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Grado en Farmacia

Use of biohydrogels for the treatment of brain injuries

(Uso de biohidrogeles para el tratamiento de lesiones cerebrales)

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Abstract

Stroke is one of the leading causes of death and long-term disability in adults. Eighty-five percent of this event is due to ischemic origin; it occurs due to an occlusion in the blood supply that causes tissue hypoxia, resulting in the nucleus of the lesion. Current treatments are aimed at restoring blood perfusion; however, they are only effective in the first hours after the stroke, which is a narrow therapeutic window that leaves a large number of patients out and consequently with neuronal sequelae that are often irreversible.

Due to the need for a therapy that promotes the regeneration of damaged tissue, a promising option has been developed with the transplantation of exogenous stem cells. Nevertheless, it poses several problems such as poor cell survival in the hostile environment of stroke, difficulty in crossing the blood-brain barrier or difficulty in cell differentiation among others. The use of hydrogels offers the possibility of obtaining a protective vehicle that provides an adequate environment for cells to survive, proliferate and differentiate. They also allow the encapsulation and release of different drugs or growth factors to improve the treatment of stroke.

Resumen

El accidente cerebrovascular es una de las principales causas de muerte y discapacidad a largo plazo en adultos. El 85 % de este evento es de origen isquémico; se produce debido a una oclusión en el riego sanguíneo que provoca hipoxia tisular dando lugar al núcleo de la lesión. Los tratamientos actuales estan dirigidos a restablecer la perfusión sanguínea sin embargo, solo son efectivos en las primeras horas tras el ictus lo que supone una estrecha ventana terapéutica que deja a una gran cantidad de pacientes fuera y en consecuencia con secuelas neuronales que en muchas ocasiones son irreversibles.

Debido a la necesidad de una terapia que promueva la regeneración del tejido dañado se ha desarrollado una opción prometedora con el transplante de células madre exógenas. Sin embargo, plantea una serie de problemas como son la escasa supervivencia celular en el ambiente hostil del ictus, la dificultad para atravesar la barrera hematoencefálica o la dificultad para la diferenciación celular entre otros. El uso de hidrogeles brinda la posibilidad de obtener un vehículo protector que proporcione un entorno adecuado para que las células sobrevivan, proliferen y se diferencien. Además permiten la encapsulación y liberación de diferentes fármacos o factores de crecimiento para mejorar el tratamiento del ictus.

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1. Introduction

The Stroke is one of the leading causes of death and severe disability in adults worldwide [7,14], is caused by local oxygen deprivation in the brain due to either hemorrhaged or occluded blood vessels [17]. Due to the limited capacity of the damaged brain to regenerate tissue de novo and the problems of current treatments, the need has arisen to investigate effective and accessible alternatives.

The objective of this work is to make a literature review of biohydrogels formulations used or stroke recovery reported during the last five years. We will discuss the main physical and mechanical properties, biocompatibility and degradation profiles of these biomaterials.

1.1 Pathophysiology of stroke

Ischemic stroke is caused by the occlusion of a vascular structure within the brain [22]. Upon injury, the neurons suffer massive degeneration and necrosis by primary and secondary damage, resulting in a deficit of neurological function [24]. Additionally, pro-inflammatory responses contribute to permanent tissue loss [12]. After the stroke occurs the massive cell death that extracellular matrix (ECM)-degrading [17]. The ECM is a three-dimensional, non-cellular structure composed of collagens, elastin, proteoglycans (including hyaluronan), and non-collagenous glycoproteins in healthy conditions [7]. They are primarily composed of two different cell types, neurons and glia and there are three main types of glial cells: astrocytes, microglia and oligodendrocytes that provide support to resident neurons [26]. In order to limit this matrix degradation to the boundaries of the stroke astrocytes and microglia are activated for guiding the stem cells to the site of injury and extend processes around the lesion to form a scar that compartmentalizes the degraded tissue within a physical empty cavity, that filled with extracellular fluid and form a physical barrier that hinders the infiltration and migration of regenerative cells [8, 6] (**Figure 1**). Glial cells alter the pH of the medium and produce an exacerbated inflammatory response by secreting pro-inflammatory cytokines, tumor necrosis factor

(TNF-alpha), and interleukin (IL1). Furthermore, they require a long period to phagocyte and degrade the wastes of dead cells [22, 43,45].

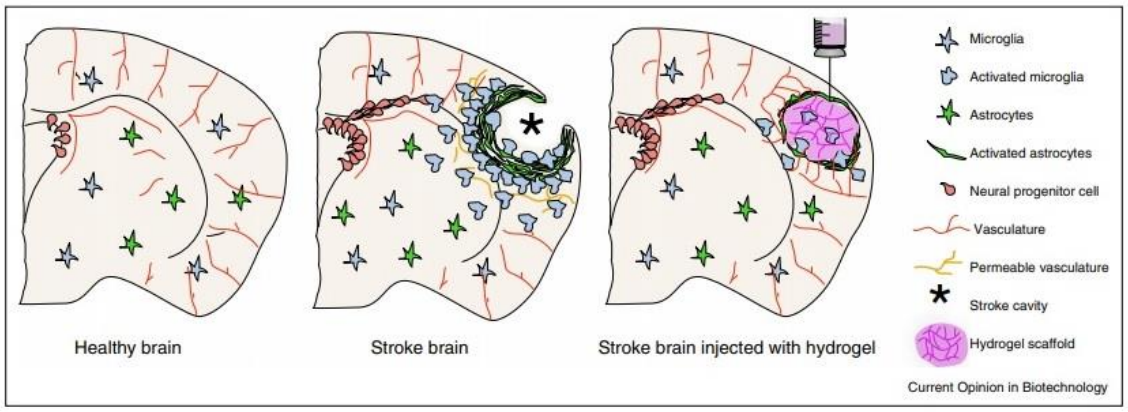


Figure 1: Schematic illustration of a coronal brain section and the major physiopathological events occurring after an ischemic stroke. Reproduced with permission from reference [8]. Copyright 2016. Elsevier.

The brain has a limited capacity for recovery, the processes it carries out in its attempt to recover damaged tissue are:

a) Neurogenesis: the process by which new neurons are generated from a stem or neural progenitor cells (NPCs) [45]. Under normal conditions, these newborn neurons migrate towards the hippocampus and the olfactory bulb. However, stroke damage was shown to increase NPC proliferation and re-route them towards the damaged site but the problem is the majority of newborn neuroblasts die prematurely within their migratory path [8,17].
b) Angiogenesis: the process by which blood vessels are formed [8].

1.2 Current therapy

At present, stroke treatments target tissue sparing with pharmacological or surgical thrombectomy [4]. The only FDA-approved treatment for ischemic stroke is tissue plasminogen activator (tPA), a thrombolytic drug [17,22,43], must not be given more than 4.5 hours post symptom onset as the potential damage for promoting hemorrhagic transformation outweigh any benefits of the treatment after this time, leaving many patients outside the therapeutic window [22,26]. Another option approved is the surgical endovascular thrombectomy. Both interventions focus on restoring blood flow by removing blood clots within the first few hours of stroke onset to prevent further infarct damage [4,22]. Although the availability of these treatments has been effective for reperfusion, these interventions do not address the long-term neurological deficit

resulting from stroke [44]. Patients who do not arrive on time for these therapies end up with long-term functional deficits, whose only effective means of recovery are physical and occupational rehabilitation therapies [16] and even so it is not always successful.

