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Hydrogel-mediated drug delivery for treating stroke

Wenyan Yu[a,b,c], Enpeng Gong[a], Bingbing Liu[a], Lei Zhou[a], Chengyuan Che[a], Shu Hu[a], Zhenzhong Zhang[a,b,c,*], Junjie Liu[a,b,c,*], Jinjin Shi[a,b,c,*]

[a] *School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China*
[b] *Key Laboratory of Targeting Therapy and Diagnosis for Critical Diseases, Zhengzhou 450001, China*
[c] *Collaborative Innovation Center of New Drug Research and Safety Evaluation, Zhengzhou 450001, China*

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ABSTRACT

Stroke is a common disease and is the major cause of death and disability. It occurs and generates devastating neurological deficits when cerebral blood vessel is blocked (ischemic stroke, IS) or ruptured (hemorrhagic stroke, HS). Hydrogel, being biodegradable and biocompatible, have shown attractive advantages in stroke therapy as a new biomaterial with desirable mechanical properties and tunability of structure, owing to special ability to load different cargoes for multiple treatment strategies, such as pharmacotherapy based on drug-delivery systems and cell therapy including mesenchymal stem cells (MSCs) and neural progenitor cells (NPCs) for improving functional outcomes. However, a comprehensive review of the functional hydrogel for treatment of stroke is still lacking. Therefore, in this work, the main pathological mechanisms of stroke including IS and HS are comprehensively described. The benefits of hydrogel for stroke treatment are also summarized regarding the natural advantages and the delivery advantages. Simultaneously, the application development of hydrogel for treatment of stroke is highlighted. Finally, the unique considerations and challenges in the design and application of hydrogel is discussed for treatment of stroke and clinical application in the future.

1. Introduction

Stroke, as a brain attack, remains the second leading cause of death and third leading cause of disability worldwide. Moreover, based on the current data, stroke carries a worse prognosis and most survivors suffer from long-term and severe neurological impairment [1]. According to differences in pathogenesis, stroke is broadly classified into two categories: ischemic stroke (IS) and hemorrhagic stroke (HS). Etiology of IS is driven towards blockage of blood vessels as the preliminary cause whereas HS involves rupture of blood vessels and leakage of blood [2]. However, the damage mechanism caused by the hindrance in blood flow displays many common features. For example, after stroke, the increase of intracellular calcium triggers mitochondrial dysfunction [3] and the production of reactive oxygen species (ROS) [4], phospholipases [5] and proteases [6], consequently resulting in neuronal apoptosis. In addition, microglia constitute 5%–10% of the total cellular population within the normal brain [7]; they act as the first and main form of active immune defense intrinsic to the central nervous system. The central nervous system-resident microglia are activated after stroke. In response to pathology signals, they can change morphologically and functionally, and migrate towards these signals. Activated microglia increases production of numerous pro-inflammatory factors and potentially neurotoxic mediators which also result in neuronal injury [8]. Moreover, inflammatory cells in the blood after stroke are also activated by pro-inflammatory factors released from the brain and then recruited to the brain *via* recruiting factors. Subsequently, the activated inflammatory cells release a variety of cytokines, chemokines, free radicals and other potentially toxic chemicals in brain, which can further damage the brain tissue. Meanwhile, some differences also need to be noted in IS and HS, such as the brain damage caused by hemoglobin (Hb) and iron in hematoma of HS [9]. These pathological mechanisms including common and unique features will cause severe damage to brain tissue at different stages.

Hydrogel is 3D crosslinked polymer matrices, having a colossal tendency to imbibe water, and exhibit swelling under physiological conditions without deformation in their hydrophilic network (Fig. 1) [10–22]. Hydrogel can perform multi-functions by encapsulating different drugs, providing a promising therapeutic strategy in the complex pathological environment of stroke [23]. After stroke, it generally takes a long time to treat and recover. Owing

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* Corresponding authors.
E-mail addresses: zzuzhangzhenzhong@163.com (Z. Zhang), liujunjie@zzu.edu.cn (J. Liu), shijinyxy@zzu.edu.cn (J. Shi).

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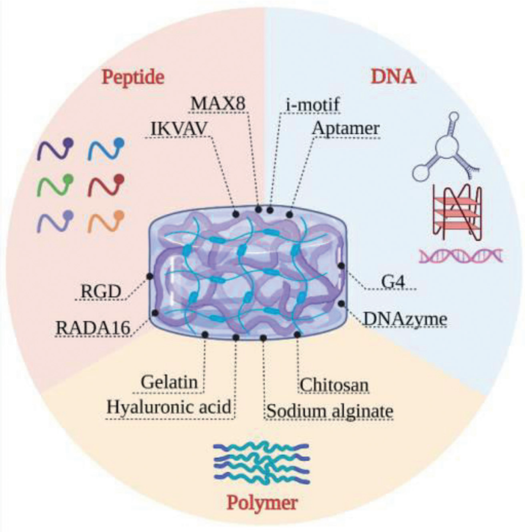

Fig. 1. Schematic illustration of key component units in hydrogel. Hydrogel could be divided into three classes based on the different components. (1) Peptide: peptide hydrogel is a kind of hydrogel cross-linked with peptide such as IKVAV (laminin peptide for neuronal growth-stimulating of stroke) [11], RGD (short amino acid sequences for cell adhesion of peripheral artery) [12], RADA16 (ionic self-complementary peptide for reparative and regenerative tissue treatment of ischemic heart) [13] and MAX8 (shear-thinning peptide for encapsulating growth factors of stroke) [14]. (2) DNA: DNA hydrogels are composed of the functional DNA motif with the unique properties such as i-motif (a special cytosine-rich tetraplex structure for tumor microenvironment of pH response) [15], G4 (four-stranded structures for biological colorimetric analysis) [16], DNA enzyme (a biocatalyst for enzymatic reaction) [17] and aptamer (single-stranded oligonucleotides for binding toward the specific targets of tumor) [18]. (3) polymer: various polymers could be harnessed for constructing the skeleton of hydrogel such as hyaluronic acid (an immunoneutral polysaccharide for cartilage repair and regeneration) [19], gelatin (a collagen hydrolysate for anti-inflammation of stroke) [20], chitosan (a natural polysaccharides with positive charge for adjuvant hemostat) [21] and sodium alginate (a naturally linear anionic polysaccharide for chronic wound healing) [22].

to the tunable physical properties, controllable degradability and capability to protect labile drugs from degradation, hydrogel serve as a platform on which various physiochemical interactions occur with the encapsulated drugs to control drug release [24]. Moreover, the metabolic rate of hydrogel can be adjusted *in vivo* with the different composition materials to obtain different retention times for meeting the treatment of different stages of stroke [25], which significantly increase the compliance of patients and provide a potential therapeutic approach for the long-term treatment of stroke. In addition, the emergence of hydrogel offers an unprecedented opportunity for cell therapy [26]. Hydrogel is very beneficial to the survival of cells to make up the limitation of cell therapy since it can mimic the three-dimensional extracellular matrix [27]. Designer-injectable gels to deliver the cell and neurogenic biomolecules have neurodegenerative capabilities [28]. Therefore, the cell therapy with hydrogel scaffolds has potential therapeutic significance for the structural remodeling and functional recovery following stroke. In this review, the main pathological mechanisms of stroke including the commonalities and differences in IS and HS are comprehensively described. The advantages of hydrogel for stroke treatment are also summarized. Simultaneously, the application progress of hydrogel for treatment of stroke is highlighted. Finally, the unique considerations and challenges in the design of hydrogel is discussed for treatment of stroke and clinical application in the future.

