● **REVIEW**

Hydrogels for neuroprotection and functional rewiring: a new era for brain engineering

Rocío Fernández-Serra[1], Rebeca Gallego[1], Paloma Lozano[1], Daniel González-Nieto[1, 2, 3, *]

1 Center for Biomedical Technology, Universidad Politécnica de Madrid. Madrid, Spain
2 Departamento de Tecnología Fotónica y Bioingeniería, ETSI Telecomunicaciones, Universidad Politécnica de Madrid, Madrid, Spain
3 Biomedical Research Networking Center in Bioengineering Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain

Abstract

The neurological devastation of neurodegenerative and cerebrovascular diseases reinforces our perseverance to find advanced treatments to deal with these fatal pathologies. High-performance preclinical results have failed at clinical level, as it has been the case for a wide variety of neuroprotective agents and cell-based therapies employed to treat high prevalent brain pathologies such as stroke, Alzheimer's and Parkinson's diseases. An unquestionable reality is the current absence of effective therapies to neuroprotect the brain, to arrest neurodegeneration and rewire the impaired brain circuits. Part of the problem might arise from the lack of adequate *in vitro* and *in vivo* models and that most of the underlying pathophysiological mechanisms are not yet clarified. Another contributing factor is the lack of efficient systems to sustain drug release at therapeutic concentrations and enhance the survival and function of grafted cells in transplantation procedures. For medical applications the use of biomaterials of different compositions and formats has experienced a boom in the last decades. Although the greater complexity of central nervous system has probably conditioned their extensive use with respect to other organs, the number of biomaterials-based applications to treat the injured brain or in the process of being damaged has grown exponentially. Hydrogel-based biomaterials have constituted a turning point in the treatment of cerebral disorders using a new form of advanced therapy. Hydrogels show mechanical properties in the range of cerebral tissue resulting very suitable for local implantation of drugs and cells. It is also possible to fabricate three-dimensional hydrogel constructs with adaptable mesh size to facilitate axonal guidance and elongation. Along this article, we review the current trends in this area highlighting the positive impact of hydrogel-based biomaterials over the exhaustive control of drug delivery, cell engraftment and axonal reinnervation in brain pathologies.

Key Words: *advanced therapies; Alzheimer's disease; biomaterials; brain; hydrogels; neurological diseases; Parkinson's disease; polymers; stroke*

Introduction

Brain pathologies are the leading causes of disability in humans. Due to demographic changes, a further increase in the incidence of neurodegenerative and cerebrovascular disorders is expected. Because the injured brain has a limited ability to repair itself, the structural and functional rewiring of damaged networks represents one of the most difficult challenges that humanity has had to face. While we study the yet unknown mechanisms causing Alzheimer's or Parkinson's in healthy brains or establish prevention programs to minimize risk factors in stroke, we are simultaneously exploring novel paradigms to provide neuroprotection and repair the damaged brain. At present there is a lack of effective treatments to stop neuronal degeneration and death and reconstruct impaired brain circuitry. In ischemic stroke, the most common form of disabling pathology, pharmacological and endovascular therapies are currently used in neurology care units to clear the occluded vessels (Saver et al., 2015). However, few patients benefit from recanalization approaches because delayed interventions are associated with a high risk of hemorrhagic transformation and increased oxidative stress due to ischemia/reperfusion. In Parkinson's disease,

levodopa and deep brain stimulation have been used for years to attenuate clinical symptoms in patients, but their efficacy declines as neurodegeneration progresses (Milosevic et al., 2018). In addition, many Parkinson's patients suffer disabling side effects and the on-off phenomenon where mobility and improvement in symptoms alternate with tremor, rigidity and bradykinesia. In Alzheimer's disease the outlook is much more devastating. Promising preclinical therapies, such as some beta-secretase inhibitors, have not shown clinical efficacy (Egan et al., 2018). The lack of effective treatments in Alzheimer's disease has driven the search for novel targets and squeezing the older ones to develop more efficient therapeutic compounds. Since the deposition of amyloid-β is an established hallmark in Alzheimer's pathology, many efforts have been focused to exploiting antibody-based immunotherapies against amyloid-β. By contrast to the accumulation of amyloid-β in surrounding neurons, intracellular aggregation of microtubule-associated protein tau into neurofibrillary tangles and transneuronal spreading of tau has been found in Alzheimer's brains. Thus, molecules that target Tau have been assayed preclinical and clinically (Congdon and Sigurdsson, 2018). Additional strategies have focused on

targeting inflammation and microglial dysfunction or improvement in lymphatic drainage and clearance of amyloid-β (Louveau et al., 2016). The discovery of the transmissible character of amyloid-β pathology might have implications in the development of novel therapies to prevent/treat this fatal disease (Purro et al., 2018). Much work has been done to identify and exploit targets involved in secondary damage after stroke. Hundreds of molecules have been tested to target central and peripheral inflammation, excitotoxicity, oxidative stress, matrix metalloproteinases dysregulation and, in less depth, spreading-depolarization waves and their related inverse hemodynamic response (Marshall et al., 2003; Rothwell, 2003; Sakowitz et al., 2009; Bratane et al., 2011). Unfortunately, most of these compounds have failed in clinical trials, reducing our expectations of quickly discovering effective neuroprotective therapies.