As an alternative, new therapies based on stem cell or growth factors transplantation are being developed. Unfortunately, several drawbacks remain to develop long-term, cell-based therapies. First, typically only 1-8% of transplanted stem cells survive, due in part to the highly hypoxic and inflamed post-stroke environment. Second, the time, cost, and infrastructure required to prepare an adequate number of stem cells for transplantation limits the ability to maintain a large-scale, stable product [21], Surviving stem cells have poor migration and differentiation to the site of injury. Other drawbacks lie in the short half-life and systemic effects of injectable growth factors. Finally found the blood-brain barrier (BBB), which prevents most therapeutics from freely diffusing into the brain parenchyma [36] and limits the diffusion of systemically delivered therapeutics [33] (**Figure 2**).

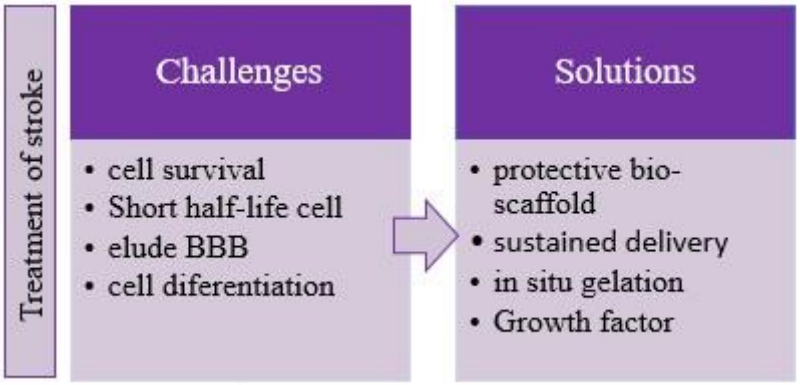


Figure 2: Challenges that must be overcome in order to tailor stem cell therapies for stroke.

One of the most promising approaches to address these issues is associated with the use of hydrogels. A hydrogel is, a hydrophilic, three-dimensional polymeric matrix, that is capable of imbibing large quantities of water, and tends to simulate biological tissues when swollen [10,15], act as a protective vehicle and that provide an appropriate environment for cells to attach, proliferate, and differentiate to facilitate the formation of the extracellular matrix. Similarly, they act protecting grafted cells from the immune response [6]. The requirements for designing hydrogels suitable for these applications will be discussed in section 3.

2. Methodology

In this work, a bibliographic review of scientific articles on the subject in question has been carried out, both in English and in Spanish. First of all, a search has been carried out in the main scientific databases such as Scopus, SciFinder, PubMed, Google Scholar and Point Q.

The keywords used were the following: hydrogel, drug delivery, stroke recovery, brain injury, stroke brain, ischemic stroke.

The literature inclusion and exclusion criteria have been based mainly on the last 5 years, thus limiting the search to all those articles published from 2016 to the present; all those articles that were outside our date range and that were in a language other than those already mentioned were excluded. To limit the search a little more and avoid finding publications that are too long and that do not interest us, such as patent conferences, letters, etc. Only original or review articles published in scientific journals included in Journal Citation Reports (JCR) were included.

The selection of the articles was made based on the reading of the abstracts and, if necessary, a quick reading of the complete article in order to check whether the information it contained was related to our topic and the main objective of the bibliographic review.

Finally, from the selected articles, each of the citations as well as the bibliographical references were analyzed to ensure that we did not exclude any other relevant studies in our work (**Figure 3**).

In addition, it was necessary to include four articles whose date is prior to 2016 since they are from which the central idea was drawn to carry out our review.

This procedure was repeated a few days before submitting the report to ensure that no other paper had been published during the development of the review.

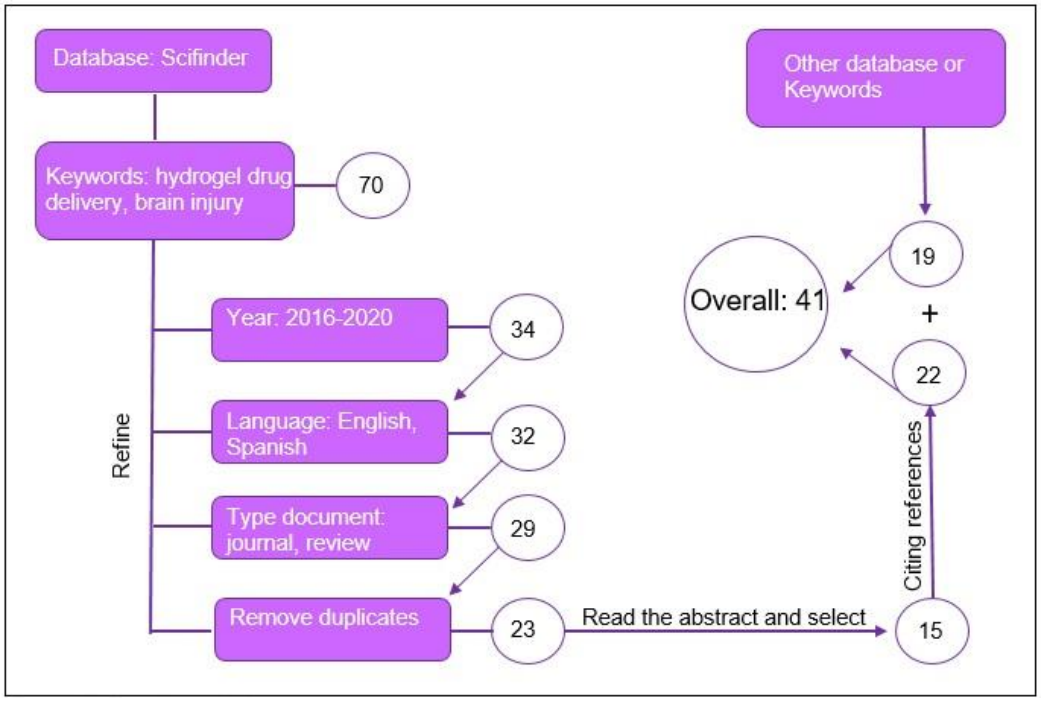


Figure 3: Shows a representative flow chart detailing the literature research methodology using SciFinder. The number of items selected in each step is represented in circles.

3. Requirements for designing suitable hydrogels

Injectable biomaterials can be used as a scaffold to fill the stroke cavity and promote interactions between transplanted material and host tissue, deliver drugs or growth factors to the damaged tissue, promote the attachment and engraftment of transplanted cells, and help recruit host cells to repopulate the lost tissue. Also, can be administered easily using a minimally invasive technique, and the minimal surgical wounds [30] and circumvents the BBB. Additionally, can activate endogenous repair processes, such as neurogenesis, that can potentially be harnessed to support tissue reconstruction in the stroke-damaged brain [5].

The choice of biomaterial compounds should be adapted for the different applications, structure targets, delay of administration, gravity and volume of lesion, zone and objective of treatment [7]. The following table lists some of the chemical and mechanical properties to take into account in the formulation of a hydrogel (**Table 1**). Generally, before testing a hydrogel in vivo, these characteristics have already been previously evaluated in vitro.

