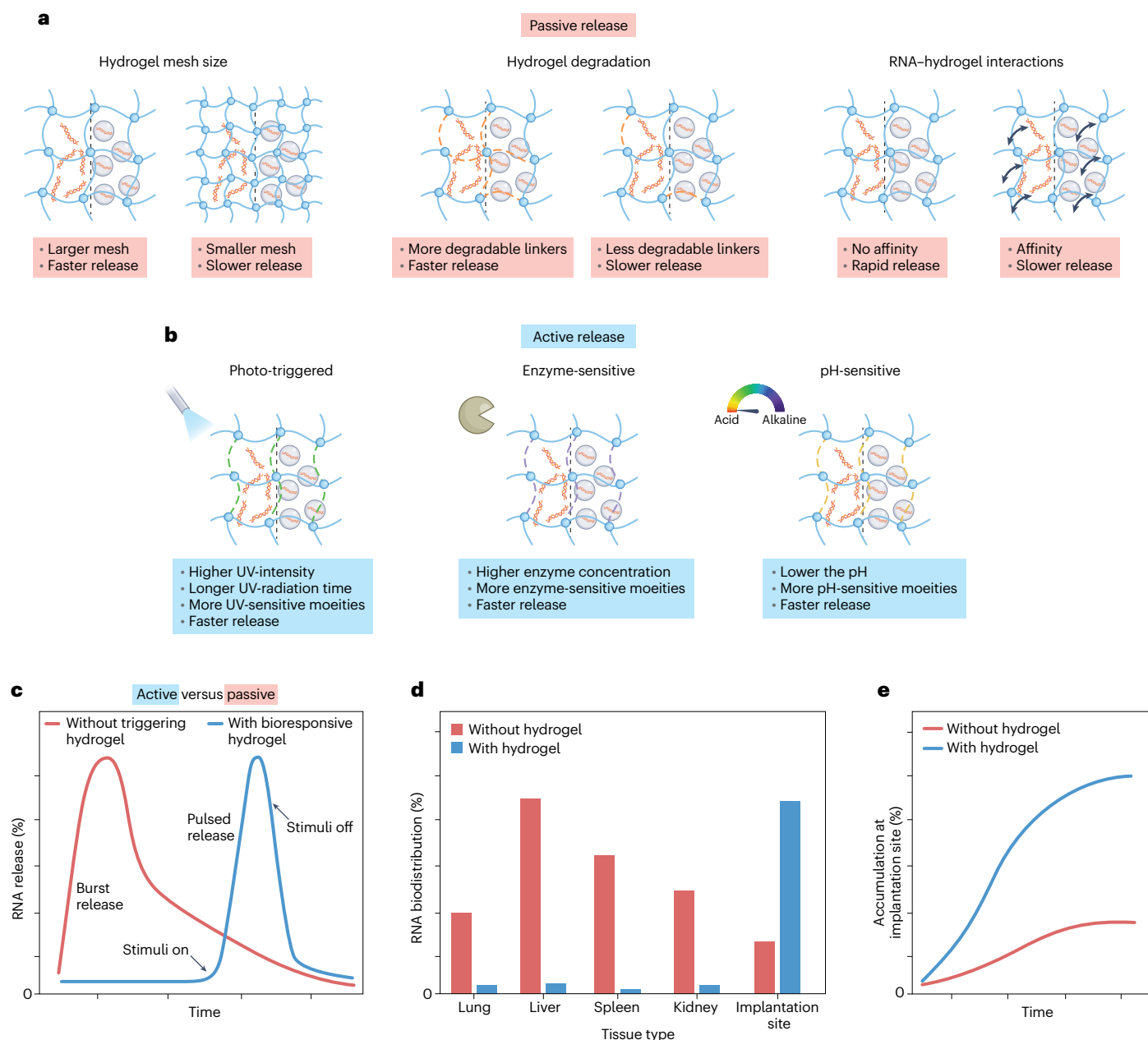
Fig. 5 | Hydrogel functional properties for controlled RNA delivery. a, The ultimate release profile of the encapsulated naked RNA and/or RNA nanocarriers is determined by the hydrogel's physical features and RNA–hydrogel interactions. **b**, Upon local administration, the release of the encapsulated RNA can be triggered by external or internal stimuli. **c**, Illustrative release profiles of encapsulated naked RNA and/or RNA nanocarriers. **d**, Illustrative biodistribution profiles of RNA therapeutics administered in the naked form or in combination with a hydrogel system. **e**, Illustrative local accumulation profiles of payload at the implantation site in the naked form or in combination with a hydrogel system.