By contrast, repairing the already damaged brain adds another level of complexity. The formation and integration of newborn neurons in brain circuits occurs actively during embryonic and neonatal development. However, in the adult and aging brain, this process is incredibly inefficient (Sorrells et al., 2018). Recent studies have proposed the opposite idea, stating that neurogenesis is very active in healthy brains at all stages of life, while this process is drastically reduced in Alzheimer's brains (Moreno-Jimenez et al., 2019). Repair strategies have been exercised through different approaches, most of them focused on stimulating neurogenesis and production of newborn neurons for subsequent incorporation in noninjured and injured areas, obviously if the pre-existing circuitry is preserved at a minimal level of structural/functional organization to foster new neuronal integration. Direct administration of neurotrophic factors (such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF)) or their release (secretome) from exogenously transplanted multi- and pluri-potent stem cells (MPSCs) has been reported to efficiently enhance neurogenesis (Steinbeck and Studer, 2015; Baez-Jurado et al., 2019). Other approaches have focused on the direct or cell-mediated administration of angiogenic factors such as vascular endothelial cell growth factor to stimulate vascular network formation in peri-lesional and lesional tissue. In parallel, cell replacement strategies have been carried out through the direct implantation of MPSCs, terminally differentiated cells (for example, dopaminergic neurons in Parkinson's models), or *in situ* reprogramming to promote the conversion of glial cells into neurons (Steinbeck and Studer, 2015; Li and Chen, 2016).

Search Strategy and Selection Criteria

Database used to indentify the most relevant papers included in this article: https://www.ncbi.nlm.nih.gov/pubmed/. 1) keywords for searching (selection criteria): Alzheimer's, Biomaterials, brain, hydrogels, ischemia, materials, polymers, neurogenesis, plasticity, remapping, neurological diseases, Parkinson's disease, stem cells, stroke; 2) Dates of searching: 2000–2019.

Hydrogel Biomaterials to Support Therapeutics

Although promising results have been achieved at the preclinical stage, there has been an undeniable lack of clinical translatability to treat central nervous system (CNS) disorders. Several factors might contribute to this discouraging scenario, such as inadequate animal models, reduced reproducibility among studies, heterogeneity and a lack of standardization of clinical procedures. Other possibilities include poor control of drugs/factors kinetics at effective doses after systemic/cerebral administration and low survival/engraftment of transplanted cells. Even assuming similarity of molecular and cellular pathways and targets between human and other mammalian species, the restrictive nature of the blood-brain barrier, the rate of drug degradation and activity decay, local drug concentration, number of donor cells and time required to achieve the desired benefit might be different between species. The use of polymeric materials to provide better control of drug/cell delivery improves classical pharmacological approaches; engineering and characterizing advanced materials and formats, analyzing their capacity to deliver different cells and compounds with precise control of release kinetics, and testing their therapeutic potential in animal models (**Figure 1**).

Biomaterials have been widely used for decades in many medical applications but their use for neurological diseases has been more restricted, probably due to the complexity of the CNS. Biomaterials for drug/cell delivery have been used in different formats, such as liposomes, nanoparticles, micelles, dendrimers and hydrogels. For medical use, biomaterials should be adaptable, biocompatible, non-inflammatory and biodegradable. In addition, they should not show toxic effects during the therapeutic use and subsequent degradation. Due to the small size (nano-scale) some biomaterial formats have been specifically employed as drug release systems for intra- and extra-cellular delivery of bioactive compounds including neuroprotective and neuroregenerative drugs/factors/recombinant proteins, DNA or small interfering RNAs. This nanometric format helps therapeutic compounds to cross the blood-brain barrier minimizing the usual fast degradation ascribed to classical approaches of drug systemic administration (Orive et al., 2009). Among the different biomaterial formats, hydrogels are very adequate for both, drug and cell delivery respectively. For example, in the context of cell-based therapies, different cells can be enclosed in the particular and adaptable three-dimensional (3D) hydrogel structure. In addition, hydrogels can be implanted directly in the brain as a pregel state for delayed *in situ* gelation, providing precision of graft location and number of implanted cells in cortical and subcortical structures.

Hydrogels can be produced by immersing a particular polymer or a blend of materials in aqueous solutions to produce an insoluble 3D gel state. The water content (> 90%) is adjustable as well as the gelation time and degradation. Because of the high water content and their physical and mechanical properties, hydrogels are very appropriate for

soft organs such as the brain. Different natural and artificial hydrogels have been used for cell/drug delivery applications into the brain, including hyaluronan, chitosan, collagen, silk fibroin, isopropylacrylamide, methylcellulose, alginate, Matrigel, poly-lactic-co-glycolic acid (PLGA) or polyethylene glycol (PEG) (Potjewyd et al., 2018). Most of these biomaterials can be formulated alone or in mixture at different ratios to improve their physical, chemical, mechanical and biological properties to increase their integration capacity with the host tissue by mimicking the extracellular matrix. It is remarkable the utility of hydrogels to increase cell survival and engraftment after transplantation in the CNS, given the hostility of injured and noninjured brain to transplanted cells (Collier et al., 1999; Ohtaki et al., 2008). The 3D hydrogel structure might foster therapeutic cells in a permissive environment where the entry of oxygen/nutrients and the output of waste products are guaranteed to assure the survival of grafted cells, at least during the therapeutic window of opportunity. In parallel, the porous hydrogel structure might create a restrictive barrier to distinct inflammatory cells that are present in the host microenvironment as consequence of injury. The mechanical properties of the hydrogel can be pre-defined by changing the polymer concentration and the cross-linking density (non-covalent and covalent interactions/bonds between chemical groups) to match the stiffness of brain tissue. The cross-linking influences the pore size and 3D structure, which might control the release of distinct biomolecules/factors with anti-inflammatory and anti-oxidant properties, secreted from the encapsulated cells. Some of these secreted factors may also stimulate specialized niches in the brain to enhance angiogenesis and neurogenesis leading to structural plasticity (i.e., axonal sprouting, dendritic branching, increasing spines turn over) that can support functional rewiring in damaged and non-damaged areas, thus promoting recovery. The particular hydrogel mesh structure might also act as an interface connecting the transplanted cells (i.e., neural stem cells and progenitors, dopaminergic neurons, or endothelial cells) with the host circuits creating new specialized neurovascular networks. The cross-linking density can be tuned to modify the permselectivity properties of hydrogels to specific uploaded drugs/biomolecules, controlling the releasing rate and thereby reducing the frequent administration of particular compounds with poor half-life due to degradation and decay of activity.

