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Smart/stimuli-responsive chitosan/gelatin and other polymeric macromolecules natural hydrogels vs. synthetic hydrogels systems for brain tissue engineering: A state-of-the-art review

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ABSTRACT

Currently, there are no viable curative treatments that can enhance the central nervous system's (CNS) recovery from trauma or illness. Bioengineered injectable smart/stimuli-responsive hydrogels (SSRHs) that mirror the intricacy of the CNS milieu and architecture have been suggested as a way to get around these restrictions in

Abbreviations: 3D, Three-dimensional; 3-NT, 3-Nitrotyrosine; 4-HNE, 4-Hydroxynonenal; Alg., Alginate; AMPA, Amino-3-hydroxy-5-methyl-4-isoxazole propionate; APP, Amyloid precursor protein; BBB, Blood-brain barrier; BMSCs, Bone marrow stem cells; CGP, Chain growth polymerization; CH, Chitosan; ChABC, Chondroitinase ABC; CMC, Carboxymethyl cellulose; CNS, Central nervous system; CNTs, Carbon nanotubes; CSF, Cerebrospinal fluid; CSPGs, Chondroitin sulfate proteoglycans; CST, Corticospinal tract;; dscECM, Decellularized spinal cord ECM; DTT, Dithiothreitol; ECM, Extracellular matrix; EGDMA, Ethylene glycol dimethacrylate; ERK, Extracellular signal-regulated kinase; ESCs, Embryonic stem cells; FGF, Fibroblast growth factor; FGF2, Fibroblast growth factor-2; FRP, Free-radical polymerization; GBM, Glioblastoma multiforme; GelMA, Gelatin methacryloyl; GOX, Glucose oxidase; H_2O_2, Hydrogen peroxide; HA, Hyaluronic acid; hiPSC, Human-originated pluripotent stem cell; HLB, Hydrophilic-lipophilic balance; hMSCs, Human MSCs; HP, Heparin-poloxamer; HPA-Gtn, Hydroxyphenylpropionic acid- gelatin; HRP, Horseradish peroxidase; ICH, Intracerebral hemorrhage; iPSCs, Induced pluripotent stem cells; JAK/STAT, Janus kinase/signal transducer and activator of transcription; LCST, Lower critical solution temperature; MAA, Monomer methacrylic acid; MAG, Myelin-associated glycoprotein; MAIs, Myelin-associated inhibitors; MC, Methylcellulose; MGMT, Methyltransferase; MMP, Matrix metalloproteinase; MMP-9, Metalloproteinase-9; MNHs, Macroporous nanocomposite hydrogels; MNPs, Magnetic nanoparticles; MSCs, Mesenchymal stem cells; MWCNTs, Multi-walled carbon nanotubes; NCPPs, Polylactic acid-PEG; NF, Neurofilament; NGF, Nerve growth factor; NgR, Nogo receptor; NIPAAm, *N*-isopropyl acrylamide; NMDA, *N*-methyl-D-aspartate; NOS, Nitric oxide synthases; NPs, Nanoparticles; NSCs, Neural stem cells; OMgp, Oligodendrocyte myelin glycoprotein; PA, Polyacrylamide; PAA, Polyacrylic acid; PBI, Piercing brain injury; PEDOT, Poly(3,4-ethylene dioxythiophene); PEG, Polyethylene glycol; PEGDA, Poly(ethylene glycol) diacrylate; PEGDMA, PEG dimethacrylates; PEI, Polyethylene-imine; pHEMA, Poly(2-hydroxyethyl methacrylate); PICH, Primary intracerebral hemorrhage; PLA, Poly lactic acid; PLGA, Poly (lactide-*co*-glycolide); PLL, Poly L-lysine; PN, Peroxynitrite; PNIPAM, Poly-N-isopropyl acrylamide; PTX, Paclitaxel; PVA, Polyvinyl alcohol; SCI, Spinal cord injury; SilMA, Silk fibroin forming methacrylate; SPIONs, Superparamagnetic iron oxide nanoparticles; SSRHs, Smart/stimuli-responsive hydrogels; TA, Tannic acid; TBI, Traumatic brain injury; TE, Tissue engineering; TMZ, Temozolomide; UCST, Upper critical solution temperature; US, Ultrasound; WDI, Weight drop injury.

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combination with medication and cell therapy. Additionally, the right biophysical and pharmacological stimuli are required to boost meaningful CNS regeneration. Recent research has focused heavily on developing SSRHs as cutting-edge delivery systems that can direct the regeneration of brain tissue. In the present article, we have discussed the pathology of brain injuries, and the applicable strategies employed to regenerate the brain tissues. Moreover, the most promising SSRHs for neural tissue engineering (TE) including alginate (Alg.), hyaluronic acid (HA), chitosan (CH), gelatin, and collagen are used in natural polymer-based hydrogels and thoroughly discussed in this review. The ability of these hydrogels to distribute bioactive substances or cells in response to internal and external stimuli is highlighted with particular attention. In addition, this article provides a summary of the most cutting-edge techniques for CNS recovery employing SSRHs for several neurodegenerative diseases.

1. Introduction

The central nervous system (CNS) is thought to be most affected by neurological conditions such as Alzheimer's disease, Parkinson's disease, epilepsy, brain stroke, ischemia, multiple sclerosis, and glioblastoma. Their effects are typically debilitating, and patients occasionally experience lasting disability. Glioblastoma multiforme (GBM) is an extremely malignant form of brain tumor, and at present, there is no cure for it. The conventional approach for treating GBM is surgical intervention to extract the maximum extent of the tumor, succeeded by a regimen of radiation therapy and chemotherapy [1]. The architecture of the neural tissue is drastically disrupted by various traumatic events such as spinal cord injury (SCI), traumatic brain injury (TBI), and hemorrhage (such as primary intracerebral hemorrhage, PICH), which result in the loss of parenchymal mass. Additionally, the development of cystic cavities and glial scarring disrupt axonal circuits, resulting in long-term neurological impairments and disabilities [2,3].

In addition, scarring is frequently viewed as the principal barrier preventing the recovery and/or regeneration of CNS tissues due to the CNS's lower capacity for regeneration following any type of damage compared to other organs and tissues (such as the liver, lung, skin, and bone) [4–9]. Attractive curative treatments that can fully stimulate CNS recovery are not yet available. Using novel biomolecules or mediators, such as *N*-methyl-D-aspartate (NMDA) receptor antagonists and monoclonal antibodies, a few optimistic animal experiments on CNS regeneration from the previous few decades have demonstrated improved neuronal network restoration. However, the same mediators failed to successfully regenerate the CNS when they were utilized in clinical trials [10,11]. New drug candidates currently being developed for the treatment or mitigation of CNS diseases typically have a low market entry success rate (8 %) and a failure rate of >30 % throughout all phases of clinical trials, taking 18 % longer to develop and receive approval than any other drug targeting systems [12].

Aside from this, there are a number of additional causes for the clinical transition's delay. First, the CNS is still not fully understood, and there is a considerable lack of knowledge of several important cellular and molecular pathways and healing mechanisms, which restricts the creation of new treatments. The blood-brain barrier (BBB), a highly selective semipermeable membrane that only permits the entry of low molecular weight and non-polar molecules (<400 Da) to the CNS, drastically reduces the number of promising biomolecules for novel treatments. Thirdly, the limited ability of the nervous system to regenerate is due to the intrinsic properties of the neural parenchyma, including insufficient progenitor neural cells, mature neural cells' slow ability to regenerate, proliferate, and migrate, and the microenvironment's biochemical characteristics [13]. The use of stem cells has been suggested as a modern strategy in cell-based therapeutics, disease modeling, and drug testing to enhance CNS regeneration to get around these constraints. In reality, stem cells induce neural cell differentiation and stimulate nerve regeneration, which allows them to heal damaged neurological systems [14,15].

Poor cell survival, uncontrolled cell differentiation, and inefficient integration into the host tissue, however, restrict the therapeutic effectiveness of stem cell transplantation [16,17]. The loss of parenchymal mass causes a fundamental disturbance of the brain tissue architecture in some pathological diseases like SCI, PICH, and TBI, which leads to severe and long-lasting neurological impairments and disabilities [18–21]. Recent developments in biomaterials and tissue engineering (TE) have suggested the use of implanted or injectable bioengineered biomaterials to repair the damaged brain tissue architecture [22–25]. The engineered scaffolds should mimic the native structure from nanoscale to macroscale, exhibit adequate mechanical, conductive, and structural properties, provide the right biophysical and biochemical cues inducing cell growth, proliferation, and differentiation, and ensure technical design reproducibility to represent an ideal tissue substitute. In this situation, hydrogels physiologically display the characteristics of soft tissues, possibly acting as a mimicking niche for stem cells.

One of the many benefits of natural hydrogel systems (collagen, hyaluronic acid, chitosan, gelatin, and alginate) over their synthetic alternatives is their biocompatibility. Because they are chemically and structurally comparable to natural soft tissues [26]. Also, the biodegradability of natural hydrogels can undergo decomposition by natural processes, making them crucial for applications in the field of biomedicine [27]. Natural hydrogels have minimal cytotoxicity, rendering them appropriate for a wide range of biomedical applications [26].

However, mechanical properties and batch uniformity are issues with natural hydrogels. For these limits, natural hydrogels are often mixed with synthetic polymers to make composites. Consider these factors while selecting hydrogel polymers, collagen, gelatin, hyaluronic acid, chitosan, and alginate can be classified into protein-based, polysaccharide-based, and decellularized tissue-based natural polymers. Each hydrogel variety has unique features that make it better for certain purposes. The polymer composition and molecules' arrangement affect the hydrogel's ability to interact with biological things, break down organically, and sustain mechanical pressures [26].

To fit unequal imperfections, hydrogels can be directly injected at the defect location as a viscous solution to form any desired forms. A particular application can be significantly impacted by the exact control that certain cross-linking processes can offer over the hydrogelation kinetics and structure. A hydrogel's ability to gel can also be controlled by selecting the right polymer, molecular weight, cross-linker concentration, and degree of chemical or physical cross-linking (e.g., UV light, pH, or temperature changes) [28–31]. Additionally, hydrogels can be utilized to encapsulate medications as well as cells, circumventing the drawbacks of traditional drug delivery and cell treatment systems as the smart/stimuli-responsive hydrogels (SSRHs) can improve cell interactions and maturation, enhance cell viability, and empower cell differentiation (Fig. 1) [32].

Additionally, 3D in vitro models for CNS regeneration can be created using hydrogels. Understanding the processes of various CNS illnesses, researching new medication approaches, and getting around the inherent limits of 2D cell investigations can all be aided by simulating the neuronal environment in vitro [33]. As diverse substrates for brain tissue creation, promising efforts have lately been directed at designing internal or exterior SSRHs [34]. Despite these research efforts, there have been no fully completed clinical trials using hydrogels or generally using biomaterial-based therapeutics for CNS regeneration. This review's objective is to provide an overview of the most promising

techniques for hydrogel-based brain TE. Here, we focus on the use of injectable hydrogels to deliver bioactive substances and cells to the CNS and the ability to modify the physical properties of hydrogels in response to both internal and external stimuli.

2. Pathology of brain injury

One of the leading causes of death and morbidity in those under the age of 45 globally is TBI [35]. Traumatic brain injury is described as a non-congenital, non-degenerative, damage to the brain caused by an external physical force that causes an altered or impaired state of awareness, as well as permanent or temporary cognitive and physical deficits [36]. Considerable progress has been achieved in addressing immediate consequences in patients with TBI. However, researchers still face major challenges in improving the overall functioning of severe TBI patients [35]. In this section, we discuss the primary and secondary brain injuries (Fig. 2).

2.1. Primary brain injury

Cerebral hematomas, skull fractures, lacerations, contusions, and penetrating wounds, as well as accident-related brain damage, are the main injuries. It is hypothesized that the first TBI insult occurs in <100 milliseconds. Pressing on fractured skull bones affects the skull and brain blood arteries. Mechanical traumas can cause localized or diffuse brain damage. Discovered that moderate to severe TBI patients often have both types of injuries, while diffuse axonal damage accounts for 70 % of TBI cases [37]. Closed head and penetrating traumatic brain injuries cause focal brain damage from lacerations, compression, and concussion forces, with skull fracture and localized contusion [38].

Injury severity can cause cognitive difficulties, abnormal conduct, and hemiparesis. Unlike localized damage, diffuse brain injury is caused by non-contact forces of rapid deceleration and acceleration shearing and stretching cerebral brain tissues [39]. Tensile forces injure neuronal axons, oligodendrocytes, and blood vasculature, causing cerebral edema and ischemic brain injury [40]. Diffuse TBI causes axonal injury, especially in subcortical and deep white matter tissue like the brain stem and corpus callosum, reducing axonal transit and cytoskeleton disintegration [39]. Axonal impairments may persist for months after a TBI, suggesting a link to delayed hemorrhages and cerebral edema [41]. TBI severity depends on axonal injury and neuronal degeneration. Though caused by shock waves rather than inertial forces, explosive blast TBI has widespread brain damage characteristics [41].

2.2. Secondary brain injury

Multiple factors, such as excitotoxicity, mitochondrial dysfunction, oxidative stress, lipid peroxidation, neuroinflammation, axon degeneration, and apoptotic cell death, contribute to the occurrence of secondary injuries. These injuries have been linked to higher mortality rates and a worsened neurological prognosis at the 6-month mark [42]. Animal and human research indicates that traumatic brain damage results in the excessive release of excitatory amino acids such as glutamate and aspartate from presynaptic nerve terminals [43]. Within 24 h of traumatic brain damage, the expression of astrocytic sodium-dependent glutamate transporters GLAST (EAAT1) and GLT-1 (EAAT2) decreased by 40 %, leading to a significant reduction in glutamate resorption [39].

Both ionotropic and metabotropic glutamate receptors (iGluRs and mGluRs, respectively) are activated by these excitatory amino acids (mGluRs). Members of iGluRs, such as the NMDA receptor and amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor, are ligand-gated ion channels that permit Na^{+}, $K^{+,}$ and Ca^{2+} ionic flow upon glutamate binding, resulting in membrane depolarization in neurons [44]. Excessive activation of glutamate receptors due to the massive

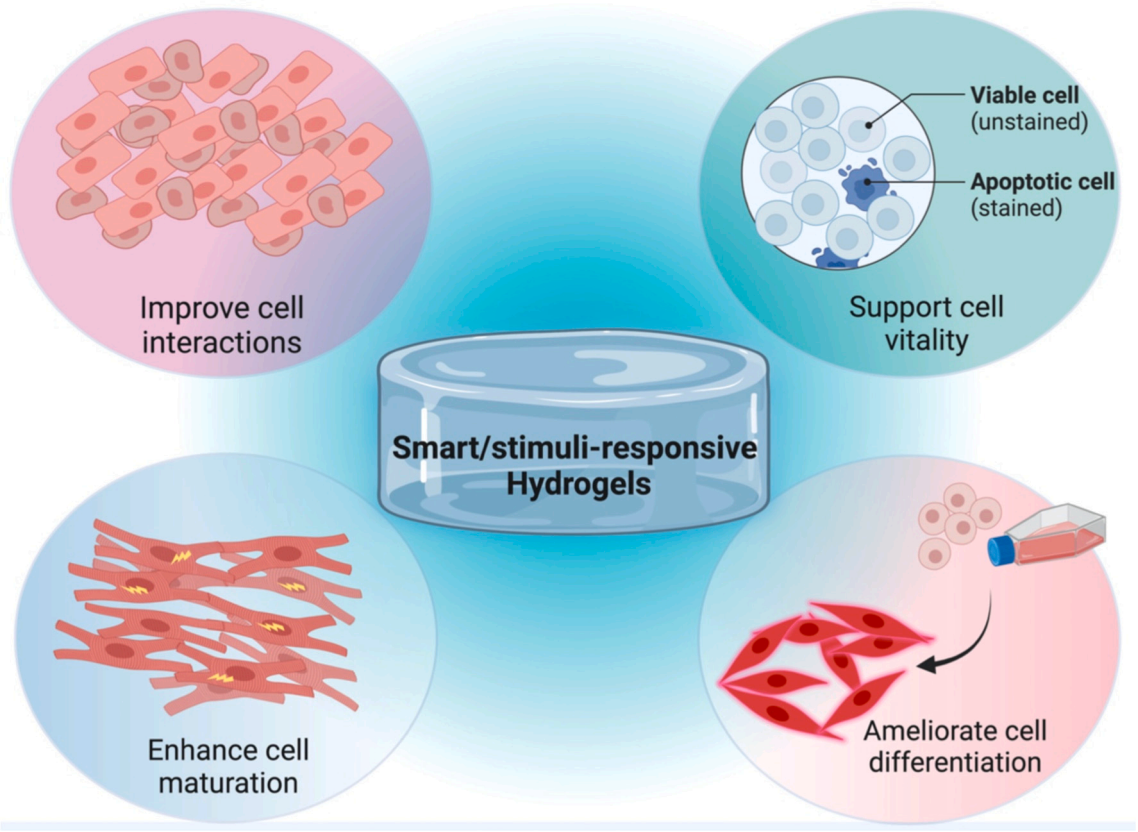

Fig. 1. Role of SSRHs to improve cell-based therapy for brain injuries.

