Hydrogel-based biomaterials for brain regeneration after stroke: Gap to clinical translation

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ABSTRACT

Due to the limited effects of current treatments on brain repair and regeneration, stroke continues to be the predominant cause of death and long-term disability on a global scale. In recent years, hydrogel-based biomaterials combined with stem cells and extracellular vesicles have emerged as promising new treatments to improve brain regeneration after stroke. However, the clinical translation of hydrogel-based biomaterials for the treatment of brain injury is still far from satisfactory. In this review, we first summarise the present status of stroke-related clinical treatments and the advantages provided by hydrogel-based materials in combination with stem cells and extracellular vesicles in preclinical studies. We then focus on the possible causes of the gap between preclinical studies and clinical translation of hydrogel-based biomaterials from the perspective of biocompatibility and safety, the choices of preclinical models, the lack of clinical noninvasive imaging methods, standardisation and quality control, manufacturing scalability, and regulatory compliance. With the progress in the abovementioned areas, we believe that the clinical translation of hydrogel-based biomaterials will greatly improve brain regeneration after stroke and that this improvement will be realised by the general public in the near future.

Keywords:
Biomaterials; Clinical translation; Hydrogel; Stroke

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1. Introduction

A stroke is caused by a disturbance of blood flow to the brain by either a rupture or an occlusion of a cerebral blood vessel.[1] Although remarkable progress has been made in stroke research over the last two decades, stroke remains the leading cause of death and disability worldwide.[2] Even with improved care, a proportion of patients die or live with significant neurological disabilities such as paralysis, aphasia, swallowing disorders, and cognitive impairment, which significantly impact their lives.[3-5]

Hydrogel-based materials, formed through physical or chemical cross-linking of hydrophilic polymers, create highly hydrophilic three-dimensional (3D) network gels. As biocompatible and regulable biomaterials, they can be engineered to deliver drugs, growth factors, or stem cells to ischaemic brain areas in a controlled manner. In addition, hydrogels provide a scaffold that mimics the extracellular matrix (ECM) to support the growth and differentiation of neural stem cells or progenitor cells. The application of hydrogel-based biomaterials in the treatment of ischaemic stroke has attracted the attention of researchers for many years. While studies have on hydrogel-based treatments for ischaemic stroke have shown promising results in animal models; however, these hydrogels have not been tested in clinical trials. In this review, we discuss the potential gap in the translation of hydrogel-based biomaterials in ischaemic stroke after introducing current clinical needs and the advantages of applying hydrogel-based biomaterials.

2. Method

Article screening was completed through PubMed, Google Scholar, and ClinicaTrials.gov with the key words 'hydrogel' and 'stroke'. Studies

that focused on poststroke treatment with hydrogels and were published in English until 2024 were selected for review.

3. The characteristics of stroke and clinical needs

3.1. Characteristics of stroke

A stroke occurs due to a disruption in cerebral blood flow, which can result from either the rupture or occlusion of a blood vessel in the brain.[1] Brain tissue damage following a stroke occurs due to a cascade of complex mechanisms, including energy failure, ion imbalance and cell swelling, excitotoxicity, oxidative stress, and inflammation etc. Hypoxia and glucose deficiency trigger a cascade of events that leads to cell death.[6] Exposure of excessive neurotransmitters causes excitotoxicity,[7] and reactive oxygen species causes oxidative stress,[8] ultimately resulting in inflammation and cellular death. Additionally, disruption of the blood-brain barrier and subsequent oedema exacerbate tissue damage.[9]

Although remarkable progress has been made in stroke research during the past two decades, stroke continues to be the leading cause of death and disability worldwide.[2] Between 2011 and 2021, the age-adjusted stroke death rate in the United States rose by 8.4%, and the total number of stroke deaths increased by 26.3%.[10] Even with improved care, a proportion of patients die or continue to live with significant neurological disabilities, such as paralysis, aphasia, swallowing disorders, and cognitive impairment, which significantly impact their lives.[4,5,10] Among survivours, 70% have reduced work capacity, and 30% require help with self-care, which highlights the significant disease burden. The cost of stroke in the U.S. was estimated at $73.7 billion in 2010, with projections reaching $1.52 trillion by 2050.[11] Current treatments for stroke are limited to thrombolysis to retrieve the clot and endovascular thrombectomy to dissolve the clot and restore blood flow. The success of the procedures is verified through imaging methods such as computed tomography (CT) or magnetic resonance imaging (MRI). National Institutes of Health Stroke Scale scores are used to monitor changes in patients' neurological function and recovery.[12] However, only a few patients are eligible for these treatments because of the short time window and strict admission criteria. In addition, these treatments do not address the existing impairment caused by haemorrhage or ischaemia. Therefore, the development of new treatments that can promote brain repair after stroke is crucial to reestablish normal brain function.

3.2. Regenerative therapies: stem cells and extracellular vesicles

3.2.1. Stem cell-based therapies

Stem cells are characterised by their ability to divide asymmetrically and differentiate into multiple cell lineages, which is necessary for continuous tissue renewal and regeneration.[13] Therefore, stem cells have demonstrated significant potential for the treatment of various diseases. In therapeutic applications, stem cells can be administered directly into damaged tissue sites, either by replacing damaged cells or by promoting endogenous cell regeneration through paracrine action.[14] Stem cells are involved in a variety of mechanisms in the treatment of ischaemic stroke, including cell migration, growth factor secretion, inhibition of cell apoptosis, inhibition of neuroinflammation, and vascular remodelling.[15] The transplantation of adipose-derived mesenchymal stem cells into a rat stroke model significantly increased functional angiogenesis and neurogenesis, leading to behavioural improvements.[16] To date, nearly 100 clinical studies involving the application of various stem cells to stroke patients have been registered at Clinicaltrials.gov.[14] A phase I/II clinical trial (NCT01297413) reported that intravenous infusion of mesenchymal stem cells enhances behavioural performance and ensures safety in patients with chronic stroke and severe functional impairments.[17]

3.2.2. Extracellular vesicle-based therapies

On the basis of their biogenesis and biophysical/biochemical properties, extracellular vesicles (EVs) can be classified as exosomes, microvesicles, or apoptotic vesicles. Research has revealed that EVs are not merely waste carriers. Current interest in this field is concentrated on their capacity to interact with cells through their internal cargoes, such as nucleic acids, lipids, and proteins.[18] EVs function as signal carriers in both normal cell homeostasis and pathological development.[19,20]

Cell-free therapies utilising EVs, especially stem cell-derived EVs (SC-EVs), offer promising avenues for the treatment of various neurological diseases and serve as potential alternatives to traditional stem cell therapies.[21,22] Currently, SC-EVs and dynamic delivery systems are being explored as direct therapeutic agents because of their ability[23] to facilitate neurogenesis and efficiently deliver highly functional small molecules into specific cells within the central nervous system. Moreover, as nonliving entities, SC-EVs circumvent the drawbacks associated with the uncontrollability of living stem cells both *in vivo* (e.g., poor survival, immune response, and tumourigenicity) and *in vitro* (e.g., limited sources, complex preparation processes, poor quality control, storage instability, and ethical controversies).[24] Many studies have demonstrated the therapeutic potential of SC-EVs in neurological diseases, including stroke,[25] Alzheimer's disease,[26] and spinal cord injury,[27] since the initial research confirming the potential therapeutic use of SC-EVs was published in 2010.[28] However, natural SC-EVs face practical

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limitations in clinical applications, including limitations in yield, bioactivity, targetability, and *in vivo* clearance.[21] Consequently, researchers are actively pursuing the development of biotechnology platforms aimed at enhancing the therapeutic efficacy of SC-EVs, such as engineering SC-EVs to improve overall yield, enhance their biological activity, optimise their targeting ability, and prolong their half-life.[21,29]

3.3. Clinical needs

SC-EVs are promising candidates for regenerative therapy due to the low immunogenicity and immunomodulatory properties of stem cells.[30] Many preclinical studies have confirmed the feasibility, safety, and reparative effects of various stem cells and SC-EVs for the treatment of ischaemic stroke. However, the clinical translation of regenerative therapy is still limited in several respects. One of the primary issues that needs to be addressed in stem cell therapy is safety. In the process of stem cell therapy, the occurrence of cell-related harmful events directly determines whether cells can be used in clinical therapy. Cell dose-dependent toxicity, allergic reactions, and side effects due to different infusion routes (e.g., pulmonary embolism due to intravenous infusion, arterial embolism due to arterial infusion) are common safety concerns.[31] In addition, for both stem cell and SC-EV therapies, increasing the targeting ability and half-life is a critical issue that requires resolution (**Figure 1**).