Hydrogels for Central Nervous System Disorders

In the context of stroke, different hydrogel biomaterials based on alginate, collagen, gelatin, chitosan, methylcellulose, hyaluronic acid and PLGA have been used as microcarriers for the release of angiogenic and neurotrophic factors such as epidermal growth factor, fibroblast growth factor-2, vascular endothelial cell growth factor, neurotrophin-3, erythropoietin, nogo-66 receptor antibodies, BDNF and ephrin A (Tian et al., 2005; Brown et al., 2009; Emerich et al., 2010; Overman et al., 2012; Wang et al., 2013; Hao et al.,

2017; Obermeyer et al., 2019). In general, the encapsulation of anti-inflammatory and neuroprotective molecules in different hydrogel formulations has been associated with better post-stroke functional outcome. For example, the immunosuppressant molecule cyclosporine-A has been delivered from PLGA dispersed on hyaluronan and methylcellulose hydrogels (Tuladhar et al., 2015). A sustained release of this drug in the brain was possible for at least 2–3 weeks. This delivery profile enhanced the survival, proliferation and migration of neural progenitor cells towards the peri-lesional tissue. When delivered from gelatin, osteopontin, another inflammatory modulator, reduced the infarction size of rats submitted to focal brain ischemia (Jin et al., 2014). Other study demonstrated the neuroprotective efficacy of ostepontin delivered from gelatin administrated via intranasal route, to bypass the blood-brain barrier, while no positive effect was noticed after the administration of free osteopontin (Joachim et al., 2014). After brain ischemia, reactive oxygen species (ROS) and free radicals produce oxidative stress that leads to irreversible damage to the salvaged penumbra (peri-lesional) exacerbating secondary damage (Chen et al., 2011). Different neuroprotective molecules with scavenging and anti-oxidant potential have been delivered through different biomaterial scaffolds. This situation applies for example to N-acetyl cysteine delivered from poly (amidoamine) dendrimers (Navath et al., 2008). In this case, N-acetyl cysteine attenuated the production of free radical nitric oxide from inflammatory microglia. Superoxide dismutase and catalase are scavenging enzymes with anti-oxidant properties. Superoxide dismutase converts superoxide anion in hydrogen peroxide and this latter can be detoxified by catalase and glutathione peroxidase producing oxygen and water. Because superoxide dismutase and catalase have very short half-life in circulation, both enzymes have been delivered from different biomaterials, for example PLGA, achieving better anti-oxidant stability and prolonged therapeutic effects *in vitro* and *in vivo* in stroke rodent models (Reddy and Labhasetwar, 2009). Another neuroprotective compound, thymoquinone, which has anti-oxidant properties by reducing ROS levels, is rapidly eliminated from plasma following systemic administration. PLGA and chitosan sustained the delivery of thymoquinone achieving better post-stroke functional outcome when this drug was administrated by intranasal route (Xiao et al., 2016). Nitrones, a family of ROS-trapping compounds have shown neuroprotective effects in neurodegenerative and cerebrovascular disorders including stroke. The half-life of nitrones is also relatively short (few hours), but it was possible to stabilize this anti-oxidant compound in chitosan-PEG (Pinarbasli et al., 2009). Edaravone, an anti-oxidant and anti-inflammatory drug is the unique neuroprotective compound approved for clinical use in stroke patients in Japan. Very recently, a proof of concept has been obtained with respect the anti-inflammatory benefit of releasing edaravone from composites of hyaluronan and chitosan (Tamer et al., 2018). In stroke, cell therapy strategies have been more effective after encapsulation of MPSCs in different hydrogel formulations. Most of these cell-hydrogel therapies reduced the

size of the lesion cavity, attenuated inflammation, promoted angiogenesis, increased neurogenesis, synaptogenesis or stimulated functional rewiring in peri-lesional and lesional areas, generally achieving functional recovery (Gonzalez-Nieto et al., 2018).

Similarly, drug and cell delivery approaches have been tested in Parkinson's models. A proof of concept for the sustained delivery of dopamine from dextran/gelatin or chitosan/gelatin hydrogels has been reported (Senthilkumar et al., 2007; Ren et al., 2017). For example, in hemiparkinsonism rats, dopamine was delivered over a period of 2 weeks from dextran/gelatin hydrogels leading to a significant behavioral improvement (Senthilkumar et al., 2007). Gelatin methacrylate hydrogels were also funcionalized with dopamine to enhance neuronal differentiation and support the formation of specialized neural networks (Zhou et al., 2018). The neuroprotective efficacy of activin-B delivered from N-isopropylacrylamide hydrogels has been tested in a Parkinson's mouse model. Because activin-B has a short half-life, this biomaterial prolonged the release of this compound in the brain promoting long-term protection of striatal dopaminergic fibers leading to better functional recovery (Li et al., 2016). Several neurotrophic factors such as BDNF, GDNF or epidermal growth factor alone or in combination with embryonic stem cell-derived dopaminergic neurons have been delivered from PLGA, polyethylene glycol, hyaluronan, xyloglucan or collagen hydrogels in the context of Parkinson's disease. For example, encapsulation of GDNF in hydrogels of collagen (Ucar and Humpel, 2019) or in composites of poly(l-lactic acid)/xyloglucan (Wang et al., 2016) enhanced dopaminergic cell survival and supported nerve fiber outgrowth and reinnervation of the striatum in a mouse model of Parkinson's disease (Wang et al., 2016). Dopaminergic axon guidance was stimulated by semaphorin 3C released from RADA hydrogels which also increased axonal length (Carballo-Molina et al., 2016). A very interesting application of hydrogels is the construction of axonal tracts for the nigrostriatal pathway (Clark et al., 2016; Struzyna et al., 2018). This strategy has been explored for example *in vivo* by encapsulating embryonic stem cell-derived dopaminergic neurons into agarose hydrogel columns formulated with extracellular matrix proteins such as collagen and laminin (Struzyna et al., 2018).