Property	**Requirements**	**References**
Biocompatibility	-Biologically inert. -Biologically similar to the host tissue. -Bioresorbable.	Gopalakrishnan et al 2019 [25]
Biodegradation	-A slow degrading to support the transplanted cells. -Faster degradation for the reduced inflammatory response in vivo. -Enzymatic or non-enzymatic hydrolysis.	Gyles et al 2017 [15]
Cellular infiltration	-Promote and guide cellular infiltration. -Promote axonal growth into a stroke cavity.	Nih et al 2016 [8]
Composition	-Natural, synthetic or hybrid.	Gyles et al 2017 [15]
Elasticity	-Stiffness in kilopascals (kPa), it should be around 3 kPa because that's the approximate stiffness of the brain	Gopalakrishnan et al 2019 [25]
Encapsulation	-Introduction of physical and/or covalent bonds between polymer chains, creating a	Ghuman et al 2016 [6]

	cross-linked network to remains drug in their structure.	
Gelation	-Moderate to rapid gelling to minimize any undesirable diffusion away from the injection site.	Ghuman et al 2016 [6]
Physic state	-Liquid state at room temperature while forming gels in situ. -Nanoparticles. -Solid scaffolds require surgery to implant.	Boisserand et al 2016 [7]
Polymerization	-Temperature -pH -Photopolymerization can lead to the formation of toxic free-radicals	Ghuman et al 2016 [6]
Swelling	-Must not swell significantly to avoid further brain damage and increasing intracranial pressure.	Tuladhar et al 2018 [17]
Toxicity	-Must not incorporate molecules that can be toxic as well as the by-products of biological compatibility must not be toxic.	Ghuman et al 2016 [6]

Table 1: Chemical and mechanical properties of hydrogels.

4. Hydrogels derived from natural polymers

In the last decades, the most widely used hydrogels for tissue engineering have been based on natural components of the ECM due to their innate biocompatibility and biodegradability. Examples include collagen, gelatin, alginate, hyaluronic acid (HA)… [26,37], even in combination, but not all are suitable. We will expose those that have been shown to have better physical-chemical characteristics and positive effects in regenerative therapy.

4.1 Hydrogels made of acellular extracellular matrix (ECM)

One of the most direct options is to directly develop ECM hydrogels. Harmanvir Ghuman and co-workers (2016) formulated a biological hydrogel produced from porcine urinary bladder ECM to replace necrotic debris and promote the infiltration of host brain cells as treatment stroke. These biomaterials were injected at different concentrations of ECM (0, 1, 2,3,4,8 mg / mL), fourteen days post-stroke into de rat brain ho were injured by the middle cerebral artery (MCA) occluded for 70 min prior to reperfusion. The hydrogel was injected using a needle and with a drainage cannula into the lesion cavity and it was waited for it to gel in situ at 37ºC before removing them. To analyze the volume of tissue loss and the distribution of the ECM hydrogel Magnetic resonance imaging (MRI) was used. Finally, the type and number of infiltrated cells were quantified and analyzed (Microglia and oligodendrocytes predominantly invade ECM hydrogel) by immunohistochemistry. Interestingly, at concentrations> 3mg / mL results in gelation and large numbers of infiltrating cells. Concentrations <3 mg / mL do not readily form a hydrogel (**Figure 4**). The 8 mg / mL concentration in the current study was the highest concentration implanted and hence also provided the highest number of invading cells (M2 phenotype predominantly) and the greatest abundance of inductive cues to elicit a regenerative response that could potentially be exploited to the treating stroke [5].

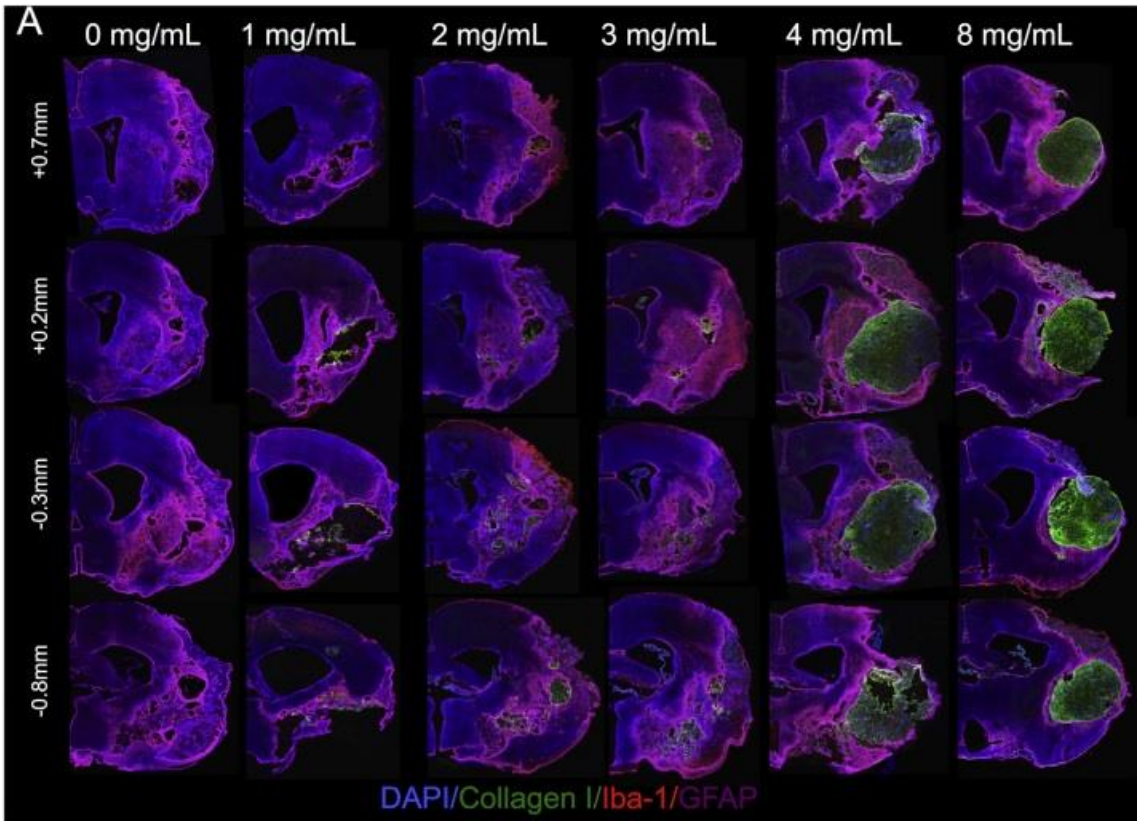


Figure 4: Histological visualization of a coronal section of the rodent brain showing gelling and retention of the hydrogel at different concentrations. Reproduced with permission from reference [5]. Copyright 2016. Elsevier.

Later it was deepened in the effects of a long-term (12 weeks) of this concentration, the result of this study indicated that an 8 mg / mL concentration of UBM-ECM hydrogel held for 12 weeks, can reduce the lesion volume cavity by 28% with minimal impact on host tissue, peri-infarct astrocytosis and glial scarring. However a battery of behavioral test in rats before and after stroke, reveal behavioral functions were not affected by the implantation or retention of the material within the cavity indicating that an 8 mg / mL preparation appears safe for the local delivery of therapeutic factors, but by itself does not appear to exert a therapeutic benefit or detrimental in stroke [9].