To achieve optimal therapeutic outcomes with EVs, maintaining high concentrations and ensuring sustained release at the lesion site are imperative. Instead of solely extending the retention time of SC-EVs, an alternative strategy is to extend the period of their action. The implantation of therapeutic EVs into various biomaterials stands out as a commonly used method to achieve precise and sustained intracerebral delivery, thereby mitigating potential side effects associated with nontargeted peripheral delivery methods.[32] Numerous studies have explored the integration of EVs into biomaterials, with hydrogels emerging as a particularly promising delivery material. For stem cells, systemic injection leads to poor targeting and the formation of emboli as mentioned above. Intracerebral delivery at the lesion site has advantages in terms of targeting; however, the harsh microenvironment in the infarct area impedes the survival of the transplanted stem cells. Hydrogel-based biomaterials can provide a scaffold that supports the survival, growth, and differentiation of stem cells, thereby enhancing their therapeutic potential.[33,34]

4. Advantages of hydrogel-based biomaterials as stem cell and extracellular vesicle carrier for stroke treatment

Hydrogels, formed through the physical or chemical cross-linking of hydrophilic polymers, constitute highly hydrophilic 3D network gels. As biocompatible and regulable biomaterials, they are usually used for drug and cell delivery. Their 3D structure and regulable physiochemical properties provide an ideal environment for stem cell and exosome loading. Technological advancements have led to the exploration of stimulus-responsive hydrogels,[35] self-healing injectable hydrogels,[36] adhesive hydrogels,[37] and conductive hydrogels for repairing damage in neurological diseases.[38]

Hydrogels can be categorised as natural or synthetic, based on their origins. Natural polymers used in hydrogel preparation include hyaluronic acid, chitosan, heparin, alginate, gelatin, fibrin, pectin, dextran, etc.[39] Among these, alginate, pectin, and dextran are plant-derived, while others like chitosan, collagen, and hyaluronic acid are sourced from animals. Natural hydrogels are typically non-toxic, biocompatible, and biodegradable, offering various benefits for medical applications. However, their low stability and poor mechanical strength compared to synthetic hydrogels, have limited their use. Synthetic hydrogels, by contrast, offer superior water absorption, mechanical strength, and long-lasting performance without degradation. Common synthetic polymers include polyvinyl alcohol, polyethylene glycol, sodium polyacrylate and acrylate-based polymers. Semi-synthetic hydrogels combine the advantages of both natural and synthetic materials, providing enhanced bioactivity and customisable properties.[40]

There are two main strategies for cross-linking hydrogels: physical and chemical methods.[41] These methods significantly influence the structure, properties, and performance of hydrogels. Chemical cross-linking involves the formation of permanent junctions between polymer chains using cross-linking agents, which results in stable structures. In contrast, physical cross-linking relies on weaker interactions, such as van der Waals forces and hydrogen bonding, to create transient junctions.[42] Compared with chemically cross-linked hydrogels, physically cross-linked hydrogels offer several advantages, including lower toxicity, responsiveness to stimuli, higher drug-loading efficiency, and the ability to form gels *in situ*.[43] Therefore, physically cross-linked hydrogels are favoured for their stimuli-responsive behaviour, injectability, and biodegradability, which increase their suitability for drug delivery, tissue engineering, and regenerative medicine where controlled response and degradation are advantageous. However, chemically cross-linked hydrogels are often selected for applications that require high mechanical strength, high stability, and resistance to degradation, such as in load-bearing structures, tissue engineering scaffolds, and long-term drug delivery systems.[44] Popular chemical cross-linking methods often utilise methacrylate precursors, whose carbon double bonds facilitate the formation of hydrogels through free radical polymerisation, which simplifies and accelerates the synthesis of complex network structures.[45]

Recent advances in hydrogels with properties such as stimulus-responsiveness, self-healing, adhesiveness, and conductivity hold great promise for treating stroke.[46] Stimulus-responsive hydrogels can encapsulate therapeutic agents and release them in a controlled fashion in response to environmental triggers, making them ideal for delivering drugs to specific areas of the brain while minimising damage to surrounding healthy tissue.[47] Jiang et al.[48] developed a dual glucose/reactive oxygen species-responsive hydrogel loaded with EVs derived from neural stem cells for treating diabetic stroke. This hydrogel extended the

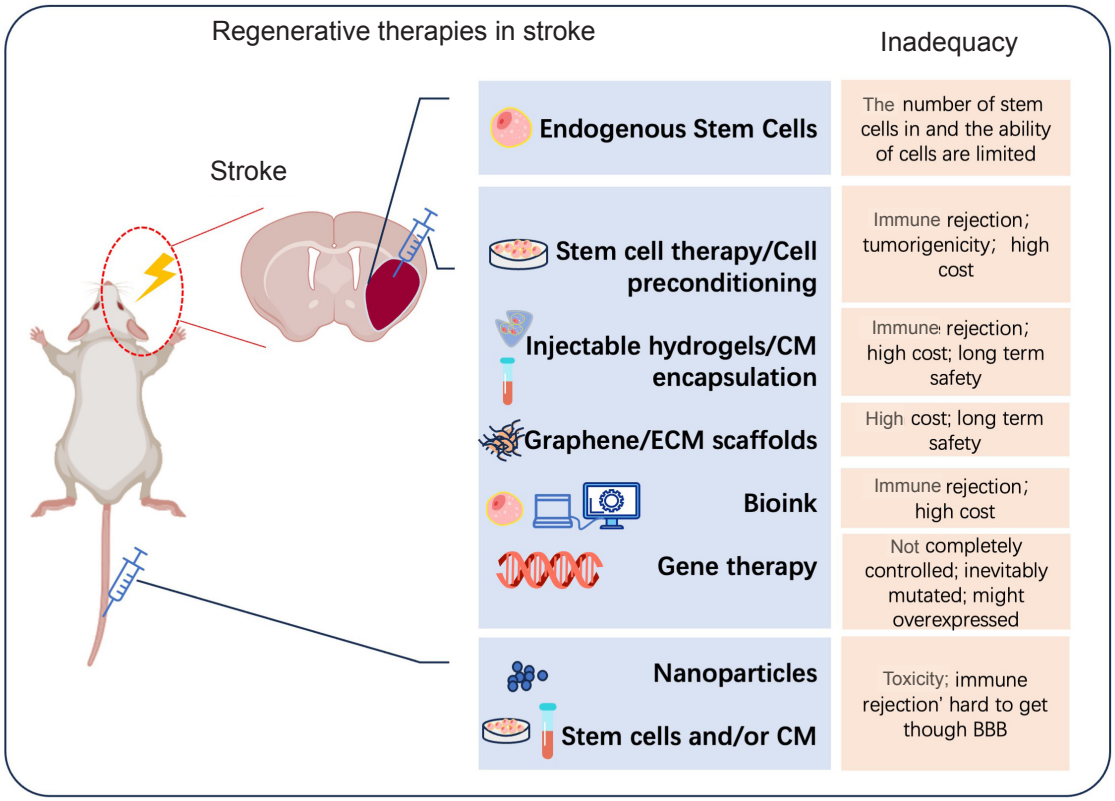

Figure 1. Summary of stem cells, biomaterials, and gene-based regenerative therapy on stroke. Stem cells and stem cell-derived extracellular vesicles have shown great potential for treating stroke. However, each of them has different or similar inadequacies, such as safety, immune rejection, cost, etc. Created with Adobe Illustrator 2021.
Abbreviations: BBB: blood-brain barrier; CM: conditioned medium; ECM: extracellular matrix.

retention and activity of the EVs in the brain, reducing brain atrophy and improving neurobehavioural recovery in diabetic stroke mice. Self-healing hydrogels are capable of repairing themselves after damage, exhibit superior fatigue resistance, reusability, and hydrophilicity.[49] Their enhanced durability and flexibility allow them to endure harsh environments and restore the structure and function of damaged neural tissue.[50] Pei et al.[51] developed a self-healing hydrogel loaded with bone mesenchymal stem cells to treat ischaemic stroke rats, resulting in a significant reduction in cerebral ischaemia. Adhesive hydrogels, which can encapsulate exosomes while preserving their activity, mimic the 3D microenvironment of tissue, promoting cell adhesion, proliferation and angiogenesis in ischaemic regions.[52] A novel injectable adhesive hydrogel significantly improved neurological function, enhanced angiogenesis, and reduced inflammation throughout the brain following ischaemic stroke, providing strong adhesion and sustained release of EVs.[53] Conductive hydrogels can restore electrical signals and promote stem cell differentiation.[54] George et al.[55] developed an electrically conductive polymer scaffold that electrically stimulated human neural progenitor cells, advancing repair processes and improving stroke outcomes.