2. Pathological mechanisms of stroke

2.1. Common mechanisms of IS and HS

Owing to its intricate pathophysiology, stroke is a serious medical condition caused by disruption or obstruction of blood vessel in the brain tissues. Despite differences in outcomes and clinical impact, two conditions share some common pathological features (Fig. 2).

2.1.1. Neuronal apoptosis

In steady states, Na^+/K^+-ATPase keeps a normal physiological membrane potential and ionic distribution. After stroke, missing energy stops Na^+/K^+-ATPase activity, ultimately leading to depolarization and change of ion concentration [29] Glutamate is the main excitatory medium in brain, which acts as a neurotoxic excitatory neurotransmitter and can produce excitotoxicity in stroke [30]. Studies have shown that the activation of the *N*-methyl-D-aspartate (NMDA) and α-amino-3–hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) of glutamate receptors can result

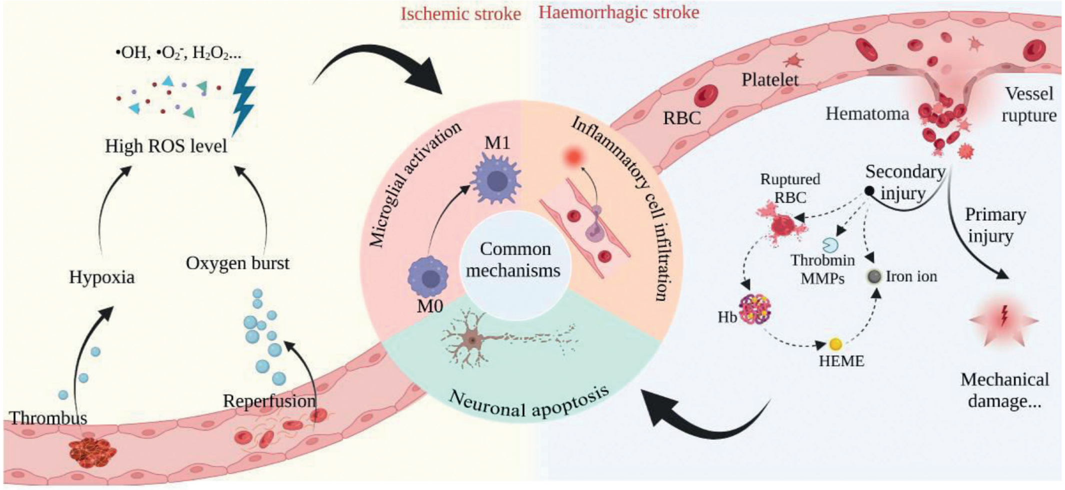

Fig. 2. Schematic representation of pathological mechanism of stroke, including the common mechanisms and the unique features between ischemic stroke and hemorrhagic stroke. The unique features of ischemic stroke are production of a large number of ROS mediated by hypoxia before thrombolysis and oxygen burst after reperfusion. The unique features of hemorrhagic stroke are: (1) Ruptured blood vessel results in leakage of blood into the brain parenchyma. (2) The blood in the brain parenchyma can immediately form hematoma, causing the primary injury, including mechanical damage, brain edema, elevated intracranial pressure and so on. And secondary injury mediated by blood components of the hematoma includes ruptured RBC (red blood cell), Hb (hemoglobin), HEME, iron ion, thrombin, MMPs (matrix metalloproteinase) and so on. The common mechanisms include the neuronal apoptosis, microglial activation and inflammatory cell infiltration.

in membrane depolarization and entry of Ca^{2+}, ultimately generating cell injury [31]. The experiment has proved that the inhibitor of AMPA and NMDA receptors prevented Ca^{2+} entry, producing neuroprotective effect in model of stroke [32]. With the dramatic influx of Ca^{2+}, mitochondria will affect neuronal apoptosis *via* releasing the proapoptotic factors such as cytochrome c, second mitochondria-derived activator of caspases, into the cytoplasm [32]. The proapoptotic factors activate the caspase-dependent mitochondrial pathway [33]. Cytochrome c binds to apoptotic peptidase activating factor 1 (Apaf-1) [34], which enables Apaf-1 to assemble into the oligomeric apoptosis complex [35]. The apoptosis complex can collect the precursor of caspase-9 and activate effector caspases such as caspase-3, which could cleave various cellular proteins, finally leading to cell death.

In addition, studies exhibited that there was inactivation of antioxidant enzymes and consumption of antioxidants after stroke, which would lead to an overproduction of ROS, including superoxide anion ($O_2{\cdot}^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), which can cause the injury to neurons and play a great role in signal transduction and metabolism [36]. The targets of signaling may include death membrane receptors [37] triggered by Fas-receptor and tumor necrosis factor (TNF) receptor.

2.1.2. Microglial activation and polarization

Microglia, as a macrophage in the central nervous system, are part of the monocyte phagocyte system. Specially, microglia are the guardian of the brain to respond to various acute brain injuries including HS and IS [38]. With the homeostasis change of the brain, microglia are activated by the various factors [39]. The phenotype of microglia changes from the static M0 phenotype to M1 (pro-inflammatory phenotype) or M2 (anti-inflammatory phenotype) [8]. Microglia with different phenotypes produce different immunomodulatory molecules such as cytokines and chemokines that are closely related to secondary damage and recovery of stroke [38]. Studies have proved phenotype of microglia appeared dynamic changes after stroke, in which microglial M1–like response showed within 6 h and microglial M2-like response presented on day 7 [40]. Classically activated M1 microglia are able to produce proinflammatory cytokines including interleukin-1β (IL-1β), IL-6, TNF and so on. The experiment showed that IL-6 knockout would decrease ischemic tissue damage [41]. The activated M2 microglia are present in an anti-inflammatory form, which generates anti-inflammatory factors (*e.g.*, IL-4, IL-10, transforming growth factor-β (TGF-β)) [8]. M2 microglia polarization can promote the repair of cells and tissues after stroke. For example, He *et al.* showed IL-4 administration reduced the markers of M1 and obviously improved markers of M2 [42]. However, the dynamic transformation between microglia M1 and M2 is not yet clear [40].

In addition, microglia also produce mutual effect with other cells [43]. For example, neurons can interact with microglia. Neurons debris released after stroke will be recognized by microglia, which promotes the migration of microglia and further promotes the polarization of microglia. Nerve cells may also produce a range of signals such as fractalkine (CX3CL1) [44], lipocalin-2 (LCN2) [45], fibroblast growth factor-2 (FGF-2), IL-34, which can also contribute to microglia-mediated phagocytosis and neuroprotective effects. For example, after IS, CX3CL1 is released in brain and involved in activation of microglial *via* CX3CR [46]. Moreover, other extracellular signals derived from microglia are of connection such as CCL20, CCL1, CCL2, CCL3. In a study, CCL2 that was released from primary astrocytes drives M1 microglial polarization [47]. This phenomenon of improvement in locomotor function and fewer M1 microglia were also found in $Ccr^{2-/-}$ mice with HS [48].