By contrast, research involving hydrogel-based therapies is much more limited in Alzheimer's disease. Peptide-amphiphile hydrogels have been used to release curcumin (Altunbas et al., 2011), an anti-oxidant and neuroprotective compound that induces amyloid-β deposits disaggregation and tau hyperphosphorylation, increasing its clearance and modulating microglia. Hydrogel formulations based on gellan gum and xanthan gum have been used to release resveratrol to favor the non-amyloidogenic cleavage of amyloid precursor protein enhancing amyloid-β clearance (Rajput et al., 2018). In the context of cell-based therapies, encapsulation of vascular endothelial cell growth factor-secreting fibroblast cells into alginate reduced amyloid-β deposition leading to an improvement of behavioral deficits in APP/PS1 Alzhei-

mer's disease mouse model (Spuch et al., 2010). In another example, the implantation of neural stem cells encapsulated in self-assembled RADA16 peptide hydrogels enhanced neural stem cell survival and engraftment, improving the learning and memory capacities of Alzheimer's disease rat model (Cui et al., 2016).

In addition to brain disorders, injectable hydrogels have been also used as scaffolds to repair traumatic spinal cord injury (SCI), filling the lesion cavity and providing a structural matrix to re-connect two nerve ends (Marchini et al., 2019). Because inhibitory signals avoid axonal tracts regrowth, hydrogels can be uploaded with stem cells and distinct biomolecules that can support the survival of grafted cells and help to organize/orient axonal growth, regeneration and plasticity in a permissive hydrogel-induced non-inflammatory microenvironment. By contrast, a material with increasing repercussion in the treatment of CNS disorders is graphene. This material has unique optical, thermal and mechanical properties and is considered to have good biocompatibility for nerve tissue engineering. A proof of concept of the strong integration and biocompatibility of graphene nanoscaffolds hydrogels has been obtained preclinically in SCI models (Palejwala et al., 2016). The delivery of several growth factors (BDNF/IGF-1) from implanted PLGA/graphene scaffolds promoted functional recovery after SCI (Pan et al., 2019). Additional evidences have supported the biocompatibility of graphene constructs with dopaminergic neurons for cell replacement strategies in Parkinson's disease (Tasnim et al., 2018). Another recent study has demonstrated the neuroprotective ability of this material to inhibit α-synuclein aggregation (Kim et al., 2018). However, only few *in vivo* studies have exploited the excellent properties of this carbon-based nanomaterial for cell/drug encapsulation in the context of neurodegeneration and cerebrovascular diseases (Menaa et al., 2015). Due to the presence of hydrophilic functional groups, graphene can be chemically funcionalized with a variety of molecules (peptides, drugs, ligands, antibodies). In addition, this material shows high electrical conductivity properties that might be exploited in combination with neurostimulation to enhance neural stem cell engraftment, axonal regrowth and networks rewiring in CNS disorders.