The integration of various hydrogel properties is crucial for effective stroke treatment, as complex conditions like stroke often require multifunctional hydrogels to address multiple pathological processes. Hydrogels that combine strong adhesiveness and self-healing capabilities have been shown to significantly enhance angiogenesis and neurogenesis after injection into damaged brain tissue.[56] Conductive hydrogels with electro-responsiveness allow electrodes to adhere to brain tissues, improving their function.[57] Panwar et al.[58] introduced a hydrogel that uniquely combines conductivity, self-healing, injectability, and tissue adhesiveness—features rarely found together. This hydrogel also promotes cell growth and tissue regeneration, while exhibiting strong biocompatibility and biodegradability, as confirmed by both *in vitro* and *in vivo* studies. Its adaptable properties and tunable conductivity make it a promising candidate for future medical applications, including stroke treatment.

4.1. Structural support

Biomaterial scaffolds have emerged as promising tools in regenerative medicine, as they exhibit considerable potential for facilitating central nervous system regeneration both in terms of tissue repair and functional recovery. In the form of hydrogels, biomaterial scaffolds have demonstrated positive outcomes in central nervous system regeneration.[59] These scaffolds provide supportive structures for cells to facilitate in their infiltration and proliferation. Additionally, when combined with stem cells, these gels contribute to the repair of central nervous system injuries.[60,61] Moshayedi et al.[62] developed a hyaluronic acid-based self-polymerising hydrogel that promoted the adhesion and survival of encapsulated human neural progenitor cells following transplantation into

the stroke brain. Similarly, Cheng et al.[63] utilised a functional self-assembling peptide 3D hydrogel, which mimicked the mechanical stiffness of brain tissue, to deliver neural stem cells. This approach not only enhances the adhesion and neuronal differentiation of encapsulated neural stem cells but also helps to fill the cavity in injured rat brains.

4.2. Targeted delivery and sustained release

Ideal drug delivery allows for the controlled and consistent release of medication at a targeted site within the body, which not only improves the efficacy of the drug, but also minimises side effects. In an ideal state, a hydrogel responds to stimulation by the external environment so that the gel can generate mechanical energy according to the environmental signals; the energy is manifested as swelling or shrinking at the macro level. By controlling the swelling kinetics of the gel, the diffusion rate of the drug is affected, and controllable release of the drug is achieved.[64] Hydrogels, which can carry hydrophilic therapeutic agents due to their water or aqueous solution content, can release these agents in a sustained manner, either for disease treatment or to mimic the structures of the ECM and tissues.[65] Lin et al.[66] developed a hydrogel that combines a hydrophilic network with hydrophobic micelle cavities that exhibited distinct release kinetics: a rapid first-order release of the hydrophilic drugs and a sustained zero-order release of the hydrophobic drugs. Therapeutic delivery to the brain is often hindered by the blood-brain barrier. Tuladhar et al.[67] engineered an injectable drug delivery hydrogel to bypass the blood-brain barrier, and for the first time, achieved local and sustained corelease of cyclosporine and erythropoietin directly into the brain.

Despite these distinctive properties, traditional prefabricated hydrogels have limited applications because they require implantation under the skin, which causes additional injury to patients. To address this issue, *in situ* forming and injectable hydrogels have been developed that transition from a sol-to-gel state after injection into the body.[68] The specific process involves mixing the gel precursor with a bioactive substance and injecting the gel into the organism through a syringe. These injected mixtures often gel as soon as they enter the body, which may be caused by pH values, temperature, and ion concentration, among other factors.[68]

4.3. Reduced inflammation

Inflammation plays a crucial role in the pathobiology of stroke. The injured brain triggers a strong immunosuppressive response, which increases the risk of fatal infections and threatens the survival of stroke patients. This response is marked by the rapid activation of microglia and astrocytes, the production of proinflammatory factors, and the infiltration of various inflammatory cells into the ischaemic brain tissue.[69] Pro-inflammatory factors such as interleukin 6, interleukin 1β, and tumour necrosis factor α stimulate the secretion of inflammasomes, which, together with these cytokines, contribute to mitochondrial dysfunction. This leads to the production of reactive oxygen species and mitochondrial-derived damage-associated molecular patterns, further aggravating inflammation.[70] This cascade intensifies brain oedema, disrupts the blood-brain barrier, and results in cell death.[71] Inflammatory signalling is central to all phases, from the initial damage caused by arterial blockage to the later regenerative processes involved in postischaemic tissue repair.[72] In recent years, considerable research has been dedicated to enhancing hydrogels to specifically counteract excessive inflammation. These efforts involve effective scavenging of surplus free radicals, sequestering chemokines, and promoting the polarisation of macrophages from the M1 to the M2 phenotypes, thereby regulating inflammation.[73]

On the one hand, hydrogels can be designed to exert anti-inflammatory effects. Researchers have developed an injectable hydrogel for use during the poststroke subacute/chronic phase that allows tannic acid to bind to carboxymethyl chitosan via polyvalent hydrogen bonding, which results in the formation of an injectable hydrogel. The tannic acid gel regulated the nuclear factor κB pathway in the peri-infarct region of stroke mice, which implies anti-inflammatory polarisation of microglia *in vivo* and enhanced neuroplasticity[74] (**Figure 2**). Another research team designed a stepwise targeting nanoplatform that, while promoting blood-brain barrier infiltration, can deliver the antioxidant resveratrol to the mitochondria, the organelles that produce reactive oxygen species. This treatment reversed mitochondrial dysfunction by clearing reactive oxygen species, regulating the microglial phenotype, relieving oxidative stress and inflammation, and achieving neuroprotective effects[75] (**Figure 3**).

On the other hand, encapsulating anti-inflammatory and neuroprotective molecules within various hydrogel formulations is associated with improved functional outcomes after stroke. When carried with gelatine and after its release inside the body, the inflammatory regulator osteopontin decreased the infarct size in rats with focal cerebral ischaemia,[76] whereas no beneficial effect was observed with free osteopontin administration.[77] In stroke treatment, cell-based therapies are more effective when stem cells are encapsulated in different hydrogels. These cellular hydrogel therapies reduce lesion cavity size and inflammation, promote angiogenesis, enhance neurogenesis and synapse regeneration, and stimulate functional rewiring in the lesion area, thus achieving functional recovery.[78] Similarly, the efficacy of exosomes encapsulated in different hydrogels has also significantly improved. Neural stem cell-derived exosomes coated with a catecholamine-grafted hyaluronic acid adhesive hydrogel were used as a delivery scaffold to continuously deliver and release exosomes in the ischaemic area to treat mice with ischaemic stroke. As these gels maintain the biological activity of exosomes, they can enhance angiogenesis and anti-inflammatory effects in the ischaemic area, decrease the expression of tumour necrosis factor α and interleukin 1 to varying degrees, and ultimately improve cerebral infarction and nerve function.[53]

4.4. Injectability

One distinct advantage of hydrogel-based biomaterials is their injectability. Traditional scaffolds without gel structures must