Notwithstanding, when another study evaluated the biodegradability during the same time of hydrogels of 3 and 4 mg / mL compared to that of 8 mg / mL **(Figure 5),** it was observed that, although a concentration greater than 8 mg / mL of ECM induces a greater cellular infiltration acutely due to its higher inductive content as already demonstrated in previous studies, but over time this relationship was reversed. This is because 8 mg / ml of ECM remained constantly present 90 days after implantation, reducing the number of infiltrated cells and interfering with tissue repair, while the less

concentrated gels (3 and 4 mg / mL) revealed an extensive degradation at 14 days and almost complete reabsorption of the implanted material at 90 days, increasing its cellular infiltration over time and displaced smaller amounts of damaged tissue. Specifically, the 4 mg/mL hydrogel has the most favorable characteristics for brain regeneration [19].

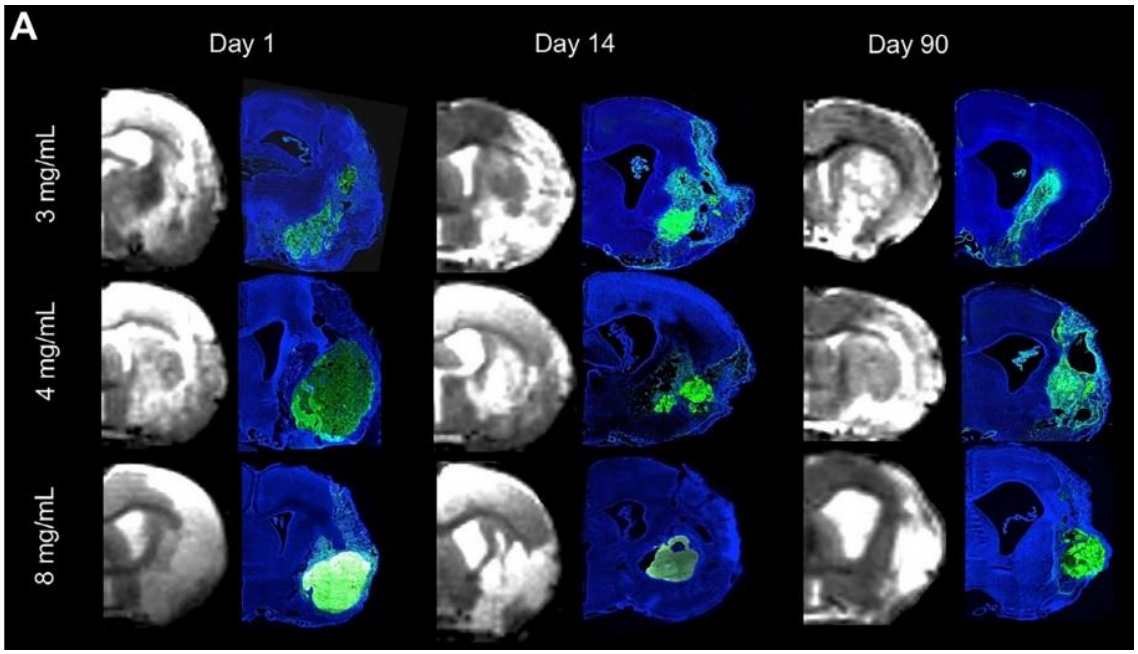


Figure 5: ECM degradation at different concentrations. Reproduced with permission from reference [19]. Copyright 2018. Elsevier.

ECM hydrogels were not only prepared from the porcine bladder but also other organs such as the esophagus, tendon, or central nervous system (CNS). A study was carried out in ECM was implanted (One hour after) in rats with traumatic brain injury (TBI) that was induced by a controlled cortical impact (CCI). As expected, it was concluded ECM treated brains showed a 25% reduction in tissue loss at 35 days. In comparison, both UBM-ECM and brain ECM increased the number of cells expressing neurites but only the brain ECM increased neurite length, mitigated glial scar formation and pro-inflammatory microglial responses, there by promoting tissue remodeling and repair [12].

4.2 Hyaluronic acid/ methylcellulose-based hydrogels

It has been shown that both hyaluronic acid and methylcellulose are the polymers that have been most developed for their compatibility with the ECM. Shoichet`s lab developed a hydrogel composed of hyaluronan and methylcellulose (HAMC) [33]. HA is a linear copolymer of 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid, its physical structure permit to exist as either firm hydrogels; by itself promote proliferation and migration, angiogenesis, as well as inflammatory response control. Methylcellulose is a cellulose-based molecule; is considered thermo-responsive as it undergoes gelation upon exposure to particular temperatures but these gels had a relatively low mechanical strength limiting its application possibilities. This problem was alleviated when methylcellulose was combined with other polymers [15]. These hydrogels were injected directly into the stroke-injured site of the mouse brain, demonstrating the survival and distribution of transplanted NPCs, improved locomotor function and attenuate the inflammatory response in the CNS. The properties of both polymers allow the gel to be bioresorbable. HAMC is a versatile drug delivery vehicle enabling several different methods of localized, sustained biomolecule release, for example, biomolecules have been released from HAMC after being solubilized, incorporated as particulates, or encapsulated in poly (lactic-co-glycolic acid) (PLGA) nanoparticles among others that we will mention[1] **(Figure 6)** [33].

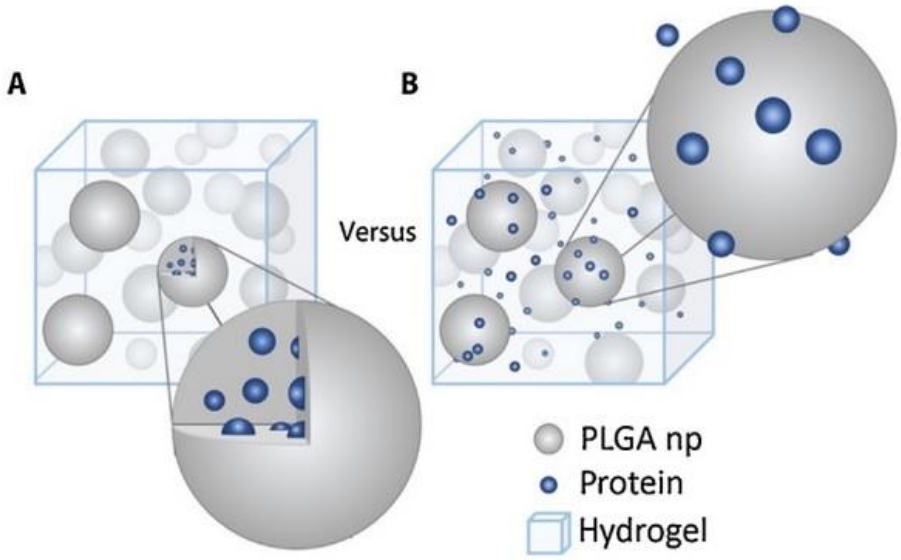


Figure 6: A) Example of encapsulation of biomolecules within PLGA nanoparticles dispersed in a hydrogel and B) Electrostatic adsorption of biomolecules onto the surface of PLGA nanoparticles dispersed in a hydrogel. Reproduced with permission from reference [33]. Copyright 2019. Elsevier.