2.1.3. Inflammation

Following the disruption or eruption of blood flow, neural cells release damage-associated molecules that stimulate drastic inflammation in the focal region within minutes [49]. Inflammatory cell infiltration significantly contributes to inflammation. For example, monocytes are the largest leukocytes circulating in the bloodstream but can infiltrate into tissues in response to the received signals, where they differentiate into macrophages or dendritic cells. Macrophages become activated when triggered by a stimulus, which produces distinctive patterns of gene and protein expression [50,51]. During cerebral ischemia-reperfusion (I-R) injury, the infiltration of macrophages into the ischemic penumbra causes inflammatory damage [52] but also regulates tissue repair in the penumbra. In addition, neutrophil infiltration also develops within 2 days in rats. Additionally, the inflammatory process involves adhesion molecules [53], inflammatory cytokines [54], enzymes [55] and so on (Table S1 in Supporting information).

Adhesion molecules: The recruitment and infiltration of leukocytes in the brain is triggered *via* inflammation-induced adhesion molecules. These adhesion molecules, such as the intercellular cell adhesion molecule-1 (ICAM-1), P-selectin, E-selectin and integrins on endothelial cells and leukocytes are promoted expression by TNF, IL-1β and IL-6 and so on, which drives the adhesion and migration of leukocytes [56]. Above all, the activated leukocytes will aggregate in brain capillaries to further damage the brain tissue [57]. Manthe *et al.* suggested that the nanocarriers targeting to ICAM-1 could recede endothelial release of soluble ICAM-1, which would inhibit the brain inflammatory reaction [58].

Cytokines and chemokines: Cytokines and chemokines play a crucial role in the inflammatory response. In the case of stroke, several cytokines are upregulated in immune cells. Some pro-inflammatory factors, such as IL-1β and IL-6, cause great damage to the brain [59]. In addition, during in the inflammatory responses, there are also some anti-inflammatory factors, such as IL-10 and TGF-β, which may have neuroprotective effects. They derive from different cells including microglia, astrocytes, endothelial cells and neurons [60]. Chemokines are regulatory polypeptides that are involved in cell communication and leukocyte recruitment during inflammatory responses [61]. Upregulation of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1) messenger RNA (mRNA) has been revealed in the rat brain after stroke [62]. Chemokines are proved to boost tissue damage by recruiting inflammatory cells. In addition, nuclear factor kappa B (NF-κB) is a heteromeric transcription factor which maintains close relationship with inflammatory responses following stroke [63]. It is related to activation of several pro-inflammatory genes including TNF-α, interferon-γ (IFN-γ), IL-6 [64]. Collectively, chemokines are proteins with low molecular weight that can recruit inflammatory cells to migrate to the lesion site and play an important role in inflammation. Cytokines are proteins or small peptides that can transmit information between cells and have the function of immune regulation.

Enzymes: Researches suggested that many enzymes also participated in inflammatory responses such as cyclooxygenase-2 (COX-2) and matrix metalloproteinases (MMPs) [65]. COX-2 is an enzyme implicated in inflammation through the production of toxic proteinoids and superoxide. COX-2 in nerve cells after stroke will rise sharply. A study showed that in the rat model of middle cerebral artery occlusion (MCAO), the expression of COX-2 mRNA was gradually upregulated as early as 6 h following ischemia and reached a five-fold induction at 12 h [66]. Studies have also shown that COX-2 inhibitors could reduce the inflammatory response [67]. Ghazanfari *et al.* demonstrated that COX-2 inhibitor NS398 could cut down the expression of inflammation factors and glial cell activation in the brain. They also found COX-1 would be a potential target that mediated brain inflammation [68]. Toxic nitric oxide (NO) could also be release through nitric oxide synthase (iNOS) by infiltrated neutrophils, endothelial cells, *etc.* Overproduction of NO results in restraining the activity of adenosine triphosphatase and facilitating pro-inflammatory enzymes to produce toxicity [69].

There are also studies show that the MMPs are part of the neuro-inflammatory process and they are novel targets in stroke treatment [70]. After stroke, the release of NO, ROS and chemokines at the lesion site contributes to the activation of MMPs. Romanic *et al.* proved that MMP-9 was significantly upregulated within 12 h and reached maximum levels by 24 h after MCAO. Five days after MCAO, MMP-9 was also detected in macrophages at the infarct [71]. Wu *et al.* found that in 2 h and 5 days after HS, the mRNA of MMP-9 presented the same trend with MCAO model [72]. Elective inhibition of MMP-9 activity would notably enhance the recovery after stroke [73].

2.2. Unique pathological features of IS and HS

Despite the many common features in stroke, there are still some unique pathophysiological features between IS and HS.

Thromboembolism is the unique pathological characteristics of IS. Glycoprotein IIb (GPIIb) and IIIa (GPIIIa) that expressed at high density on the platelet membrane [74] engage in the formation of thrombus [75]. In normal platelets, GPIIb/IIIa does not bind to target compound. Once platelet activation, cellular signals will be produced and integrated at checkpoints including calcium and diacylglycerol (DAG)-regulated guanine nucleotide exchange factor I (CalDAG-GEFI) [76]. CalDAG-GEFI is a prime candidate to integrate signaling downstream of the rise in calcium and DAG levels after activation of multiple receptors. Study has proved that relative to control group, CalDAG-GEFI-knockout mice appeared severe bleeding characteristics [77]. In the end, the talin1, an integrin-binding cytoplasmic adaptor that is a central organizer of focal adhesions, will be activated and mediates integrins to form [78]. *In vitro* studies revealed that missing of talin1 gave rise to critical damage integrin αIIbβ3-mediated platelet collection [79]. Thrombogenesis will give rise to the permanent impaired of blood flow, which leads to rapidly evolving infarctions. Another characterization of IS is cerebral ischemia-reperfusion injury. Studies have proved that the early recovery of blood flow was beneficial and could decrease the infarct, whereas, the late reperfusion would cause the progressive damage [80]. What induces reperfusion injury may be relevant to upregulation of various redox enzymes such as iNOS, nicotinamide adenine nucleotide dinucleotide phosphate oxidase (NOX) [81]. The reactions between enzymes and substrates will aggravate cell death and a greater infarct. Studies have shown that after reperfusion, upregulated expression of iNOS *via* the NF-κB pathway leads to excessive amounts of NO [82]. Besides, in a study, the infarct area of nNOS$^{-/-}$ knockout (KO) mice were smaller than wild-type mice in the model of cerebral ischemia-reperfusion. Nitric oxide (NO) is also involved with the disruption of blood-brain barrier and formation of edema, which is closely related to changes of tight junction (TJ) proteins [83]. NO is one of the most important signal molecules, which is involved in physiological and pathological processes of stroke. On the one side, NO is a potent vasodilator that will increase the blood flow and regulate blood pressure, which is beneficial for restoration of neurological function. On the other side, high concentrations of NO will have an effect on nerve death by caspase-dependent apoptosis pathway and promotion of inflammation. In addition, after reperfusion, platelets also accumulate at the site of the responding tissue, and also release a large number of inflammatory factors, resulting in re-damage to the tissue. Inflammation caused by reperfusion can cause activation of the complement system and also worsen the damage [84].