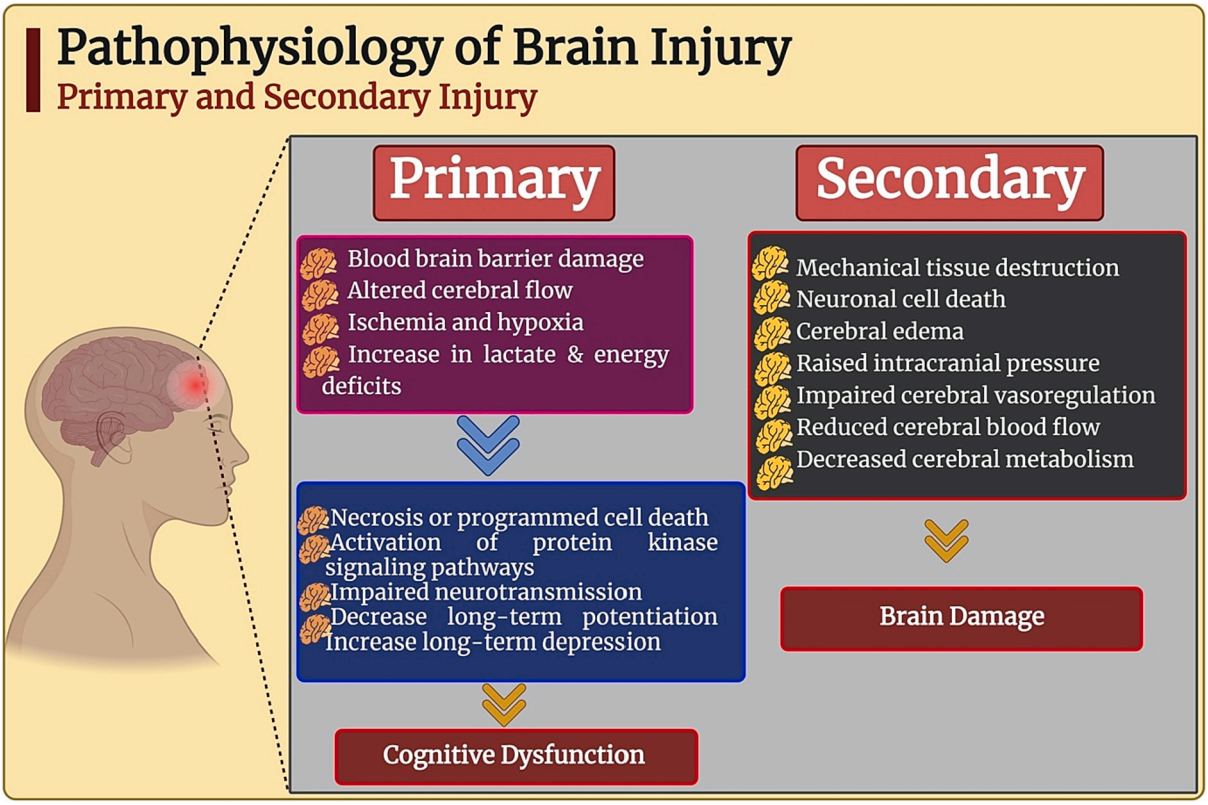

Fig. 2. Schematic illustration of the pathology of primary and secondary brain injuries.

release of excitatory neurotransmitters causes extended post-traumatic oxidative stress and cell death, which corresponds with an increased mortality rate and a worsened 6-month neurological prognosis [44]. The Ca^{2+} accumulation after traumatic brain damage increases the activity of nitric oxide synthases (NOS), hence facilitating NO production. The combination of excessive NO with free radical superoxides creates peroxynitrite (PN), which causes oxidative damage that may be measured by detecting oxidative markers such as 3-nitrotyrosine (3-NT) and 4-hydroxynonenal (4-HNE) [44].

It has been shown that persistent overexpression of several cytokines is associated with increased BBB permeability, edema development, and neurological deficits. As a member of the Fas superfamily, TNF-α interacts closely with the Fas ligand to activate caspases required for programmed cell death. Post-trauma, chemokines such as MIP-1, MCP-1, IL-1β, and IL-8 (CXCL8) are drastically elevated, attracting more leukocytes and neutrophils to the damage site [39]. Delays in neuroinflammation attract macrophages, activate local microglia, and cause astrogliosis. TBI survivors' macrophages and activated microglia show growing phagocytosis and chronic inflammation years after injury [39].

2.2.1. *Glial scar formation and the response of axons*

Frequently, CNS injuries elicit astrocyte activation and proliferation. As a result, reactive astrocytes infiltrate the lesion site and undergo reactive astrogliosis, which is characterized by increasing hypertrophy and the complexity of their processes. Interaction between astrocytic and oligodendrocyte processes, the slow development of meningeal cells, microglia, and fibroblasts. A scar-like development that has been historically associated with a major cause of mortality Fig. 3. TBI is treated by eliminating physical barriers to axonal regeneration recovery [39].

However, glial scar chondroitin sulfate proteoglycans (CSPGs) like neurocan and versican hinder axonal regeneration following CNS damage, according to recent research [39]. These substances, together with other inhibitory molecules in the glial scar, including tenascins and semaphorin 3 A, provide an environment that is not favorable to axonal growth [39]. Intriguingly, the RhoA pathway mediates their inhibitory effects, since the suppression of RhoA activity or its downstream effectors improves permissive axonal growth on these substrates [45].

The signaling cascades formed by semaphorin 3 A in glial scar include the neuropilin-plexin receptor complex and the activation of Rho GTPases, which are hypothesized to induce growth cone collapse via controlling the F-actin cytoskeleton [46]. In addition, damaged myelin in a severed axon exposes axon outgrowth inhibitors, including myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), and Nogo-A [47]. These myelin-associated inhibitors bind solely to the neuronal membrane Nogo receptor (NgR) complex, which consists of the co-receptors p75NTR, Troy, and LINGO-1. The activation of RhoA GTPases and Rho kinases by these inhibitors may induce growth cone collapse and neurite retraction [39].

2.2.2. *Inhibitors of regeneration in myelin*

Given the large number of myelin inhibitors and receptors, scientists have wondered if they work similarly. To study Nogo/MAG/OMgp triple knockout mice, two groups generated them. [48]. In a previous review, they talked in detail about the different mutations and genetic backgrounds that were used [48]. What happened is explained in a few words below. Using in vitro neurite growth assays, both studies found that Nogo has a big effect on how inhibiting CNS myelin is. In one study, getting rid of Nogo-A, MAG, OMgp, or all three did not help corticospinal tract (CST) grow back after a dorsal hemisection, and getting rid of all three did not help 5-HT grow back after a complete cut [49].

After a pyramidotomy, deleting Nogo helped CST grow, and after a lateral hemisection, deleting MAG or OMgp helped 5-HT grow. Myelin inhibitors' effects on axon sprouting were neither additive nor synergistic in any of the cases. This shows that myelin inhibitors may have a limit to how much they can be changed [48]. In the other study, researchers confirmed what they had already found, which was that deleting Nogo alone helped CST grow back. MAG or OMgp deletions alone

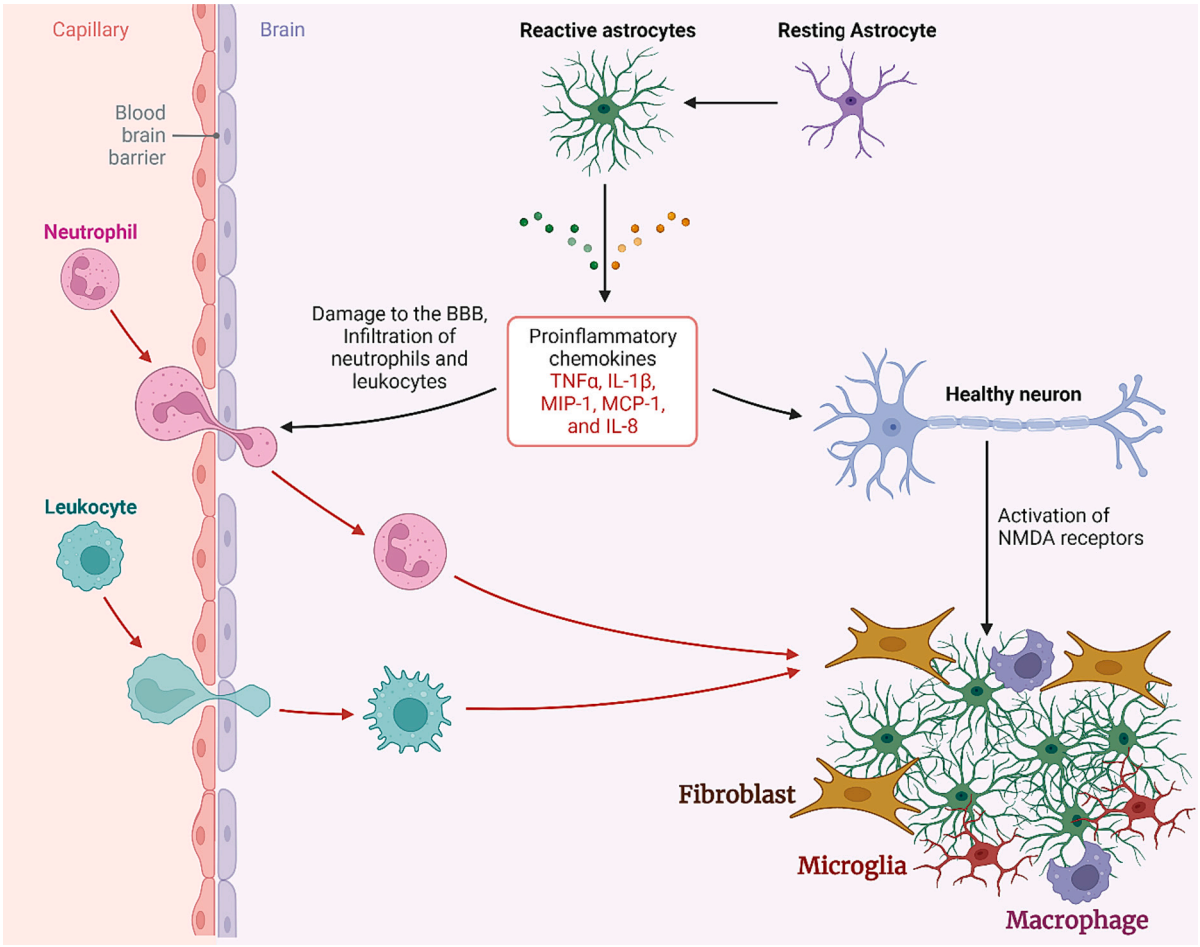

Fig. 3. Reactive astrocytes cause glial scarring and secondary brain injury.

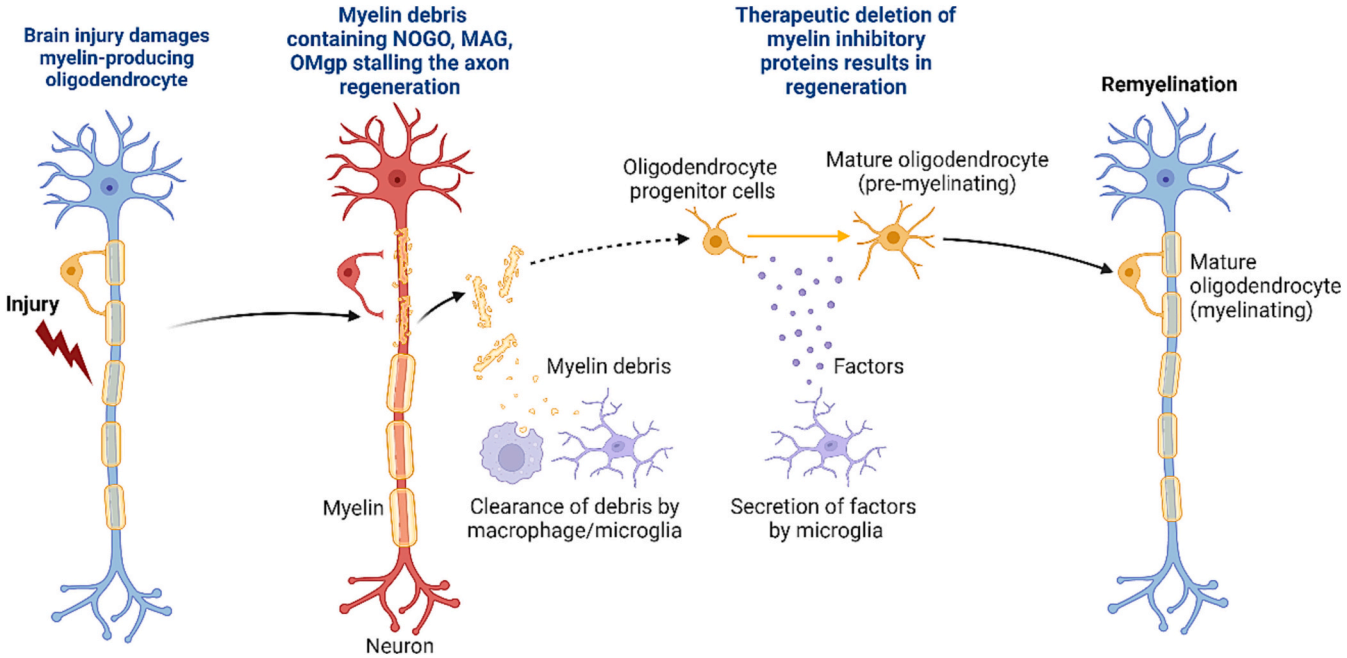

Fig. 4. Therapeutic neutralization of myelin-associated inhibitory proteins following serious brain injury facilitated permissive oligodendrocyte proliferation.

did not cause CST regeneration, but MAG or OMgp deletions plus Nogo deletion caused more CST regeneration than Nogo deletion alone [50] (Fig. 4). Targeting Nogo or all three inhibitors also made 5-HT grow in a model with a partial lesion. Together, these two studies back up the idea that manipulating myelin-associated inhibitors (MAIs) has a more consistent and repeatable effect on axon sprouting than regeneration [50].

Inhibitors of myelin and CSPGs may potentially be functionally redundant. Two different research using NEP1–40 and chondroitinase did not see a synergistic effect on axon growth in a slice culture or organotypic co-culture system [51]. In contrast, acute Nogo-A antibody and delayed Chondroitinase therapy enhance CST development additively and improve functional recovery when combined with a rehabilitation program. Targeting Nogo produced bigger axons while targeting CSPGs produced finer processes with varicosities. The chemical mechanism behind this phenomenon needs further study [51].

2.2.3. *Oligodendrocyte apoptosis and Wallerian degradation*

Apoptosis of neurons and oligodendrocytes characterizes secondary brain injury demonstrating that neuronal cell death is detectable in the human hippocampus for up to a year after TBI [52]. These apoptotic events involve the activation of cysteine proteases such as caspases and calpain and can be triggered by the interaction of various neurochemical, cellular, and molecular pathways, such as extracellular signal-regulated kinase (ERK), p38 MAPK, Janus kinase/signal transducer and activator of transcription (JAK/STAT) Mori dependent cell death may be mediated by either the death receptor pathway or the mitochondrial system [53].

The extrinsic pathway involves the interaction of TNF and Fas with their specific cell surface receptors, while the intrinsic pathway is triggered by the release of cytochrome *c* after mitochondrial depolarization [54]. Cytochrome c, apoptotic activating protein-1, and ATP create an ATP-dependent complex in the cytosol. Both techniques increase caspase-dependent downstream signaling by upregulating and activating caspases 8 and 9, which cleaves and activates caspase 3. Alternately, calpains can activate caspase-independent apoptosis in TBI by proteolyzing cytoskeletal proteins like spectrin and NF and releasing mitochondrial proteins like AIF, Smac/DIABLO, Omi/HtrA2, poly (ADP-ribosyl) polymerase. These mitochondrial proteins enter neuronal and glial cell nuclei and activate downstream signaling molecules, damaging DNA and condensing chromatin. Bcl-2 family proteins and Bax may influence apoptosis. Bcl-2 protein expression is significantly increased in TBI brain tissues, according to studies. Traumatized rats' brains have 25 % more Bax protein [39].