[1] We will see the PLGA hydrogels better in the synthetic hydrogels section

4.3 Gelatin hydrogels

Yao et al., (2019) synthesized a gelatin hydrogel dual-enzymatically cross-linked by glucose oxidase (GOX) and horseradish peroxidase (HRP), encapsulated with bone (Mouse) mesenchymal stem cells (BMSC) for TBI model treatment. GOX was used to oxidate D-glucose and generate H2O2 for the gelation of hydrogel (**Figure 7**). Gelatin (the porcine skin), a product of collagen hydrolysis have remarkable advantages including excellent biocompatibility, low-cost, mediated degradability, easy chemical modification, natural cell adhesion motif retention and the digestion process confers gelatin low antigenicity and minimal inflammatory response. The hydrogel was injected into the brains of rats that were induced to head trauma, 7 days after injury. The neurological motor function of the rats was evaluated as well as learning and memory through the Morris water maze test (MWM). The results were compared with control rats, rats to which only the scaffold was administered or rats to which only the cells were administered and they observed that scaffold + BMSC transplantation ameliorated the learning and memory in rats after TBI and promoted the recovery of neurological function, besides that prevents cell death from injury by neurotrophic factors and inhibits cell apoptosis (**Figure 8**) [24].

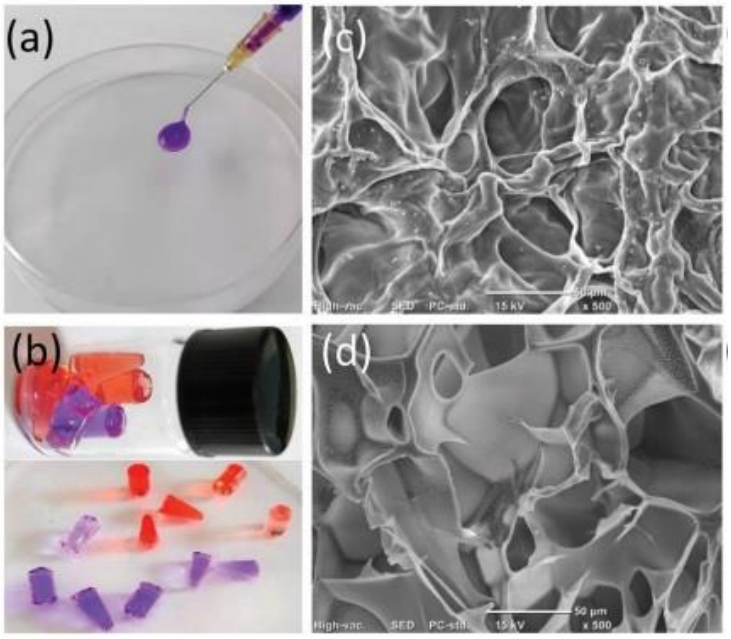


Figure 7: (a) Injectability of the Gelatin-Hydroxyphenyl (GH) hydrogel; (b) GH hydrogel with different shapes and colors; (c) surface and (d) internal microtopography of the hydrogel. Reproduced with permission from reference [24].

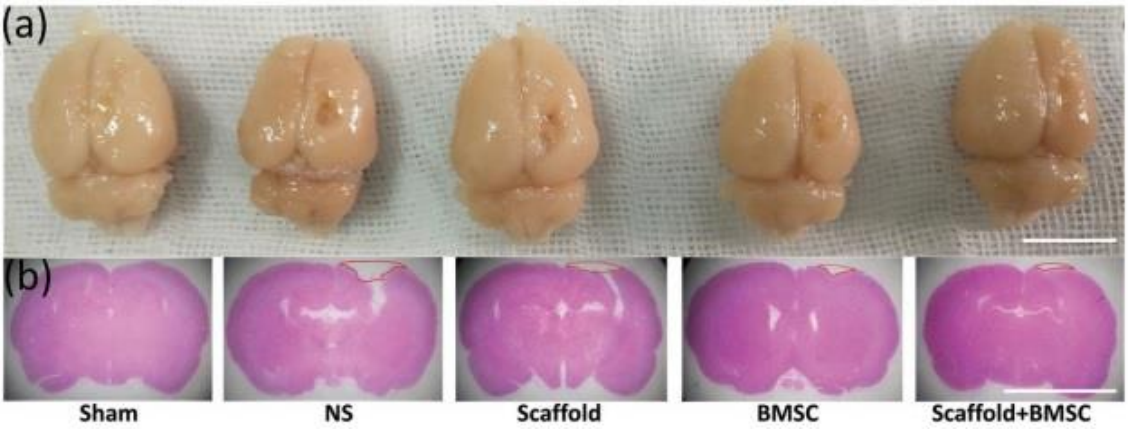


Figure 8: (a) images and (b) staining of the damaged areas in the sham, NS, scaffold, BMSC, and scaffold + BMSC. Reproduced with permission from reference [24].

4.4 Alginate hydrogels

Singh et al., (2019) designed alginate, sterculia gum polysaccharide base hydrogel. Alginate extracted from the sea weeds is a hydrophilic, heteropolysaccharideand and is an effective wound and skin healing agent. In this study only trials of degradation, biocompatibility and evaluated physic and mechanical properties. Also, they studied release dynamics of citicoline drug, a nerve regenerating agent but not tested on animal models. Overall, these observations indicated that the hydrogel of sterculia/alginate could be used as material for brain drug delivery and wound dressings [40].

4.5 Growth factors delivery from natural hydrogels

Growth factors involved in neuronal recovery such as the endothelial growth factor (EGF) to induce proliferation and migration of endothelial cells, the fibroblast growth factor (FGF) to induce local angiogenesis, the vascular endothelial growth factor (VEGF) that stimulates angiogenesis too, platelet-derived growth factor (PDGF) used to stimulate migration of some stem cells [27], have also been investigated for their encapsulation and release in hydrogels in order to enhance their activity.

In recent years it have been demonstrated on Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is to play a role in brain plasticity that regulates a wide spectrum of neuronal function including neuronal development, survival, neurite extension and synaptic remodeling [11,31] and promotes recovery of function in several preclinical models in stroke. However, BDNF injected does not penetrate the BBB well. For this reason it is the most studied in its incorporation into hydrogels.

Cookand and co-workers tested a hyaluronic acid hydrogel + BDNF, at different concentrations, 7 days after of photothrombotic stroke was induced mouse and non-human primate [13]. The results were evaluated by behavioral tests and visualized by magnetic resonance. These results suggest that Sustained BDNF release from an HA hydrogel inside the infarct cavity at a time point of subacute stroke promotes motor recovery, axonal, induces the initial migration of immature neurons and increases neurogenesis [13].

These results have been reinforced by another study in which examined the effects of BDNF, delivered via an extended-release hystme-C hydrogel (HyStem®-C hydrogels are composed of biocompatible materials and contain both a thiol-reactive cross-linked and thiol-modified HA) implanted (after eight days) into a model of an MCA stroke in rats. In this study, rats received BDNF only, hydrogel only, hydrogel + BDNF LOW (0.057 µg/µL) or hydrogel BDNG HIGH (0.167 µg/µL) **(Figure 9).** The result has been evaluated with a behavioral test and compared the effect of different hydrogels two months post-ischemia. As a result, infarct volume was reduced in rats treated with hydrogel + BDNF HIGH in addition to an improved sensorimotor function and mitigated glial-scar formation [16].