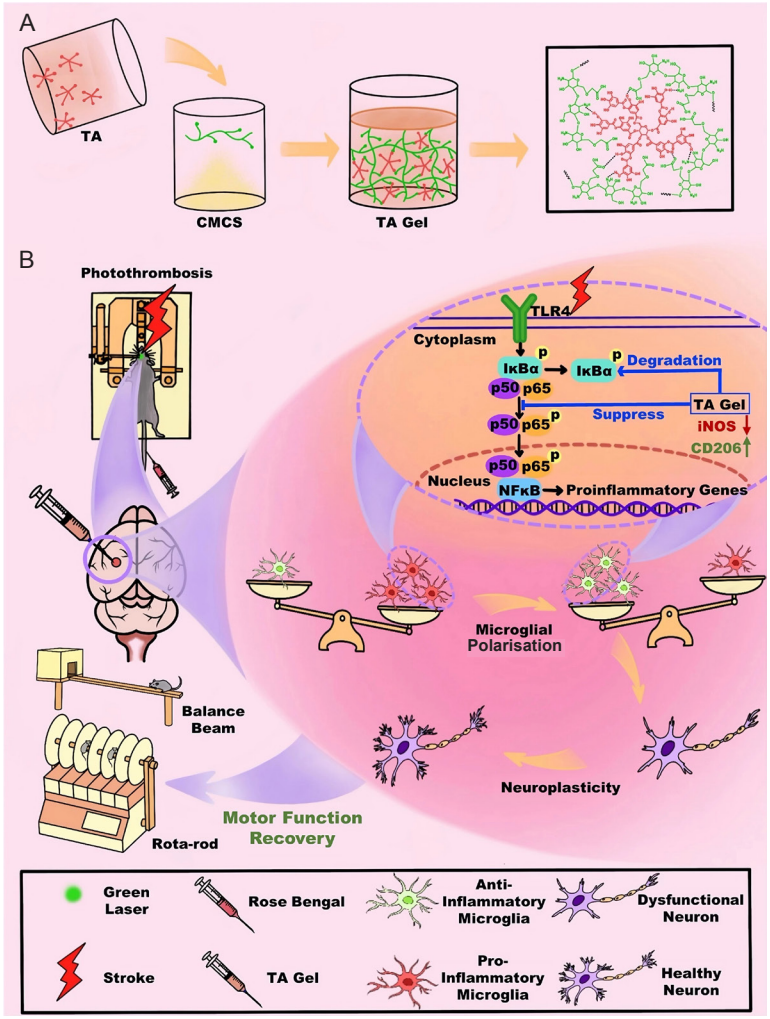

Figure 2. (A, B) The preparation protocol (A) and the proposed mechanism (B) by which TA gel promotes post-stroke rehabilitation. Reprinted from Liu et al.[74] Abbreviations: CMCS: carboxymethyl chitosan; iNOS: inducible nitric oxide synthase; IκBα: inhibitor of κBα; NFκB: nuclear factor κB; TA: tannic acid; TLR4: Toll-like receptor 4.

be molded and surgically implanted to fit the damaged area. In contrast, hydrogels with shear-thinning and self-healing properties can be injected directly in their gel state.[79] Other injectable hydrogels exhibit fluidity when administered into the body but form a solid gel *in situ*.[80] This *in-situ* gelation preserves the hydrogel's structure and properties, enhancing therapeutic performance. Wang et al.[81] studied the effects of an injectable hydrogel that undergoes a sol-to-gel transition under physiological conditions, such as specific pH levels and ion concentrations. This property makes the hydrogel ideal for filling lesion cavities after injection, offering an effective treatment for brain diseases like stroke.

4.5. Treatment efficacy

Recent studies have demonstrated the advantages of hydrogel-based treatments for stroke. Here, we review several key findings that highlight their effectiveness. In animal models of stroke, hydrogel treatments significantly reduce the size of the infarct area. For example, Shen et al.[82] developed a topological hydrogel probe to conduct neuromodulation. The infarct volume in rats with stroke at the beginning of the experiment reached 22%. However, after 12 days of continuous optogenetic modulation, the infarct volume decreased to 4%. In comparison, the infarct volume of untreated rats with stroke decreased from 22% to 15% after 12 days of self-recovery.[82] Hydrogels have also shown promise in improving neurological functions. The modified neurological severity score values of the rats treated with a neural stem cell-laden hydrogel were significantly lower than those of the control rats, which indicates better athletic movement.[83] Another study revealed that, 14 days after hydrogel injection, stroke-affected rats exhibited greater than 70% recovery of behavioural function, a significant improvement compared with the control group.[84] Notably, a neutrophil membrane-camouflaged polyprodrug nanomedicine devised by Zhao et al.[85] not only enhanced behavioural functions and neurofunctional recovery in mice with middle cerebral artery occlusion but also markedly increased the survival rate of these mice to 100%.

5. Time to clinical translation

5.1. Clinical applications

Due to their numerous advantages, hydrogels have led to remarkable progress in various clinical applications. They are now widely used in fields such as wound care,[86] tissue