Wallerian degeneration occurs minutes after DAI. Mechanical damage immediately disorganizes the axonal cytoskeletal network's longitudinally oriented neurofilaments and microtubules. Myelin sheath breakdown, axonal transport impairment, and protein accumulation can lead to delayed and secondary axotomy days or months after acute axonal injury. As axonal connections dissociate and transport proteins accumulate in the node, retraction bulbs may cause damaged axons to bulge and neurons and oligodendrocytes to die [39]. In animal models of

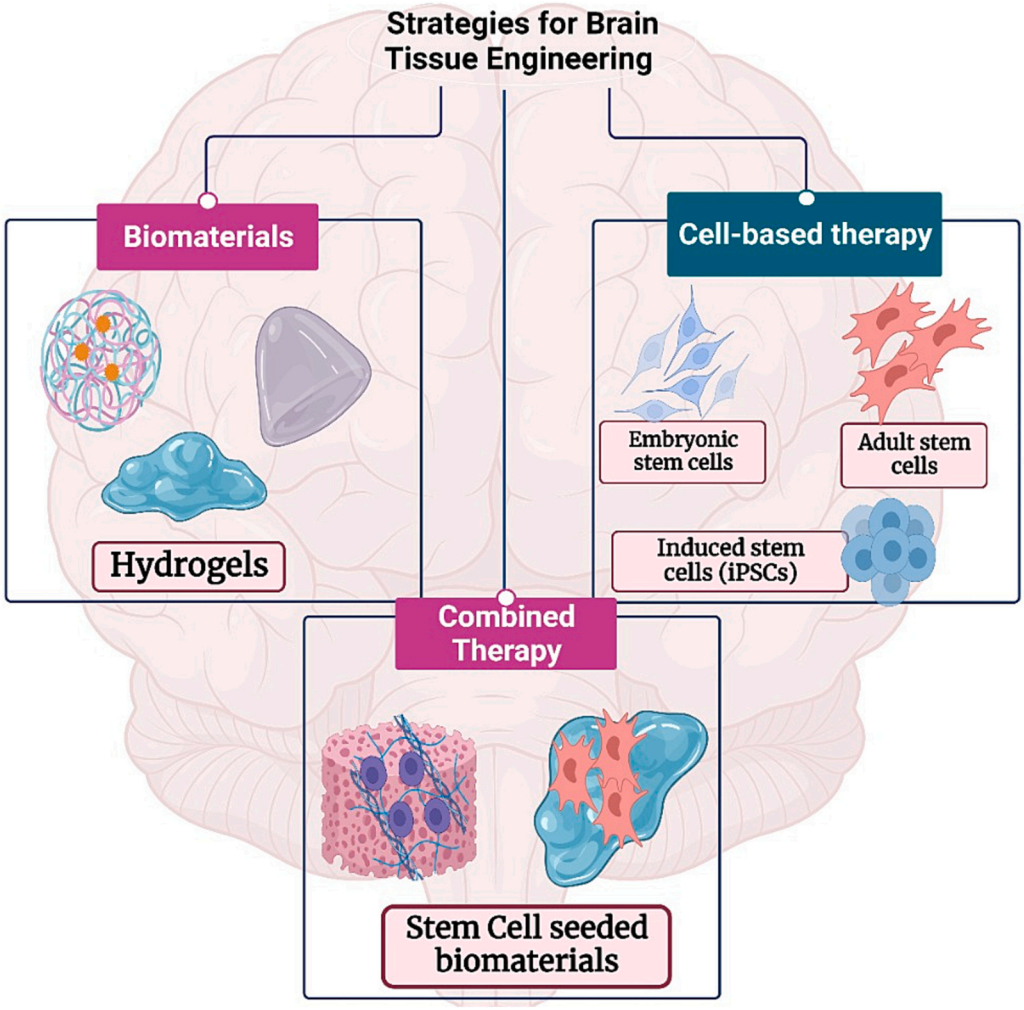

Fig. 5. Current regeneration strategies employed for brain tissue engineering.

widespread TBI, the axonal markers -amyloid precursor protein (-APP) and neurofilament (NF) may identify these retraction bulbs as early as 1-day post-TBI and for up to 2 weeks. Retraction bulbs are most common in the corpus callosum and pyramidal tracts of the brain stem, but they have also been documented in the hippocampus, cortex, cingulum, and internal and external capsule [55]. Hellewell and co-workers demonstrated the relationship between axonal damage in the corpus callosum and the infiltration of neuroinflammatory cells (microglia and macrophages), which results in disruption of blood vessels, degradation of axons, damage to oligodendrocytes, and deformation of white matter [55].

3. Current regeneration strategies for brain TE

There are three principal strategies for treating brain disease or injured brain tissues in patients by brain TE: (a) cell-based repair by implantation of freshly isolated or cultured cells; (b) biomaterials; and (c) combination therapy [56] as illustrated in Fig. 5.

3.1. Cell-based repair

The most basic regenerative medicine strategies depend on the actions of cells, which can be transplanted alone or within a biomaterial like a hydrogel. These cell therapies include injecting or implanting healthy cells to replace populations of cells that have stopped working due to.

disease or injury [57–59]. These therapies can involve autologous cells generated in culture from a tissue biopsy [60]. Stem cells are attractive prospects in regenerative medicine, and they have a big role in developing new therapies for tissue regeneration, especially in brain TE. There are three types of stem cells, embryonic stem cells (ESCs), adult stem cells, and induced pluripotent stem cells (iPSCs) [61]. The iPSCs are a new cell source in TE that eliminates the problems of ESCs as well as the constraints of human tissues. It provides an alternate and limitless source, avoiding the drawbacks of embryonic stem cells while providing significant benefits [62]. Somatic cells are reprogramed to generate iPSCs. Their derived cells can be directly injected, or by injecting neural subtypes like neural precursor cells, glia, and neurons (Fig. 6). Bioprinting can be used to create the expected structure for the tissue. Undifferentiated and differentiated iPSCs applied with 3D bioprinting are the two types of iPSCs created, depending on their intended use [63].

To aid nerve regeneration, many live cell types with or without scaffolding have been used, including mesenchymal stem cells (MSCs), neural stem cells (NSCs) [64], Schwann cells, and olfactory ensheathing cells [65]. MSCs, when cultured with differentiation factors, can differentiate into neurons in vitro in an experimentally controlled manner [66,67]. They can replace damaged cells and maximize their intrinsic regenerative capacity by producing growth factors and cytokines [68]. Using mesencephalic fetal tissue implanted into the putamen in treating neurodegenerative illnesses has also shown encouraging outcomes, particularly in younger patients [69]. Clinical trials were recently completed for treating Alzheimer's disease with mesenchymal stem cell therapy [70]. Bone marrow stem cells (BMSCs) can be employed in nerve regeneration because the production of fibroblast growth factor 2 by BMSCs derived from male Lister rats model of sciatic nerve transection causes a rise in glial cell proliferation, which in turn aids in nerve regeneration [71]. Other studies employing a rat sciatic nerve model revealed that using BMSCs increased axonal regeneration and encouraged remyelination [72]. During the scaffold production process, living cells can be planted into scaffolds or incorporated into printing media [73,74]. Ectopically inducing the formation of neuronal (glial) cells to facilitate brain healing is an alternative strategy [75–77]. The main challenge remains to discover a suitable cell source that might be employed in clinical trials [78]. Cell-based therapy has been widely explored in various strands of TE to overcome the risks of immunogenicity and cell collection. Also, to eliminate tumorigenic factors and improve growth techniques and cell culture [79].

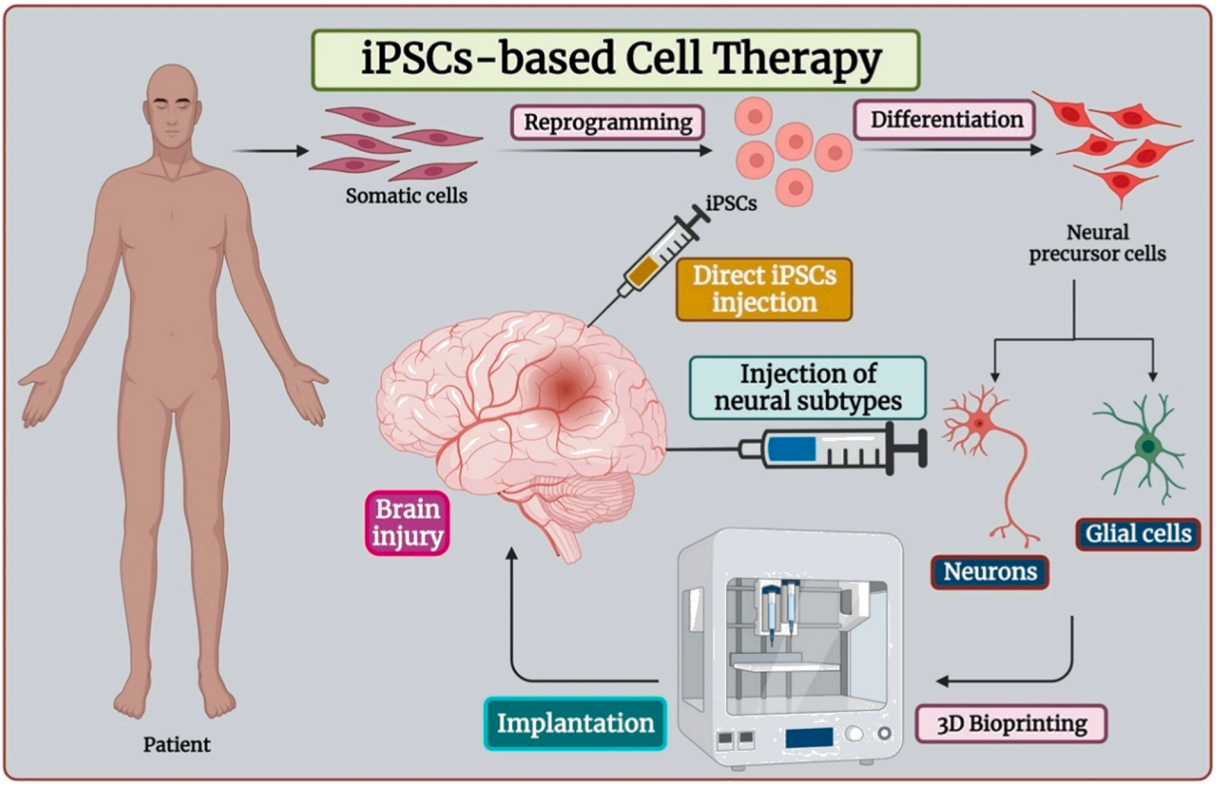

Fig. 6. Induced pluripotent stem cells (iPSCs) based cell therapy for the engineering of the brain tissue.

3.2. Biomaterials

In recent years, one of the greatest challenges has been synthesizing and characterizing biomaterials for novel uses in regenerative medicine or controlled release [22–25]. Tissue engineering is among the most researched biomedical fields where hydrogels are recommended for optimal applications [80]. Several studies have implanted biomaterials into damaged brain regions to boost neuronal regeneration by repairing localized, damaged brain cells [81], and improving the effectiveness of cell transplant and medication delivery [82]. Different types of biomaterials have been utilized for the engineering of the CNS. Among them are the following:

3.2.1. Hydrogels

Hydrogels are hydrophilic polymer networks that can absorb up to 30 % of their dry weight in water (as a minimum) [83]. Among the several biomaterials available, they provide the most flexibility and simplicity of modification of material properties to meet neural regenerative needs. Hydrogels are appealing for use in the brain because of their softness, which resembles soft brain tissues, and high permeability, allowing oxygen and nutrients to pass through [84,85]. Hydrogels are divided into naturally derived hydrogels and synthetic hydrogels [83].

3.2.1.1. Naturally derived hydrogels. Due to their greater biocompatibility, hydrogels derived from natural sources are a good alternative, but their poor stability and mechanical characteristics are frequently limiting [86].

Due to similarities with polymers found in the body, biologically generated (natural) polymers have improved biocompatibility [87]. Alginate (Alg.), hyaluronic acid (HA), chitosan (CH), gelatin, and collagen are most widely used in natural polymer-based hydrogels [88,89] (Table 1 and Fig. 7).

3.2.1.1.1. Alginate. Alginate is a natural marine anionic polysaccharide derived from brown seaweed. The monomeric units of alginate are β-(1,4) linked d-mannuronic acid and α-(1,4)-linked l-guluronic acid [90]. Because of its biocompatibility, low toxicity, low cost, and gelation properties, Alg. is an abundant and readily available biopolymer gaining popularity in TE [91]. It's employed in several biomedical applications, including medica/tion, wound healing, protein delivery, and as a cell culture substrate. Tissue engineering has also found Alg. gels to be extremely beneficial [90]. It is anticipated to function as a pH-responsive hydrogel for controlled medication release in specific circumstances [92].

3.2.1.1.2. Hyaluronic acid. Hyaluronic acid (HA) is a linear non-sulfated glycosaminoglycan and an anionic, nonsulfated linear disaccharide consisting of d-glucuronic acid and d-*N*-acetylglucosamine that has been widely explored for TE applications owing to its adaptive properties, including biocompatibility, hydrogel-forming capability, and biodegradability owing to its prominent constituent of the extracellular matrix (ECM) and is present in nearly all bodily tissues and fluids [93]. In brain TE, HA has been shown to be effective in promoting differentiation and proliferation on various substrates [94].

3.2.1.1.3. Chitosan. Chitosan is a linear cationic polymer produced by the deacetylation of chitin to improve cell adhesion, cell survival, interaction, and neurite outgrowth [95,96].

In more specific terms, it is a polymer made up of two repeating units: (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (*N*-acetyl D-glucosamine) and (1 → 4)-2-amino-2-deoxy-β-D-glucan (*N*-acetyl-glucosamine) and an important component in the ECM hence it is biocompatible and biodegradable through the action of lysozyme, acid, and colonic bacteria, various processes occur within the human body [92].

3.2.1.1.4. Gelatin. Gelatin is a denatured protein made from animal collagen that has been hydrolyzed thus the utilization of hydrogels based on gelatin offers numerous benefits owing to their inherent biocompatibility, biodegradability, and non-toxic characteristics. Gelatin consists of monomeric units that are composed of a repeating sequence of amino acids, which is analogous to the structure of other proteins. Gelatin is obtained from the collagen found in animals such as pigs, cows, or fish. It consists of a significant amount of glycine, proline, and 4-hydroxyproline residues. The substance is a combination that contains different types of polypeptides, either single or multi-stranded. Each polypeptide has extended left-handed proline helix conformations [97]. Gelatin can have either anionic or cationic characteristics, which are determined by the pH and chemical alterations it experiences. Below its isoelectric point, gelatin can exhibit cationic polyelectrolyte behavior, as seen in a study where gelatin was observed to possess cationic polyelectrolyte properties at a pH 1.9 units lower than its

Table 1
Merits and demerits of hydrogels employed for brain tissue engineering.