Biomedical applications

Hydrogel delivery of RNA therapeutics has been applied in diverse biomedical applications (Table 2). Hydrogels are primarily used to facilitate local administration of RNA therapeutics to the disease site and to protect the RNA from innate immune responses. However, depending on the disease pathology, hydrogels can be modified to yield various release profiles to maximize the efficacy of RNA therapeutics. Below, we primarily highlight the use of RNA delivery hydrogels in cancer therapy, bone regeneration, cardiac repair and wound healing (Fig. 6).

Cancer therapy

Systemic cancer treatment is useful for metastasis but is also associated with systemic toxicity and possible immunogenicity due to leakage/ accumulation in major organs. In this context, hydrogels could facilitate long-term and sustained local delivery of RNA therapeutics to reduce potential side effects while attacking the primary tumour[24,67], reprogramming the primary tumour to prevent metastasis[81] and/or inhibiting the recurrence of the primary tumour after surgical resection[68]. Accordingly, different types of activated oncogene mRNA or miRNA can be effectively inhibited by RNAi technology to inhibit tumour growth. For example, NPs containing RNA therapeutics (for example, miRNA) can be embedded in a hydrogel matrix, which is in turn implanted next to the tumour[24,81]. Remarkably, a hydrogel network composed of a two-component system, namely Schiff-base interactions between an oxidized polysaccharide and an amine-containing dendrimer, demonstrated the use of the ratio of aldehyde to amine groups to control the

Table 2 | Biomedical applications of hydrogel-based RNA delivery

Application	RNA	Hydrogel	Reference
Cancer	siRNA	PEG-PEI; poly(organophosphazenes); PAMAM-dextran; mPECT(D)/GDDC-4(R); PFF; Collagen; Chitosan	19,21,22,67,68,104
	shRNA	PLGA-PEG-PLGA	35
	miRNA	PAMAM-dextran	24,81
Immunomodulation	siRNA	Chitosan	105
	miRNA	GelMA	28
	mRNA	Chitosan-Alginate	60
	Plasmid DNA/siRNA	Dextran-PEG	96
Bone regeneration	miRNA	HyStem-HP; PEG; PEG-PLGA-PNIPAM; PEG-GelNB	39,72,106
	siRNA	PEG; PLA-DX-PEG; PEI; DEX-MAES; Fibrin	16,59,73,80,107
	mRNA	Fibrin; Collagen	108,109
Angiogenesis	siRNA	PTK-UR; PEUR; PUR; Agarose	98,110
Cardiovascular disease	siRNA	PEI-PEG; HA; Pullulan	31,44,78
	miRNA	HA; Elastin-like protein-HA; HyStem-HP	40,111
Spinal cord injury	miRNA	Collagen	112
Intervertebral disk degeneration	miRNA	PEG-thiol	113
Chronic rhinosinusitis	siRNA	Chitosan	114
Allergic rhinitis	miRNA	Chitosan	115
Rheumatoid arthritis	siRNA	Sericin	116
Fibrosis	siRNA	Agarose	117
Fibrous encapsulation	siRNA	PEG	18
Ventral root avulsion	shRNA	Pluronic F-127	118
Tendon adhesions	miRNA/Plasmid DNA	HA/PEG	65
Ageing-induced vascular dysfunction	miRNA	Silk fibroin	26
Atopic dermatitis	siRNA	Sericin	119

DEX-MAES, dextran-mono(2-acryloyloxyethyl) succinate; FA, folic acid; PAMAM-dextran, amide and ester conjugates of aceclofenac with polyamidoamine dendrimers-dextran; PEO, poly(ethylene oxide); PFF, precursor fluid formulation.