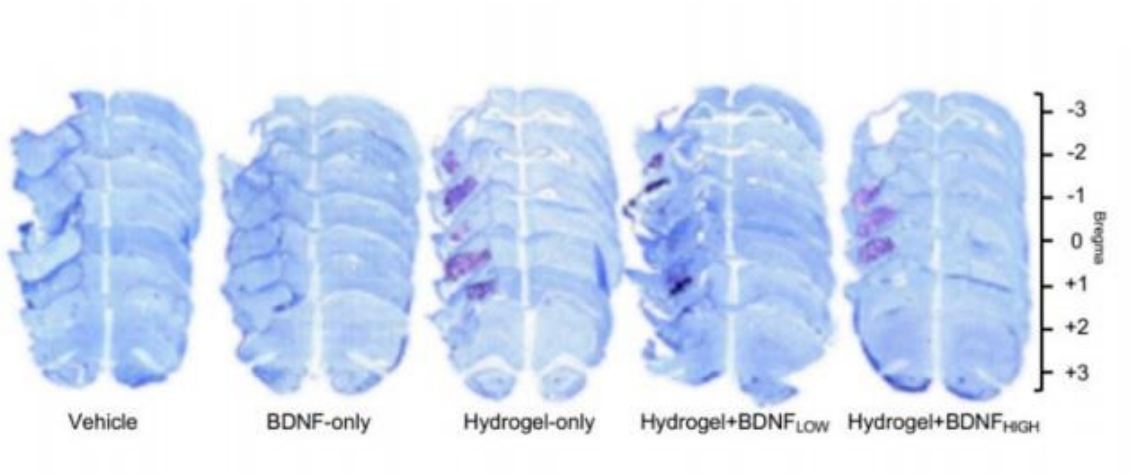


Figure 9: Coronal sections of rat brain where the different hydrogels tested are compared. Reproduced with permission from reference [16].

Obermeyer et al. substantiated the role of BDNF and obtained similar results to the studies already cited above. In this case, BDNF was dispersed in a hydrogel composed of hyaluronan and methylcellulose (HAMC) with PLGA nanoparticles, a hybrid hydrogel of natural and synthetic polymers. In addition, this hydrogel was deposited directly onto the surface of the cortex (of rat´s brain) in the space created by the skull and polycarbonate

disk with a concentric hole. A second polycarbonate disk with no opening was secured over the top of the first disk **(Figure 10**) [32].

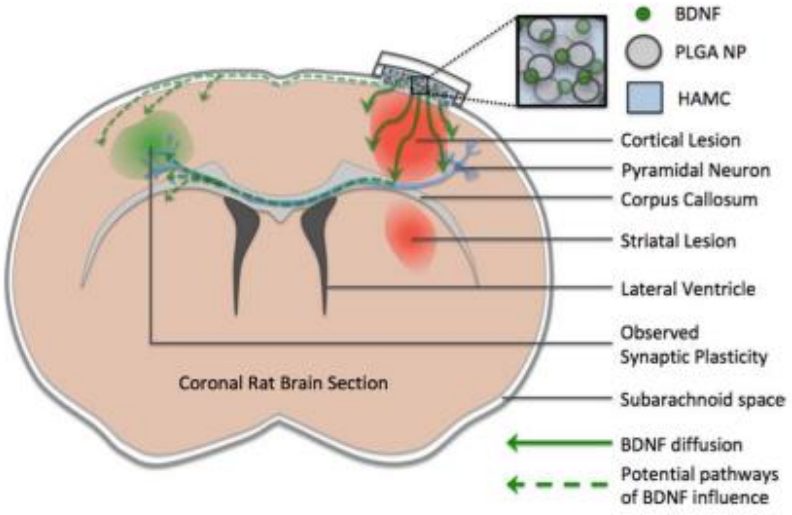


Figure 10: Implantation of a HAMC hydrogel of BDNF. Reproduced with permission from reference [32].

5. Hydrogels derived from synthetic polymers

Synthetic hydrogels have not been independently studied in our review papers due to limited ability to induce endogenous repair responses [45]. However, have been used as microcarriers for the release of angiogenic and neurotrophic factors [37] and have been efficiently tailored with the incorporation of biological signals to support cell growth and proliferation [25]. For this reason we will see them in the next section where we will talk about the most common such as PEG and PLGA.

5.1 Growth factors delivery from synthetic hydrogels

In 2018 George et al synthesized a hydrogel of polyethylene glycol (PEG)-peptide polymers and C7 recombinant protein in which they encapsulated VEGF-A and matrix metallopeptidase (MMP-9). The hydrogel was tested in an animal model as we have seen previously. Simple mixing of the two components at constant physiological conditions forms a hydrogel. The biodegradable hydrogel incorporated with VEGF-A and MMP-9 was designed to be injected into the cortex similar to stem cell therapy to determine its efficacy on functional recovery after stroke. The animals that received hydrogel + VEGF-A and MMP-9 or two factors separately it was observed that when the factors were administered simultaneously this hydrogel mimics stem cell effects on functional recovery after ischemia and promotes recovery post-stroke which does not happen if they are administered separately [21].

One of the most recent and interesting studies is that of Thuladhar et al., that demonstrated, for the first time, that the combination of two clinically used drugs, Cyclosporine (CsA) and Erythropoietin (EPO), but not each drug alone, accelerates functional recovery and promotes tissue repair after stroke in an endothelin-1 rat model. CsA is a common immunosuppressant and has been shown to stimulate the endogenous stem cells in the rodent brain and EPO stimulates red blood cell production and has been shown to promote neurogenesis in the rodent brain. They synthesized a composite hydrogel of drug-loaded poly (lactide-coglycolide) (PLGA) particles that were then dispersed in HAMC. CsA and EPO were independently encapsulated in separate PLGA particles. The gel was implanted onto the surface of the rat brain, 4 days after injury and was enclosed in a polycarbonate casing. The result of this study indicated that stroke lesion volume is decreased by combined delivery and accelerated functional recovery and improved tissue repair [35].

6. Other Approaches

Although in this review we focus on polymer-based hydrogels, other materials are also studied, an example of this is the injectable hydrogel with Choline-functionalized graphene oxide and poly (acrylic acid) (CFGO) (**Figure 11**). Neurotransmitter choline is known to exert neurogenesis in an injured brain and has been evaluated for its potential of neuro-recovery in the treatment of stroke as well as TBI and together with graphene oxide have neuroprotective roles [29].

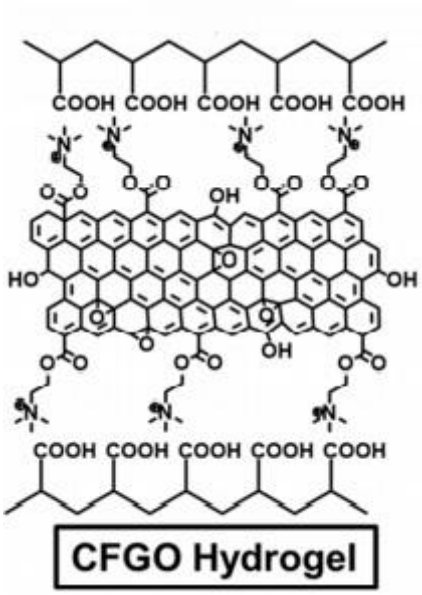


Figure 11: structure of CFGO hydrogel. Reproduced with permission from reference [29]. Copyright 2019. American Chemical Society.