Hydrogel		Advantages	Disadvantage	Ref.
Natural hydrogels	Alginate	Water-solubility, cross-linking at mild conditions, biodegradability, biocompatibility, suitability for in situ injections, non-antigenicity, and chelating properties.	Unstable mechanical properties, difficulties in sterilization, handling, storage in solutions, and lack of specific cell-recognition signals.	[112–115]
	Hyaluronic acid	Water-solubility, biocompatibility Biodegradability, limited immunogenicity, and increased cell proliferation and differentiation. Safe degraded products, viscoelastic properties, and ability to influence wound healing.	Mechanical weakness, non-adherence of cells, and high costs.	[116–119]
	Chitosan	Excellent host response, biodegradability, outstanding biocompatibility, antimicrobial activity, hydrophilic surface, provides cell proliferation, adhesion, and differentiation.	Mechanical weakness, highly viscous, soluble in acidic solutions, and costly to purify	[120–123]
	Gelatin	Water-solubility can be derived from a variety of animal by-products. Forms hydrogels with high mechanical and thermo-revisability, as well as matrix hydrogels and films with low antigenicity, and good cell recognition.	Extremely viscous, quickly biodegrades, and loses thermal stability as the temperature rises	[124–126]
	Collagen	Biocompatibility, good water uptake capacities, availability of numerous isolation methods, and capacity to modify mechanical and cross-linking properties.	Weak mechanical and structural stability upon uptake of water.	[107–111,127]
Synthetic hydrogels	Poly-N-isopropyl acrylamide (PNIPAM)	Water-soluble, temperature-responsive polymer with exceptional mechanical characteristics and biocompatibility that can be employed for tissue engineering and controlled medication delivery	Requests chemical cross-linking, poor thermal stability, and cytotoxicity	[128–132]
	Polyethylene glycol (PEG)	Water-soluble, low toxicity, repeatable production, and good mechanical properties	Low cell adhesion, poor cell affinity, and reduced cellular responsiveness	[133–135]
	Polyvinyl alcohol (PVA)	Water-soluble, non-toxic, good mechanical properties, film-forming ability, and biocompatible	Limited hydrophilicity and poor flexibility do not enable cell proliferation and attachment.	[136–140]

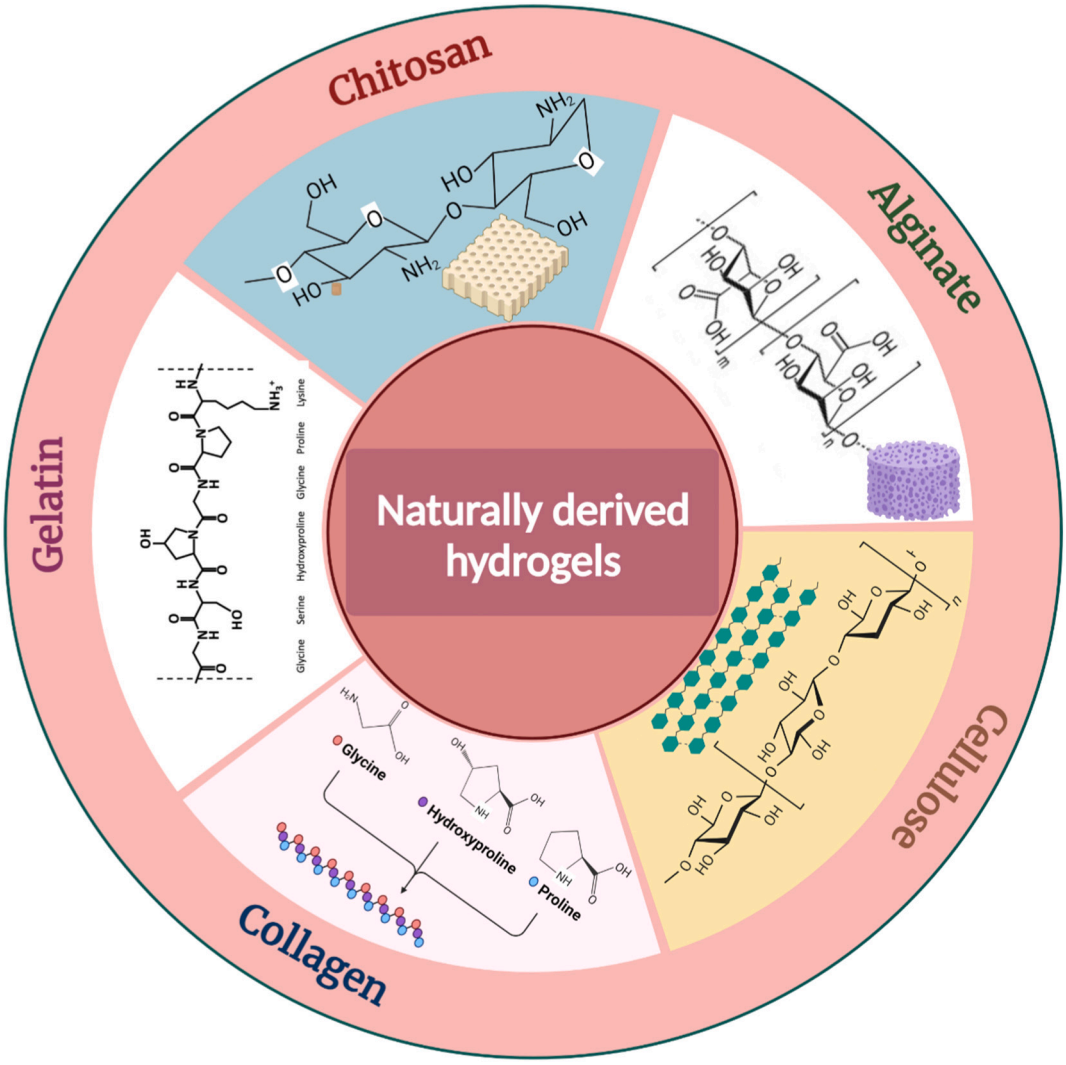

Fig. 7. Various types of naturally derived hydrogel.

isoelectric point [98]. Furthermore, gelatin that has been treated with amino compounds, known as cationized gelatin, exhibits cationic characteristics and has been employed in several applications. Thus, gelatin can exhibit both anionic and cationic properties depending on the prevailing conditions [98].

Electrospun gelatin is commonly used in conjunction with other polymers. In vitro, for example, the combination of gelatin with PCL promotes neurite outgrowth and SC proliferation [99,100], and in vivo [101]. In addition, gelatin nanoparticles for brain TE have been produced [102]. Gelatin becomes less antigenic, and its chemically changeable structure enables cell adherence and proliferation regulation, improving polymeric device biocompatibility [103].

3.2.1.1.5. Collagen. Collagen, as the principal protein constituent of the ECM, accounts for approximately 25 % of the total protein content in the majority of mammalian organisms. The structure is distinguished by a triple helix of elongated fibrils, composed of amino acids such as glycine, proline, hydroxyproline, and arginine [104]. Collagen hydrogels serve as drug-delivery devices. Collagen can exhibit both anionic and cationic characteristics, depending on the specific substances it interacts with and the conditions present [105]. Due to their flowability and injectability, biocompatible drug delivery matrices are easily administered. The effective pore size of collagen gels is in the range of several tens of nanometers, rendering it insufficient for precise control of drug release by impeded diffusion. The regulation of drug release can be achieved by the interaction between the active agent and collagen, facilitated by either covalent or noncovalent bonding [106]. Collagen is a promising natural biomaterial with a wide range of applications that has the potential to further the development of treatment techniques for injuries and degeneration of the CNS [107]. Nerve guide conduits made of collagen have been utilized to heal minor nerve damage (5 mm gap) [108]. However, They have shown promise in regenerating greater distances, such as a 15 mm gap in rat sciatic nerve regeneration [109]. Collagen is also combined with other polymers and proteins in some cases [110], and it's the only biopolymer approved for clinical application in brain engineering [111].

3.2.1.2. Synthetic hydrogels. To address the limitations associated with naturally generated hydrogels, one alternative approach is to create a framework using a modified synthetic bioinert polymer [141]. Due to their biologically inert nature, synthetic hydrogels exhibit little cell adherence. In contrast, synthetic hydrogels possess high chemical stability and can be specifically designed for applications in neural engineering [83]. Synthetic polymers offer superior mechanical strength and consistency, reduced costs, and the flexibility to manipulate their composition over time, hence enabling biodegradation and hydrolysis processes [80] (Fig. 8 and Table 1).

3.2.1.2.1. Poly-N-isopropyl acrylamide (PNIPAM). A subset of smart hydrogels known as poly-N-isopropylacrylamide (PNIPAM) hydrogels

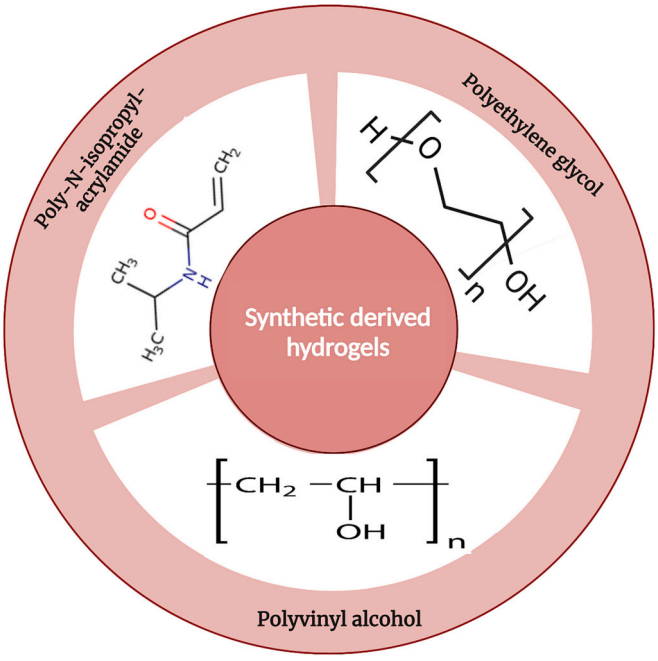

Fig. 8. Various types of synthetic-derived hydrogel.

show unique thermo-responsive characteristics in the vicinity of a lower critical solution temperature (LCST). These hydrogels' distinct temperature behavior and biocompatibility have drawn a lot of interest in a variety of biomedical applications [142]. The solubility of PNIPAM hydrogels in specific organic solvents is one of its constraints, which may limit their usage in various applications [142].

The PNIPAM hydrogel was synthesized to establish the intracerebral hemorrhage (ICH) animal model with different mass effects. The role of mass effect on early erythrolysis after experimental ICH was investigated based on the poly-N-isopropyl acrylamide [143,144].

3.2.1.2.2. Polyethylene glycol (PEG). Hydrogels based on polyethylene glycol (PEG) have been investigated for brain tissue engineering applications. Because of their high modularity and customizable nature, these hydrogels are synthetic matrices that can be used to support the proliferation of neuronal cells and aid in the restoration of brain tissue. According to a study, electrically charged PEG-based hydrogels can act as scaffolds for brain parenchymal abnormalities. Moreover, the hydrogel can promote the regeneration of brain tissue when neural stem cells are transplanted into it. PEG-based hydrogels' biocompatibility in the brain has also been examined, demonstrating its potential for medication release and tissue engineering in the brain. PEG hydrogels therefore show promise for a number of uses in neural tissue engineering, such as the controlled release of medications and the restoration of brain tissue [145].

The PEG helps reseal axonal membranes in several in vivo and in vitro injury models [146]. The PEG-based core-shell NPs were created to develop high-quality medication delivery systems [147]. The cross-linkers utilized in their production are cytotoxic. As a result, recent research has focused on reducing the toxicity of cross-linking agents by replacing them with more natural molecules such as citric acid [148].

3.2.1.2.3. Polyvinyl alcohol (PVA). Polyvinyl alcohol (PVA) hydrogels have demonstrated potential in the field of brain tissue engineering, specifically in the areas of nerve regeneration and neural cell growth support. To maximize the mechanical characteristics, biocompatibility, and design standards of self-healing hydrogels for use in brain tissue engineering applications, more investigation is required [149]. PVA is a typical TE polymer with superior biocompatibility, solubility, and excellent mechanical qualities and is non-toxic and non-carcinogenic [149]. PVA is made by hydrolyzing poly(vinyl acetate) completely or partially [150].

4. SSRHs for CNS TE

4.1. Synthesis scheme of stimuli-responsive hydrogels for CNS TE

The synthesis methodology for SRHs was divided into two categories: first-generation, which included polymerization, then second-generation, which included a more sophisticated approach using physical or chemical cross-linking.

4.1.1. Polymerization

Hydrogels are three-dimensional (3D) polymeric network systems that can entrap and retain water because of the hydrophilic polymeric groups existing within their components. The low-extent aqueous hydrophilic solution of polymer-free from entangled structures exhibits Newtonian tensile behavior. The linkers in hydrogels can give remarkable viscosity and suppleness (shear strain >2000 Pa) represented by the network [151,152].

Hydrogels are often made using water-loving monomers, although hydrophobic monomers can also be used to adjust tensile and physicochemical properties to specific applications, because of their low water solvation, such hydrogels are the fundamental focus of swollen polymeric matrix, in addition to their practical research [153]. Naturally derived or fabricated polymeric matrices can be used to make hydrogels. Naturally derived polymeric hydrogels are rhythmic with the body, biodegradable, and include physiologically recognizable moieties that assist biological processes [154]. As a result, naturally derived polymeric hydrogels have aroused the curiosity of biomaterials researchers [155].

Oligosaccharides or proteins are two naturally derived polymers that have sparked the most attention due to their unique usage in the creation of hydrogels. Moreover, synthetic polymers have stronger lipophilicity and chemical durability than natural polymers. They provide a mechanically powerful framework that breaks down at a slightly slower pace [156]. Combining monomers with varied behaviors in response to distinct external stimuli is perhaps the most feasible method for creating SRHs. Any technology capable of forming a cross-linked structure between polymers can be used to make hydrogels. Free-radical polymerization (FRP) approaches, which utilize either naturally derived or artificial water-loving monomers plus cross-linkers with a variety of functionalities, are frequently used to produce hydrogels [157]. The polymerization methodology adopted has a significant impact on the properties of the resulting hydrogels [158]. Hydrogels can be made in a solitary step by copolymerization and cross-linking of different multifunctional monomers at the same time, or in a sequential method by producing polymers containing substituents reacting groups capable of interrelating with cross-linking incentive or self-cross-linking [159] (Fig. 9).

Hybrid hydrogels are made by mixing different types of organic, polymeric blends, or inorganic materials to achieve the best synergistic effect for certain applications. For example, including lipophilic polymer parts in the presence of cross-linkers might result in the desired mechanical toughness and adaptability to a specified form [160].

4.1.1.1. Chain growth polymerization. Chemically cross-linked hydrogels are made via chain-growth polymerization (CGP), additive polymerization, and condense polymerization, as well as electron and gamma irradiation polymerization. Chain-growth polymerization includes FRP, controllable FRP, and ionic (cationic or anionic) polymerization. Chain initiation, propagation, and termination are the three procedures that are used to achieve this. A free-radical reactive position is created during initiation, that joins monomers together in a chain-link pattern [161]. In the synthesis of PNIPAM hydrogel, usually, PVA FRP is usually utilized. Using ethylene glycol di-methacrylate (EGDMA) for

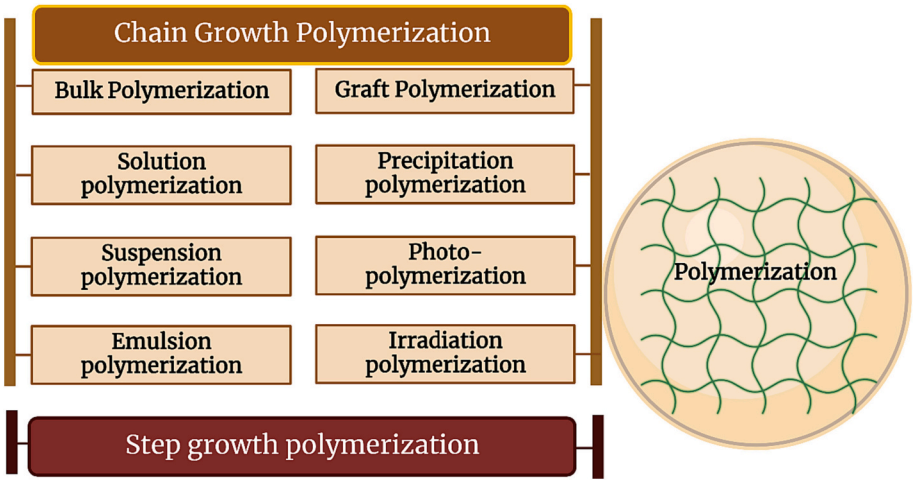

Fig. 9. Chart representation of polymerization methods used in the synthesis of SSRHs.

linking and benzoyl peroxide as a chemical activator, PVA was chemically connected with monomer methacrylic acid (MAA) through an aqueous system. The MAA monomer affords pH-responsive properties. This method of chemically cross-linking pH-responsive PVA hydrogels appeared to be promising [162]. Controlled FRP offers increased growing chain longevity than conventional FRP for supramolecular production. Because ionic polymerization processes are so sentient of water content, they were not used to make polymeric hydrogels. The following are some of the FRP methodologies for making hydrogels:

4.1.1.1.1. Bulk polymerization. Bulk polymerization is frequently used in the production of hydrogels because of its versatility. The polymerization procedure is generally started using ultraviolet rays, chemical accelerators, and/or irradiation [163]. Bulk polymerization possesses a speedy rate of polymerization with poor heat control, resulting in a rapid increase in reaction density. As a result, regulating its transformation rate seems crucial for fine-tuning the properties of the resulting hydrogel. The resultant hydrogels contain a crystalline but transparent polymeric framework that stretches and turns out rubbery when submerged in water [157] (Fig. 10).