A Known Biomaterial to Drive Post-stroke Recovery

Our group has been pioneering in implanting silk fibroin hydrogels as an adjuvant to potentiate the therapeutic effect of stem cells. We fabricated silk fibroin hydrogels through the sonication-induced gelation of regenerated silk fibroin solutions. This natural and nonimmunogenic material was intracerebrally injected in a pregel state, achieving in situ gelation within a period of 10–15 minutes (Fernandez-Garcia et al., 2016). Several weeks after implantation, we did not observe marked cognitive, sensorial and motor deficits. Concurrently, the sleep wakefulness cycle and electroencephalogram activity dependent of behavioral contexts were within the normal range, supporting the biocompatibility of this material with
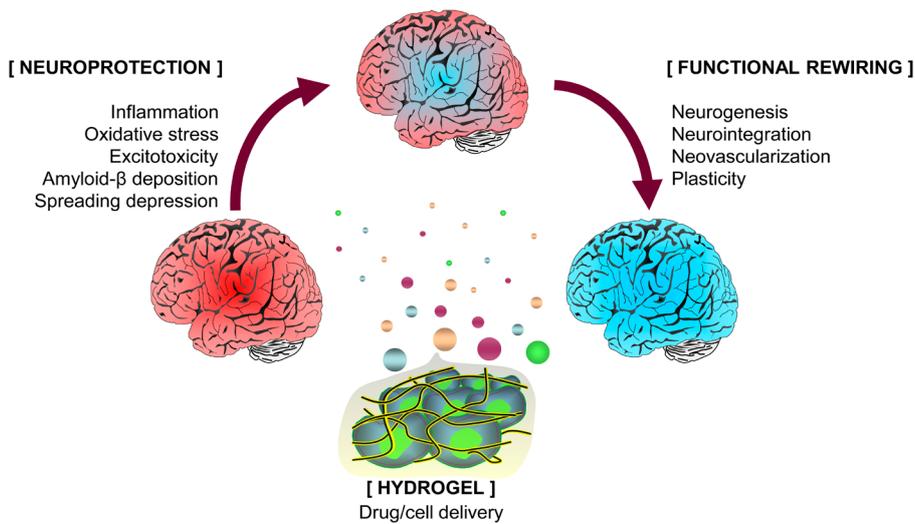
Figure 1 Hydrogel scaffolds for brain engineering.
Hydrogel-based therapeutics sustains drug delivery and support cell survival and engraftment after implantation. Similar to classical approaches, hydrogels target inflammatory, excitotoxicity and oxidative stress pathways to exert neuroprotection on the brain or mitigate pathological symptoms (for example, releasing dopamine for Parkinson's disease). Other approaches are focused on stimulating neurogenesis and angiogenesis, pursuing the re-establishment of brain circuitry, creating new networks or modifying the pre-existing ones through uncertain endogenous mechanisms of structural and functional rewiring.
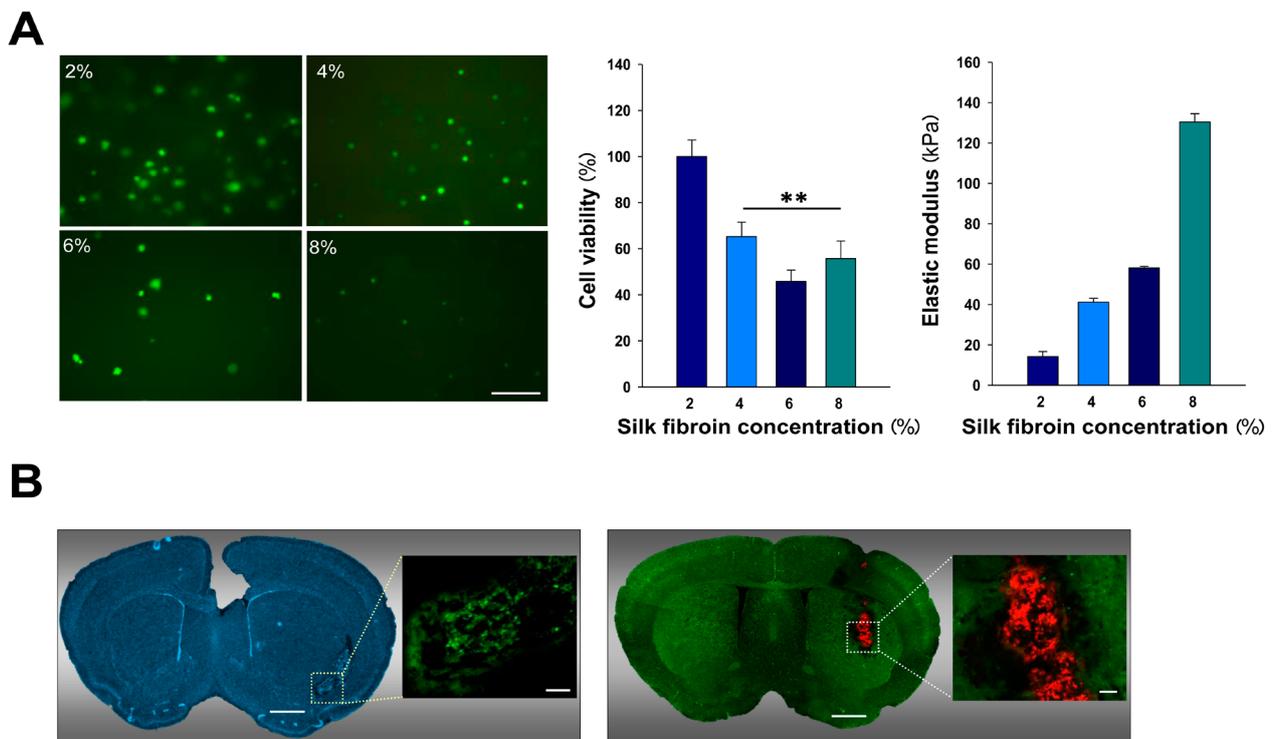
Figure 2 Viability of mesenchymal stem cells encapsulated in silk fibroin hydrogels.
(A) On the left, representative images of calcein-positive mesenchymal stem cells encapsulated in silk fibroin hydrogels of 2, 4, 6, and 8% polymer concentration (scale bar: 200 μm). In the middle, quantification of mesenchymal stem cell viability forty-eight hours after encapsulation in silk fibroin hydrogels of different concentration. Asterisks denote significant differences between 4–8% and 2% silk fibroin concentrations (data are shown as the mean ± the standard error of the mean with a minimal of 3 samples per each fibroin concentration, one-way analysis of variance test followed by Tukey's test; $P < 0.01$). Delayed gelation was induced using a single sonication pulse on silk fibroin solutions with a Branson 450 Sonifier coupled to a 3 mm diameter Tapered Microtip (parameters: sonication time 15 minutes; 15% amplitude; 40–45°C temperature). Note how cell survival is higher at lower hydrogel polymer concentrations, which show stiffness properties (right panel) close to the human brain (variable stiffness ranges have been reported for the rodent (25–50 kPa) and human (2–10 kPa) brains). (B) Images of coronal brain sections at high (scale bar: 1 mm) and low magnification (Scale bar: 100 μm) showing the location of mesenchymal stem cell populations encapsulated into 2% silk fibroin hydrogels twenty hours after implantation into the striatum of non-EGFP (left panel) and EGFP expressing mice (right panel). Previously to the implant, mesenchymal stem cell populations were loaded with CFSE (pseudocolored green, left panel) or Vybrant Dil (red, right panel) fluorochromes. In the left panel (non-EGFP), nuclei were labeled with DAPI (blue). Note in the right panel, EGFP is expressed throughout all brain regions although more intensely in cerebral vessels. CFSE: Carboxyfluorescein succinimidyl ester; DAPI: 4′,6-diamidino-2-phenylindole; EGFP: enhanced green fluoresent protein.

cerebral tissue. For several reasons this polymer concentration (2%) is very appropriate for cerebral applications. First, these hydrogels have elastic moduli that match the stiffness properties of brain tissue. Second, this specific concentration is optimal for cell survival because higher polymer concentrations lead to stiffer hydrogels with degrees of extreme confinement that compromise cellular viability (**Figure 2**). This biomaterial and polymer concentration enhanced the engraftment of mesenchymal stem cells implanted in the brain, promoting functional recovery after stroke (Fernandez-Garcia et al., 2018). The improvement was first associated with a reduction in extended damage and partial preservation of function in the affected cortical territory. This positive effect was probably due to the neuroprotective and anti-inflammatory properties of this stem cell population and their secretome capacity (Menaa et al., 2018; Martin-Martin et al., 2019). The anti-inflammatory and anti-oxidant properties of hydrogel-encapsulated MSC have been also exploited in other contexts, such is Parkinson's disease, using composites of collagen/PEG and collagen/hyaluronan hydrogels (Chierchia et al., 2017). In our study, several weeks after silk fibroin implantation, we could detect cortical reorganization events related to emerging sensory activity in peri-lesional motor areas. This emergent functional rewiring caused a significant improvement of sensorimotor dexterity, which had been initially impaired after cerebral infarction. This type of cortical reshaping between sensory/motor areas linked with recovery has been observed previously in stroke monkeys and humans after rehabilitative training (Nudo et al., 1996; Jaillard et al., 2005), although in our study, cortical remapping was favored by a cell therapy approach that resulted in more efficient after cell encapsulation in silk fibroin hydrogels.