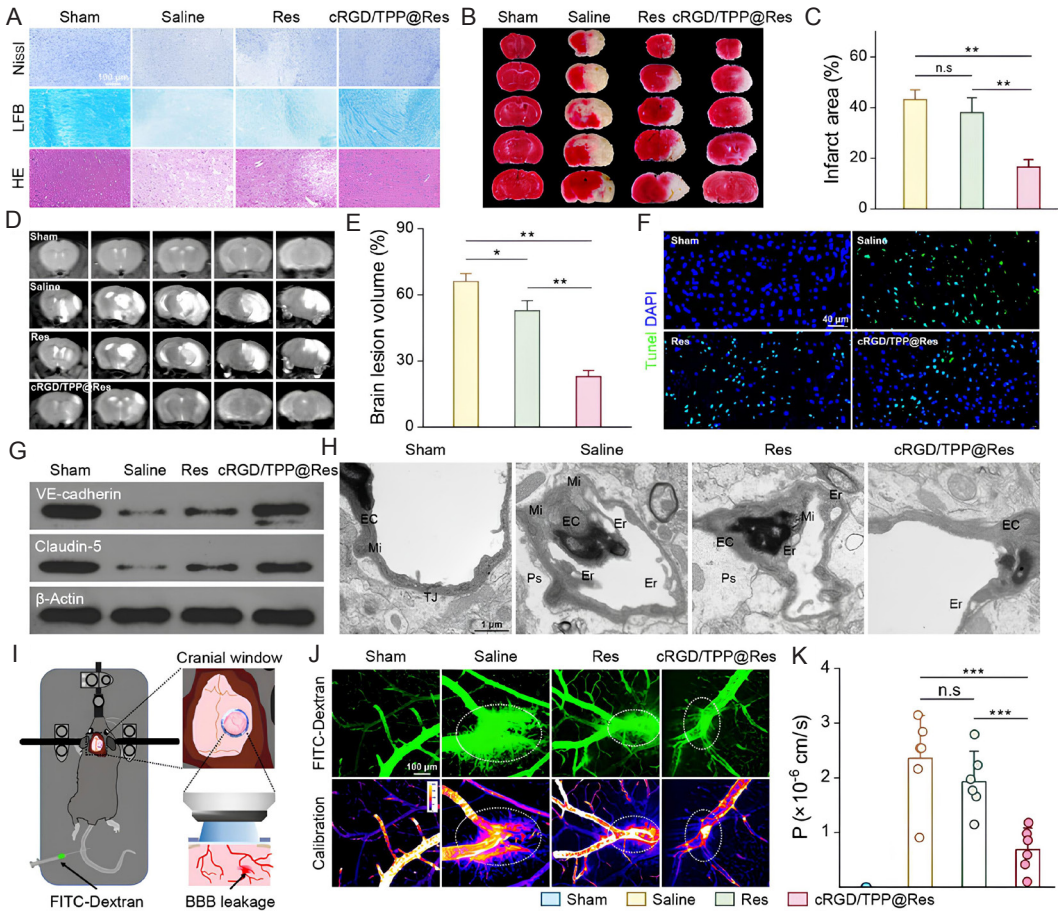

Figure 3. *In vivo* therapeutic efficacy of cRGD/TPP@Res for ischaemic stroke. (A) Nissl, LFB, and H&E staining in different groups ($n = 3$). Scale bar: 100 μm. (B, C) TTC staining (B) and infarct ratio (C) of tMCAO mice ($n = 3$). (D, E) T2-weighted MRI (D) and infarct volume (E) of tMCAO mice ($n = 3$). (F) TUNEL staining of ischaemic brain in different groups. TUNEL-positive cells were stained green, and nuclei were stained with DAPI (blue). Scale bar: 40 μm. (G) Western blot analysis of VE-cadherin and Claudin-5 in ischaemic brain of different mice ($n = 3$). (H) TEM images of BBB in different groups ($n = 3$). Scale bar: 1 μm. (I) Schematic diagram showing *in vivo* vascular permeability observation from the cranial window by two-photon microscope. (J) *In vivo* two-photon imaging showing FITC-dextran (MW = 3500 Da) penetrating from damaged vessels into brain parenchyma. Scale bar: 100 μm. (K) The vascular leakage rate (P) in different groups ($n = 6$). Data are presented as mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way analysis of variance). Reprinted from Wang et al.[75] Copyright 2023 American Chemical Society. Abbreviations: BBB: blood-brain barrier; cRGD: cyclo(Arg-Gly-Asp-d-Tyr-Lys) peptide; DAPI: 4′,6-diamidino-2-phenylindole; EC: endothelial cells; Er: endoplasmic reticulum; FITC: fluorescein isothiocyanate; H&E: haematoxylin-eosin; LFB: Luxol fast blue; Mi: mitochondria; MRI: magnetic resonance imaging; n.s: not significant; Ps: perivascular space; Res: resveratrol; TEM: transmission electron microscopy; TJ: tight junction; tMCAO: transient middle cerebral artery occlusion; TPP: triphenylphosphine; TTC: triphenyl tetrazolium chloride; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; VE: vascular endothelial.

engineering,[87] and drug delivery.[88] The hydrogel market is projected to expand from a value of USD 22.1 billion in 2019 to USD 31.4 billion by 2027. The versatility of commercial hydrogels is evident in diverse drug delivery applications, including transdermal patches with controlled release, stimuli-responsive hydrogels for oral delivery, and localised delivery through parenteral methods.[89] They also play a crucial role in ophthalmology, where they are used in devices, such as contact lenses, and in procedures such as eye surgeries.[90] More than 30 injectable hydrogel-based products, including those for facial aesthetics, cancer treatment, and spinal cord injuries, have received U.S. Food and Drug Administration approval.[91] Given their broad range of applications, it is not surprising that hydrogels are currently being tested in numerous clinical trials across diverse fields, including regenerative medicine, urinary incontinence, and cancer care.[91]

Despite the current status of hydrogel materials used clinically, the clinical translation of hydrogel-based therapies for ischaemic stroke is very limited. Only two registered clinical trials can be found at ClinicalTrials.gov. One of these studies was designed to implant allogenic mesenchymal cells encapsulated in alginate in the brain cavity after haematoma removal and is now registered as terminated because of the "need to improve the design of study medication" (NCT01298830). The other study was designed to test a medical device composed of synthetic polysaccharides that mimic the ECM component heparan sulphates (NCT04083001), which can be delivered by intra-arterial injection. However, recruitment has not been started for this trial. The following section discusses the possible reasons for the low rate of translation of hydrogel-based materials for stroke therapy from the perspectives of biocompatibility and safety, selection of preclinical models, noninvasive imaging

modalities, standardisation, and manufacturing, as well as what can improve their translational potential.

5.2. Biocompatibility and safety

The biocompatibility and safety of hydrogel-based materials for stroke treatment are crucial considerations for their development and potential clinical translation. Hydrogels can be engineered to resemble the natural ECM of the brain to create a favourable environment that promotes cell proliferation and differentiation.[92] This helps reduce the risk of inflammation and immune responses that could hinder recovery. Generally, the biocompatibility of hydrogel-based materials is evaluated in terms of their mechanical properties, *in vitro* cytotoxicity, and *in vivo* immunogenicity.

A close match between the elastic modulus of the biomaterial and that of brain tissue is desirable to minimise inflammation, scarring, and other adverse effects. The elastic modulus of brain tissue ranges from approximately 150 Pa to 7 kPa according to previous studies with different methods and sample preparations.[93,94] Therefore, biomaterials with elastic moduli within this range are often considered suitable for brain implantation. However, within this range, hydrogel-based biomaterials with different elastic moduli can differentially interact with the host tissue. Lam et al. reported that, compared with softer hydrogels with an elastic modulus of approximately 300 Pa, stiffer hydrogels with an elastic modulus of approximately 1300 Pa lead to more reactive astrocytes in the naïve brain and more inflammatory cells in the stroke brain.[95] However, thorough studies to determine the optimal elastic modulus within this range that induces a minimal inflammatory response and supports regeneration and repair to the greatest extent are still lacking. In addition to the elastic modulus, other mechanical properties, which could also play essential roles in the application of biomaterials, are rarely considered when designing brain-implanted biomaterials; these other properties include tensile strength, viscoelastic responses, energy dissipation, conductivity, and mass diffusivity[96] (**Figure 4**). The way in which these properties can be manipulated and how they affect the interaction between biomaterials and the brain are worth investigating to improve the integration of biomaterials after brain implantation.

Most hydrogels are composed of biologically inert materials, and thus they pose minimal risks of direct cytotoxicity to cells. Therefore, *in vitro* seeding or coculture of neurons/neural stem cells within hydrogels usually results in excellent cell viability, even in long-term testing[97,98] (**Figure 5**). *In vivo* immunogenicity is usually estimated according to the number of reactive astrocytes and microglia/macrophages surrounding the lesion area.[99] Due to their high water content and soft consistency, hydrogels generally elicit fewer foreign body reactions *in vivo* than traditional implantable devices. Nevertheless, the inflammatory response after long-term hydrogel implantation is still worth investigating. Modo et al.[100] used ferromagnetic resonance imaging to longitudinally track the infiltration of perfluorocarbon-labelled blood-circulating immune cells into peri-infarct tissues and an ECM bioscaffold.[100,101] In that study, acute infiltration of neutrophils and macrophages occurred within 6 hours after implantation and peaked between 12 and 18

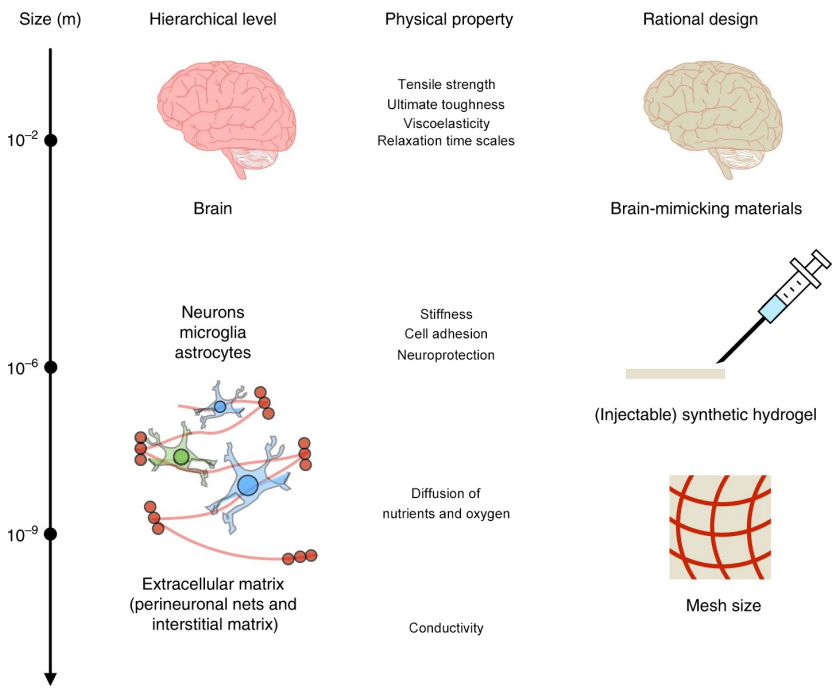

Figure 4. The extracellular matrix of the brain (perinueonal net and the neural interstitial matrix) and its protein nanoscale building blocks give brain tissue its physical properties that differ from region to region. Resembling at different scales mechanical properties such as the tensile strength, ultimate toughness, viscoelasticity, relaxation times, stiffness, cell adhesion, and well as other physical features such diffusion of nutrients and oxygen and conductivity are key for the rational design of brain tissue mimicking hydrogels. Biomaterials comprehensively capturing the diverse properties of brain tissue can propel applications such as neural probes for brain–machine interfaces, brain organoids for studying neurogenesis or drug screening and injectable materials to substitute current hard materials to treat certain types of brain tumours such as glioblastoma multiforme. Reprinted from Axpe et al.[96] Copyright 2014 Acta Materialia Inc.