As the temperature of the reaction and initiator quantity rise, the percentage of transformation, as well as bulk polymerization, rises [164]. Another option is to cease the reaction at a low transformation rate, although this is considered uneconomical for large-scale processes. As a result, several polymerization techniques, including solution, emulsion, and suspension polymerization, are routinely utilized to make hydrogels [163]. The creation and characterization of a bulk polymerized hydrogel based on PEG and Poly(L-lysine (PLL) polymers using UV radiation was reported. It was basic neural progenitor cells NPCs derived from mice models that were cultivated in vitro and exhibited their viability among these gels. Moreover, NPC transformation into a matured neuronal lineage is aided by PEG/PLL gels. NPC persistence and transformation may be critical in the management of CNS injuries, as

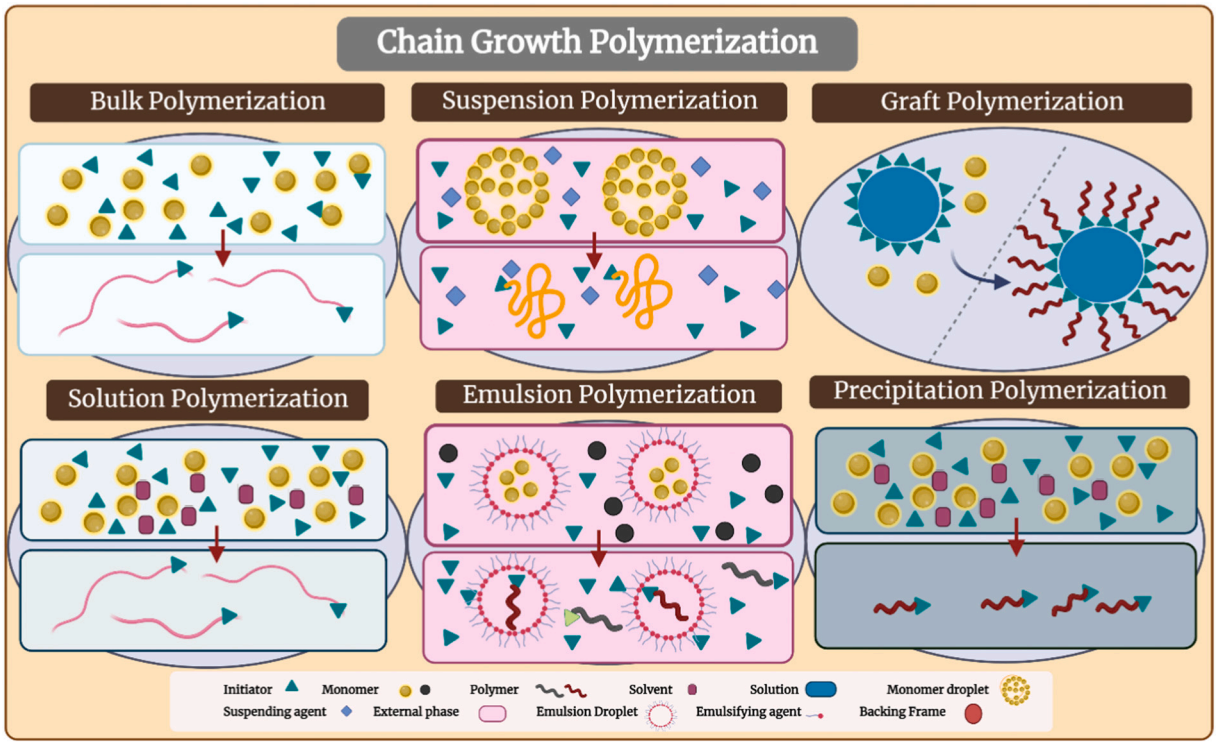

Fig. 10. Diagrammatic representation of several chain-growth polymerization techniques used to create intelligent, stimuli-responsive hydrogels for the brain.

well as providing sites enabling chemical modification, including the conjugation of cell-adhesion proteins [165].

4.1.1.1.2. Solution polymerization. The most common approach for achieving CGP hydrogels is solution polymerization. It is a redox or UV induction uniform polymerization wherein most of the components (neutral or charged monomers, initiator or activators, and cross-linkers) remain soluble inside the medium (ie, water, benzyl alcohol, ethanol, or hydroalcoholic blends). After isolating the hydrogel, distilled water splashing is used to remove any remaining monomers, activators, cross-linkers, or impurities [157]. To create organic-inorganic framework hydrogels, some inorganic constituents, such as clays, could be dispersed into a polymerization medium throughout this process (Fig. 10). This approach can result in a relatively homogenous hydrogel. Yet, drying, smashing, and granulating the resultant bulk hydrogel might take a lot of energy [166].

The solution polymerization technology is simple to make and an inexpensive process, and it allows for better control of heat transmission during polymerization. It's also safe because it happens in an aquatic environment [167]. Solution polymerization is used to create super absorbency cellulose hydrogels. Polymerization proceeds quickly, and the operation is completed at room temperature. Solution polymerization generates stronger heat flux and convection over bulk polymerization since the generated solution has a lower thickness and spinning the reaction mix increases quicker [167]. Solution and bulk polymerization approaches are relatively homogenous polymerization processes, however, they could turn heterogeneous if the resultant polymer is insoluble [168].

4.1.1.1.3. Suspension polymerization. In this form of polymerization, usually insoluble monomers are combined with initiators inside an aqueous phase with the aid of a suspending agent that has a low hydrophilic-lipophilic balance (HLB). Initiation occurs within the monomer molecule with even more than one radical per molecule at any given time [169] (Fig. 10). The existence of many radicals per molecule causes kinetic termination, which is analogous to solution polymerization. They are continually agitated to generate monomer molecule sizes ranging from 0.1 to 5 mm. As the polymerization process continues, droplets of the polymeric hydrogel form and these may be removed from the liquid of the reaction by filtration [170].

Single monomers are then bulk polymerized on a microscopic level. Because the aqueous environment is the most commonly utilized, it is an excellent heat transfer medium [168]. A preventive colloidal agent [e.g., PVA, methylcellulose (MC), or carboxymethyl cellulose (CMC)] is widely used to protect against droplet coalescence. [171]. A gelatin methacryloyl (GelMA) enhanced transwell hydrogel-based BBB system comprising endothelial and astrocyte layers of cells was developed for assessing the efficiency of anti-metastatic medicines against breast carcinoma cells in the brain [172]. Inverse suspension polymerization is frequently used in the production of hydrogels [173,174]. It is used to produce N-(3-aminopropyl) methacrylamide gel particles with small particle sizes and glob shapes [175]. AM, N-succinyl CH, and APT were used to construct a new N-succinyl CH-graft-PAM/APT composite hydrogel using inverse suspension polymerization. The resulting white granulated hydrogel seems to be rounded particles with rough surfaces. A closer look reveals that every particle contains a huge number of micropores. Such a structure may allow water to enter the network more easily, increasing swelling capacity and, as a result, adsorption effectiveness [176].

For optimal distribution in suspension polymerization, an adequate stabilizer is necessary. Span is a well-known W/O stabilizer [177]. Stabilizers including Span 40, Span 60, and Span 80 are routinely employed [178]. Researchers employed a combination of Span and Tween. Span was used as a stabilizer to synthesize microgels, which had homogenous smooth consistency microparticles [176]. Its continuous phases are commonly composed of paraffin oil, heptane, cyclohexane, and hexane [174,179]. *Comb*-type grafted and ordinary NIPAM hydrogel may be created with or without integration of NIPAM supramonomer previously obtained by radical polymerization of NIPAM monomer employing Span 80 as surfactant and cyclohexane as the continuous phase [180]. FRP using suspension polymerization in a continuous vehicle of water was used to develop the pH-responsive hydrogel. In a 1:2 mol fraction, several M.W. PEG dimethacrylates (PEGDMA) and methyl acrylic acid were employed separately. The size of the particles of hydrogel increases as the M.W. of PEGDMA increases [181].

4.1.1.1.4. Emulsion polymerization. In the polymerization process, a water-soluble initiator, cross-linkers, a surfactant, and monomers are usually used. The monomer's restricted solubility in the dispersion media distinguishes this sort of polymerization, although initiation happens exterior of the monomer droplets. If surfactants are present, the initiator initiates chain development of the solvated monomer in the dispersion media or the monomer contained in micelles [182]. In monomer-rich drips, the radius of the resultant hydrogel droplet is governed primarily by the number of polymerization sites formed out of the monomer units, rather than through droplet size [166]. The created microparticles have a size of 100 nm, which is tiny in comparison to that generated by suspension polymerization, but they're often of low polydispersity. Seeded emulsion polymerization is employed to produce larger particles using emulsion polymerization [183] (Fig. 10).

4.1.1.1.5. Graft polymerization. When grafting is applied, the bulk polymeric hydrogel has insufficient mechanical characteristics, especially when grafted toward a more appropriate substrate frame. Generally, free radical sites are formed on the surface of the substrate. Where monomers could be polymerized instantly to form stronger covalent bonds with the substrate structure Grafting vinyl monomers upon polysaccharides, for instance, is a common procedure [184] (Fig. 10).

Photo-*co*-polymerized gradually disintegrating PEG with swiftly degrading PLA-b-PEG-b-PLA using grafted MAA groups and Irgacure 2959 was developed using a photoinitiator to control its biodegradability. The researchers looked at how the resultant hydrogel affected the enclosed rat prime embryonic neurons: polylactic acid (PLA) amendment enabled hydrogel breakdown, but unmodified PEG caused cell mortality, oxidative stress, and delayed growth [185].

4.1.1.1.6. Precipitation polymerization. Precipitation polymerization has lately sparked a lot of attention, and it is likely the most commonly employed procedure for creating hydrogels. Precipitation polymerization makes use of differences in the capacity of monomers and polymers to solvate in water to create microparticles. Larger chains of polymer have lower solubility than smaller ones. Over a particular M.W., phase separation is preferred. Polymerization by precipitation begins as a sole phase, homogeneous solution polymerization. Even though the starting monomer solution is limpid and homogenous, the propagated polymerized chain phase isolates again from the solvent immediately after polymerization commences, forming a nucleus that obstructs the newly formed polymer chains. Because the temperature might be altered to impact overall precipitation/deswelling propensity, precipitation polymerization is particularly successful for the production of thermo-responsive hydrogels with a tight dispersity size distribution in the submicron range of sizes [186] (Fig. 10).

The precipitation polymerization of N-isopropyl acrylamide, acrylic acid, and *N*, *N*′-methylene bisacrylamide resulted in PNIPAM microgels. The vinyl groups were then connected to the microgel frameworks by reacting carboxylic acid groups in microgels with 3-butene-1-amine. It was established that this hydrogel is ideally suited for fabrication into wearable and implanted sensors capable of obtaining physiological pressure gestures, recording local range prospects in rat brains, and transmitting signals across rats' wounded peripheral nerves [187].

4.1.1.1.7. Photopolymerization. In situ, type of cross-link framework, photopolymerization includes the activation of radicals coupled with radiations such as UV, visual, or IR. Although photoresponsive monomers respond to light radiation and create radicals, this process occurs after radical polymerization. [188]. Initiating radical generation can be done through photo-cleavage, cation photopolymerization, or hydrogen abstraction. These radicals behave similarly to FRP in terms of

propagation and termination. In terms of directed gelation, this polymerization method surpassed others. Depending on the quantity and strength of UV light, photopolymerization is used to make hydrogels from Alg., guar gum, and PEG [189].

The Darocur 2959 activator was employed to photopolymerize PEG-PLA hydrogel, which was then modified with collagen and bFGF-2 for the treatment of brain injury. The hydrogel biodegradation was provided by PLA. Without collagen, PEG-PLA hydrogel had been reported to support significant cell growth, which was further enhanced with the addition of bFGF-2. Collagen, on the other hand, did not improve cell viability, limiting the effects of bFGF-2 [190]. Hynes et al. used Irgacure 2959 photo activator to modify the PLL hydrogel with both a linear or four-armed acrylated PEG photopolymerization. After 24-h contact with trypsin, linear PEG altred polymer maintained undegraded, whereas four-arm PEG alterations resulted in increased stiffness. After photopolymerization, included mouse postnatal NPCs revealed a low level of apoptosis. By an unknown mechanism, a four-arm PEG hydrogel supported cell survival and promoted primarily neuronal development [191]. A pool of 52 different hydrogels made up of linear or four-armed PEG and PLL with various molecular weights was created by the same group [165]. An oligo-(PEG) fumarate had been photo cross-linked with [2-(methacryloyloxy)ethyl]-trimethylammonium chloride (Irgacure 2959 as photo activator) to create a cationic hydrogel. It was discovered that seeding rat embryonic DRG cells upon these hydrogels increased neurite extension [192]. A new gelatin methacryloyl (GelMA) augmented transwell implant-based BBB model comprising endothelial, as well as astrocyte cell layers, was developed using UV photopolymerization technique for evaluating the ability of anti-metastatic medicines against cancer metastasis to the brain [172].

4.1.1.1.8. Irradiation polymerization. High-energy radiations are used in irradiation polymerization techniques, which are not used in photopolymerization. In various research, microwave radiation was used to create cross-linking within hydrogels [167]. Ionizing or elevated energy radiation, including electron beams and gamma rays, are used to liberate radicals in unsaturated monomer constituents during irradiation polymerization. To make cross-linked polymeric materials, these radicals exploit the chain expansion process. The privilege of this technology above previous technologies is that it requires no initiator or modifying agents, and the swelling properties may be controlled by varying degrees of cross-linking or the hydrogel's composition [193].

4.1.1.2. Step growth polymerization. Polymerization of poly-functional monomers happens in stages, and poly-functional monomers interact with others via covalent bonds. Covalent bonds were produced as a result of the release of water or HCl molecules throughout this kind of polymerization, which is usually self-catalyzed [194]. Step growth polymerization results in a progressive increment of molecular weight, though lengthier chains necessitate a high rate of reaction. The terminal point of the step-growth polymerization operation stays active, while the chain-growth polymerization reaction stops when all active regions are chemically bonded [195].

The tensile modulus of photodegradable biopolymer hydrogel manufactured using step-growth and chain polymerization techniques has been investigated [196]. Compared to chain-growth hydrogels, step-growth polymerization exhibited more pliability, tensile tenacity, shear force to yield, and structural stability. The degree of degradation produced by exposure to light is diminished in chain-growth hydrogels since the network connection is firmer [197].

4.1.2. Cross-linking

Cross-linkers are either physical or chemical binding agents that link two types of polymers together via physical or chemical interactions such as ionic interactions or the formation of hydrogen bonds. The rheological properties of the polymers employed as construction blocks as well as the cross-linkers utilized to govern them [198]. The 3D scaffolds that are created can traverse through the damaged areas. Due to its environmentally responsive character, in situ, gelations take place following injection to the interior parts of the wounded tissue in vivo [199].

There are two types of cross-linking methods: physical and chemical.

4.1.2.1. Physical cross-linking. Physical or reversal gels are simple to make and have the benefit of not needing the usage of cross-linkers, allowing for the addition of active pharmacological components or the removal of them before use. The correct selection of hydrocolloid, amount, and pH may contribute to the production of a wide range of gel structures, and it is presently attracting a lot of attention, especially in brain engineering. Heating or chilling a liquid solution, ionic interaction [200], complex coacervation [201], hydrogen bond development [202], and ripening or aggregation produced via heat and freeze-thaw processes are all examples of approaches to producing physically cross-linked hydrogels [203]. Hydrogels made through physical cross-linking procedures are more delicate than those made with covalent cross-linking processes. These complexes become reliable to the comparably weak interactions among polymer chains, such as hydrogen bonds, hydrophobic or electrostatic contacts, thermal, and freeze-thaw cycles [203].

4.1.2.1.1. Crystallization (freeze-thawing) method. Crystallization, which entails a series of freeze-thaw cycles, can result in a solid, elastic gel. The formation of that architecture was first noticed in hydrogels made of synthetic PVA. These are currently widely utilized in the biotechnology industry, particularly in the production of peptides and proteins. The freeze-thawing hydrogels of PVA/CH, PVA/starch, and PVA/gelatin have been demonstrated to have applications in brain engineering [204]. A hybrid hydrogel of PVA and Phytagel prepared with freeze-thaw cycles could simulate soft tissues with adequate rigidity. The polymer mix may also replicate the brain's relaxed state better than other imitating compounds (including gelatine, agar hydrogel, and polyacrylamide (PA). A freeze-thaw sequence is required for PVA hydrogel to produce physically cross-linked hydrogen bonds. As a result, this ink works in tandem with a cryogenic approach to deliver a straightforward solution that integrates hybrid hydrogel cross-linking in a single step [205,206].

4.1.2.1.2. Stereocomplex development method. This approach employs the formation of stereo-complexes among polymers exhibiting conflicting steric effects to produce hydrogel that is physically cross-linked and capable of delivery of therapeutics. These hydrogels are easily made by dissolving each component in water and then mixing the solution. However, for this kind of structure, only modest polymer compounds are used. Physically connected dextran hydrogels generated by the stereo complex fabrication of lactic acid oligopolymer are one instance of a class. However, hydrogel production in the grafts needed at least 11 lactic acid monomers [207]. A nanofiber scaffold bionics dura matter replacement was created by the stereo-complex method via electrospinning depending on enantiomeric poly(lactic acid) with poly (D-lactic acid)-grafted tetra calcium phosphate [208].