Conclusion

The era of natural and synthetic biomaterials to potentiate the therapeutic effects of drugs and cells for brain disorders has only just begun. While we seek and exploit promising targets and develop/improve tools to validate the potential of different therapies at the molecular, cellular, structural and functional levels, we are racing to develop tailorable hydrogels of increasing sophistication in terms of composition, structure, mechanical properties and biocompatibility to rationally cross the preclinical/clinical transition. For drug/cell delivery in cerebral disorders, only a few biomaterials have reached the clinics, such as gliadel, a polyanhydride polymer created to deliver anti-tumoral compounds. However, there are no doubts regarding the opportunity that this area provides in developing new forms of sophisticated therapies to advance the treatment of brain disorders.

References

Altunbas A, Lee SJ, Rajasekaran SA, Schneider JP, Pochan DJ (2011) Encapsulation of curcumin in self-assembling peptide hydrogels as injectable drug delivery vehicles. Biomaterials 32:5906-5914.

Baez-Jurado E, Hidalgo-Lanussa O, Barrera-Bailon B, Sahebkar A, Ashraf GM, Echeverria V, Barreto GE (2019) Secretome of mesenchymal stem cells and its potential protective effects on brain pathologies. Mol Neurobiol doi: 10.1007/s12035-019-1570-x.

Bratane BT, Cui H, Cook DJ, Bouley J, Tymianski M, Fisher M (2011) Neuroprotection by freezing ischemic penumbra evolution without cerebral blood flow augmentation with a postsynaptic density-95 protein inhibitor. Stroke 42:3265-3270.

Brown CE, Aminoltejari K, Erb H, Winship IR, Murphy TH (2009) In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J Neurosci 29:1719-1734.

Carballo-Molina OA, Sanchez-Navarro A, Lopez-Ornelas A, Lara-Rodarte R, Salazar P, Campos-Romo A, Ramos-Mejia V, Velasco I (2016) Semaphorin 3C released from a biocompatible hydrogel guides and promotes axonal growth of rodent and human dopaminergic neurons. Tissue Eng Part A 22:850-861.

Chen H, Yoshioka H, Kim GS, Jung JE, Okami N, Sakata H, Maier CM, Narasimhan P, Goeders CE, Chan PH (2011) Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. Antioxid Redox Signal 14: 1505-1517.

Chierchia A, Chirico N, Boeri L, Raimondi I, Riva GA, Raimondi MT, Tunesi M, Giordano C, Forloni G, Albani D (2017) Secretome released from hydrogel-embedded adipose mesenchymal stem cells protects against the Parkinson's disease related toxin 6-hydroxydopamine. Eur J Pharm Biopharm 121:113-120.

Clark AR, Carter AB, Hager LE, Price EM (2016) In vivo neural tissue engineering: cylindrical biocompatible hydrogels that create new neural tracts in the adult mammalian brain. Stem Cells Dev 25:1109-1118.

Collier TJ, Sortwell CE, Daley BF (1999) Diminished viability, growth, and behavioral efficacy of fetal dopamine neuron grafts in aging rats with long-term dopamine depletion: an argument for neurotrophic supplementation. J Neurosci 19:5563-5573.

Congdon EE, Sigurdsson EM (2018) Tau-targeting therapies for Alzheimer disease. Nat Rev Neurol 14:399-415.

Cui GH, Shao SJ, Yang JJ, Liu JR, Guo HD (2016) Designer self-assemble peptides maximize the therapeutic benefits of neural stem cell transplantation for Alzheimer's disease via enhancing neuron differentiation and paracrine action. Mol Neurobiol 53:1108-1123.

Egan MF, Kost J, Tariot PN, Aisen PS, Cummings JL, Vellas B, Sur C, Mukai Y, Voss T, Furtek C, Mahoney E, Harper Mozley L, Vandenberghe R, Mo Y, Michelson D (2018) Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. N Engl J Med 378:1691-1703.

Emerich DF, Silva E, Ali O, Mooney D, Bell W, Yu SJ, Kaneko Y, Borlongan C (2010) Injectable VEGF hydrogels produce near complete neurological and anatomical protection following cerebral ischemia in rats. Cell Transplant 19:1063-1071.

Fernandez-Garcia L, Mari-Buye N, Barios JA, Madurga R, Elices M, Perez-Rigueiro J, Ramos M, Guinea GV, Gonzalez-Nieto D (2016) Safety and tolerability of silk fibroin hydrogels implanted into the mouse brain. Acta Biomater 45:262-275.

Fernandez-Garcia L, Perez-Rigueiro J, Martinez-Murillo R, Panetsos F, Ramos M, Guinea GV, Gonzalez-Nieto D (2018) Cortical reshaping and functional recovery induced by silk fibroin hydrogels-encapsulated stem cells implanted in stroke animals. Front Cell Neurosci 12:296.

Gonzalez-Nieto D, Fernández-García L, Pérez-Rigueiro J, Guinea GV, Panetsos F (2018) Hydrogels-assisted cell engraftment for repairing the stroke-damaged brain: chimera or reality. Polymers 10:184.