release rate of RNA therapeutics. However, highly crosslinked hydrogels can hinder the release of embedded RNA therapeutics, reducing therapeutic efficacy. To address this issue, one-component injectable polyplex hydrogels were used to deliver RNA therapeutics (for example, siRNA) with higher efficiency[19,21]. Typically, these polyplex systems contain a thermo-sensitive moiety that allows sol-to-gel transition upon injection into the target tissue. Subsequently, the release rate of RNA-containing polyplexes from these gels depends on their dissolution rate, which can be further controlled by incorporating degradable linkers sensitive to hydrolysis or enzymatic activity. Consequently, hydrogel-mediated delivery of RNA therapeutics for cancer therapy entails prolonged retention of the nano-vectors around the tumour, which enhances uptake by the particular cancer cell population. This becomes crucial when dealing with brain tumours, as such platforms are capable of overcoming biological barriers (mainly the blood–brain barrier) to deliver therapeutics to the brain tissue. In general, hydrogels for the delivery of RNA therapeutics in cancer have demonstrated enhanced bioavailability and increased tumour accumulation with less homing in non-target tissues. In the context of cancer, RNA hydrogel delivery is not limited to silencing of oncogenic genes, and the next great achievement on the horizon could be manipulation of immunomodulatory factors to recruit immune cells for cancer immunotherapy[82].