And for HS, erythrocyte not only aggregate into hematoma, but also degrade into other harmful substances that can cause damage to brain cells and tissues. Study has shown that erythrocyte lysis can happen within 24 h after HS [85]. And a few days after the injection of erythrocyte into the brain, severe edema and neurological dysfunction were caused in the brain, which suggested that there was potential toxicity [86]. An experiment has shown that the perfusion of lysed erythrocyte into the basal ganglia of rats also induced the destruction of the blood-brain barrier and cell damage [87]. In addition, *in vivo* and *in vitro*, neurotoxicity is observed after intracerebral injection of Hb [88]. Hb is also one of the important mediators that promote the occurrence of inflammation. The pro-inflammatory response of Hb is mediated through a variety of pathways. Methemoglobin and heme are ligands of Toll-like receptor-4 (TLR4), which is expressed by microglia and macrophages. Activation of TLR4 causes the secretion of TNF, triggering nuclear NF-κB activation and inflammation [89]. Heme deriving from Hb degradation in the brain will be degraded into iron, carbon monoxide and bilirubin by heme oxygenase [90]. Injecting these degraded substances into brain can cause damage to brain tissue. Experiments have proved that heme-oxygenase inhibition reduces HS-induced brain injury. Heme will have a very serious toxicity to the nerves. The main reason is attributed to the production of iron by heme metabolism. Iron will also produce Fenton reaction caused by oxidative stress. Thus, timely removal of a large amount of iron can reduce secondary damage after HS. Experiments have shown that deferoxamine can reduce the amplification of hematomas in HS and reduce cerebral edema [91]. Above all, these indicate that both Hb and its degradation products can cause serious damage to the brain.

3. The advantages of hydrogel in stroke

3.1. The natural advantages of hydrogel in stroke

Hydrogel is three-dimensional crosslinking networks of water-soluble polymers, which can be made from almost any water-soluble polymer, including a wide range of chemical compositions [92]. Hydroxyl, primary amine and other chemical residues constitute the crosslinking point of hydrogel by forming physical or chemical bonds [93].

First of all, hydrogels are ideal biomaterials for nerve repair. They are able to be designed to imitate the native extracellular matrix (ECM). Importantly they provide structural support for repair processes [94]. There are all kinds of materials to compose the skeletal networks of hydrogel (*e.g.*, collagen, hyaluronic acid (HA), gelatin) [95]. They can also be functionalized with cell adhesion motifs requiring for the attachment of transplanted cells or recruitment of endogenous brain cells [96]. Additionally, basing on the same property with microenvironmental, the hydrogel that made up with ECM, barely lead up to immune responses [97]. Wu *et al.* designed ECM hydrogel which could reduce lesion volume, improve neuro-behavioral function and ameliorate pro-inflammatory responses [98]. Hydrogel also possesses the characterization of biocompatibility that it gradually degrades over time within implant site and cannot cause tissue scarring and formation of glial scar.

Biomaterials based on organism have been receiving much attention to construct hydrogel scaffolds. For example, the hydrogel made of hyaluronic acid, fibrin has been used to enhance the treatment of stroke. These biomaterials are biocompatible and mechanically stable with nontoxic degradation products, which can reduce infiltration of activated macrophages and microglia in the site of injury. What is more, these biomaterials possess suitable elasticity, which will significantly regulate the fate of encapsulated cells and the distribution of hydrogel at the injury site. In general, these biomaterials based on organism are suitable for the therapy of stroke.

Secondly, the mechanical properties of human tissues are different relying on their formation and character. The brain is a susceptible part. Brain tissue has a relatively consistent elastic modulus (several hundreds of Pa to several tens of kPa) [99]. Thus, time-independent mechanical properties such as stiffness and viscoelasticity are regarded as vital terms of brain cell behaviors. Based on the particularity of hydrogel skeleton, hydrogel is allowed to adjust its own mechanical properties by changing the crosslinking point or gradual degradation to integrate into brain tissue, which cannot induce rejection reaction. What is more, the suitable mechanical properties will be beneficial for the encapsulated cells to differentiate [100]. In general, the mechanical properties of hydrogel are regulable, which is meaningful not only in adjusting the destiny of encapsulated cells and integrating the host tissue but also in the distribution of hydrogel at the site of injury.

For minimally invasive administration, these biomaterials are injectable so as to fill various shaped pathological cavities including HS. Engineered hydrogels are of permeability that can release encapsulated therapeutics in a controlled manner, which prolongs drug presence time around the target tissue, reduces the dose needed and avoids systemic side effects [101].

3.2. The delivery advantages of hydrogel in stroke

Hydrogels are biomaterials that are intensively studied in drug delivery applications. Polysaccharide-based hydrogel is paid attention because of their non-toxicity, biocompatibility, degradability and sustained release. At present, the therapy of stroke can be treated with small molecule drugs, proteins, stem cells and so on. Hydrogel also have unique advantages in delivering these goods.

3.2.1. Enhancing the efficacy and reducing toxicity of drugs

The purpose of designing drug carriers is to greatly improve the treatment efficacy of drugs. First of all, hydrogel can be designed to encapsulate small molecule drugs *via* covalent or non-covalent interactions, which refrains from accumulation of drugs in non-targeted organs [102]. Meanwhile, in the design of hydrogel, the pore size of hydrogel would be crucial to adjust the release of small molecule drugs [103]. The interaction between hydrogel and drug, consisting of electrostatic, hydrophobic, hydrogen-bond, van der Waals, or other specific and nonspecific interactions, can also make a significant impact on release kinetics. These effects can avoid producing the toxicity by reason of a burst release of drugs. Yang showed that a pH-response hydrogel could decrease the toxicity of chemotherapy medication including doxorubicin and paclitaxel. This study also expounded how the hydrogel influences drugs release kinetics [104].

3.2.2. Achieving controlled release of drugs

An attractive therapy being proceed for stroke includes the delivery of growth factors such as FGF and brain-derived neurotrophic factor (BDNF) [105]. However, retention time of these factors in the brain is restricted [106]. Hydrogel has the advantages that it can control the sustainable release of cargo though gel degradation and diffusion [107]. Particularly, the hydrogel, made up with ECM, can regulate the diffusion of signaling through non-covalent interactions between those proteins and the ECM [108]. Lots of the biomaterials such as heparin and heparan sulfate that are natural constituents of the ECM, have been applied [109]. One novel study for a poly(2-hydroxyethyl methacrylate)-heparin functionalized hydrogel was developed by Nilasaroya. The study clearly demonstrated that hydrogel was capable of retaining FGF-2 and subsequently exhibited sustained penetration of the growth factor to mesenchymal stromal cells [110]. Besides, other constituents of the ECM that have the same function with heparin/heparan sulfate, have also been explored (*e.g.*, collagen, fibronectin, vitronectin). Loading growth factors in the hydrogel to achieve a sustainable release can contribute to promoting the directional neurite extension of the ganglion. Although the growth factors of high concentrations are allowed to cross the blood brain barrier (BBB), they may cause toxicity on account of their off-target distribution. Thus, controlled release of growth factors may need to be required at the site of injury. In conclusion, the variable mechanical properties of hydrogel can improve the release kinetics of growth factor to achieve specific delivery.