Hao P, Duan H, Hao F, Chen L, Sun M, Fan KS, Sun YE, Williams D, Yang Z, Li X (2017) Neural repair by NT3-chitosan via enhancement of endogenous neurogenesis after adult focal aspiration brain injury. Biomaterials 140:88-102.

Jaillard A, Martin CD, Garambois K, Lebas JF, Hommel M (2005) Vicarious function within the human primary motor cortex? A longitudinal fMRI stroke study. Brain 128:1122-1138

Jin Y, Kim IY, Kim ID, Lee HK, Park JY, Han PL, Kim KK, Choi H, Lee JK (2014) Biodegradable gelatin microspheres enhance the neuroprotective potency of osteopontin via quick and sustained release in the post-ischemic brain. Acta Biomater 10:3126-3135.

Joachim E, Kim ID, Jin Y, Kim KK, Lee JK, Choi H (2014) Gelatin nanoparticles enhance the neuroprotective effects of intranasally administered osteopontin in rat ischemic stroke model. Drug Deliv Transl Res 4:395-399.

Kim D, Yoo JM, Hwang H, Lee J, Lee SH, Yun SP, Park MJ, Lee M, Choi S, Kwon SH, Lee S, Kim S, Park YJ, Kinoshita M, Lee YH, Shin S, Paik SR, Lee SJ, Hong BH, Ko HS (2018) Graphene quantum dots prevent alpha-synucleinopathy in Parkinson's disease. Nat Nanotechnol 13:812-818.

Li H, Chen G (2016) In vivo reprogramming for CNS repair: regenerating neurons from endogenous glial cells. Neuron 91:728-738.

Li J, Darabi M, Gu J, Shi J, Xue J, Huang L, Liu Y, Zhang L, Liu N, Zhong W, Xing M (2016) A drug delivery hydrogel system based on activin B for Parkinson's disease. Biomaterials 102:72-86.

Louveau A, Da Mesquita S, Kipnis J (2016) Lymphatics in neurological disorders: A neuro-lympho-vascular component of multiple sclerosis and Alzheimer's disease? Neuron 91:957-973

Marchini A, Raspa A, Pugliese R, El Malek MA, Pastori V, Lecchi M, Vescovi AL, Gelain F (2019) Multifunctionalized hydrogels foster hNSC maturation in 3D cultures and neural regeneration in spinal cord injuries. Proc Natl Acad Sci U S A 116:7483-7492

Marshall JW, Cummings RM, Bowes LJ, Ridley RM, Green AR (2003) Functional and histological evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 hours after occlusion. Stroke 34:2228-2233.

Martin-Martin Y, Fernandez-Garcia L, Sanchez-Rebato MH, Mari-Buye N, Rojo FJ, Perez-Rigueiro J, Ramos M, Guinea GV, Panetsos F, Gonzalez-Nieto D (2019) Evaluation of neurosecretome from mesenchymal stem cells encapsulated in silk fibroin hydrogels. Sci Rep 9:8801.

Menaa F, Abdelghani A, Menaa B (2015) Graphene nanomaterials as biocompatible and conductive scaffolds for stem cells: impact for tissue engineering and regenerative medicine. J Tissue Eng Regen Med 9:1321-1338.

Menaa F, Shahrokhi S, Prasad Shastri V (2018) Impact and challenges of mesenchymal stem cells in medicine: An overview of the current knowledge. Stem Cells Int 2018: 5023925.

Milosevic L, Kalia SK, Hodaie M, Lozano AM, Fasano A, Popovic MR, Hutchison WD (2018) Neuronal inhibition and synaptic plasticity of basal ganglia neurons in Parkinson's disease. Brain 141:177-190.

Moreno-Jimenez EP, Flor-Garcia M, Terreros-Roncal J, Rabano A, Cafini F, Pallas-Bazarra N, Avila J, Llorens-Martin M (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med 25:554-560.

Navath RS, Kurtoglu YE, Wang B, Kannan S, Romero R, Kannan RM (2008) Dendrimer-drug conjugates for tailored intracellular drug release based on glutathione levels. Bioconjug Chem 19:2446-2455.

Nudo RJ, Wise BM, SiFuentes F, Milliken GW (1996) Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. Science 272: 1791-1794.

Obermeyer JM, Tuladhar A, Payne SL, Ho E, Morshead CM, Shoichet MS (2019) Local delivery of brain-derived neurotrophic factor enables behavioral recovery and tissue repair in stroke-injured rats. Tissue Eng Part A 25:1175-1187.

Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, Prockop DJ (2008) Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci U S A 105:14638-14643.

Orive G, Anitua E, Pedraz JL, Emerich DF (2009) Biomaterials for promoting brain protection, repair and regeneration. Nat Rev Neurosci 10:682-692.

Overman JJ, Clarkson AN, Wanner IB, Overman WT, Eckstein I, Maguire JL, Dinov ID, Toga AW, Carmichael ST (2012) A role for ephrin-A5 in axonal sprouting, recovery, and activity-dependent plasticity after stroke. Proc Natl Acad Sci U S A 109:E2230-2239.

Palejwala AH, Fridley JS, Mata JA, Samuel EL, Luerssen TG, Perlaky L, Kent TA, Tour JM, Jea A (2016) Biocompatibility of reduced graphene oxide nanoscaffolds following acute spinal cord injury in rats. Surg Neurol Int 7:75.

Pan S, Qi Z, Li Q, Ma Y, Fu C, Zheng S, Kong W, Liu Q, Yang X (2019) Graphene oxide-PLGA hybrid nanofibres for the local delivery of IGF-1 and BDNF in spinal cord repair. Artif Cells Nanomed Biotechnol 47:651-664.

Pinarbasli O, Aktas Y, Dalkara T, Andrieux K, Alonso MJ, Fernandez-Megia E, Novoa-Carballal R, Riguera R, Couvreur P, Capan Y (2009) Preparation and evaluation of alpha-phenyl-n-tert-butyl nitrone (PBN)-encapsulated chitosan and PEGylated chitosan nanoparticles. Pharmazie 64:436-439.