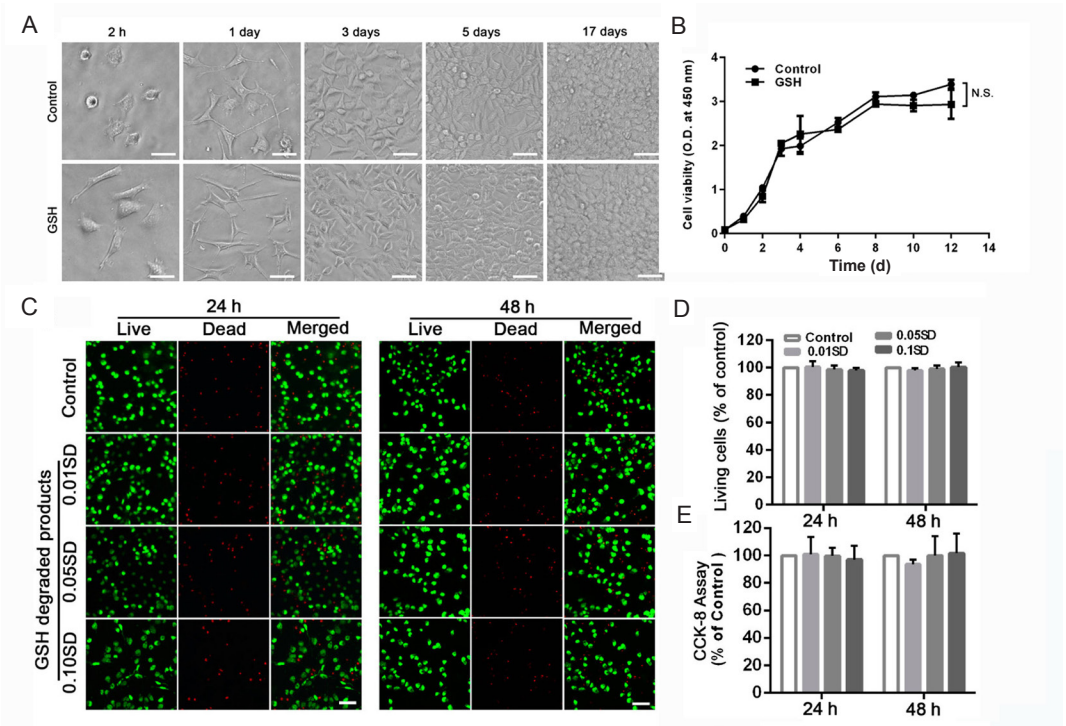

Figure 5. The GSH supports human neuronal cells (SHSY-5Y cells) long-term *in vitro* growth. (A). The representative phase-contrast images showing that the cells grew on the wells of a 24-well culture plate (control) or the GSHs formed at the bottom of the wells of a 24-well plate for 20 days. Scale bars, 50 μm. (B) Quantification of the O.D. value at 450 nm for CCK-8 assay that was used for analysing cell viability. Data were analysed by Student's *t*-test. The cyto-compatibility of the sericin solution and the GSH degraded products with mouse primary cortical neurons. (C) The Live & dead staining for primary cortical neurons 24 and 48 hours after treated with the sericin solution (SS; mg/mL) or the GSH degraded products (SD; mg/mL) at the indicated concentrations. The cells received no treatment were the controls. The green staining indicates living cells while the red staining indicates dead cells. Scale bars: 50 μm. (D, E) Quantification of the percentage (normalised to the control) of living cells in C examined by live & dead staining (D) or CCK-8 assay (E). Reprinted from Wang et al.[98] Copyright 2015 American Chemical Society. Abbreviations CCK-8: Cell counting kit-8; GSH: genipin-cross-linked sericin hydrogel; N.S.: not significant; O.D.: optical density.

hours post-implantation.[100] Fernández-García et al.[102] reported acute accumulation of microglia/macrophages 72 hours after the implantation of silk fibroin hydrogels, which decreased over time, and revealed no significant inflammation one month after implantation. The immune response induced by biomaterial implantation plays a complex role in regeneration and repair after stroke. Adaptive immune responses were found to be involved in the degradation of D-peptide cross-linked microporous annealed particle hydrogel scaffolds through the recruitment of myeloid cells. A lack of adaptive immune system activity hinders skin regeneration induced by the D-peptide cross-linked microporous annealed particle hydrogel.[103] Therefore, proper immune responses help to coordinate the degradation of hydrogel scaffolds and the infiltration/regeneration of host brain cells in the stroke cavity. Clarifying host tissue inflammatory responses and their impact on neural regeneration after stroke within a longer timeline is critical for the clinical translation of hydrogel-based therapy. In addition, the degradation of most hydrogel-based materials *in vivo* can occur over several months.[104] Studying how host tissues react to degraded products over the long term is therefore essential for determining whether the degraded products of hydrogels pose any risk of toxicity to neighbouring cells or if they adversely impact neuronal survival, proliferation, and differentiation, which remains poorly understood *in vivo*. Moreover, it is important to investigate whether any leachable substances or byproducts generated during hydrogel manufacturing, sterilisation, or storage procedures might compromise long-term safety.

5.3. Selection of preclinical models

When selecting preclinical models for the evaluation of hydrogel-based materials for stroke treatment, it is essential to choose animal species and experimental designs that closely recapitulate the pathophysiology and progression of stroke in humans. Animal models of stroke include distal middle cerebral artery occlusion, photothrombosis, endothelin-1 induced vasoconstriction, transient middle cerebral artery occlusion (tMCAO) with intraluminal suture, and embolic middle cerebral artery occlusion (eMCAO).[105] Among them, distal middle cerebral artery occlusion and photothrombosis are the most common disease models in stroke research involving biomaterial implantation because of their relatively stable lesion areas and ease of operation compared with the tMCAO and eMCAO models. While considering the resemblance to the pathophysiology of human stroke, tMCAO and eMCAO are the best choices. These methods result in large cavities of liquefactive necrosis which is also observed in human patients. For the eMCAO model, thrombosis with tissue plasminogen activator can serve as a positive control or as a model of combination therapy with a proposed new therapy. However, the high degree of variation among animals in the tMCAO and eMCAO models

also prompts greater requirements for the stability of operation and the number of animals used (**Table 1**). Thus, careful considerations should be given so that the most relevant model can be selected according to the study objective.

Considering age and sex differences is vital for designing preclinical stroke studies involving hydrogel-based materials.[106] Ageing affects stroke susceptibility, lesion size, and recovery patterns. The incidence of stroke doubles every decade in individuals over the age of 45 years, and 70% of stroke patients are older than 65 years.[107] In addition, ageing exacerbates stroke outcomes. For example, increased permeability of the blood-brain barrier has been reported in aged mice compared with their younger counterparts.[108] Therefore, it is crucial to incorporate aged animals in experiments whenever possible. Similarly, sex differences also affect stroke incidence, severity, and outcomes. For example, elderly women older than 85 years have a higher stroke incidence than elderly men.[109] In terms of therapeutics, caspase inhibitors are beneficial for female mice but not male mice.[110] However, the inhibition of poly(adenosine 5'-diphosphate-ribose) polymerase 1 and nitric oxide only works for male animals, with no benefits for female animals.[111,112] Hence, an ideal study should include both male and female animals to account for potential variations.