3.2.3. Improving transplantation efficiency for the stem cells

Neural stem cells (NSCs) and mesenchymal stem cells (MSCs) are common cell sources in neural tissue engineering [111]. However, ill-defined culture strategies for pluripotent stem cells (PSCs) expansion emerge huge challenge to the application of PSCs. Hydrogel-based technologies to guide cell expansion would be a newly-developing exploration to solve the issue [112]. Hydrogel biomaterials can maintain the function of stem cell *in vivo*, which is of great importance in the cell-based therapy [113]. Firstly, the hydrogel is porous enough to allow nutrients to reach the cells within it, while allowing waste to flow out as those cells continue to proliferate. Secondly, the hydrogel containing adhesion motifs can provide mechanical forces to promote stem cells migration and viability. Besides, it is also significant for the hydrogel to protect exogenous cells from immune rejection. These attributes endow hydrogel the benefits not only in the endogenous cells surrounding the implant site but also in the transplanted cells. Overall, stem cell-based therapy has been bringing a glimmer of hope for stroke treatment. Meanwhile, designing applicable hydrogel scaffolds is also crucial, which could promote stem cell therapy efficiency for stroke.

4. The applications of hydrogel for stroke

At present, hydrogel has been applied in the treatment of stroke because of its various advantages (Fig. 3).

4.1. Hydrogel for the drug delivery

Stroke is currently the leading cause of adult disability worldwide, of which pathological microenvironment is very complex. At present, various clinically used drugs based on different pathological mechanisms have been developed for the treatment of stroke (Table 1).

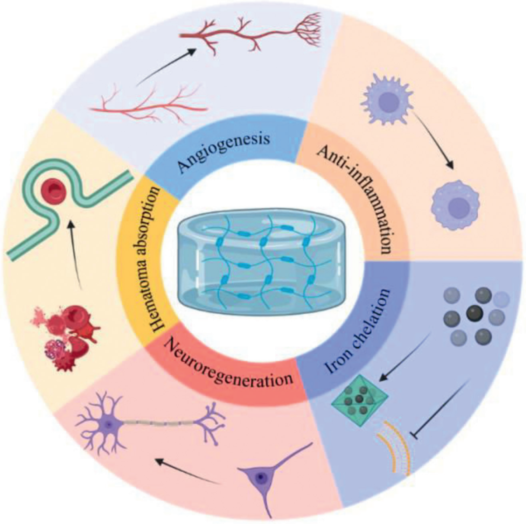

Fig. 3. The application development of hydrogel for treatment of stroke.

Table 1
The clinically used drug against stroke.

Drug	Disease	Mechanism
Alteplase	IS	Plasminogen activators
Cilostazol	IS	Platelet aggregation inhibitors; type 3 cyclic nucleotide phosphodiesterase inhibitors
Clopidogrel	IS	Platelet ADP receptor antagonists; platelet aggregation inhibitors; purinergic P2 receptor antagonists
Recombinant activated factor VIIa	HS	Activate thrombin
Tranexamic acid	HS	Restrain fibrinolysis
Aspirin	IS	Platelet aggregation inhibitors
Celecoxib	HS	Selective inhibitor of COX-2
Aspirin	IS	Cyclooxygenase inhibitors; nitric oxide stimulants; platelet aggregation inhibitors
Valsartan	HS	Antihypertensives

However, the inherent defects of free drugs, such as short half-life and poor bioavailability, will not only cause serious side effects, but also significantly reduce the therapeutic effect. The emergence of drug delivery systems marks an unprecedented opportunity to develop mainstream solutions to a variety of healthcare dilemmas. Numerous drug delivery systems have been developed to improve the efficacy and to reduce the side effects, since well-designed drug delivery systems possess many unique advantages, such as increasing the solubility of poorly soluble drugs, improving the stability and extend the half-lives of drugs *in vivo*. Moreover, targeted modified drug delivery systems could assist drugs to cross the BBB which is a main barrier for most drugs to reach the brain, and realize accumulation at the desired site to avoid non-specific distribution. Numerous drug delivery systems have been developed to validate site-specific targeting of lesions and to make dramatic improvements in stroke injury. In addition, cell therapy is also a potential therapeutic strategy of delivery system. More importantly, many delivery systems based on cell therapy have been investigated in clinical trials (NCT01678534, NCT01501773, NCT01845350) [114].

During the past few decades, hydrogels have been in use for biomedical applications such as drug delivery systems, *in situ* gels, and tissue scaffolds. Hydrogels have a unique three-dimensional cross-linked network of natural polymers, which have the ability to imbibe large amounts of water. As an intriguing material, hydrogels are smart, environmentally sensitive and compatible with biological systems, and can be made degradable and responsive to various stimuli. These hydrogels were found to be competent in the loading of non-steroidal anti-inflammatory drugs *viz*., ibuprofen, ketoprofen, and diclofenac. The use of naturally modified hydrogels has also given rise to a new area of research. Moreover, the drug delivery systems based on hydrogel have been an emerging treatment strategy. For example, liposome-based [115–118] and polymeric micelles (PMs)-based [119–122] delivery of biological drugs [123] has shown desirable therapeutic potential [124–126]. Moreover, a variety of liposome- and PMs-based drug delivery have been successfully used in clinical. Therefore, on this basis, the loading of liposomes and PMs with hydrogels has been gradually developed.

4.2. Hydrogel for the anti-inflammation

The inflammatory response is the result of a joint action after stroke, including glial cell activation, recruitment of peripheral immune cells and release of cytokines and chemokines, *etc.* [64]. In the initiation of inflammation, the microglia will be activated and polarized to the M1 phenotype. The current treatment strategy is to regulate microglia M2 polarization. M2 microglia phenotype conversion inhibits inflammation and promotes the repair of the brain. In addition, various evidences suggest that inflammatory factors including IL-1β, TNF-α and IL-6 play a key role in the pathological process [127]. With double action in inflammatory response, they have a harmful function in the early stages and a repair function in the later stages so that the appropriate treatment time is extremely vital. Inflammatory responses can exacerbate brain damage by inducing excessive nerve death, enhancing excitatory toxicity and oxidative stress, disrupting the integrity of BBB, promoting cerebral edema dilation and leading to subsequent brain damage.

One advantage of hydrogel is that they possess the excellently biocompatibility that barely induces immune response [24]. Moreover, the skeleton materials of hydrogel also have the anti-inflammatory properties [128]. Based on these characteristics, hydrogel provides new ideas for the treatment of stroke. Liu *et al.* designed engineered HA hydrogel for targeted dual drug delivery to realize combined treatment. In this hydrogel system, 6-bromoindirubin-3′oxime (BIO), a glycogen synthase kinase 3β inhibitor, was loaded in Pluronic F127 nanoparticles. Meanwhile, vascular endothelial growth factor (VEGF) was loaded in poly(lactic-*co*-glycolic acid) (PLGA) porous microspheres. BIO could be initially released to reduce the inflammatory response, while VEGF was enabled sustained release to induce angiogenesis (Fig. 4) [129].