In recent years, injectables self-assembling peptide-based hydrogels (SAPH) have been developed to encapsulate growth factor SAPH is composed of chemically synthesized oligopeptides Self-assembling that can promote cell-specific signaling [42].

Other examples is the Chondroitinase ABC (ChABC) (bacterial enzyme) has been encapsulated in a methylcellulose hydrogel. One study determined that sustained delivery of this stabilized enzyme in a methylcellulose hydrogel is a viable strategy for degrading the inhibitory glial scar in stroke and other CNS injuries [34].

When intracerebral hemorrhage (ICH) occurs, there is no necrosis and loss of tissue ipso facto as in stroke, however, if not treated properly, Iron overload can lead to injury and damage to the brain tissue. Very recently, Zhu et al., synthesized a deferoxamine mesylate (DFO) (the iron chelator) loaded thermosensitive keratin hydrogels (TKGs) (**Figure 12**), that effectively reduced the iron overload after ICH to enhance the neurological deficits recovery [30].

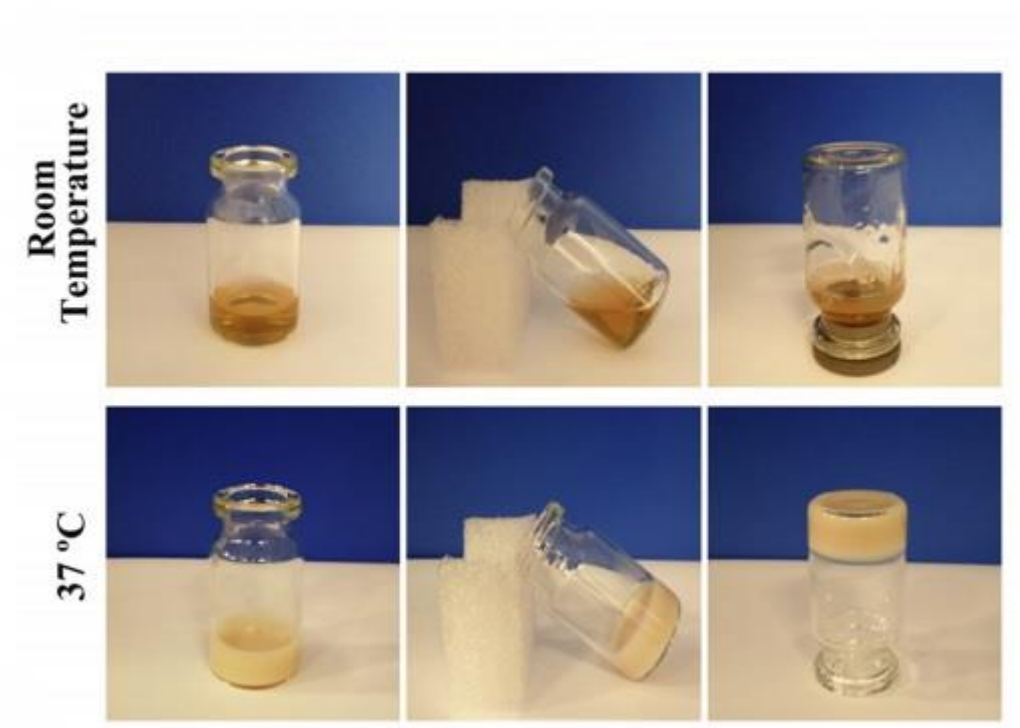


Figure 12: Photographs of the thermo-sensitivity of the TKG at room temperatures and 37 °C. Reproduced with permission from reference [30]. Copyright 2019. Elsevier.

Curiously, nanoparticles of Progesterone (PG) loaded N, N, N-Trimethyl chitosan (TMC) hydrogel, was prepared by this hydrogel was administered by Cardia et al. very recently for inhalation via an aerosol apparatus for brain delivery. Progesterone is a sex hormone that shows neuroprotective effects in stroke and TMC is a hydrophilic polymer obtained by chitosan methylation but it is necessary to carry out more studies in order to identify the main route involved in PG delivery to the brain tissue as well as its effect on stroke [28].

The following table summarizes the hydrogels mentioned in this review (**Table 2**):

Composition of hydrogel	Type	Growth factor or drug	Results	Model of stroke	References
ECM bladder: Extracellular, acellular matrix + variety of growth factors	Natural	Transforming growth factor-b, vascular endothelial growth factor-A, basic fibroblast growth factor, and nerve growth factor	Reduces the volume of the lesion but doesn`t modify motor functions	MCA occluded	Ghuman et al 2016 [5]
ECM brain: Extracellular, acellular matrix + variety of growth factors	Natural	Transforming growth factor-b, vascular endothelial growth factor-A, basic fibroblast growth factor, and nerve growth factor	Increased neurite length, mitigated glial scar formation and pro-inflammatory microglial responses, there by promoting tissue remodeling and repair	MCA occluded	Wu et al., 2017 [12]
Hyaluronic acid + BDNF	Natural	BDNF	Modify motor functions	Phototrombotic	Cook et al., 2017 [13]
HyStem®-C **HA + Thiol**	Natural	BDNF	Modify motor functions in high concentrations	MCA occluded	Ravina et al., 2018 [16]

HAMC: hyaluronan + methylcellulose	Natural	-	Survival and distribution of transplanted cells, improved locomotors function and attenuate the inflammatory response	MCA occluded	Ho et al., 2019 [33]
Gelatin + GOX + HRP	Natural	-	Ameliorated the learning and memory, promoted the recovery of neurological function	Traumatic lesion	Yao et al., 2019 [24]
Alginate + sterculina	Natural	Citicoline	More studies	-	Singh et al., 2019[40]
TKG	Natural	DFO	Reduced the iron overload	ICH	Zhu et al., 2019 [30]
TMC	Natural	PG	More studies	-	Cardia et al., 2019[28]
Methylcellulose + Chondroitinase ABC	Natural	Chondroitinase ABC	More studies	-	Hettiaratchi et al., 2019 [34]
PEG + C7 + VEGF-A + MMP-9.	Synthetic	VEGF-A and MMP-9.	Promotes recovery after stroke		George et al., 2018 [21]

HAMC + PLGA + BDNF	Hybrid	BDNF	Modify motor functions	MCA occluded	Obermeyer et al., 2019 [32]
HAMC + PLGA + CsA + EPO	Hybrid	CsA + EPO	Lesion volume decreased , accelerated functional recovery, improved tissue repair	Endothelin-1	Thuladhar et al., 2020 [35]
CFGO	Others	Choline	More studies	-	Pradhan et al., 2019 [29]

Table 2: shows a scheme with the main synthetic, natural, and mixed biomaterial compositions have been described in this review.

7. Conclusions

Advances in the development of hydrogels for neuronal regeneration after stroke are being a promising therapy. Nevertheless, the application of biomaterial strategies for brain repair is in its nascent preclinical stages and applying it to humans remains a great challenge today.