With respect to the selection of animal species, rodents, particularly rats and mice, remain the primary choice for preclinical stroke studies because of their well-characterised genetics, availability of numerous transgenic lines, ease of handling, low cost, and established surgical techniques. Nevertheless, the use of nonhuman primates offers certain advantages, especially when pharmacokinetics, haemorrhagic transformation, and cognitive behavioural assessments are studied.[113-115] In particular, in terms of biomaterial implantation, when considering the large infarct size of human patients during clinical translation, nonhuman primates have natural superiority in providing a comparable infarct cavity to that of human brains.[116,117] Cook et al.[116] used a hydrogel as a carrier of brain-derived neurotrophic factor for the sustained release of brain-derived neurotrophic factor in the infarct cavity in *Macaca fascicularis*. When combined within hydrogels, brain-derived neurotrophic factor was released 2 cm from the infarct, which is comparable to the infarct size of human stroke patients. However, very few studies have tested hydrogel-based biomaterials as therapies for stroke in nonhuman primates. Future studies investigating the biocompatibility and long-term efficacy of hydrogel-based therapy in nonhuman primates will increase confidence in its clinical translation.

By considering these factors when selecting preclinical models, researchers can maximise the relevance and applicability of their findings, which would expedite the transition from foundational scientific discoveries to clinical applications.

5.4. Optimisation of noninvasive clinical methods for tracking implanted biomaterials

The implantation of biomaterials into the brain can be achieved through either craniotomy or stereotaxic injection, depending on the physical properties of the biomaterial. For bulk hydrogel scaffolds, craniotomy is needed for implantation, which might result in additional comorbidities. For *in situ* hydrogel and hydrogel microspheres, stereotaxic injection can be utilised with minimal invasiveness. However, regardless of the implantation method, it is necessary to monitor the biomaterials *in vivo* after implantation.[118]

Various imaging modalities are clinically available for monitoring implanted biomaterials *in vivo*, including

Table 1. Models of focal ischaemic stroke

Type	Model	Description	Advantages	Disadvantages
Transient model	Intraluminal filament model	Occlusion of the MCA by inserting filament into the MCA	Controlled occlusion and reperfusion time	Skills needed for the surgery and complications induced by the surgical manipulation
	Endothelin-1 model	Use of endothelin-1 as a vasoconstrictor in the middle cerebral artery after local application	Can induce brian ischaemia in conscious animal, which is a character of human patients. Non-surgical and minimally invasive with high reproducibility	Limited clinical relevance. The timing and degree of reperfusion can be variable and less controlled
	Embolic model	The introduction of a clot or embolic material occlude the MCA	Autologous blood clot can be used. Reperfusion can be induced in this model through pharmacological thrombolysis (e.g., with tissue plasminogen activator, tPA) or mechanical thrombectomy, allowing for the study of both ischaemic injury and reperfusion injury. Closely mimic the pathophysiology of human thromboembolic stroke	Limited control in consistent embolus delivery and in embolic duration and location of ischaemia; high mortality
Permanent model	Distal coagulation model	Coagulating the distal part of the MCA after craniotomy	Localised ischaemia with high reproducibility	The lesion is limited to the cortex. Limited penumbra and surgical skills needed
	Photothrombotic model	Form thrombus through a light-sensitive dye, which can generate ROS, leading to platelet aggregation after activation by a specific wavelength of light	Non-invasive to the major blood vessels, localised lesions and high reproducibility	Lack of collateral circulation and limited penumbra

Note: MCA: middle cerebral artery; ROS: reactive oxygen species; tPA: tissue plasminogen activator.

ultrasound, CT, MRI, positron emission tomography, single-photon emission CT, and optical coherence tomography[119] (**Figure 6**). Each modality offers unique advantages depending on factors such as spatial resolution, depth penetration, and specificity. However, these methods have limitations considering their application in the brain, where the soft brain parenchyma is encapsulated by the thick cranium. This feature excludes the use of ultrasound and optical coherence tomography, which have limited depth penetration, for *in vivo* monitoring of hydrogel implants in the brain.

Positron emission tomography/single-photon emission CT are nuclear imaging techniques that utilise radioactive tracers to visualise metabolic activity, molecular targets, and regional physiological functions in living organisms. Theoretically, labelling biomaterials with radioactive tracers can enable imaging of the implanted biomaterials *in vivo*. However, the degradation of hydrogel-based biomaterials to fulfil their scaffold function is usually a slow process, and long-term monitoring of the implanted biomaterials requires repeated imaging. The presence of radioactive tracers in the brain over a long-time frame or repeated exposure to radioactive tracers can impose a severe radiation burden. Therefore, positron emission tomography/single-photon emission CT is not suitable for monitoring biomaterial distribution, degradation, and interaction with host tissues in the brain.

CT and MRI are the most common imaging methods used in the clinic for brain imaging. CT scans utilise X-rays to generate detailed cross-sectional images of the brain, which involves ionising radiation. Compared with MRI, CT is much faster, and therefore, CT is ideal for emergencies such as stroke and brain trauma, where rapid diagnosis is crucial. Nevertheless, repeated ionising radiation exposure should be avoided as much as possible. In addition, in terms of imaging quality, CT does not provide good soft tissue contrast compared with MRI. MRI is therefore the preferred choice for long-term monitoring of implanted biomaterials in the brain because of its superior soft tissue contrast and the absence of radiation exposure.

MRI has been widely used in the monitoring of hydrogel-based biomaterials in the brain, especially for stroke research, in which the position and size of the infarct cavity should be determined before the implantation of hydrogel-based biomaterials.[104,116,119,120] Diffusion imaging combined with T2-weighted imaging can be employed to visualise the infarcted area *in vivo*.[120] Bible et al.[120] used a ^{19}F-MRI contrast agent to label neural stem cells so that their distribution could be monitored when they were transplanted together with an ECM hydrogel. However, direct monitoring of hydrogel-based materials in the brain *in vivo* is challenging. Chemical exchange saturation transfer (CEST) MRI has been used to visualise hydrogel-based materials in the brain[121,122] (**Figure 7**). An individual component of a hydrogel can cause a diamagnetic CEST effect, which can be used to analyse its distribution and biodegradation *in vivo*. Jin et al.[121] reported that the diamagnetic CEST signal remains stable despite variations in pH and temperature. However, the CEST signal is not a direct detection of hydrogel molecules, but rather, is a measurement of the labile proton content, which may also be produced by other molecules. Therefore, the CEST signal is not specific to hydrogel-based materials. Contrast agents with

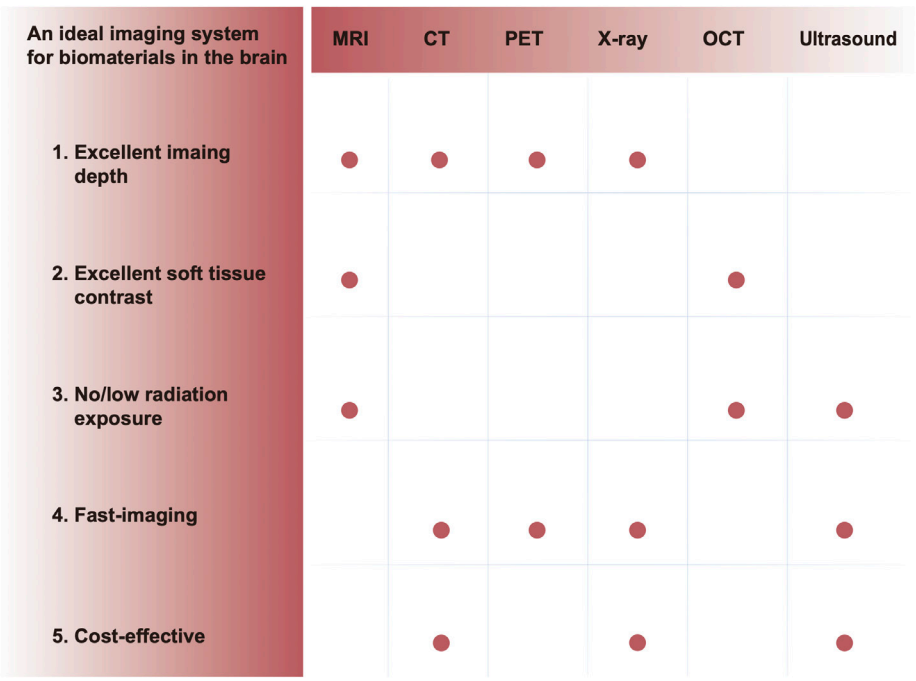

Figure 6. Comparison of clinically available imaging techniques for imaging of biomaterials in the brain. An ideal imaging system for biomaterials in the brain should have excellent imaging depth and soft tissue contrast, with no/low radiation exposure, be capable of fast-imaging and cost-effective. MRI, CT, PET, X-ray, OCT, and ultrasound have advantages in some of these aspects but not all of them. Created with Adobe Illustrator 2021. Abbreviations: CT: computed tomography; MRI: magnetic resonance imaging; OCT: optical coherence tomography; PET: positron emission tomography.