In addition, there are plenty of studies that injectable hydrogel has been applied to promote nerve repair and functional recovery [130]. Hydrogel using specific biomaterials such as gelatin and silk fibroin [131,132] can bind specifically to receptors on the cell surface to promote polarization of the immune cells (Fig. S1 in Supporting information) [20]. Gorenkova *et al.* proved that self-assembling silk fibroin hydrogel did not present an overt microglial/macrophage response and it had interference with inflammatory response. The self-assembling silk fibroin hydrogel also offered a support matrix that is propitious to microenvironment in the lesion location of stroke [133].

Currently, various hydrogels are gradually explored to apply for anti-inflammation in stroke (Table S2 in Supporting information). Based on the exploration of anti-inflammatory drugs, many studies showed the excellent neuroprotective effect of multiple traditional Chinese medicines (TCMs) can against nod-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome activation. These TCMs may be in the different forms of TCM prescriptions (*e.g.*, Dong Chong Xia Cao, He Shou Wu, Deng-Zhan-Xi-Xin) [134]. Hydrogel as the vehicles for delivering the TCMs has great potential for stroke treatment. In addition, a study also suggested NSCs were explored to exhibit strong anti-inflammatory functions.

4.3. Hydrogel for the neuroregeneration

The treatment of stroke is very difficult [135]. Nerve destruction is the most direct damage that occurs in stroke. Therefore, promoting nerve regeneration may be a potential therapeutic strategy (Table S3 in Supporting information). In recent years, because of the continuous in-depth study of the versatility and plasticity of hydrogel, a new scaffold has been provided for cell transplantation, which can imitate the microenvironment of the ECM and promote wound healing and nerve regeneration [136]. In addition, nerve growth factors can hitch a ride to stimulate nerve regeneration by hydrogel. Jian *et al.* designed nanohybrid hydrogel making

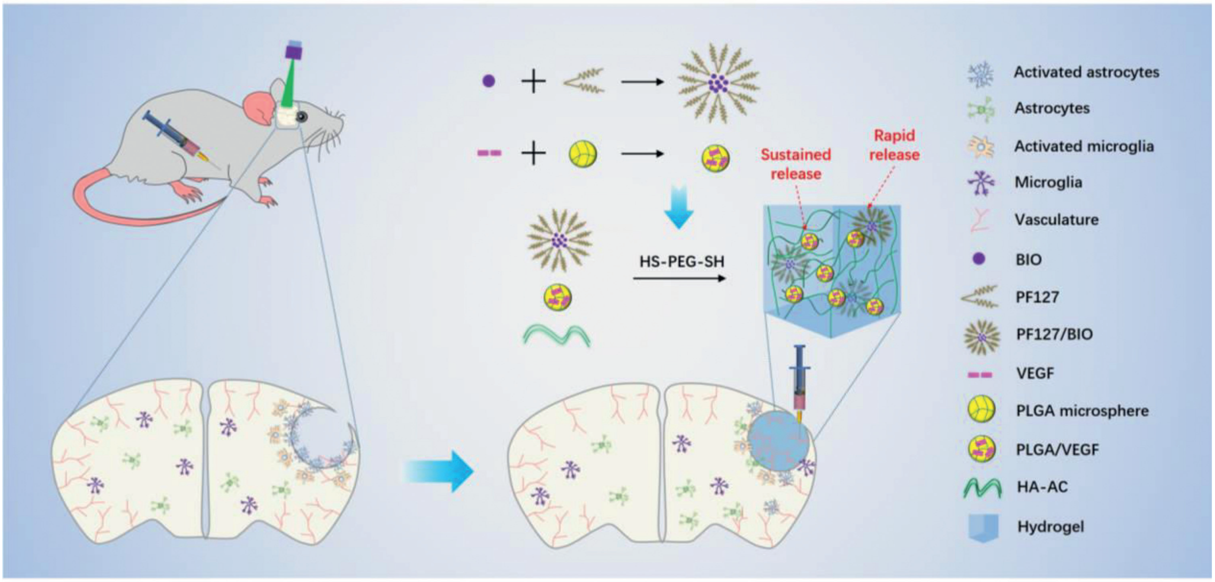

Fig. 4. Schematic illustration of engineered hyaluronic acid hydrogel for treatment of ischemic stroke. Copied with permission [129]. Copyright 2022, Elsevier.

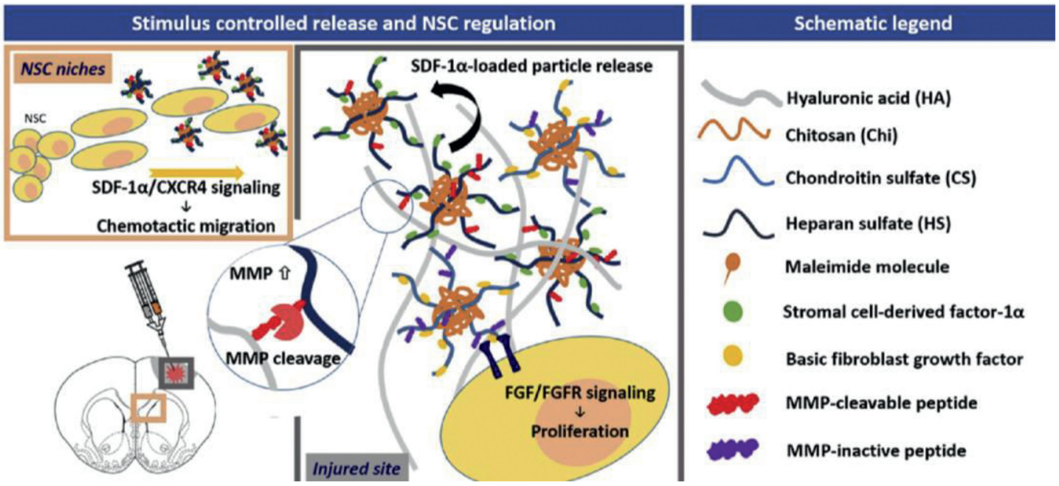

Fig. 5. The nanohybrid hydrogel with GAG-based PCN implants ischemic stroke model through *in situ* gelation. Reproduced with permission [137]. Copyright 2018 Elsevier.