Bone regeneration

Bone regeneration and repair is another arena in which hydrogel-mediated RNA delivery has shown promise[72,75]. Bone healing relies on several dynamic and spatiotemporal mechanisms, including inflammatory, repair, and remodeling phases at crucial cellular (that is, inflammatory cells, vascular cells, osteochondral progenitors, and osteoclasts) and molecular levels (that is, pro-inflammatory cytokines, growth factors, and angiogenic and pro-osteogenic factors)[83]. As a result, it is crucial for the hydrogels to modulate the spatio-temporal and dose-controlled release of RNA therapeutics to meet the highly complex microenvironment present during bone regeneration. Correspondingly, if the hydrogels are to be implemented as a scaffold, their degradation should correlate with the rate of ingrowing tissue to provide sufficient mechanical support. Given such requirements, photodegradable hydrogels have shown immense potential to facilitate the on-demand release of RNA therapeutics[80]. In these systems, the hydrogels contain both hydrolytically degradable linkages (for example, disulfide and/or ester bonds) as well as photolytically degradable sites (thiol-acrylate bonds). Therefore, UV radiation induces photodegradation of photolabile linkages in the hydrogel network, affecting the hydrogel's physiochemical properties such as swelling and degradation rate. The results showed that UV radiation can lead to faster release of siRNA from these hydrogels, and that the associated release rate can be further modified by adjusting the ratio of photolabile groups in the hydrogel composition. Hydrogels of this sort allow temporal tuning of RNAi presentation directly at the diseased site, which is known to enhance bone formation[84]. Although research studies have explored the delivery of osteogenesis-inducing therapeutic agents, it is also possible to study additional cell responses, including angiogenesis and cell infiltration.
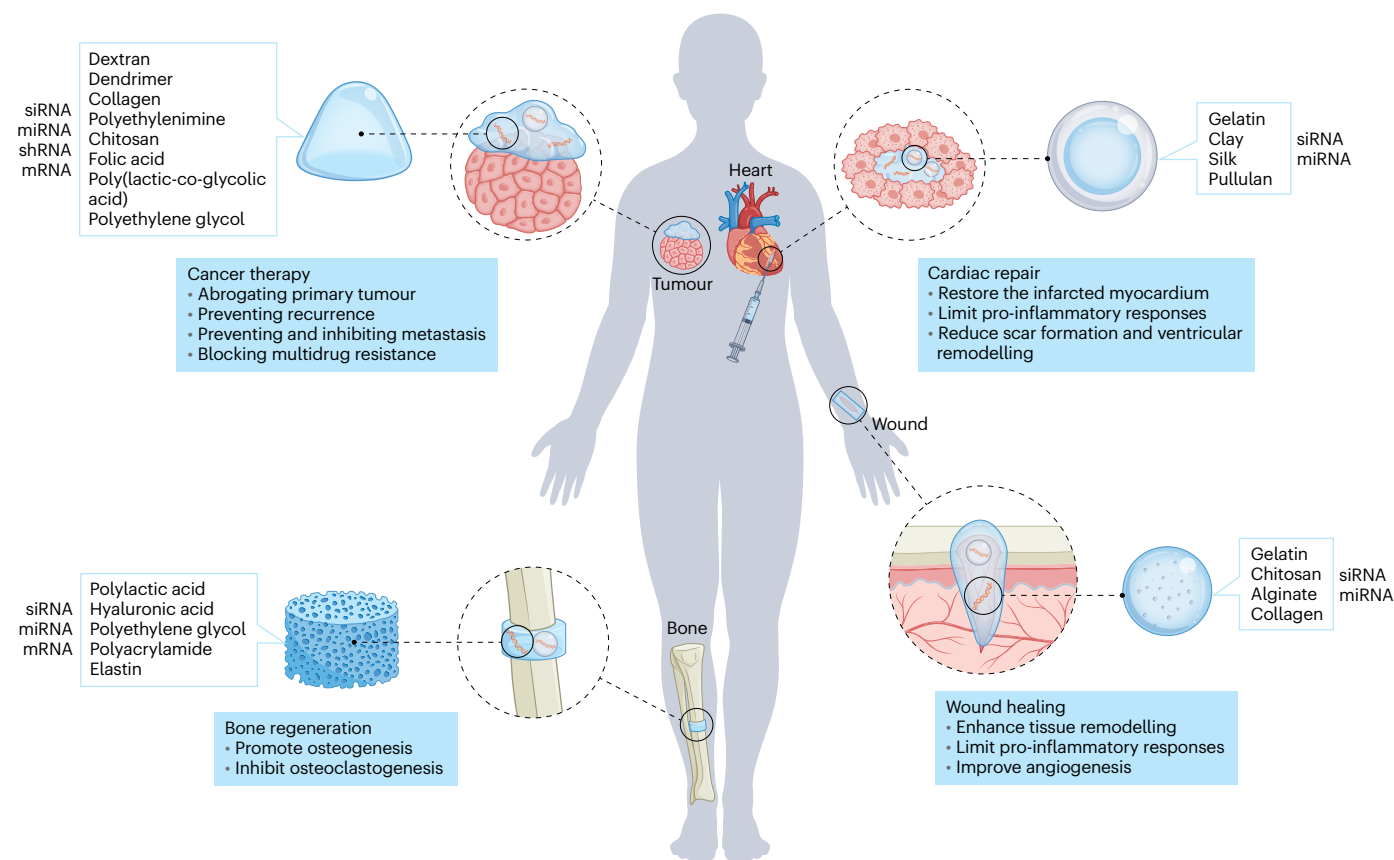
Fig. 6 | Biomedical applications of hydrogel-based RNA delivery. The conjunction of naked RNA or RNA nanocarriers with multifunctional hydrogels can find multiple biomedical applications, such as cancer therapy, wound healing, bone regenearation and cardiac repair.