4.1.2.1.3. Electrostatic interaction (ionic) method. Electrostatic interconnection in polymers does not need the presence of charged reactive groups and can even be cross-linked under normal circumstances, i.e. at normal temperature and biological pH. Alg., which contains mannuronic plus glucuronic acid sequences and may be linked with Ca^{2+} ions, is indeed a famous example. These gels were utilized as a matrix for cell trapping and peptide delivery, it is used to mimic brain tissue because of their minimal cytotoxicity and capability to gel under moderate circumstances [209]. Cross-linked CH to glycerol-phosphate disodium-salt, on the other hand, leads to the creation of a hydrogel. If CH solutions are treated with this salt, they remain liquid at a tepid temperature but immediately convert into a gel once heated.

Carrageenan, a polysaccharide including an irregular fraction of sulfate groups, readily forms a gel in the presence of potassium ions or

the absence of potassium ions. Tougher hydrogels may be generated by the incorporation of metallic ions [210]. A 3D hydrogel scaffolding that is cross-linked among the physical matrix of calcium Alg., as well as the chemical framework of polyacrylic acid (PAA), was created to aid in the treatment of brain trauma in 8 weeks and then degrades safely [155]. Hyaluronic acid is an anionic polysaccharide found inside the brain's extracellular environment, and its dearth of inherent immunogenicity makes it a perfect foundation for grafting electrical copolymers onto neural probe coatings [211].

4.1.2.1.4. Hydrogen bonding method. Hydrogel is formed into gelatin hydrogel through the creation of hydrogen bonds among minor electron affinity hydrogen with major electron affinity functional groups. Some of the elements influencing hydrogen bonding include the polymer molar ratio and its quantity, the temperature utilized, and the sort of solvent [208]. Examples of cross-linking through hydrogen bonding include agar with gelatin, CMC blended with starch, and MC mixed with HA [212]. Walter et al. investigated the impact of PNIPAM hydrogel conformation transitions on the solvent constitution in water-methanol mixtures, the robust connection of methanol units to the polymeric chains via hydrogen bonding appears to have influenced the cononsolvency.

The alignment of methanol units, i.e., the hydrophobic effect caused by the alignment of methyl groups of methanol toward the bulk solution, therefore encouraged hydrogel disintegration [213].

An injected hydrogel with hydrogen bonding cross-linkage employing imidazole groups-amended gelatin methacrylate and Polydopamine NPs were employed as stromal-cell derived factor-1 (SDF-1) carriers were utilized to treat focal brain damage [214]. The effect of graphene oxide content on the characteristics and susceptibility to electric fields of a PAA hybrid hydrogel was reported. The carboxyl and hydroxyl groups on graphene oxide sheets hydrogen bind with the PAA matrix, making GO integration possible in the formation of hydrogel for brain injury [215]. The researchers examined bioadhesive pectin hydrogels mixed with drug nanocrystals covered with polylactic acid-PEG (NCPPs) to be delivered directly into the tissue of the brain for post-surgical localized delivery to brain tumors. Greater pectin levels were observed to result in better adhesion due to a higher number of hydrogen bonding sites [216].

4.1.2.1.5. Heat induction cross-linking. The gelation cross-linking in gum Arabia is caused by a heat process that promotes the protein constituent to accumulate, resulting in an increase in molecular weight as well as the creation of a high hydrophilic affinity hydrogel with structural rigidity [210]. The thermoplasticity of the ether-based polyurethane hydrogel also was employed because it permits the steady production of the hydrogel fibers to be transplanted within brain tissue [217]. Collagen-based bioink cross-linking is often thermally regulated, and collagen-based bioprinting has been employed in biomedical applications. The elastic modulus of hydrogels may be modified to be appropriate for brain TE [218].

4.1.2.1.6. Hydrophobized polysaccharides. The hydrophobic alteration enables polysaccharide polymers including CH, methylcellulose, and dextran to be physically coupled. Hydrophobized polar glycol CH substituted with palmitoyl moieties has been demonstrated to be degrading and biocompatible, as well as capable of encapsulating water-soluble pharmaceuticals [155]. The MC has been used as a polysaccharide in the manufacture of hydrogels enabling controlled delivery methods of chondroitinase ABC (ChABC) toward functional restoration within the stroke-injured brain. Protein-peptide affinity associations fixed ChABC inside the hydrogels, while electrostatic affinity associations entrapped SDF [219].

4.1.2.2. Chemical cross-linking. It entails the formation of a covalent connection between polymers, that is more stable and strong than physical linkage [220]. Chemical cross-linking includes specific structural, photoinduction, and enzymatically induced cross-links.

4.1.2.2.1. Tiny cross-linking agent. Tiny cross-linking agents are tiny molecules with numerous reactive ends that chemically connect with polymer functional groups to connect them. To alter the functional and mechanical qualities of gels, small cross-linkers including tannic acid (TA), glutaraldehyde, dopaminergic, and genipin have typically been used. The use of glutaraldehyde is restricted, among other items, since it has bad impacts on a cellular level [221]. As a result, TA, genipin, dopaminergic, and caffeic acid have aroused attention as ideal substitutes and are routinely used to affect the function of polymer architecture. Dopamine hydrochloride-coupled carbonized HA was used to create a chemically cross-linked fluorescent nano hydrogel DDS as a brain antitumor that controls the release of an anticancer medication and NO in acidic cancer cells and allows fluorescence-based cellular imaging [222]. The pH-dependent liberation of doxorubicin from such hydrogel technology resulted in 80 % of doxorubicin discharged in 30 h at pH 5.0. At higher pH hydrogels discharged below 20 % of the doxorubicin. The breaking of the boronate ester link between catechol with boronic acid under acidic circumstances induced this pH dependence [222].

4.1.2.2.2. Photoinduced cross-linking. Photoinduced hydrogels have sparked considerable attention because of their in-situ gel formation and easy formulation processes; they are triggered by light of a certain wavelength (UV and visible light). can result in gel morphological changes as well as the formation of a 3D matrix [223]. Because of their strong cytocompatibility, PEG and PVA are often utilized as photo-cross-linkers in hydrogel synthesis. Furthermore, natural polymers including HA, collagen, or gelatin had been modified to form this hydrogel structure. Hydrogel polymerization requires the addition of photoinitiators which are responsive to light irradiation. As a result, photoinitiators play an important role in the course of photo cross-linking because they influence the consistency of the gel produced. Photoinitiators are classified into two types based on their respective reaction mechanisms.

When exposed to oxygen, type I, commonly known as “cleavage,” could create initiating radicals via breaking intermolecular connections [224]. Type II photoinitiators demand smaller activation energy than type I photoinitiators. It is known as hydrogen abstraction. Eosin Y is a Type II photoinitiator that is commonly employed with either amines or thiols to promote radical polymerization. Riboflavin is also a type II initiator, has good biocompatibility, and is widely used to create hydrogels under visible light [225]. Local drug distribution Photoinduced PEG-DMA-based hydrogel had been used to deliver temozolomide (TMZ) to the brain. Where glioblastoma patients were managed with an injectable hydrogel containing TMZ-loaded micelles photoinduction was promoted using UV radiation, which kills cancer cells [226]. Another research coupled the hydrogel and paclitaxel (PTX) NPs in the management of brain malignancies (glioma). An MRI picture was developed to construct the tumor's 3D shape for local administration of paclitaxel. Finally, it was discovered that nanocomposite hydrogel may be employed as a possible solution for improving the therapeutic effectiveness of PTX [227]. The impact of chain length of HA photo cross-linked with GelMA matrix on GBM39 brain cancer cell invasion was explored, and the findings revealed an inverse relationship between invasion and matrix-immobilized HA molecular weight [228]. Similarly, the effect of the quantity of HA on the invasion of U251MG brain cancer cells has been studied. The scientists found that hydrogels containing HA had much shorter mean invasion distances than hydrogels without matrix-immobilized HA [229].

4.1.2.2.3. Enzyme induced cross-linking. Enzymatic cross-linking approaches for hydrogel production are rapidly gaining popularity to improve material properties such as gelling time and cytocompatibility. The hydrogels generated have great stiffness and a short gelation period but the danger of cytotoxicity from residual chemicals is still a challenge [155]. Injectable enzyme-induced hydrogels have been investigated for their ability to reduce local recurring after glioma potential therapeutic repair surgery by utilizing the availability of matrix metalloproteinase (MMP) enzymes in vivo. MMP enzyme-induced hydrogel was loaded

with TMZ and O6-benzylamine (O6-methylguanine-DNA methyltransferase inhibitor) for glioma recurring reduction [230].

An injectable ROS-induced hydrogel (TM) containing curcumin (C) and poly(propylene sulfide) was reported to reduce ROS levels in injured brain tissue and promote neuron renewal and restoration. In in vivo weight drop injury (WDI) and piercing brain injury (PBI) animal models, the hydrogel dramatically decreased brain edema [231]. An injectable gelatin hydrogel had been synthesized via in situ dual-enzymatically cross-linked by glucose oxidase (GOX) and horseradish peroxidase (HRP), and the clinical efficacy of such a hydrogel embedded with BMSCs for brain trauma rats was explored [232].

4.2. Fabrication of hydrogel scaffolds for brain engineering applications

4.2.1. Emulsification

Emulsification is a multi-stage agitation process that produces tiny droplets of hydrophilic hydrogel precursors inside a hydrophobic media such as organic solvent and oil. The diameter of the hydrogel droplet may be adjusted by viscosity, dynamic agitation rate, and surfactants, and it is utilized to modulate surface tension among phases as well as to avoid hydroparticle aggregation. For the hydrogel droplets, several cross-linking processes are utilized to form particles such as spherical or NPs from a variety of synthetic or natural polymers such as PLGA, PLA, CH, Alg., collagen, and agarose.

Cells are incorporated into the aqueous medium that contains the hydrogel precursor, and gel NPs of cell-laden might be formed as a result of this addition. Emulsification as in vitro cultivation within hydrogel tiny particles could be used to encapsulate embryonic stem cells; such an approach can create a more regulated environment for development [233]. It was claimed that multimodal conducting macroporous nanocomposite hydrogels (MNHs) were developed using an air-in-water emulsion matrix supported by colloidal hybridization comprising carbon nanotubes (CNTs) with gelatin methacryloyl. In vitro cell, culture experiments revealed that MNH hydrogels might stimulate the spread and development of neural cells [206].

4.2.2. Lyophilization (freeze-drying)

Lyophilization is a low-temperature dehydration process that involves freezing the product. This process required a vacuum process and then undergoing sublimation of the solvents, leaving pores, and voids in the hydrogel structure. As this strategy has been widely employed in TE, such a technique creates porous hydrogel frameworks [233]. The research indicated that agarose hydrogel scaffolds with channels, such as straight porous channels, were created using a modified lyophilization approach. In this approach, in the first phase, one lateral pillar containing agarose gel was exposed to a dried ice block and immersed in a liquid nitrogen lake. Ice crystals were generated due to a uniaxial thermal gradient, which is associated with the orientation of that slope, but in the second phase of lyophilization, solvent sublimation, a network of extremely straight porous channels with dimensions suitable for cell infiltration is formed. These scaffolds, agarose-based hydrogel scaffolds made using that method, were exposed for axonal regeneration in a spinal cord damage model [234].

4.2.3. Porogen leaching

As porous scaffolds with approximately consistent pore size, this is the most basic approach for scaffold production. In this approach, molding of the solution, i.e. organic solution containing polymers and salt particles with cross-linker, followed by subsequent dissolution of the salt and evaporation of solvent that is engaged in the salt particulates in solution. However, this method has certain drawbacks since some leftover salt particles remain in the scaffold. This approach is also the sole way to create thin-film scaffolds. This technology has been claimed to be employed for the fabrication of thin scaffold membranes with relatively high porosity and open-cell morphogenesis, and films of porous scaffolds have been coated with diverse anatomical structures to emerge 3D scaffolds [233].

4.2.4. Gas foaming

This method employs effervescent salt as a gas-foaming agent in order to facilitate the formation of the porous structure of the scaffolds. A polymeric gel, which includes evenly distributed salt particles like ammonium bicarbonate, is formed by pouring it into a suitable mold and then submerging it in hot water. The release of carbon dioxide and ammonia gases, along with the removal of remaining ammonium bicarbonate particles from the solidifying hydrogel, results in the creation of a porous matrix with strong interconnections. The scaffolds produced using this method exhibited a macro-porous structure with open cells that had consistent pore diameters ranging from 100 to 200 μm. Before immersing the gel mold, citric acid or acidic salt has been mixed with water to further change the operation. Citric acid salt combines with ammonium bicarbonate, allowing gas generation and the formation of macroporous scaffolds having high porosity. Mechanical properties and porosity may be regulated using this modified approach by adjusting the rate of the reaction among salts as well as the degree of generation of foaming gases. Highly open porous scaffolds were created, which were considered encouraging for seeding and cellular uptake [235].

4.2.5. Electrospinning

This approach is one of the most significant fabrication procedures to produce highly porous scaffolds, employed in TE. An exterior electric field has been required in electrospinning techniques, and capillary tube end microfibers are removed from the charged polymeric solution. After charging the polymer with a high voltage, it is stressed as a thin filament guided toward the revolving collector with an oppositely charged surface in the intended way of fiber collection. The morphology, porosity, and size of the fibers are the results of processing variables like heat, conductivity, provided voltage, fluidity, and polymer solution [236]. Submicron, spongy fibrous, and polyacrylic acid/PVA hydrogels are among the several hydrogel scaffolds made using this method. Interfiber pores in produced fibrous hydrogel scaffolds became linked, a disadvantage of synthetic tissue that allows motility and cell-to-cell contact. Another study used a salt-leaching approach in conjunction with electrospinning to create collagen–HA hydrogel nanofibers [237].

4.2.6. 3D bioprinting

A novel technique for TE involves stacked strands of either cell-laden hydrogels or dropwise seeding [74]. This method is useful for creating 3D scaffolds, including a fast prototyping-derived process wherein internal morphology has been predesigned and outward form is comparable to specific cell settling. A computer-aided model of the graft is translated by a prototyping machine into a stratified specified internal morphology and exterior form cell-laden hydrogel created utilizing either in vivo patching or an in vitro model [238]. Granular hydrogel scaffolds had been implemented to improve 3D human-originated pluripotent stem cell (hiPSC)-obtained neural systems. A custom-built 3D printed toolset was created to exude HA hydrogel through a perforated nylon fabric to manufacture hydrogel granules. Cellular hydrogel granules were joined utilizing a weaker subsequent gelation process, resulting in self-supporting cell-laden scaffolds. Granular scaffolds outperformed bulk hydrogels in terms of cell viability at 3 and 7 days [239].

4.2.7. Photolithography

This approach was created specifically for nanotechnology and electronics [240]. Recently, this approach has been employed to manufacture a wide range of biomedical uses using hydrogel scaffolds, including micro-engineered scaffolds. In these procedures, several synthetic and naturally derived photo-cross-linkable polymers were employed, and all these cross-linkers are cross-linked to generate hydrogels. This method is based on the interaction of a photo-cross-linkable polymer thin layer with UV light as a substrate. The photo-responsive polymer is blinded by light while passing through a

transparent region, and photochemical reactions take place allowing cross-linking of the polymers. Hydrogel matrices have recently been constructed utilizing a similar technology that just focuses and scans light. Photo-cross-linkable materials, such as blue light, are employed; additional innovation and refinement were added expressly for the aim of TE to ensure the safety of this technique [233].

Laser lithography is a similar photolithography technique. Laser light is employed in this process to cross-link the photoresponsive hydrophilic polymers at certain locations. Similar technologies were used to build 3D complicated tissue scaffolds single layer-by-layer. Conjugated bioactive moieties had been shown using concentrated light and/or premade hydrogel scaffolds. This technology, for example, has been applied inside agarose-based hydrogel to prepare adhesive paths; these pathways permitted concentrate cell migration into a hydrogel, although this photolithography has relevance as a production procedure to build hydrogel scaffolds [242]. Some problems have been observed, such as the demand for photo-cross-linkable polymers as well as UV light's detrimental impact on human cell activity and cytotoxicity due to the usage of photoinitiators. Moreover, this is primarily a 2D process, hence matrices are necessary for further assembly to make 3D scaffolds [233].

4.3. SSRHs for CNS TE

The SSRHs employed for the engineering of the brain tissue are illustrated in Table 2.