up with sulfated glycosaminoglycan-based polyelectrolyte complex nanoparticles (PCN) that could deliver bioactive factors by electrostatic interaction. The hydrogel not only realized the controlled release of basic fibroblast growth factor (bFGF) and stromal-derived factor-1α (SDF-1α) but also recruited endogenous NSCs and regulate cellular fate. In IS model, the factors are beneficial to restoration of neurological by enhancing neurogenesis and angiogenesis (Fig. 5) [137]. In addition, the shape of hydrogel is plasticity so that hydrogel can accommodate the cavity. Wang *et al.* developed a carbon-nanotubes-doped sericin scaffold (CNTs-SS). They showed that CNTs-SS could be injected into the cavity and restored its preprocessed shape to fill the cavity well (Fig. S2 in Supporting information) [138]. Self-assembling hydrogels are also receiving increasing attention on account of the emergence of various biological materials, especially self-assembling peptides (SAPs) by the twisting and winding of these nanofibers or nanotubes. These eventually turn into a stable meshwork pattern similar to the fibrillar proteins of the ECM [139]. With strong hydration, the self-assembling hydrogel is biocompatible with amino acids as the basic crosslinking and they do not produce an immune response and can also be well integrated into the pathological regions that dovetail well with the host, creating a good microenvironment. Lindsey *et al.* indicates that the MAX8 hydrogel has bright prospect in delivery of NGF and BDNF for the treatment of stroke (Fig. S3 in Supporting information) [14]. Moreover, the SAPs hydrogel not only can load NGF but also encapsulate NSCs. The hydrogel functionalized with a bioactive ligand such as IKVAV (cell adhesion motif) possesses high affinity with cells that is good for cell adhesion [140]. Thus, the SAPs hydrogel offer a novel supporter for cell transplantation. Sun *et al.* reported a self-assembling-peptide nanofiber hydrogel for nerve regeneration, carrying IKVAV and RGD peptide, which self-assembled into a nanofiber hydrogel at suitable pH (Fig. S4 in Supporting information) [141].

Stem cell transplantation is one of the most promising methods for the treatment of stroke which has been proved that it can promote neurogenesis and functional recovery. Hydrogel as the carrier to deliver stem cells not only improve the transplantation rate but also make stem cells effectively touch microenvironment. However, there are still all kinds of obstructions from bench to clinic, which needs us to explore further.

4.4. Hydrogel for revascularization

The neoangiogenesis includes angiogenesis, arteriogenesis, and vasculogenesis [142]. Studies have shown that the production of

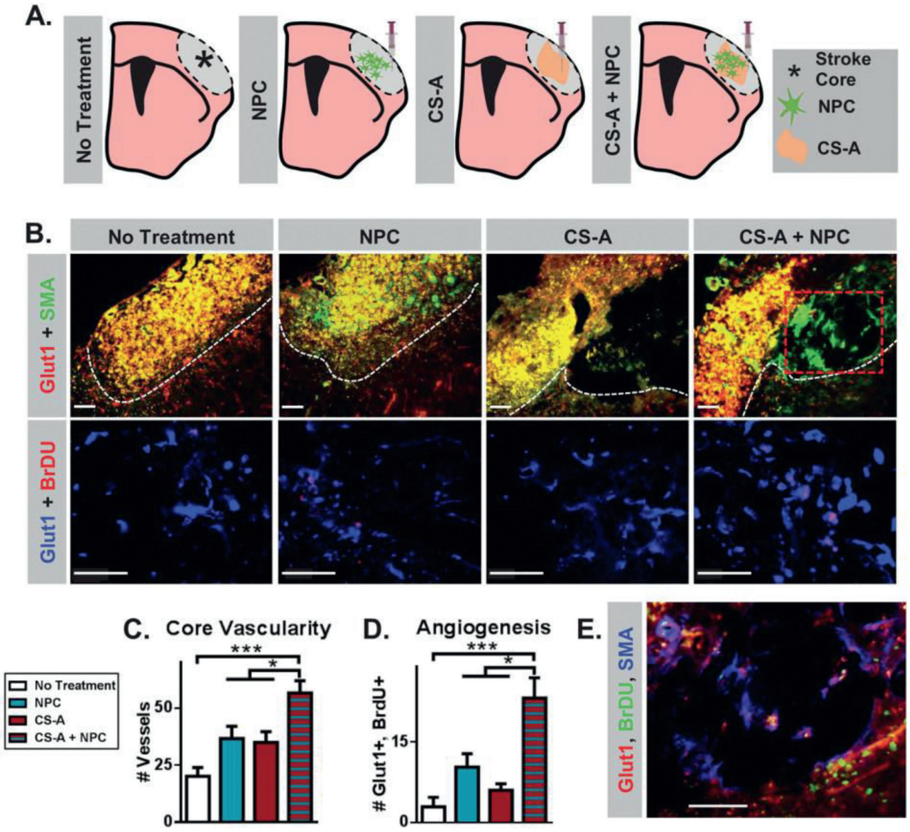

Fig. 6. Schematic illustration of reparative and regenerative effects of treatment groups in stroke. Copied with permission [147]. Copyright 2020, John Wiley and Sons.

neovascularization can promote the regeneration of nerve cells and reduce secondary damage in IS [143]. Neovascularization can provide enough blood for the diseased area to provide enough energy for the cells, so proper reconstruction of blood vessels is a key way to promote nerve recovery [142]. At present, the strategies for blood vessels repair are delivery of VEGF or angiogenic hormone that stimulates angiogenesis and neural regeneration [144]. However, the challenge that how to deliver them into the pathological cavity is necessary to be considered. Given that pathological microenvironment is complex, the hydrogel basing on HA has shown the enormous potential to deliver VEGF, which would serve as the initial physical support for migration of cells, angiogenesis and axonogenesis. Ju *et al.* designed a HA-based biodegradable hydrogel scaffold, mixed with PLGA microspheres containing VEGF and angiopoietin-1, which are two factors to stimulate angiogenesis. This hydrogel exhibited excellent biocompatibility with the environment of the brain. Particularly, angiogenesis could be observed around the implanted HA–PLGA hydrogel. Meanwhile, the animal models manifested motor recovery [145]. In addition, Nih *et al.* also found that heparin nanoparticles (nH) could hold the ability to bind VEGF, but the function of reducing blood coagulation did not take effect. Theses exhibited that HA gel not only promoted the angiogenesis but also decreased the amount of microglial and macrophage in MCAO model [146]. With the development of delivery systems of stem cells, the transplantation of PSCs also provides a kind of manner for the regeneration of blood vessels and the restoration of motor function. McCrary *et al.* showed the neural progenitor cells (NPCs) was encapsulated in a bFGF binding chondroitin sulfate-A (CS-A) hydrogel that was used to improve the injury in a mouse stroke model. It showed that CS-A hydrogel enhanced the remodeling of vascular and recovery of motor function after stroke (Fig. 6) [147].

Hydrogel is a new strategy for revascularization (Table S4 in Supporting information). Currently, delivering VEGF is the common approaches to constitute a potential target for restoring vascularization. Besides, many nucleic acid-based therapeutics also showed huge potential. Qu *et al.* found that microRNA-126 could regulate angiogenesis and neurogenesis by the proliferation and migration of endothelial cells [148]. Li *et al.* proved that microRNA-124 was able to regulate cerebromicrovascular impairments [149]. Nucleic acids can be entrapped within hydrogel, either as conjugates or as polyplex particles, for local and controlled release. Besides, hydrogel shows excellent feature of kinetics of *in vivo* that is suitable for further clinical translation.