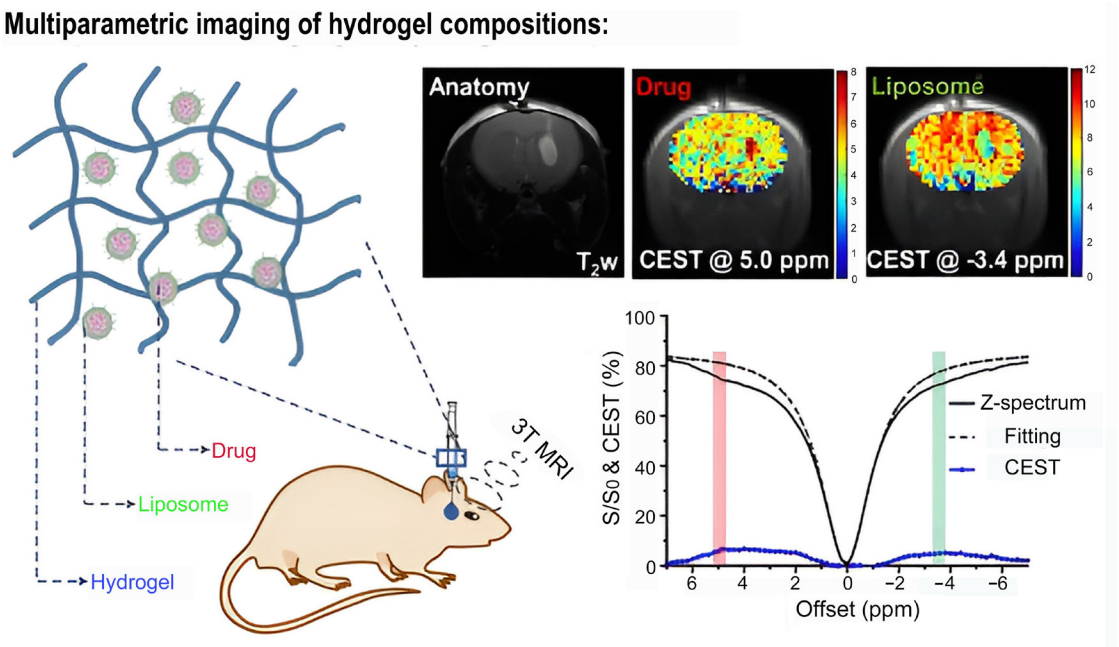

Figure 7. Chemical exchange saturation transfer (CEST) magnetic resonance imaging (MRI) was developed to detect and monitor injectable liposomal hydrogel *in vivo* at 3T clinical field. Mechanical attributes of these hydrogels and their *in vitro* and *in vivo* CEST imaging properties were systematically studied. Reprinted from Han et al.[122]

no risk of radiation exposure could be used to label individual components of hydrogel-based materials. However, the incorporation of contrast agents into hydrogels might affect the mechanical and physiological properties of the material, which should be carefully investigated before any translational study.

In summary, although multiple noninvasive brain imaging techniques are available, current clinical options for noninvasive tracking of implanted biomaterials are limited. Ongoing efforts in developing novel imaging techniques, targeted probes, spectroscopy methods, and biochemical marker assessments hold great promise for future advances in this field.

5.5. Standardisation and quality control

Limited clinical evidence currently supports the safe adoption of hydrogel-based therapies in stroke patients. Large-scale randomised controlled trials with extended follow-up durations are needed to validate the safety of hydrogels in human subjects. Factors such as patient selection criteria, dosage, administration routes, and combination strategies with conventional treatments must be carefully evaluated before these novel technologies can be translated into standard care. In addition, as new hydrogel formulations emerge, ensuring consistent production methods, characterisation protocols and rigorous testing will become increasingly critical for standardised production and quality control of new hydrogel materials. The development of industry standards and regulatory guidelines will facilitate the development of safer and more reliable hydrogel-based products suitable for stroke therapy.

5.6. Manufacturing scalability and regulatory compliance

Hydrogel-based materials present exciting opportunities for stroke treatment because of their adjustable physical properties, biocompatibility, and ability to mimic native tissue environments. However, manufacturing scalability and regulatory compliance are critical considerations when hydrogels are translated from benchtop experiments to clinical applications. To ensure consistent production quality and large-scale manufacturability, it is essential to establish robust fabrication protocols suitable for industrial settings. This includes the identification of appropriate raw materials, optimisation of synthesis conditions, and the design of reproducible processes amenable to automation. Common fabrication techniques include bulk polymerisation, microfluidics, electrospinning, photolithography, and 3D printing. Among these, 3D printing has gained significant attention because it allows precise control over hydrogel architecture and enables personalised medicine tailored to individual patients' needs. Nevertheless, scaling up hydrogel production requires careful optimisation and validation of sterilisation procedures to ensure consistency across batches.

In addition, obtaining approval from regulatory agencies such as the U.S. Food and Drug Administration, is crucial for bringing any therapeutic product to the market. Key requirements include demonstrating safety, efficacy, and quality control during the entire lifecycle of the hydrogel-based material. Preclinical studies involving animal models should follow Good Laboratory Practice guidelines. It is also important to engage with regulators early in the development process to facilitate a smooth transition through different phases of regulatory approvals.

6. Limitations

This review has several limitations. We concentrated on hydrogel-based materials, highlighting their properties and advantages. However, we did not summarise whether

hydrogels made from different materials, such as chitosan and hyaluronic acid, might have varying therapeutic effects for stroke treatment. Due to space constraints, we did not cover hydrogels with different moduli, pore sizes, or degradation rates. Furthermore, protein-based hydrogels and their potential biomedical applications for stroke treatment were not addressed.

7. Conclusions

Hydrogel-based biomaterials hold great potential for clinical translation in the field of regenerative therapy for stroke. However, few clinical studies have been conducted thus far. In this review, we first summarised the regenerative therapies for stroke followed by the current clinical needs; we then discuss the functions of hydrogel-based biomaterials in promoting brain repair and regeneration after stroke. We focused on the gap that needs to be filled by preclinical studies before safe clinical trials are conducted, including clarification of the long-term host responses induced by biomaterials and their degradation products. In the future, to advance the clinical application of hydrogels, ensuring that hydrogels are biocompatible and do not elicit adverse immune responses. Careful selection of appropriate pre-clinical stroke models and animal species, with consideration for sex as well as age, always needs to be addressed. Hydrogels must be designed to withstand the conditions within the body and maintain safe and functional over time. The optimisation of noninvasive biomaterial imaging methods is advancing, which enables the monitoring of hydrogels' long-term effects in the brain. Finally, as with any emerging medical technology, hydrogels must navigate regulatory pathways before widespread clinical use. Regeneration of the central nervous system is a hot topic in both academia and industry, and filling the gap in clinical translation should be an effort in future studies of hydrogel-based biomaterials.

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Conflicts of interest statement
Zhibin Wang, Qianqian Liu, and Jie Chen are employees of Lingyi iTECH Manufacturing Co., Ltd., Ankerui (Shanxi) Biological Cell Co., Ltd., and Jiangsu Charity Biotech Co., Ltd., respectively. There is no conflict of interest for this review.

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Author contributions
Conceptualization: HHL, TTG, YHT; *Writing-original draft:* HLL, TTG, JJX, LG. *Writing-review & editing:* All authors. All authors read and approved the final manuscript.

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Consent for publication
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Availability of data
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