Cardiac repair

MI results from coronary artery occlusion, producing local ischemia, tissue damage, and ultimately, heart failure. There are interesting approaches that have been applied to enhance ECM homeostasis and angiogenesis or to prevent fibrosis and calcium imbalance, namely the delivery of siRNA, miRNA and short hairpin RNA (shRNA)[85]. Remarkably, the use of self-healing hydrogels that are injected into the infarct site using minimally invasive approaches (for example, catheters) and are able to reform after this shear cessation has been a promising option to the challenge of delivery and retention of RNAi therapeutics[86]. To this end, a variety of chemistries, including ionic bonds[87] and dynamic covalent bonds (hydrazone linkages[25] and guest–host interactions[40]), have been implemented to yield injectable self-healing hydrogels for RNAi delivery. Among them, guest–host hydrogels (involving CD molecules) can facilitate a slower release of cholesterol-modified RNAi via hydrophobic interactions. Stimuli-responsive linkages (for example, protease-sensitive[78] and pH-responsive[76]) were also utilized as other means to achieve on-demand release from injectable self-healing hydrogels. Particularly, these two stimuli were chosen because MI is associated with changes in the tissue microenvironment, including a reduction in pH (from 7.4 to 6.8) and local upregulation of proteolytic activity. This emphasizes the importance of considering disease pathology when designing hydrogels for RNA delivery. In general, a significant advantage of RNAi delivery using injectable self-healing hydrogels is that single-dose administration of such systems can significantly and continuously restore the infarcted myocardium and enhance cardiac function over an extended period (from one to three months). Given the role of the immune response in MI disease progression and repair, hydrogels can play an essential role in delivering RNA therapeutics

for manipulation of macrophages and regulatory T cells, limiting pro-inflammatory responses and increasing regenerative cytokines in the infarct region.

Wound healing

Wound healing is a well orchestrated and regulated process that can be divided into three overlapping phases: haemostasis and inflammation, proliferation, and tissue remodeling[88]. However, this process becomes severely dysregulated by pathophysiological conditions. RNAi therapeutics have already shown potential in addressing all three phases and therefore facilitating functional tissue regeneration. Hydrogels can play a central role in both the local delivery of RNAi therapeutics and providing an artificial matrix to aid in the healing process. For instance, thermo-responsive hydrogels (for example, pluronic F-127, methylcellulose and agarose) have commonly been used to deliver miRNA or siRNA to accelerate wound healing[89]. One disadvantage of such hydrogels may be the inability to control the release rate of the encapsulated RNAi therapeutics.

To address this issue, both physically and chemically crosslinked hydrogels have been employed, and the degree of crosslinking was used to control the release rate of RNAi therapeutics. A common example of physically crosslinked hydrogels is the layer-by-layer assembly of two oppositely charged biopolymers[90]. Increasing the number of layers led to a slow release of RNAi therapeutics from these hydrogels. Given their physical nature, these gels are capable of releasing RNAi over a span of 2 weeks. For chemically crosslinked hydrogels, other variables can also affect the release rate of RNAi therapeutics. This generally depends on the entities involved in forming the chemical crosslinks within the hydrogel. For instance, the crosslinking could be a result of Schiff base bonds between aldehyde and amine groups

on polymer matrixes[91]. These hydrogels often degrade slowly over a period of a month, and hence they yield a longer release profile for RNAi therapeutics. Conversely, hydrogels formed as a result of interactions between the polymer matrix and the RNAi nano-vector are degraded more quickly (maximum, seven days), yielding a shorter release profile[92,93]. In these cases, there is a direct correlation between the number of active functional groups on the nano-vector and the corresponding hydrogel crosslinking density. As a result, the release rate of encapsulated RNAi can be simply tuned by adjusting the concentration of nanocarrier in the hydrogel. Notably, nanocarriers with active surface functionalities can be incorporated into chemically crosslinked hydrogel networks as a way to provide more control over the release rate of RNAi therapeutics[28]. The miRNA-laden hydrogels drive wound healing by triggering resolution of the inflammatory phase. The results showed elevated macrophage infiltration and effective local polarization of macrophages toward the M2 phenotype in vivo. Hydrogels, with their remarkably high water uptake, support cell attachment and growth, leading to better wound healing. These hydrogel examples yield a spectrum of different temporal release profiles for RNAi therapeutics, which is particularly beneficial when considering that wound healing consists of a carefully orchestrated sequence of biological events.

Notably, the risk of infection or localized trauma is commonly associated with the use of conventional methods for wound closure (for example, sutures). Therefore, the use of hydrogel-based adhesives, which strongly attach to the wound, can be used as a physical barrier to protect the wound from the above-mentioned risks that will strongly affect re-epithelialization and healing rate[94].