Potjewyd G, Moxon S, Wang T, Domingos M, Hooper NM (2018) Tissue engineering 3D neurovascular units: A biomaterials and bioprinting perspective. Trends Biotechnol 36:457-472.

Purro SA, Farrow MA, Linehan J, Nazari T, Thomas DX, Chen Z, Mengel D, Saito T, Saido T, Rudge P, Brandner S, Walsh DM, Collinge J (2018) Transmission of amyloid-beta protein pathology from cadaveric pituitary growth hormone. Nature 564:415-419.

Rajput A, Bariya A, Allam A, Othman S, Butani SB (2018) In situ nanostructured hydrogel of resveratrol for brain targeting: in vitro-in vivo characterization. Drug Deliv Transl Res 8:1460-1470.

Reddy MK, Labhasetwar V (2009) Nanoparticle-mediated delivery of superoxide dismutase to the brain: an effective strategy to reduce ischemia-reperfusion injury. FASEB J 23:1384-1395.

Ren Y, Zhao X, Liang X, Ma PX, Guo B (2017) Injectable hydrogel based on quaternized chitosan, gelatin and dopamine as localized drug delivery system to treat Parkinson's disease. Int J Biol Macromol 105:1079-1087.

Rothwell N (2003) Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. Brain Behav Immun 17:152-157.

Sakowitz OW, Kiening KL, Krajewski KL, Sarrafzadeh AS, Fabricius M, Strong AJ, Unterberg AW, Dreier JP (2009) Preliminary evidence that ketamine inhibits spreading depolarizations in acute human brain injury. Stroke 40:e519-522.

Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, Albers GW, Cognard C, Cohen DJ, Hacke W, Jansen O, Jovin TG, Mattle HP, Nogueira RG, Siddiqui AH, Yavagal DR, Baxter BW, Devlin TG, Lopes DK, Reddy VK, du Mesnil de Rochemont R, Singer OC, Jahan R (2015) Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. N Engl J Med 372:2285-2295.

Senthilkumar KS, Saravanan KS, Chandra G, Sindhu KM, Jayakrishnan A, Mohanakumar KP (2007) Unilateral implantation of dopamine-loaded biodegradable hydrogel in the striatum attenuates motor abnormalities in the 6-hydroxydopamine model of hemi-parkinsonism. Behav Brain Res 184:11-18.

Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, James D, Mayer S, Chang J, Auguste KI, Chang EF, Gutierrez AJ, Kriegstein AR, Mathern GW, Oldham MC, Huang EJ, Garcia-Verdugo JM, Yang Z, Alvarez-Buylla A (2018) Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 555:377-381.

Spuch C, Antequera D, Portero A, Orive G, Hernandez RM, Molina JA, Bermejo-Pareja F, Pedraz JL, Carro E (2010) The effect of encapsulated VEGF-secreting cells on brain amyloid load and behavioral impairment in a mouse model of Alzheimer's disease. Biomaterials 31:5608-5618.

Steinbeck JA, Studer L (2015) Moving stem cells to the clinic: potential and limitations for brain repair. Neuron 86:187-206

Struzyna LA, Browne KD, Brodnik ZD, Burrell JC, Harris JP, Chen HI, Wolf JA, Panzer KV, Lim J, Duda JE, Espana RA, Cullen DK (2018) Tissue engineered nigrostriatal pathway for treatment of Parkinson's disease. J Tissue Eng Regen Med 12:1702-1716.

Tamer TM, Valachova K, Hassan MA, Omer AM, El-Shafeey M, Mohy Eldin MS, Soltes L (2018) Chitosan/hyaluronan/edaravone membranes for anti-inflammatory wound dressing: In vitro and in vivo evaluation studies. Mater Sci Eng C Mater Biol Appl 90:227-235.

Tasnim N, Thakur V, Chattopadhyay M, Joddar B (2018) The Efficacy of Graphene Foams for culturing mesenchymal stem cells and their differentiation into dopaminergic neurons. Stem Cells Int 2018:3410168.

Tian WM, Zhang CL, Hou SP, Yu X, Cui FZ, Xu QY, Sheng SL, Cui H, Li HD (2005) Hyaluronic acid hydrogel as Nogo-66 receptor antibody delivery system for the repairing of injured rat brain: in vitro. J Control Release 102:13-22.

Tuladhar A, Morshead CM, Shoichet MS (2015) Circumventing the blood-brain barrier: Local delivery of cyclosporin A stimulates stem cells in stroke-injured rat brain. J Control Release 215:1-11.

Ucar B, Humpel C (2019) Therapeutic efficacy of glial cell-derived neurotrophic factor loaded collagen scaffolds in ex vivo organotypic brain slice Parkinson's disease models. Brain Res Bull 149:86-95.

Wang TY, Bruggeman KF, Kauhausen JA, Rodriguez AL, Nisbet DR, Parish CL (2016) Functionalized composite scaffolds improve the engraftment of transplanted dopaminergic progenitors in a mouse model of Parkinson's disease. Biomaterials 74:89-98.

Wang Y, Cooke MJ, Sachewsky N, Morshead CM, Shoichet MS (2013) Bioengineered sequential growth factor delivery stimulates brain tissue regeneration after stroke. J Control Release 172:1-11.

Xiao XY, Zhu YX, Bu JY, Li GW, Zhou JH, Zhou SP (2016) Evaluation of neuroprotective effect of thymoquinone nanoformulation in the rodent cerebral ischemia-reperfusion model. Biomed Res Int 2016:2571060.

Zhou X, Cui H, Nowicki M, Miao S, Lee SJ, Masood F, Harris BT, Zhang LG (2018) Three-dimensional-bioprinted dopamine-based matrix for promoting neural regeneration. ACS Appl Mater Interfaces 10:8993-9001.