Despite the encouraging results of these strategies in tackling brain repair after stroke and the evidence obtained from the improvement in neurogenesis and angiogenesis as well as in motor function, it is difficult to predict whether documented functional recovery is found in rodent models can be translated in humans. This is mainly due to none of the known stroke animal models are a perfect representation of human disease. Moreover, although most of the hydrogels tested appear to be harmless in animal tests, there is an unknown horizon regarding their safety and tolerability in the human brain, which is why it is necessary to carry out more tests of toxicity, biodegradability and biocompatibility. However, the existence of bio-scaffold with a continuous supply of neural progenitor, growth factors or drug cells presents opportunities to harness the regenerative capabilities of the brain that is why we can conclude that biomaterial-based hydrogels will exert significant influence as regenerative therapeutics in stroke.

Glossary

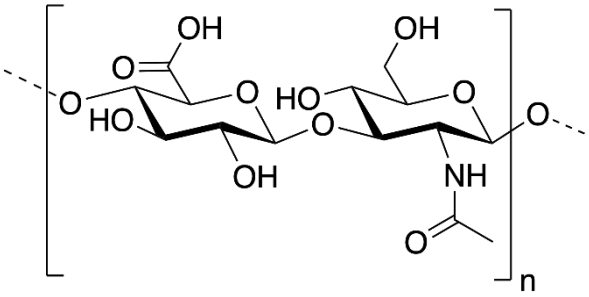
BBB	Blood-brain barrier.
BDNF	Brain-derived neurotrophic factor.
BMSC	Bone mesenchymal stem cells.
CCI	Controlled cortical impact.
CFGO	Choline-functionalized with Graphene oxide.
ChABC	Chondroitinase ABC.
CNS	Central nervous system.
CsA	Cyclosporine.
DFO	Deferoxamine mesylate.
ECM	Acellular extracellular matrix.
EGF	Endothelial growth factor.
EPO	Erythropoietin.
FGF	Fibroblast growth factor.
GH	Gelatin-hydroxyphenyl.
GOX	Glucose oxidase.
Gtn-EGF	Gelatin hydrogel with epidermal growth factor.
HA	Hyaluronic acid.
HAMC	Hyaluronan and methylcellulose hydrogel.
HRP	Horseradish peroxidase.
ICH	Intracerebral hemorrhage.
IL1	Interleukin.
JCR	Journal Citation Reports.
kPa	Kilopascals.
MCA	Middle cerebral artery.
MMP-9	Matrix metallopeptidase.
MRI	Magnetic resonance imaging.
MWM	Morris water maze test.
NPCs	Neural progenitor cells.
PDGF	Platelet-derived growth factor.
PEG	Polyethylene glycol.
PG	Progesterone.
PLGA	Poly (lactic-co-glycolic acid).

SA	Sodium alginate.
SAPH	Self-assembling peptide-based hydrogels.
TBI	Traumatic brain injury.
TKGs	Thermo sensitive keratin hydrogels.
TMC	N, N, N-Trimethyl chitosan.
TNF-alpha	Tumor necrosis factor.
t-Pa	Tissue plasminogen activator.
UBM-ECM	Urinary bladder matrix- acellular extracellular matrix.
VEGF	Vascular endothelial growth factor.

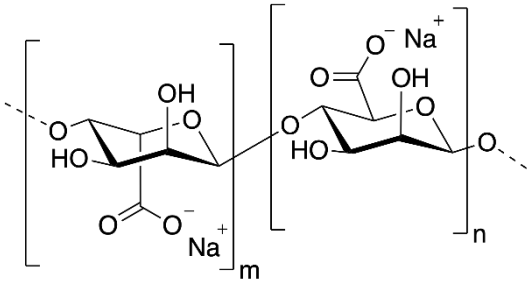
Annex 1

The chemical structures of some biopolymers and synthetic polymers with stroke application.

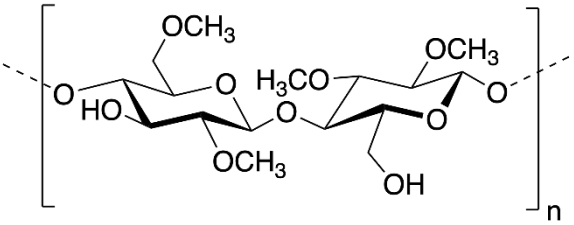
Hyaluronic acid



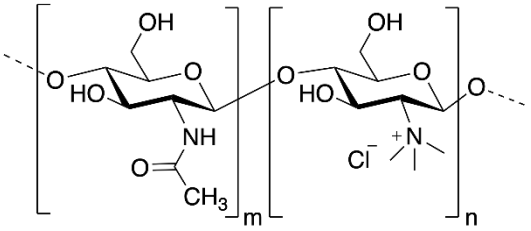
Sodium Alginate



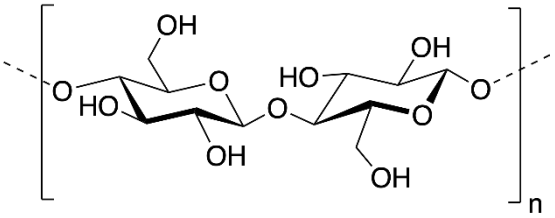
Methylcellulose



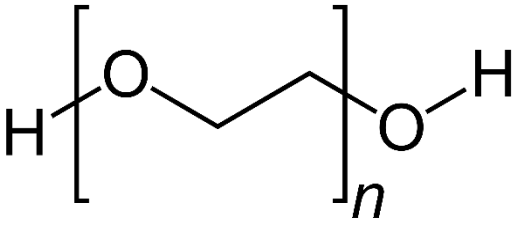
Trimethyl chitosan



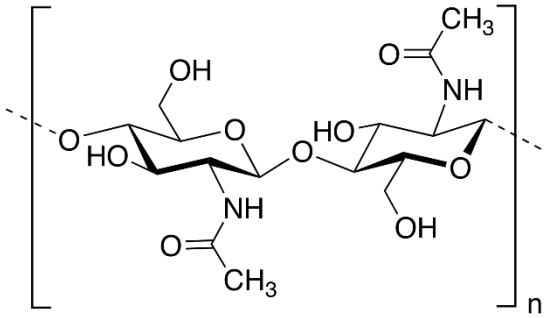
Cellulose



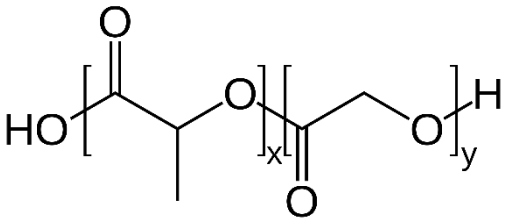
PEG



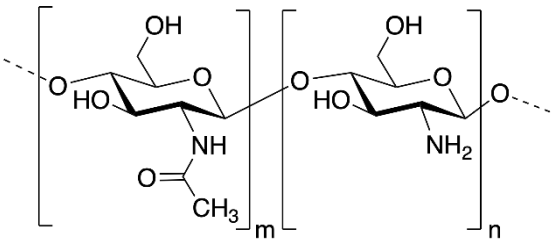
Chitin



PLGA



Chitosan



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