Other applications

RNA molecules have received a great deal of attention for immunomodulation, mainly due to RNA silencing of crucial factors in immune cells[95]. Adhesive hydrogels have been used as immunomodulatory dressings. For the combined delivery of dendritic cell (DC) chemo-attractants (MIP3a) and pDNA-siRNA-loaded microparticles to antigen-presenting cells, an in situ crosslinkable and fast-degrading hydrogel was developed[96]. DCs were able to infiltrate the hydrogels and efficiently phagocytose the microparticles carrying pDNA-siRNA. The gels attracted four- to sixfold more DCs compared with an equivalent bolus dose. A more recent study showed that, upon the in vivo delivery of mRNA–lipoplexes loaded into chitosan–alginate hydrogel, T-cell proliferation and interferon-γ secretion were both increased[60]. At week 1, a humoral response was observed for lipoplex-loaded hydrogels, while protein-based vaccines did not elicit IgG production until 2 weeks post-injection, which potentiated their applications as a viable immunization method against multiple diseases.

The process of angiogenesis is closely tied to wound healing and tissue regeneration. Stimulating both the formation and maturation of blood vessels is therefore of great interest, especially in the treatment of some chronic skin wounds. Localized silencing of ubiquitously expressed genes (for example, *mapk-1*) in an open wound bed was demonstrated using an agarose hydrogel loaded with liposomal siRNA[97]. Delivery of siRNA NPs via hydrogels incorporating polyurethane (PUR) and its derivatives, polyester urethane (PEUR) or poly(thioketal urethane) (PTK-UR), was also explored for angiogenesis[98]. Modulating the release rate caused changes in the in vivo silencing profile. Silencing prolyl hydroxylase domain protein 2 (PHD2) resulted in the expression of vascular endothelial growth factor and fibroblast growth factor, while vascular volume and thickness within the hydrogels also increased. The use of these hydrogels for local PHD2 siRNA delivery showed excellent promise for promoting angiogenesis for wound healing.

Some other reported applications, such as spinal cord injury, fibrosis and inflammatory diseases, are summarized in Table 2.

Summary and future directions

Macroscale delivery systems that can be locally implanted on the disease tissue while avoiding the complications associated to the systemic delivery of RNA therapeutics, have captured the attention of researchers in the field in the past decades. Particularly, hydrogels can be used to efficiently deliver both small and macro molecules, like chemotherapeutics, proteins and genetic materials (such as RNA), along with nanoparticle-based therapies. Hydrogels as a three-dimensional matrix framework have gained attention in therapeutics due to their biocompatibility, biodegradability, drug loading ability and controlled drug release. Compared with systemic administration, hydrogel systems have many advantages, such as locally controlled RNA delivery, low blood RNA concentration, high permeability, few toxic side effects, avoidance of first-pass hepatic metabolism, and minimal pain and discomfort[70,75]. The combination of a local platform to treat and re-educate the disease tissue along with systemic administration to treat an existing distant disease niche would impart highly efficacious translational therapeutic platforms with improved clinical outcomes[81]. To take this to the next level, macroscale hydrogels have recently been developed with nano/micro hydrogel building blocks[99]. Hydrolytic degradation of macroscale hydrogels then allowed for the gradual release of RNA-loaded nano/micro hydrogels without leaving any residual biomaterial at the disease site after treatment. The balance between hydrogel-RNA design complexity, manufacturing costs, regulatory policies and the effective release into the target tissue needs to be evaluated in detail so these strategies can be commonly applied in clinical procedures.

Disease location and targeted tissue will dictate the necessary physical attributes of the hydrogel while disease type will determine the suitable spatiotemporal RNA release profile. Injectable hydrogels with self-healing properties are extremely useful for heart delivery, while topical hydrogels with adhesive properties are preferred for RNA delivery to the skin. For instance, self-healing hydrogels can withstand the shear forces during injection as well as the dynamic forces generated by the beating muscles after myocardium injection. Further parameters must be taken into consideration when hydrogels are also intended to serve as a scaffolding matrix to promote tissue regeneration. This includes parameters such as mechanical robustness and degradation rate of the hydrogels, which can inevitably affect the release rate of encapsulated RNA therapeutics. For example, RNA delivery to injured bone tissue must be commensurate with the healing timeline (approximately 3–4 weeks)[33]. Hence, depending on the type of RNA and its association with a specific healing phase, the delivery timeline could vary from several days to several months[100]. Additionally, disease-associated changes to a tissue microenvironment, such as altered pH or upregulation of certain enzymes, can be implemented in the hydrogel design to trigger RNA release.