4.5. Hydrogel for the iron chelation and hematoma absorption

Iron is a metabolite of Hb and has certain effects on brain. Studies have shown that the accumulation of iron in HS wound cause edema and brain atrophy. At present, the treatment of iron overload is to decrease iron concentration mainly through some iron chelating agent drugs, such as deferoxamine or minocycline. However, these drugs also have many deficiencies, such as toxicity and un-controlled release. With the development of biomaterials, keratin was gradually discovered and it has excellent biological activity, biocompatibility, which exhibits significant biological and biomedical application potentials [150]. Its applications cover hemostasis, targeted drugs release and so on. He *et al.* found that *in situ* delivery of the keratin-gels restrained hematoma expansion, relieved neuroinflammatory reactions and neurological deficits when rebleeding occurred [151]. However, the traditional keratin hydrogel leaves much to be desired such as injectable property. *In situ* the sol–gel phase transition can be achieved by injectable hydrogel, which is able to realize the minimally-invasive

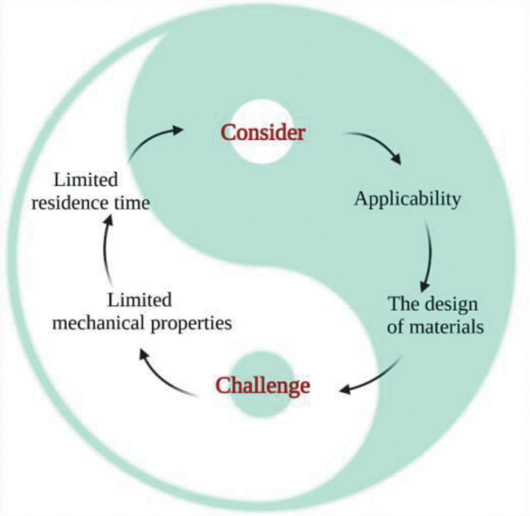

Fig. 7. The consideration and challenge of design for treatment of stroke.

delivery. Luo *et al.* developed human hair keratose hydrogel loading with minocycline hydrochloride (MH) to reduce iron overload after HS for enhancing postoperative functions (Fig. S5 in Supporting information) [152]. In addition, Zhu *et al.* designed a thermal keratin hydrogel that was prepared by redox reaction of a thermal material poly(*N*-isopropylacrylamide) (PNIPAM) and keratin, which solved the problem of keratin injectability (Fig. S6 in Supporting information) [153].

Open craniotomy is the most studied approach in surgical treatment of HS, which can directly remove harmful hematoma to prevent of mass effect [154]. However, the rate of postoperative rehemorrhage is still high, which can cause serious secondary damage particularly mediated by iron overload. A study showed 548 patients with HS underwent surgery, of which 116 developed postoperative rehemorrhage [155]. Therefore, reducing the HS postoperative iron overload is necessary to improve the postoperative functional recovery. Hydrogel packaging iron chelator can keep sustained release of the iron chelator and will contribute to fast reduction of iron overload, which is extremely conducive to functional recovery after HS surgery (Table S5 in Supporting information).

In general, newly developed hydrogel will play a vital role in future treatment strategies for stroke in which it is crucial to consider the biomaterials for therapeutic applications. The hydrogel has the potential to surmount the present therapeutic challenges.

5. Conclusion and perspectives

Hydrogel with biocompatible skeleton has also been widely used in the treatment of stroke research which has been proved that hydrogel for *in situ* injection has become a very good treatment strategy for stroke diseases. Basing on the injectability and adaptability of the hydrogel, the skeleton can be combined with stem cells and drugs to promote the repair of the nervous system and the reconstruction of brain tissue.

Although tissue engineering hydrogel has many applications in stroke treatment, it still has many problems to deal with (Fig. 7). For example, the biodegradability, elasticity and pore size still need further exploration to achieve better treatment efficiency. In addition, the brain itself is also a complex system. Subtle changes in the brain will also have a great impact on the microenvironment. Therefore, it would be best for hydrogel to have the following characteristics: (1) Injectability. Hydrogel is injected in a liquid state and then form a solid gel *in situ*. This type of hydrogel will be crosslinked due to the influence of different covalent bonds or non-covalent bonds between biological materials [156]. It has appropriate mechanical stability and biological stability, which can protect the encapsulated growth factors and stem cells from injury caused by injection. If the hydrogel is difficult to inject, then it will be difficult for the hydrogel to fit into the cavity of the stroke. The brain will be unable to adapt to mechanical damage. (2) Biocompatibility. The successful delivery of hydrogel to the brain should not only consider that the delivered hydrogel is compatible with the host tissue, avoiding the immune response of the implanted posterior brain tissue, but also make the cells in the hydrogel adapt to the brain environment [157] for improving the survival rate of the transplantation. Above all, many biomaterials, such as HA, alginate, collagen and the like, can be designed to construct the skeleton of hydrogel, and these biomaterials are the components of ECM and they can be better removed after degradation and will not cause secondary damage to the tissue. (3) Elasticity. Elasticity is a very important parameter of hydrogel for delivering stem cells. The native brain tissue is soft (elastic moduli, $E = 0.4–1.5\,\text{kPa}$) [158]. Suitable elasticity is conducive to regulating stem cell differentiation [159]. Studies have shown that the soft matrixes are suitable for growth of NSCs, neurons, and glia cells. Flexible hydrogel matrices ($E = 0.5–1.5\,\text{kPa}$) can favor neuronal attachment and growth, while tough hydrogel ($E = 7.2\,\text{kPa}$) is beneficial to growth of astrocyte [159]. Meanwhile, a study explored the changes of adult NSCs (aNSCs) in alterable moduli polymer hydrogel. The results showed that aNSCs can self-renew and differentiate on RGD peptide-modified variable moduli interpenetrating polymer networks ($E \geq 100\,\text{Pa}$) [99]. (4) Pore size. Stem cells and other substances in hydrogel will migrate through the pore to brain tissue. Large pore size could cause the leakage of cargos, nevertheless it is difficult to release the cargos with small pore size. Thus, the appropriate pore size is contributed to the effective contact between the wrapped cargos and the outside microenvironment [160].

For clinical transformation, several major challenges still have to be addressed. Biocompatibility and biodegradability must be considered for clinical conversion of hydrogel. The degradation products of the hydrogel on normal physiological behaviors of cells/tissues are still to be investigated. Attention should be paid to the toxicity of materials used in both preclinical and clinical studies. In addition, the appropriate mechanical property for human brains is another key factor to consider, which will ensure an appropriate tissue response and prevent damage to healthy tissue. Moreover, large-scale production of hydrogel remains a big challenge. Ideally, hydrogel should have the desired physicochemical properties high safety and controlled pharmacokinetic.

In the end, it is noteworthy that DNA-based hydrogel is currently widely used in both nanotechnology and materials. DNA hydrogel possesses many excellent features such as biocompatibility, controlled biodegradability, adjustable mechanical properties, stability against proteases, the ability for self-healing, and responsiveness to various stimuli. Compared with DNA hydrogel, many other hydrogels such as alginate or gelatin, lack these features of versatility and structural programmability. So far, the applications of DNA-based hydrogel have been covered from biosensing to delivery of drugs. Finally, DNA-based hydrogel present great potential for the treatment of stroke, which may bring a silver lining for the healing of stroke.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108205.

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