4.3.1. Temperature-responsive hydrogels

Thermo-responsive class of hydrogels is one of the most valuable categories of SSRHs which has various applications and significant results in biomedical fields [243]. Phase conversion of thermo-responsive hydrogels that follow temperature variation occurs due to the inclusion of thermo-responsive polymers having lower critical solution temperature (LCST) or upper critical solution temperature (UCST). Temperatures exceeding the LCST lead to the collapse of polymers forming a reversible denser form that returns to its normal liquid form below the LCST [244]. The temperature-based conformational change in the hydrogel is attributed to the interaction between polymer chains and water molecules [243]. In vivo, the administration of thermo-sensitive hydrogels makes them prone to gelation upon meeting physiological conditions with suitable temperatures [245]. Repair of neurological defects may be carried out through the inclusion of growth factors, cells, and drugs in the systems intended for CNS delivery. Temperature stimulus-responsive hydrogels provide the advantage of undergoing volume and structural transformations in response to stimuli, allowing for a wide range of multidimensional applications [246]. Additionally, they have the potential to be employed in regulated drug delivery [247]. Nevertheless, temperature stimuli-responsive hydrogels have drawbacks such as a restricted temperature vary and the requirement for meticulous temperature regulation [31].

Thermo-responsive hydrogels were suggested to be a promising candidate for the incorporation of these materials and for the treatment of various CNS injuries [248]. Biocompatibility of the prepared systems, particularly injectable ones, needs to be taken into account. This is accomplished by proper selection of the polymers used and fabrication conditions.

The inclusion of easily breakable weak bonds in the prepared system facilitates the design of biodegradable thermo-responsive hydrogels [245]. Sufficient mechanical strength is also required to endure in vivo stresses, ensure structural stability, lower immunological reactions, and control CNS attachment and differentiation. The density of cross-linking and polymer amounts can be adjusted to acquire the most suitable mechanical features for neural applications [271]. The mechanical stiffness of temperature-responsive hydrogels for the CNS should be similar to that of normal neural structures (0.4–1.4 kPa for systems targeting the brain and 5–42 kPa for those targeting the spinal cord) [21].

Thermo-responsive hydrogels for CNS applications may be composed of natural, synthetic, or both types of polymers. Despite the desirable characteristics of synthetic polymers including adjustable and controllable mechanical features, constitution, and breakdown rate compared to natural polymers [21], the similar tensile features to CNS structures, appropriate porosity, and good inclusion of growth factors and therapeutics made the natural polymers hydrogels an appealing choice for CNS as well [272]. Combining both natural and synthetic polymers in one hydrogel was reported to exploit the advantages of both types [248]. Zhang et al. developed thermoresponsive hydrogel composed of β-phosphoglycerate, collagen, hydroxyethylcellulose, chitosan (β-GP/Col-HEC-CH), and incorporating BMSCs to enhance neural regeneration in SCI mouse model. The hindlimb motor function was restored after 28 days of BMSC-loaded hydrogel treatment [273].

Incorporation of nerve growth factor (NGF)-overexpressing human adipose-derived MSCs (hADSCs) into (β-GP /HEC/ CS) hydrogel was also developed by Alidazeh et al. for SCI treatment in a rat model. Substantial enhancement of cell differentiation and improved locomotor features were observed in the group that received hADSCs/ β-GP /HEC/ CS hydrogel proving the good potential of the system in neural TE [250]. Injectable imidazole-poly (organophosphazenes) (I-5) thermo-responsive hydrogel developed by Hong et al. could create a desirable fibrotic ECM transformation as a result of the hydrogel-inflammatory cell interactions. The cystic cavities, that occur after CNS injuries and are regarded as a major hurdle to tissue regeneration, were subsequently reduced by the developed ECM [274]. Xu et al. designed thermo-responsive hydrogel comprising heparin-poloxamer (HP), decellularized spinal cord ECM (dscECM), and fibroblast growth factor-2 (FGF2) for prolonged protein-dependent treatment in SCI. The prepared hydrogel exhibited rapid gelation at body temperature and FGF2 sustained release. Significant recovery of neural functions and neuroprotection were achieved upon using HP-dscECM-FGF2 hydrogel compared to other groups [275]. Coupling heparin with poloxamers was reported to enhance their efficacy, controlled-release features, and growth factors binding affinity [276]. Abbasi Aval et al. designed *N*-isopropyl acrylamide (NIPAAm)-HA-peptide interpenetrating network (Puramatrix®) thermo-responsive hydrogel for brain TE. In vitro, assessment of the prepared hydrogel exhibited comparable elasticity to brain tissues and important biological features needed for tissue repair [251]. Depending on the potential of M2 microglia-derived extracellular vesicles (EVs) to enhance the regeneration of neural tissues and decrease inflammation, M2-EVs- loaded poly (d, L-lactide)-poly (ethylene glycol)-poly(d,L-lactide) (PLEL) thermo-responsive hydrogel was designed by Zhang et al. to treat SCI injuries. Rapid conversion into gel form once the system has been administered, achieved efficient maintenance, and prolonged release. Besides the restoration of motor neural functions, a neural protective effect was also noticed due to the polarization of M2 microglia and suppression of neural apoptotic damage [249].

4.3.2. Light-responsive hydrogels

Stimulation of sol-gel conversion in light-responsive hydrogels depends mainly on the ability of light (visible light or UV) to make certain alterations in gel precursors. Light stimuli-responsive hydrogels offer many advantages for controlled drug delivery precisely and quickly [277]. Besides its flexibility and focusability, light intensity, wavelength, exposure duration, and beam diameter can be adjusted to control and guide it. This does not injure or invade the subject. Light-responsive hydrogels react reversibly or irreversibly depending on the photosensitizer. Light-responsive hydrogels can be used in various biomedical applications due to their volume and structure alterations. Nevertheless, additional investigation is necessary to comprehensively comprehend their enduring stability and possible obstacles in clinical application [277].

Response to different sorts of light is based on the presence of certain photoinitiators which are classified into two main categories according

Table 2
SSRHs employed for the engineering of the brain tissue.

Type of smart/stimuli-responsive hydrogel		Smart/stimuli-responsive hydrogel polymer (main component)	Smart/stimuli-responsive hydrogel system	Stimuli	Study Model	Purpose of use	Year	Ref.
Physical-responsive hydrogels	Temperature-responsive hydrogels	-poly (d, L-lactide) -poly (ethylene glycol -poly(d,L-lactide) (PLEL)	M2 microglia-derived extracellular vesicles (EVs)-loaded PLEL hydrogel	-Temperature	Rat SCI model	Enhance neural regeneration and decrease inflammation of SCI	2022	[249]
		-Chitosan -β-glycerophosphate -Hydroxyethylcellulose	Human adipose-derived mesenchymal stem cells (hADSCs) / Chitosan / B-glycerophosphate / hydroxyethylcellulose thermoresponsive hydrogel	-Temperature	Rat SCI model	Regeneration of injured spinal cord	2020	[250]
		-N-isopropylacrylamide (NIPAAm) -Hyaluronic acid (HA)	NIPAAm- HA- Puramatrix	-Temperature	In vitro	Brain tissue engineering	2022	[251]
		-Chitosan -Pluronic	Chitosan /pluronic/aniline-based hydrogel containing vascular endothelial growth factor (VEGF)	-Temperature -Electric	Hippocampus ischemia rat model	Treatment of CNS disorders	2020	[252]
		-Chitosan	Chitosan hydrogel incorporating FK506-loaded polymeric micelles and ciliary neurotrophic factor (CNTF)	-Temperature	Traumatic optic neuropathy rabbit model	Treatment of traumatic optic nerve injury	2020	[253]
	Light/photo-responsive hydrogels	-Methacrylate -Silk fibroin	Basic fibroblast growth factor (bFGF)-loaded methacrylate-silk fibroin (SilMA) photoresponsive hydrogel	-Light	Rat SCI model	SCI repair	2022	[254]
		-Gelatin -Hyaluronic acid (HA)	Gelatin (GL)/ hyaluronic acid (HA)-based light responsive hydrogel	-Light	Rat SCI model	SCI repair	2022	[255]
		-Poly-(N-isopropylacrilamyde) (pNIPAM) -Polypyrrole -Gelatin	Poly-(N-isopropylacrilamyde)(pNIPAM)/ polypyrrole photothermal hydrogel	-Light	Rat model	Deliver various biomolecules (e.g neurotransmitters and proteins) into CNS through controlling light stimulation	2015	[256]
		-Chondroitin sulfate -Polypyrrole	Gelatin/chondroitin sulfate/ polypyrrole	-Electric	Rat SCI model	Neural regeneration	2022	[257]
		-Gelatin methacryloyl (GelMA)	Cobalt (Co) / multi-walled carbon nanotube (MWCNTs)- gelatin methacryloyl (GelMA) hydrogel incorporating apical papilla stem cells	-Electric	In vitro	Neuronal differentiation of apical papilla stem cells	2022	[258]
	Electric-responsive hydrogels	-Poly(citrate-maleic)-ε-polylysine (PME) -Polydopamine (PDA)	polydopamine (PDA)/ MWCNTs - poly(citrate-maleic)-ε-polylysine (PME)	-Electric	Rat SCI model	Enhance SCI Regeneration and repair	2022	[259]
		-Poly(ethylene glycol)-co-polyvaline (mPEG-PLV)	Tetraniline / poly(ethylene glycol)-co-polyvaline (mPEG-PLV)/ nerve growth factor (NGF)	-Electric -Temperature	Rat SCI model	Enhance SCI Regeneration and repair	2021	[260]
		-Chitosan-altered polypyrrole -N-carboxyethyl chitosan -polyurethane	Neural stem cells (NSCs) –based chitosan-altered polypyrrole/ N-carboxyethyl chitosan/ difunctional polyurethane hydrogel	-Electric	Zebrafish brain injury model	Motion sensing and nerve repair	2020	[261]
		-Peptide (RADA-16I)	Peptide (RADA-16I) / Neural progenitor cells (NPCs)/ human mesenchymal stem cells (hMSCs)	-Magnetic	Rat SCI model	Evaluate magnetic alignment of hydrogel and its ability to enhance tissue engineering in SCI	2022	[262]
	Magnetic-responsive hydrogels	-Poly (lactide-co-glycolide) (PLGA)	Magnetic responsive hydrogel comprising poly (lactide-co-glycolide) (PLGA) fibers/ Superparamagnetic iron oxide nanoparticles (SPIONs)	-Magnetic	In vitro	Enhance growth of nerve cells	2017	[263]
		-Collagen	Collagen / reduced graphene oxide magnetic nanoparticles-based hydrogel incorporating neuroblastoma cells SH-SY5Y	-Magnetic	In vitro	Enhance neuronal differentiation	2019	[264]

(*continued on next page*)

Table 2 (*continued*)

Type of smart/stimuli-responsive hydrogel		Smart/stimuli-responsive hydrogel polymer (main component)	Smart/stimuli-responsive hydrogel system	Stimuli	Study Model	Purpose of use	Year	Ref.
Chemical-responsive hydrogels	Ultrasound-responsive hydrogels	-Gelatin	PC12 cells incorporated into hydroxyphenylpropionic acid- gelatin (HPA-Gtn) hydrogel	-Ultrasound	In vitro	Enhance neural growth and regeneration	2019	[265]
	Glucose-responsive hydrogels	-Dithiothreitol (DTT), poly (ethylene glycol) diacrylate (PEGDA)	NSCs /endothelial cells-based (dithiothreitol (DTT)-poly(ethylene glycol) diacrylate (PEGDA)- borax) Glucose-responsive hydrogel	-Glucose	In vitro	Development of vascularized neurological tissue	2017	[266]
		-Gelatin-hydroxyphenyl copolymer	Murine bone marrow derived mesenchymal stem cells (BMSC)-based gelatin hydroxyphenyl hydrogel	-Enzymatic	Traumatic brain injury (TBI) mouse model	Neural tissue engineering following TBI	2021	[267]
Biological-responsive hydrogels	Enzyme-responsive hydrogels	-Polyethylene-imine (PEI) -PEG	Rolipram –based polyethylene-imine (PEI)/PEG nanogel	-Enzymatic	Mouse SCI model	Protection from astrocytes-induced inflammmation following SCI	2019	[268]
		-Poly[(lactic acid)- *co* -[(glycolic acid)- *alt* -(L-lysine)] (PLGL)	Hexapeptide/(PLGL –based enzyme responsive hydrogel	-Enzymatic	Cryogenic mouse injury model	Repair of brain injury	2020	[269]
		-O^6-benzylamine	temozolomide /O^6-benzylamine- based enzyme responsive hydrogel	-Enzymatic	Postoperative glioma mouse model.	Prevent temozolomide-resistant glioma after surgery	2020	[270]

to reactions' mechanisms. The formation of starting radicals following breakage of intramolecular bonds by UV light is known as cleavage type (Type 1) and involves various substances such as I2959 (2-Hydroxy-[4′-(2-Hydroxyethoxy) Phenyl] -2-Methyl Propanone, I651 (2,2-dimethoxy-2-phenyl acetophenone and LAP (lithium acylphosphinate). Riboflavin and Eosin Y are examples of Hydrogen abstraction type (Type II photoinitiators) which needs less energy than Type 1. Gelatin, collagen, PVA, PEG, and HA are examples of polymers that are modified to have light-stimulated cross-linkable groups [223]. Irradiation time, light distance, and wavelength can be modified to meet the requirements of hydrogel attributes [278]. Light-responsive hydrogels have a significant role in delivering various therapeutics in a controlled manner and designing self-healing scaffolds [279]. Unfortunately, the production of unsafe free radicals, harmful effects of included photoinitiators, and slow effect limited their biomedical applications in TE, especially for those of CNS [279]. Methacrylate is extensively used with other polymers (e.g. gelatin) forming Gelatin methacryloyl (GelMA), a common light-responsive material [224]. Silk fibroin is a silk fiber component that shows many profits when used in biomedical applications. Good safety profile, biocompatibility, improved mechanical features, and enhanced permeability made silk fibroin a promising candidate for TE purposes. Neural TE was reported to be enhanced greatly by the incorporation of silk fibroin in biomaterials [280].

Methacrylate was also combined with silk fibroin-forming methacrylate (SilMA) material that was exploited as light-responsive hydrogels in TE purposes such as cartilage and bone repair. Zhou et al. have exploited this combination to enhance tissue regeneration in spinal cord injuries by designing Basic fibroblast growth factor (bFGF)-loaded SilMA photoresponsive hydrogel. Photo cross-linking was carried out using a LAP photoinitiator and UV light with a wavelength of 405 nm. The developed hydrogel showed rapid gelation (15 s), good biocompatibility, enhancement of nerve axon regeneration, prevention of oxidative stress, and promotion of mitochondrial performance. This made it a promising option for neural regeneration [254].

Poly(2-hydroxyethyl methacrylate) (pHEMA) 3D hydrogel incorporating immobilized collagen was designed by Cai et al. for repairing spinal cord injuries. The concept of using light-immobilized protein in the hydrogel proved to be promising in the regeneration of neural cells and restoration of motor function in a rat model. Neural stem cell (NSC) proliferation and prolongation of bFGF release were also observed while applying the in vitro studies [281].

Zhao et al. developed HA/Gelatin photosensitive hydrogel for repairing spinal cord injuries. The developed hydrogel-enhanced neural stem cell regeneration decreased inflammatory reactions and restored motor function within 60 days in the rat model [255]. Poly-(N-isopropylacrilamyde)(pNIPAM)/polypyrrole photothermal hydrogel was developed by li et al. to deliver various biomolecules (e.g. neurotransmitters and proteins) into CNS. The use of near-infrared stimulation at a wavelength of 980 nm offered a great opportunity for remote controlling of neural activities due to its potential to penetrate different living tissues deeply. The pattern, amount, and speed of substances' release were easily adjusted by controlling light stimulation. Avoidance of both chemical cross-linking and degradation after photostimulation was also achieved [256].

4.3.3. Electric-responsive hydrogels

Electric current's ability to promote structural changes in specific hydrogels was exploited to fabricate electric-sensitive hydrogels. A mixture of electro-osmotic, Coulombic, and electrophoretic processes has been expected to be responsible for the sol-gel conversion of these hydrogels [282]. Adjustable electric current makes it feasible for the hydrogel to achieve the desired response which makes electric-sensitive hydrogels an attractive choice for biomedical applications including CNS TE [283]. However, there is insufficient data available on the long-term durability of electric-responsive hydrogels, which is a critical aspect of their practical utilization. Translating the findings of electric-

responsive hydrogel research into practical use in clinical settings presents difficulties, and further investigation is required to comprehensively grasp their potential advantages and constraints [246].

Osmotic pressure variation following the use of external electric current and electrolyte-based media stimulates water migration and leads to morphological alterations in the hydrogel system [282]. Moreover, nerve cells' electrical characteristics and their good responsiveness to electrical stimulation, supported the development of these scaffolds [284]. Electric-sensitive hydrogels are widely synthesized with the aid of electroconductive substances including polypyrrole, carbon nanotubes, polyaniline, graphene, and polythiophene [285].