As mentioned above, biomedical applications of hydrogel-mediated RNA delivery can range from tissue regeneration to cancer therapy. However, one unexplored area is the employment of hydrogel scaffolds to support RNA–cell interactions. Here, hydrogels function as a staging area for gene regulation and engineering as cells migrate into the hydrogels for interaction with RNAs. This concept has been utilized in incorporating previously gene-modified human mesenchymal stromal cells into a cryogel scaffold[101]. These genetically modified cells can release certain antibodies capable of triggering T-cell-mediated anti-tumour responses. Ultimately, hydrogel scaffolds for RNA-cell interactions could have a combinatorial effect by simultaneously editing certain genes in the cells and supporting their proliferation and survival, ensuring the constant release of effective levels of antibodies.

Another promising application of hydrogels in this field is the delivery of RNA nano-vaccines. The coronavirus disease 2019 (COVID-19) pandemic is still raging all over the world, and vaccination is the best defence. After unremitting efforts, two mRNA vaccines based on lipid NPs (BNT162b2 by Pfizer/BioNTech and mRNA-1273 by Moderna)

are now in clinical use. Injectable hydrogels were recently used for local delivery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymeric nano-vaccines (containing the SARS-CoV-2 virus spike protein with/without adjuvant) in animal models[102]. Interestingly, the results show that sustained delivery of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein nano-vaccine in an injectable hydrogel depot formulation achieved higher total anti-RBD IgG titres compared to bolus vaccine controls. A similar concept can be applied to current SARS-CoV-2 mRNA vaccines by incorporating them into hydrogels. Currently, these mRNA nano-vaccines require two doses separated by 3-4 weeks. The use of hydrogel capsules with pulsatile release could release the vaccines in pulses over weeks 3–4, which if injected along with free nano-vaccines, might provide one-shot vaccination[103]. However, it should also be noted that little has been done to examine the in vivo stability of mRNA in hydrogels at physiological conditions for such a long period of time, which will be an important area for future studies. Indeed, for larger RNAs such as mRNA, suboptimal stability elicits only short-term transient protein expression and requires delivery vehicles like NPs to protect them from enzymatic degradation and improve their transfection efficiency. The use of hydrogels for naked mRNA delivery has rarely been reported. Thus, for future efforts to modify large RNAs to improve stability and transfection, the application of hydrogels for naked RNA delivery will be more fruitful. Another issue with mRNA nano-vaccines is that they must be stored and shipped at low temperatures. Given the current scant evidence, identifying hydrogels suitable for long-term mRNA storage at 4 °C or even room temperature will be important. With further research, hydrogel-mediated mRNA vaccine delivery may become a viable alternative to traditional nucleic acid immunization methods.

In this Review, we have discussed the use of hydrogels as RNA delivery systems from design to biomedical applications. The research discussed herein demonstrates that hydrogel systems are capable not only of sustained local delivery of RNA (which avoids repeated administration) but also of spatial and temporal control over release rate. Further investigation in vivo of the characteristics of RNA-loaded hydrogels, such as degradability, clearance, controlled release and foreign body response, is urgently needed. It is expected that continuous improvements in hydrogel design and fabrication will bring these exciting materials ever closer to clinical applications of RNA therapy.

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Competing interests

R.L. declares the following financial interests: Alnylam Pharmaceuticals, Inc. and Moderna, Inc. For a list of entities with which R.L. is involved, compensated or uncompensated, see the Supplementary Note. J.C. is a co-founder and shareholder of TargTex S.A. - Targeted Therapeutics for Glioblastoma Multiforme. The other authors declare no competing interests.

Additional information

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