Gelatin/chondroitin sulfate/polypyrrole-based injectable hydrogel was developed by Luo et al. to rejoin the cavity spaces following spinal cord injuries. The developed electro-responsive hydrogel exhibited good mechanical strength, self-healing features, and neural proliferation ability upon in vitro assessment. In vivo performance of the hydrogel demonstrated the stimulation of MEK/ERK and PI3K/AKT pathways in the SCI rat model, resulting in enhanced neural regeneration and recovery of motor function [257]. Neural stem cells –based CH-altered polypyrrole/N-carboxyethyl CH/difunctional polyurethane electric-responsive hydrogel was developed by Xu et al. for neural regeneration. In vitro tests of prepared hydrogel showed good conductivity, self-healing, flexibility, and motion-sensing features.

The biocompatibility of hydrogel was proved using a rat subcutaneous implantation model. In vivo study using a zebrafish brain injury model exhibited neural proliferation and restoration of motor function [261]. Coupling the benefits of electro-responsive polymers with bioactive moieties in one system, seemed to be an appealing choice for CNS TE. Owing to the remarkable role of HA in TE, wound healing, and angiogenesis, it was employed to enhance the biological performance of electroconductive polymers [286].

HA-Poly(3,4-ethylene dioxythiophene) (PEDOT)/gelatin/CH-based hydrogel was developed by Wang et al. and monitored for NSC differentiation. The developed hydrogel showed a higher degree of NSC proliferation and expression genes upon applying different in vitro studies [287]. Diverse TE applications have used carbon nanotubes due to their distinctive structure, good mechanical features, and remarkable electrical conductivity [259].

Besides the electrical conductivity features of multi-walled carbon nanotubes (MWCNTs), they also have a vital role in enhancing neural proliferation. Unfortunately, their low solubility and safety hinder their use as electrolyte-based media in CNS engineering applications so they were combined with certain hydrophilic moieties to enhance their electric responsiveness, biocompatibility, and stability [258,259]. Examples of reported hydrophilic moieties are cobalt metallic moieties [258] and polydopamine (PDA) [259]. Apical papilla stem cells were incorporated into electro-responsive hydrogel composed of cobalt (Co)/(MWCNTs)- GelMA for neural regeneration purposes. Co/MWCNTs-GelMA hydrogel showed good biocompatibility and rapid enhancement of papilla stem cell differentiation. Greater electric responsiveness was observed in Co/MWCNTs-GelMA compared to MWCNTs-GelMA.

Moreover, hydrogels exposed to electric stimulation showed higher neuronal alterations and higher amounts of neuronal-related markers compared to non-exposed ones [259]. PDA/ MWCNTs - poly(citrate-maleic)-ε-polylysine (PME) electro-responsive hydrogel was developed by Wang et al. to enhance Traumatic SCI repair. The developed hydrogel showed good electro-responsiveness, self-healing features, and antibacterial effect. In vivo assessment of PDA/MWCNTs – PME hydrogel in SCI rat model exhibited better regenerative properties, decreased inflammation, and restoration of motor function [259].

4.3.4. Magnetic-responsive hydrogels

The use of magnetic nanoparticles (MNPs) (e.g. oxides, metallic NPs, and coated oxides) integrated into a hydrogel and driven by additional magnetic waves, makes up the magnetic-responsive hydrogels [288]. The quantity of incorporated MNPs influences not only the magnetic characteristics of the hydrogel but also the susceptibility of the cultured cells and the therapeutics 'release pattern [289]. Magnetic stimuli-responsive hydrogels possess several advantages, including minimal invasiveness, excellent tissue permeability, controlled drug migration, and versatile characteristics. Nevertheless, it is imperative to tackle obstacles such as the aggregation of nanoparticles and their possible toxicity to fully exploit their biological capabilities [290].

The rapid effect, good biocompatibility, and attractive mechanical properties were reported upon using magnetic responsive hydrogels [291]. Moreover, the magnetic field has a higher penetration ability through body tissues than that of light, permitting the development of more tunable scaffolds [262]. Co-precipitation, microemulsion, thermal decomposition, high-intensity ultrasound (US), and sol-gel formation are among the methods used to synthesize MNPs [288]. The pronounced magnetic features and biocompatibility of iron oxide NPs in magnetic-based hydrogels expanded their applications in TE [283]. However, toxicity may arise when used in higher quantities due to their higher content of iron [292]. A magnetic responsive hydrogel comprising poly (lactide-*co*-glycolide) (PLGA) fibers/Superparamagnetic iron oxide nanoparticles (SPIONs) was fabricated by Omidinia-Anarkoli et al. using the micro-cutting/electrospinning method in the presence of external magnetic source. SPIONs were employed to decrease the amount of iron oxide needed. PLGA was reported to have a supportive role in neural proliferation. The prepared hydrogel enabled unidirectional signal propagation and linear growth of nerve cells [263]. The 3D magnetic responsive hydrogel was fabricated by Santhosh et al. and composed of collagen/reduced graphene oxide MNPs.

Orientation of MNPs into collagen hydrogel was done by applying a low external magnetic source, this enabled the entrapment of neuroblastoma cells and the proliferation of neuronal cells [264]. To enhance the exosome delivery and cellular uptake, fluorescently tagged PC12 cell-based- exosomes were combined with magnetic nanogel and permitted to be aligned by a magnetic field. Magnetic stimulation promotes the proliferation of adipose stem cells into neuron-like cells [293]. Depending on the potential of the magnetic field to orient fibers, Tran et al. designed injectable peptide (RADA-16I) hydrogel to repair spinal cord injuries. Neural progenitor cells (NPCs) were added to the outer layer of the hydrogel to simulate infiltrating axons. Peptide hydrogel/NPCs/human MSCs (hMSCs) system exhibited better proliferation and orientation upon application of a magnetic field and the presence of magnetic carbonyl iron particles. In vivo studies proved the efficacy of the prepared scaffold showing enhanced axon proliferation and reduced inflammation in magnetic responsive systems compared to non-magnetically oriented ones [262].

4.3.5. Acoustic/ultrasound-responsive hydrogels

Controlling the delivery of various therapeutics and their penetration through different tissues can be accomplished by the use of US waves. The ease of direction of these waves, non-invasiveness, and good safety profile made US waves an appealing choice for not only drug delivery but also for the diagnosis of several medical conditions [294]. Moreover, BBB penetration, which represents a major challenge in delivering therapeutics to the brain, is facilitated by directing US waves through the targeted part of the brain [295].

Two implications of US waves have been reported to be responsible for their action. The first one is the thermal effect which depends on obtaining thermal energy through the transformation of US energy. This improves blood vessel permeability and disrupts cell membranes due to the remarkable increase in temperature. Cavitation is responsible for the second effect, non-thermal, which leads to nanobubbles and microbubbles formation. Cavitation can also generate sonoporation, an enhancement of cell membranes' porosity and permeation, achieving enhanced uptake of therapeutics [294]. Burst and pulsatile release of many bioactive moieties and cellular organization can be obtained through the use of US waves [296]. Cell transfection, sorting, and trapping were applied successfully. The orientation of cells and

therapeutics can be easily accomplished as well. According to the compressibility and concentration of cells concerning the surrounding environment, the pattern of cell alignment may differ [265]. The use of polymers with sol-gel conversion ability was fundamental to guarantee the fixation of cells at the targeted site following their alignment by US waves.

Cheng et al. designed an inexpensive and easily replicable 3D micropattern of neural tissue for tissue regeneration purposes [265]. Ultrasound standing waves were exploited to orient PC12 cells into a hydroxyphenylpropionic acid-gelatin (HPA-Gtn) hydrogel. Diluted hydrogen peroxide (H_2O_2) and HRP were used for cross-linking the HPA-Gtn precursor solution to settle the aligned cells. The HPA-Gtn combination was selected due to its suggested potential to manage the neurogenesis of MSCs, biodegradability, and structural similarity to neural tissue. The study ensured that US-exposed scaffolds did not cause a decline in neural cell differentiation and exhibited higher directional uniformity compared to non-exposed ones. However, in vivo, studies should be carried out to confirm these results.

4.3.6. Glucose-responsive hydrogels

Hydrogels developed to react to varying amounts of glucose are known as glucose-responsive hydrogels. Accordingly, they were considerably employed to manage diabetes and monitor blood glucose levels. Glucose-responsive hydrogels have been fabricated by incorporating several moieties including phenylboronic acid, glucose oxidase, lectin, and concanavalin A [288]. Free-radical cross-linking and covalent bonding are among the reported methods used to design glucose-responsive hydrogels [297]. The employment of diverse systems of glucose-responsive hydrogels such as micelles, vesicles, mesoporous NPs, and nanogels may be achieved [298]. Glucose-responsive hydrogels, on the other hand, have been exploited to design vascularized tissue models [266]. One of the vital issues that should be considered in designing TE scaffolds, is proper Vascularization. Owing to the diffusion difficulty, cells placed into hydrogels often suffer from nutrition and oxygen deficiency. As a result, this problem must be solved if engineered constructs are to be used for therapeutic purposes. Using sacrificial materials to create vascularized structures is an outstanding approach to overcoming this problem [299].

Tseng et al. fabricated a glucose-responsive hydrogel to serve as a vascularized neurological tissue. By combining dithiothreitol (DTT), poly(ethylene glycol) diacrylate (PEGDA), and borax solution, a boronate ester linkages were created and provided the desirable glucose-sensitive and self-healing features. The fabricated vascular-shaped structures of glucose-responsive hydrogels were incorporated into NSC-based non-glucose-responsive hydrogels. After 3 days, Vascular endothelial cells, placed into hydrogel channels, were moved into the non-glucose-responsive hydrogel. After a longer period (14 days), Vascularized neurological tissue was obtained by the generation of vascular connections from endothelial cells and the formation of neurosphere-like structures from NSC. Moreover, the presence of both NSCs and endothelial cells in the developed scaffold led to an enhancement in the expression of angiogenesis-related genes and neural proliferation. The results also suggested the promising potential of the developed glucose-responsive hydrogel in reconstructing vascularization in the brain [266].

4.3.7. Enzyme-responsive hydrogels

Stimulation of certain biological responses at specific body sites can be accomplished by changing the levels of particular enzymes performed by the application of enzyme-induced hydrogels [300]. Enzyme-responsive hydrogels can be engineered to selectively release pharmaceuticals by utilizing enzymatic reactions, enabling accurate regulation of drug administration [301]. The user's text is very short and does not provide any information. Hydrogels can react to enzymes and other stimuli, making them appropriate for tissue engineering by creating an optimal environment for cell proliferation and tissue creation. Enzyme-responsive hydrogels can self-repair, making them advantageous in several medicinal contexts [301]. However, it is necessary to investigate the intricate process of hydrogel production and gather more data on its long-term stability to comprehensively comprehend its possible advantages and drawbacks [301].

Enzyme-induced hydrogels offered various appealing features including high biocompatibility, rapid sol-gel conversion, good strength, and avoidance of chemical cross-linking-based cytotoxicity [224]. Enzymatic cross-linking using choline oxidase and horse radish peroxidase was exploited to develop an injectable murine BMSCs-based gelatin-hydroxyphenyl hydrogel for treating TBI. Incorporated BMSC showed enhanced proliferation, cell viability, and release of neurotrophin upon applying in vitro studies. In vivo studies proved the efficacy of the BMSC-based gelatin-hydroxyphenyl hydrogel group in improving neural function and regeneration compared to other groups, making it an attractive therapeutic option for TE purposes in CNS [267].

Activated astrocytes may represent a deleterious factor that enhances the development of spinal cord secondary injury. As a result, a protective rolipram–based polyethylene-imine (PEI)/PEG nanogel was designed to decrease astrocytes-motivated inflammatory reactions. The release of rolipram was aided by the degradation of nano gel caused by lysosomal enzymes. The prepared hydrogel showed good stability, considerable compatibility, reduced inflammatory reactions, and decreased Lcn2 and iNOS levels. In vivo, studies confirmed the efficiency of the developed system in the restoration of motor function in the early stage and maintenance of neural viability [268]. Metalloproteinase-9 (MMP-9) is an endopeptidase substantially elevated in macrophages which is prevalent in the newly reconstructed fibrotic ECM. Based on the ability of MMP-9 in wound healing and reconstruction of the fibrotic matrix, Hong et al. designed an Imidazole-poly (organophosphates) injectable hydrogel to fill the cystic gaps and enhance neural tissue repair. The potential of developed hydrogel to stabilize macrophages in the injury site led to fibronectin/MMP-9/fibroblasts-based interactions with subsequent improvement of neural tissue repair [274]. Removal of glioma, a highly dangerous brain tumor, is still a difficult issue despite the use of TMZ after surgery. The elevated expression of O^6-methylguanine-DNA methyltransferase (MGMT) in the glioma microenvironment reduces the efficiency of TMZ and increases the incidence of drug-resistant glioma cells. By incorporating of MGMT inhibitor (O6-benzylamine) with TMZ in a hydrogel system, Zhao et al. managed to prevent the expression of MGMT, improve the efficiency of TMZ, and decrease the presence of drug-resistant cells.

The higher amount of MMP enzymes that existed after neurosurgery, motivated the release of O6-benzylamine and TMZ [270]. Furthermore, an enzyme–responsive hydrogel comprising neuroprotective hexapeptide and poly (lactic acid)- co -[(glycolic acid)- alt -(L-lysine) (PLGL) was designed by Adak et al. to create an environment mimicking that of brain ECM and improve brain tissue regeneration. The release of hexapeptide was stimulated by the presence of MMP-9 in injured tissue [269].

5. Conclusion and future perspectives

Everyday occurrences like trauma, cancer, and neurodegenerative diseases can have a major impact on the CNS, rendering novel therapeutic approaches more necessary. Because of its intricate anatomy and physiology, the CNS has been studied for many years but is still a relatively unexplored area. This study covers the most recent advancements in bioactive SSRHs for the regeneration of CNS, which will play a major part in the field of neuroregeneration in the upcoming years. As extensively covered in this study, innovative methods involving the use of intelligent injectable hydrogels have several benefits, not the least of which is the opportunity to achieve a new level of customized therapies, specially fabricated to modulate specific faults and disorders. The combination strategies that allow concurrent utilization of structural, biochemical, and cellular manipulation are essential.

Stimuli-responsive hydrogels are intelligent materials capable of reacting to several stimuli, including temperature, pH, light, and biomolecules. They have demonstrated significant promise in the fields of tissue engineering, medication delivery, and various other biomedical applications. As a result, stimuli-responsive injectable hydrogels may soon be used on humans due to their capacity to replicate injured tissue with workable regeneration results, as shown in numerous pre-clinical trials. Additionally, engineering hydrogels with increased heterogeneities and complexities with similar CNS-ECM is crucial for better tissue integration. Despite the significant research advances, there are still some limitations that remain in clinical translation.

The manuscript highlights several gaps in existing studies, including the lack of fully completed clinical trials using hydrogels or biomaterial-based therapeutics for CNS regeneration. It also mentions the limitations that remain in the clinical translation of stimuli-responsive injectable hydrogels despite significant research advances. Additionally, it emphasizes the challenges faced in improving the overall functioning of severe TBI patients. To address these gaps, the manuscript suggests the need for further research to enhance the design of stimuli-responsive hydrogels for specific purposes, such as heart tissue engineering and oral disease treatment. It also emphasizes the importance of future advancements in the utilization of hydrogels, including enhancing their mechanical properties, biocompatibility, and stability, as well as exploring novel techniques for their production and analysis. Furthermore, the manuscript underscores the necessity of conducting fully completed clinical trials using hydrogels and biomaterial-based therapeutics for CNS regeneration. By addressing these gaps, researchers can advance the development of hydrogel-based systems for CNS regeneration and brain tissue engineering, potentially leading to improved treatment techniques for CNS injuries, degenerative diseases, and traumatic brain injuries.

As a concluding observation, the development of injectable smart hydrogel-based approaches may have a transformative positive impact on a number of life-threatening and debilitating pathologies (such as myocardial infarction, skeletal muscle injuries, cartilage, and tendon regeneration), where remission is incomplete, or progress is very slowly despite the use of current pharmacological and/or surgical methods. Future advancements in the utilization of these hydrogels involve enhancing their mechanical properties, biocompatibility, and stability, while also exploring novel techniques for their production and analysis. Furthermore, additional study is required to enhance the design of stimuli-responsive hydrogels for specific purposes, such as heart tissue engineering and oral disease treatment. In summary, stimuli-responsive hydrogels have great promise as a research area and provide numerous possible applications in the realm of biomedicine.

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Declaration of competing interest

The authors declare they have no conflict of interest.

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